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Study protocol for a double-blind, randomised trial of low-dose naltrexone for post-COVID fatigue syndrome

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Abbreviations

ACTH	Adrenocorticotropic hormone
AE	Adverse events
BC	British Columbia
CCDP	Complex Chronic Diseases Program
CK	Creatinine kinase
CRP	C-reactive protein
C&W	Children's and Women's Hospital and Health Centre of British Columbia
DSMB	Data Safety and Monitoring Board
EQ-5D	EuroQol 5 dimensions
FDA	Food and drug administration
FM	Fibromyalgia
FSS	Fatigue severity scale
GAD-7	Generalized anxiety disorder-7
HC	Health Canada
HRQOL	Health-related quality of life
IL-6	Interleukin-6
IFNγ	Interferon-gamma
IOM	Institute of Medicine
LDN	Low dose naltrexone
MCID	Minimal clinically important difference
ME/CFS	Myalgic encephalomyelitis/ chronic fatigue syndrome
MHRA	Medicines and Healthcare products Regulatory Agency
NINDS CDE	National Institute of Neurological Disorders and Stroke Common Data Elements
PCC	Post-COVID-19 condition
PC-COS	Post-COVID-19 core outcome set
PCFS	Post-COVID fatigue syndrome
PC-ICCN	Post-COVID-19 Interdisciplinary Clinical Care Network
PCP	Primary care provider
PCR	Polymerase chain reaction
PHQ-9	Patient health questionnaire-9
POTS	Postural orthostatic tachycardia syndrome
PQSymp-12	Patient phenotyping questionnaire short form
PROMIS	Patient Records and Outcome Management Information System
PROMs	Patient reported outcome measures
PVFS	Post-viral fatigue syndrome
RAT	Rapid antigen test
RCT	Randomized controlled trial
REB	Research ethics board
rT3	Reverse triiodothyronine
SAE	Serious adverse events
SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials
SQ-2	Sleep questionnaire-2
T3	Triiodothyronine
T4	Thyroxine
TNF	Tumour necrosis factor
TSC	Trial Steering Committee
TSH	Thyroid stimulating hormone
UBC	University of British Columbia
VAS	Visual analogue scale
WHRI	Women's Health Research Institute

ABSTRACT

Introduction: A significant proportion of individuals suffering from post-COVID-19 condition (PCC, also known as *long COVID*) can present with persistent, disabling fatigue similar to myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS) and post-viral fatigue syndromes. There remains no clear pharmacologic therapy for patients with this subtype of PCC, which can be referred to as post-COVID fatigue syndrome (PCFS). A low dose of the opioid antagonist naltrexone (i.e., low dose naltrexone, LDN) has emerged as an off-label treatment for treating fatigue and other symptoms in PCC. However, only small, non-controlled studies have assessed LDN in PCC, so randomised trials are urgently required.

Methods and analysis: A prospective, randomised, double-blind, parallel-arm, placebo-controlled phase II trial will be performed to assess the efficacy of LDN for improving fatigue in PCFS. The trial will be decentralized and open to eligible individuals throughout the Canadian province of British Columbia (BC). Participants will be recruited through the province-wide Post-COVID-19 Interdisciplinary Clinical Care Network (PC-ICCN) and research volunteer platform (REACH BC). Eligible participants will be 19-69 years-old, have had a confirmed or physician-suspected SARS-CoV-2 infection at least 3 months prior, and meet clinical criteria for PCFS adapted from the Institute of Medicine ME/CFS criteria. Individuals who are taking opioid medications, have a history of ME/CFS prior to COVID-19 or history of significant liver disease will be excluded. Participants will be randomised to an LDN intervention arm (n=80) or placebo arm (n=80). Participants in each arm will be prescribed identical capsules starting at 1mg daily and follow a prespecified schedule for up titration to 4.5mg daily or the maximum tolerated dose. The trial will be conducted over 16 weeks, with assessments at baseline, 6, 12, and 16 weeks. The primary outcome will be fatigue severity at 16 weeks evaluated by the fatigue severity scale. Secondary outcomes will include pain visual analogue scale score, overall symptom severity as measured by the Patient Phenotyping Questionnaire Short Form, 7-day step count, and health-related-quality of life measured by the EuroQol 5-dimension questionnaire.

Ethics and dissemination: The trial has been authorized by Health Canada and approved by The University of British Columbia / Children's and Women's Health Centre of British Columbia Research Ethics Board. Upon completion, findings will be disseminated to patients, caregivers, and clinicians through engagement activities within existing PCC and ME/CFS networks. Results will be published in academic journals and presented at conferences.

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Strengths and limitations of this study

- This is one of the first randomised, placebo-controlled trials that investigate low dose naltrexone (LDN) in adults with post-COVID-19 condition (PCC), an illness that has afflicted millions of individuals, has disabling symptoms, but no widely recognized pharmacological treatment.
- The trial will be decentralized and will recruit participants from throughout the geographically large and ethnically diverse Canadian province of British Columbia (BC). This will permit inclusion of patients from communities that do not typically have access to investigational treatments, and patients who may be too symptomatic to attend in-person assessments.
- In addition to evaluating fatigue severity as the primary outcome, the study will capture several secondary outcome measures known to be important to PCC patients, including pain, overall symptom burden, health-related quality of life, and activity levels (as measured by step count).
- The study does not have a restriction on how long a participant may have had their symptoms since COVID-19. This may limit the treatment effect if LDN efficacy is greater earlier in the disease course.
- As in-person evaluation is optional, this limits the ability to assess potentially important objective outcomes in some participants.

INTRODUCTION

Background and Rationale

Approximately 15-20% of adults with a confirmed or suspected SARS-CoV-2 infection experience long-term symptoms lasting over 3 months.[1–4] The presence of new or persistent symptoms following acute COVID-19 disease is now referred to as post-COVID-19 condition (PCC), or 'long-COVID'.[5–11] Among the hundreds of symptoms reported by PCC patients, fatigue is one of the most common and may have the greatest impact on functioning.[12–21] Given that millions of individuals may be currently affected by PCC worldwide, it has become a priority to investigate potential treatments in randomized controlled trials (RCTs).[5,22]

However, it has been challenging to identify candidate treatments for PCC as it is a heterogeneous illness, and the underlying pathobiology is poorly understood. It is suspected that different groups of PCC patients may have distinct underlying disease processes, such that the ideal pharmacologic therapy may not be the same for all.[5,22] Increasingly, studies have suggested that PCC may not represent a single disease, but rather a collection of different conditions or subtypes.[23–25]

For example, clinical experience and patient-centred studies have indicated that a proportion of PCC patients present with a symptom profile indistinguishable from myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS).[26–31] ME/CFS is characterized by persistent disabling fatigue accompanied by other symptoms including nonrestorative sleep and post-exertional malaise.[32–34] While the precise pathogenesis of ME/CFS also remains unresolved, it usually follows acute infections.[35] When provoked by a viral infection, ME/CFS is often referred to as a post-viral fatigue syndrome (PVFS).[35–38] It is believed that some PCC patients have developed a PVFS from SARS-CoV-2, and we will refer to this subset of PCC patients as having 'post-COVID fatigue syndrome' (PCFS).[36–39]

A low dose of the medication naltrexone is a potential treatment for PCFS.[40,41] Naltrexone is an opiate antagonist that is approved for treatment for alcohol and opiate use disorders.[42] For these indications, it is typically prescribed at 25-50 mg.[43] At lower doses (≤5 mg) it has been used off-label for chronic pain, multiple sclerosis, Crohn's disease, recurrent depression, fibromyalgia (FM) and ME/CFS.[44–58] Although evidence supporting the use of low dose naltrexone (LDN) in ME/CFS has been limited to case series and chart reviews,[47,55] it has been investigated in clinical trials for related conditions such as

fibromyalgia.[51,52,58,59] In these and other studies, LDN has been shown to be safe with a limited side-effect profile.[50,52–54,58]

Based on its hypothesized mechanism of action, it is plausible that LDN could be efficacious for ME/CFS and PCFS. LDN increases circulation of the endogenously produced opiate-like molecule beta-endorphin, which is reduced in ME/CFS.[60,61] Furthermore, LDN has been found to antagonize toll-like receptors on neuroglia and peripheral blood mononuclear cells, resulting in reduced production of inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor (TNF).[62–65] Increased IL-6 and TNF signalling have been implicated in PCC,[66] and studies have implicated increased neuroinflammation in ME/CFS and PCC pathogenesis.[18,67–70]

There is ongoing public interest in investigating LDN for PCC. Media outlets including *Rolling Stone, National Geographic*, *Reuters,* and *The New York Times Magazine* have all touted LDN as a potential PCC treatment, citing the anecdotal experiences of PCC patients and physicians who have used LDN.[71–78] However, published evidence for LDN in the post-COVID-19 context remains limited. In a single-centre study, 52 patients treated with LDN had, on average, overall improvement in activities of daily living, energy levels, pain, concentration, and sleep disturbance.[41] In a retrospective study, 37 of 59 (62.7%) patients treated with LDN reported improvement in at least one symptom.[79]

RCTs are required to determine whether LDN is an effective treatment for post-COVID-19 symptoms. Since there is no widely accepted pharmacologic treatment for PCFS, the ideal comparator group is a placebo. Accordingly, we have designed a double-blinded placebo-controlled trial of daily LDN vs. placebo for the treatment of fatigue severity in PCFS.

Objectives

Study objectives are outlined in **Table 1**. The primary objective is to determine whether LDN can reduce fatigue severity associated with PCFS, as measured by the fatigue severity scale (FSS). The secondary objectives are to determine whether it can reduce pain, reduce symptom severity, improve health-related quality of life (HRQOL) and increase activity levels. We have developed additional exploratory objectives that examine other patient reported outcome measures (PROMs), laboratory outcomes, and physical measurements.

Table 1: Summary of study objectives and associated outcomes

Primary objective		***Primary outcome***
To determine if LDN, administered at 1-4.5 mg/day to individuals with PCFS, reduces fatigue severity.		FSS score at 16 weeks.
Secondary objectives		***Secondary outcomes***
To determine if LDN, administered at 1-4.5 mg/day to individuals with PCFS:	Reduces pain.	Pain VAS score at 16 weeks.
	Improves severity of symptoms associated with PCFS.	PQSymp-12 score at 16 weeks.
	Increases activity levels.	Average number of steps over 7 days at 16 weeks.
	Improves self-reported quality of life.	EQ-5D-5L health utility score at 16 weeks.
Exploratory objectives		***Exploratory outcomes***
To determine if LDN, administered at 1-4.5 mg/day to individuals with PCFS:	Reduces inflammatory marker values in peripheral blood.	IL-6, IFNγ, and CRP values at 16 weeks. Cytokine profile values using Human Cytokine Proinflammatory Focused 15-plex Discovery Assay® Array at 16 weeks.
	Improves disease severity associated with PCFS.[80]	CK level at 16 weeks.
	Improves rT3 as an indirect marker of disease severity.[81]	rT3 in conjunction with TSH, free T3 and free T4 at 16 weeks.
	Improves AM blood cortisol and improves ACTH.	AM blood cortisol level at 16 weeks. ACTH level at 16 weeks.
	Reduces fatigue VAS score.	Fatigue VAS score at 16 weeks.
	Improves sleep.	SQ-2 at 16 weeks. Seep VAS score at 16 weeks.
	Improves depression symptoms.	PHQ-9 score at 16 weeks.
	Improves anxiety symptoms.	GAD-7 score at 16 weeks.
	Improves self-reported health.	Self-reported health VAS score at 16 weeks.
	Improves functional status.	Post-COVID-19 Functional Status Scale at 16 weeks.
	Reduces prevalence markers of POTS or postural hypotension*	Prevalence of POTS or postural hypotension symptoms based on serial blood pressure and heart rate measurements at 16 weeks.
	Improves clinical endurance/ strength parameters in subjects with PCFS*	Hand grip strength at 16 weeks.[82] Sit and stand test results at 16 weeks.

Legend. Abbreviations: ACTH- adrenocorticotropic hormone; CRP- c-reactive protein; EQ-5D-5L EuroQol 5 Dimension 5-level; FSS- fatigue severity scale; LDN- low dose naltrexone; PQSymp-12- Patient Phenotyping Questionnaire Short Form-12; PCFS- post-COVID fatigue syndrome; rT3- reverse triiodothyronine; SQ-2- sleep questionnaire-2; T3- triiodothyronine; T4-thyroxine; TSH- thyroid stimulating hormone; VAS- visual analogue scale. *Optional in person visits.

Trial Design

The development of this trial protocol followed the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines.[83] This trial is designed as a randomised, controlled, double-blind prospective trial with two parallel groups and primary end point of fatigue severity at 16 weeks. The intervention group will receive LDN capsules dosed at 1.0mg to 4.5mg daily and the control group will receive placebo capsules. Randomisation will be stratified by sex and performed as permuted block randomisation with a 1:1 allocation. The trial will be conducted in British Columbia (BC), Canada.

METHODS AND ANALYSIS

Study Setting

The trial will involve a collaboration between BC's Post-COVID-19 Interdisciplinary Clinical Care Network (PC-ICCN) and the Complex Chronic Diseases Program (CCDP) at BC Children's and Women's Hospital and Health Centre (C&W) located in Vancouver. The PC-ICCN was founded as a learning health system for post-COVID-19 care and research in BC.[84–87] The network previously comprised of 5 physical Post-COVID Recovery Clinics (PCRCs) but has now consolidated to a single virtual program. Adults throughout BC may be referred to this program by their primary care provider (PCP) if they have had COVID-19 and meet criteria for PCC. The CCDP is an interdisciplinary program that supports patients with ME/CFS and related conditions.[88]

Eligible participants will be recruited from throughout BC. Participants will have virtual or in-person study visits, may have their study product and pedometer delivered to them, complete questionnaires electronically, and have blood tests done at their local laboratories. Collection of exploratory data during in-person visits will be optional.

Eligibility Criteria

Inclusion and exclusion criteria are listed in **Table 2**. To be included, participants must be aged 19 to 69 years, have significant fatigue and related symptoms that started after a SARS-CoV-2 infection and meet the criteria we have developed for PCFS. These criteria are adapted from the Institute of Medicine (IOM) ME/CFS standard clinical criteria,[34] but with duration of symptoms of 3 months rather than

6 months to be consistent with PCC definitions (**Box 1**).[11] Diagnosis for eligibility purposes will be determined from clinical assessment by a study physician and supported by laboratory data and responses to the screening and baseline questionnaires. Participants who do not have a documented positive polymerase chain reaction (PCR) test will be eligible if they are determined by a physician through medical history to have had a positive rapid antigen test (RAT) or compatible symptoms following close contact with individuals who tested positive. Individuals will be excluded if they have a history of ME/CFS prior to SARS-CoV-2 infection, have significant liver disease, have taken naltrexone within 30 days, or if they have taken opioids within 15 days.

Table 2: Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
1. Male and female patients ages 19 to less than 70 years. 2. Case of SARS-CoV-2 over 3 months previously, confirmed by a positive test result or clinical confirmation by a physician. 3. Meet the clinical diagnostic criteria for PCFS (**Table 3**). 4. Agree to maintain any other regular medications at current doses for the duration of the trial (except for essential need of new medication or dose change, as prescribed by a physician). 5. Agree to use effective contraception for the trial duration, as appropriate (if female). 6. The participant resides within the delivery area for the drug as determined by FedEx Clinical Trial Services.	1. Pregnant, planning to become pregnant, or breastfeeding. 2. Use of opioid medications within last 15 days, as reported by the patient or during the trial. 3. A positive urine test for opioids (only for the first 16 participants; see below). 4. History of alcohol, opioid or other substance misuse. 5. Participation in another interventional clinical trial in the last 30 days or planned during the trial period. 6. Confirmed ME/CFS or FM existing prior to SARS-CoV-2 infection. 7. Allergy to naltrexone or medication components. 8. Acute hepatitis, liver failure, or severe kidney failure. 9. Current or recent use of naltrexone within 30 days.

Legend. Abbreviations: PCFS- post-COVID fatigue syndrome; ME/CFS- myalgic encephalomyelitis/ chronic fatigue syndrome; FM- fibromyalgia.

Box 1: Study diagnostic criteria of post-COVID fatigue syndrome

Diagnosis requires that the patient have the following 3 symptoms following a SARS-CoV-2 infection:
1. **A substantial reduction or impairment in the ability to engage in pre-illness levels of activity** (occupational, educational, social, or personal life) that: 1. lasts for more than 3 months. 2. is accompanied by fatigue that is: 1. often profound 2. of new onset (not life-long) 3. not the result of ongoing or unusual excessive exertion 4. not substantially alleviated by rest 2. **Post-exertional malaise** 3. **Unrefreshing sleep**
At least 1 of the 2 manifestations must be present:
1. **Cognitive impairment** 2. **Orthostatic intolerance**
AND
Absence of other diseases or conditions that explain symptoms, based on differential diagnosis

Legend. For this study, we have developed criteria for post-COVID fatigue syndrome, a form of post-COVID-19 condition which presents with symptoms similar to an myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS) following a SARS-CoV-2 infection. The criteria are based on the IOM for ME/CFS but requires 3 months of symptoms as opposed to 6.[34]

Interventions

Eligible participants will be randomized at a ratio of 1:1 (n=80 each) into either active treatment group with LDN or placebo. The treatment duration is 16 weeks. The LDN and placebo will be compounded by Macdonald's Prescriptions Labs Ltd. (Vancouver, BC) and dispensed at the C&W Pharmacy where the blinding will occur.

Macdonald's Prescriptions Labs Ltd. will compound the required doses of LDN from Naltrexone Hydrochloride USP supplied by MEDISCA in empty gelatin CONI-SNAP capsules.[89] The compounded LDN capsules will be filled with CELLULOSE, NF/EP (Microcrystalline) (Flocel® 101).[90] Placebo capsules will look identical to the compounded LDN capsules and filled with the same diluent and food colouring. We will complete batch testing of the LDN and placebo compounds (**Appendix 1**).

The dose-titration schedule from 1 mg to 4.5 mg is outlined in **Table 4**. The drug will be dispensed to participants by certified courier, temperature-controlled shipping, in-person pick-up, or delivered by staff. Participants will be able to adjust treatment doses by reverting to the previous well-tolerated dose if they experience persistent but minor side effects following any increase in dose. If a participant has reverted to a previous dose, that dosage will be maintained for the remainder of the study

period. Changes in doses will be documented by the participant by completing a daily dosing diary, completed for the first 4 weeks and for 7 days after any change in dose.

By allowing participants to reduce doses if experiencing any potential side effects, we expect low rates of medication use interruption. In addition to diaries, participants may also have visits or contact with the study team where adherence can be discussed. Furthermore, there are treatment compliance questions asked with each series of questionnaires. The participants will be asked to return the unused study drug, empty containers, and study drug diary sheet(s).

Participants will be asked to maintain any other regular medications at their current doses for the duration of the trial, unless there is an essential need of a new medication or dose change. Participants can withdraw from the study at any time without giving reasons. Withdrawal criteria are described in **Box S1.**

Table 3: Product supply timeline and titration schedule

Week (s)	Supply	Dose	Capsules
1	First	1 mg/day	*1 mg cap*
2	First	2 mg/day	*Two 1 mg capsule*
3	First	3 mg/day	*Three 1mg capsule*
4-6	First	4.5 mg/day	*Three 1 mg capsule, plus one 1.5 mg capsule*
7-16	Second	4.5 mg/day *	*One 4.5 mg capsule**

Legend. There will be two dispensing time points when participants will be supplied with the study product. The first supply will be for weeks 1-6 and the second supply will be for weeks 7-16 of the study. For the first supply, participants will receive 1mg and 1.5mg capsules of the study product (low dose naltrexone or placebo). They will be asked to up-titrate the dosage as tolerated and keep a diary of their dosage. In the second dispensing period, they will be supplied with capsules of their maximum tolerated dose. *Or maximum tolerated dosage (i.e. one 1mg capsule, one 2mg capsule or one 3mg capsule).

Outcome Measures

The primary outcome measure is fatigue severity, as measured by the FSS. The FSS is a 9-item PROM scored from 9 (least fatigue) to 63 (most fatigue).[91] A score of >36 is consistent with clinically significant fatigue.[92,93] The FSS has been has been validated in multiple diseases, and has been used in randomised trials for ME/CFS.[92,94–96] The FSS received the highest level of recommendation of any subjective fatigue measure for ME/CFS by the National Institute of Neurological Disorders and Stroke Common Data Elements (NINDS CDE) Project and was a recommended measure by the Post-COVID-19 Core Outcome Set (PC-COS) initiative.[19,97–101] We have previously investigated the FSS in patients with

PCC in BC, and demonstrated that the instrument has strong acceptability, internal consistency, and construct validity in this population.[99]

Secondary PROMs will include pain severity as measured by the pain visual analogue scale (VAS); total symptom score on the Patient Phenotyping Questionnaire Short Form (PQSymp-12) and HRQOL captured by the EQ-5D-5L instrument. Pain is a common symptom in PCC and studies have suggested that LDN may be an effective analgesic.[16,18,44,53,54,56,102] The pain VAS is a single-item tool that has been shown to have strong psychometric properties among patients with chronic pain.[103] The PQSymp-12 is a 12-item questionnaire which covers seven clusters of symptoms derived from the Canadian Consensus Criteria for ME/CFS; it has been recommended as a core assessment measure for ME/CFS by the European Network on ME/CFS (EUROMENE)[104] and is included in the UK ME/CFS biobank.[105] The EQ-5D-5L is a generic HRQOL instrument that was recommended by PC-COS.[101] By applying Canadian preference weights, responses to the EQ-5D will be used to derive a health utility (HU) score from 0 (dead) to 1 (perfect health).[106]

An additional secondary outcome is average step count. We will ask participants to wear a pedometer and document daily step counts for 7 days prior to starting the study drug, and again in week 16. All participants will use the same brand and type of pedometer (OZO Fitness CS1 Easy Walk Pedometer). Step counts have been used previously in randomised trials to measure change in activity levels among patients with ME/CFS.[96,107–109]

There will be several exploratory outcomes (**Table S1)** including PROMs (fatigue VAS, sleep, depression symptoms, anxiety symptoms, self-reported health, and functional status), laboratory-based (inflammatory markers, CK, thyroid profile, AM cortisol and ACTH level) and based on physical measurements (grip strength, sit and stand test, and orthostatic changes in vitals). Physical measurements will be limited to participants who choose to attend in-person visits.

MRI Study

As a sub-study of the RCT, 25 participants of each study arm are planned to have brain magnetic resonance imaging (MRI) scans at baseline prior to the intervention/placebo and at 16 weeks. A multimodal functional and spectroscopy (fMRI/MRS) protocol piloted in a ME/CFS study (REB# H20-

01804, unpublished) will be employed (**Figure S1**). MRI findings will be linked to the primary and other outcome measures.

Participant Timeline

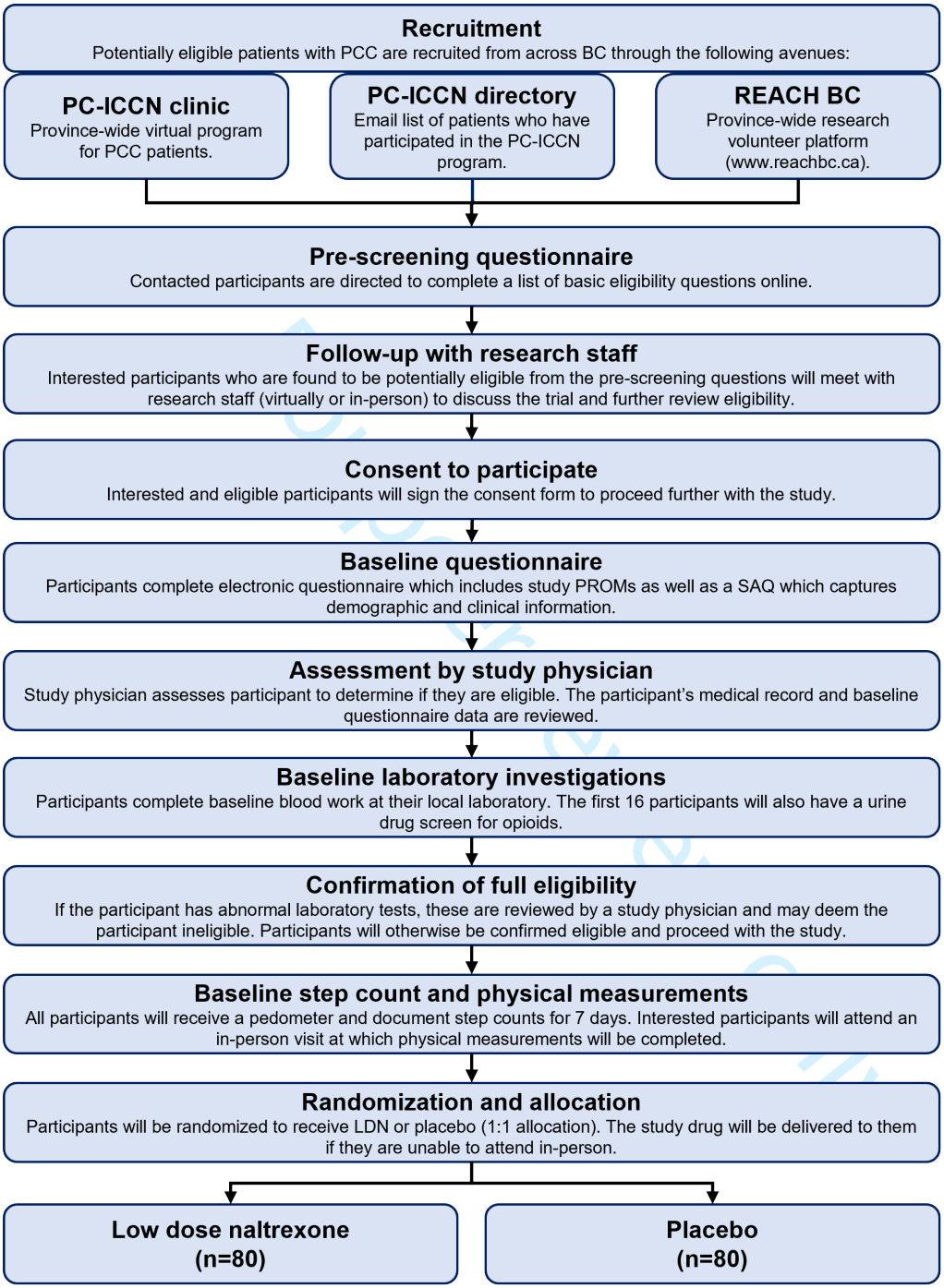
The participant timeline is detailed in **Table 4** and **Figure 1**.

Table 4: Participant timeline and schedule of study procedures

TIMEPOINT	Screening and Baseline	Week 1	Weeks 4-5	Week 6	Week 12 weeks	Week 16 weeks
ENROLLMENT:						
Eligibility screening by research staff and study physician	X					
Informed consent	X					
Allocation	X					
INTERVENTIONS:						
Study drug (LDN or placebo)	X	X	X	X	X	X
Study drug diary (daily for first 4 weeks and for 7 days after any change in dose)	X	X	X			
VISITS:						
Adverse effects check		X	X	X*	X*	X
Monitor study drug use		X	X	X*	X*	X
ASSESSMENTS:						
Questionnaires^	X			X	X	X
Laboratory investigations	X					X
Pedometer (Number of steps per day)	X					X
Hand grip (Muscle Strength) ‡	X					X
Blood pressure and heart rate‡	X					X
Sit and stand test‡	X					X

Legend. This table outlines the schedule of study procedures. See Figure 1 for timeline of recruitment, eligibility screening and baseline assessments. Abbreviations: LDN- low dose naltrexone. *Occurs as part of questionnaires if optional visit does not occur; ^Short answer questionnaire for demographic and clinical information is done at baseline only; ‡Only for those agreeing to have in person visits.

Figure 1: Flowchart of initial study procedures



Legend. This flowchart outlines the process for study recruitment, eligibility assessment and baseline assessments. A full study timeline is outlined in Table 4. Abbreviations: BC- British Columbia; LDN- low dose naltrexone; PCC- post-COVID-19 condition; PC-ICCN- Post-COVID-19 Interdisciplinary Clinical Care Network; SAQ- short answer questionnaire.

Sample Size

The sample size was calculated based on the primary hypothesis of reduction in fatigue severity with treatment. To detect a 4.7-point difference (effect size [d]=0.5) in the Fatigue Severity Scale (FSS) (9-63) between arms, we estimate a sample size of 64 participants per arm assuming 80% power, 5% significance and a pooled standard deviation of 9.4 (estimated from the CCDP Data Registry).[88] To account for possible loss-to-follow up of 20%, we estimate a final sample size of 80 per arm, for a total target sample size of 160 participants. We chose this method for sample size estimation (as opposed to use of a minimal clinically important difference (MCID)) because we believed this to be a realistic treatment effect and there were no published MCID values available for the FSS in ME/CFS or PCC.[110,111] In a sensitivity analysis, we calculated sample size using a published MCID for systemic lupus erythematosus and this yielded a similar estimate (**Appendix 2**).[112]

Recruitment and Screening

New PC-ICCN patients will be contacted regarding study participation, and the PC-ICCN directory will be used to identify other candidate PCC patients to contact. Additionally, the trial will be accessible to patients through REACH BC, a provincial online platform that facilitates connections between research studies and participants. All potential participants will be asked to complete an online pre-screening questionnaire, and those potentially eligible will meet with research staff to provide consent. Consented participants will complete baseline questionnaires and be assessed by a physician to confirm eligibility. Baseline laboratory studies for all participants will be done prior to initiation of study drug and abnormal results will be reviewed by a study physician.

Allocation

Participants will be randomised into either the LDN treatment group or placebo at a ratio of 1:1 (n=80 each), as per a computer-generated randomisation schedule stratified by sex and using permuted blocks varying between 2, 4, and 6 participants. A statistician who is not part of the study team will generate a randomisation sequence and corresponding randomization codes. The randomisation codes will be used to maintain the blinding and will be uploaded to REDCap. The randomization sequence will

be provided to the unblinded CWH Research Pharmacy. After confirmation of eligibility, research staff will randomise participants by REDCap, which will provide the randomisation code. CWH Pharmacy staff will then dispense the study drug based on the randomisation allocation sequence.

Blinding

All participants will be blinded to their treatment regimen. The placebo and intervention capsules will appear identical, and the C&W Pharmacy will distribute study drug to study staff in identical containers. Participants, their healthcare providers, and all study staff including research assistants, coordinators, statisticians, trial physicians, and investigators will be blind to allocation. Unblinding will only occur when knowledge of the actual treatment is essential for further management of the patient or investigation of serious adverse events (SAEs). If unblinding is deemed necessary by the DSMB or investigator, the C&W Pharmacy will be contacted for release of treatment allocation.

Data Collection and Management

We will use the secure REDCap platform for storage of study data.[113,114] Participants will complete questionnaires electronically, with the links provided to the participant via email. Data from other sources will be entered manually, and will include study physician assessments, laboratory results, dose diary information, step counts, physical assessment measures, and adverse events (AEs). REDCap field validation tools will be used where possible to optimise data accuracy (e.g. dates that are out of range and data that are missing). No new data will be collected from participants who withdraw, except for reason for withdrawal and details regarding AEs and SAEs.

Biological Specimens

Leftover plasma will be stored at -80C at the BC Children's Hospital Biobank for up to 10 years to allow for additional sample testing related to this protocol that may be identified from the results of this study.

Monitoring and Oversight

A Trial Steering Committee (TSC) will be formed with patient partners, investigators, and other research team members. Additionally, we have formed a Data Safety and Monitoring Board (DSMB) that is comprised of peer researchers with expertise in clinical trials and ethics and independent from the study team. Lastly, an independent study monitor from the CWH Quality Assurance Office has been hired to verify participant rights and wellbeing, data collection and compliance with regulatory requirements. Roles of the TSC, DSMB and study monitor are outlined in **Box S2.**

Statistical Methods

Primary and secondary outcomes will be analyzed by intention-to-treat. The primary outcome (FSS score at 16 weeks) will be analyzed using a linear mixed effects model adjusting for baseline level, sex (stratification factor) and other relevant prognostic factors identified *a priori*. The model will include interaction between treatment arm and time, and treatment arm and baseline level and include all post-randomization timepoints at which the FSS is captured. To assess the FSS at 16 weeks, we will calculate an estimated marginal mean difference between arms with corresponding 95% confidence interval, with statistical significance set at 0.05. Similar contrasts at each interim time point will be provided. Effect modification by baseline FSS level will be demonstrated graphically. Participants who are lost to follow up will be compared descriptively to those who remain in the trial. If selection bias occurs, we will consider inverse probability weights for censored individuals.

For secondary and exploratory outcomes, questionnaire, laboratory, and physical measure data will be analyzed similarly with generalised linear models, adjusting for baseline level, other relevant prognostic factors and using link function based on the variable type from questionnaires (e.g., logit for binary outcomes).

Effect modification by baseline factors will be considered by the inclusion of interaction terms with treatment arm in the above models. Possible effect modifiers include baseline fatigue severity, sex, gender, age, severity of and time of acute SARS-CoV-2 infection, pre-existing co-morbidities, COVID-19 vaccination, final dose, and side effects. Significance of effect modification will be based on the likelihood ratio test comparing models with and without the interaction term.

Dose response analyses will involve comparison of various dosing levels as a covariate versus control in the primary linear model. Secondary analysis will look only at dose comparisons within the intervention arm Dose will be included in these models as a nonlinear effect via restricted cubic splines.

We will conduct a per-protocol analysis to assess the expected effect of adhering to the trial protocol using G-methods, which allow for adjustment for post-randomization confounding.[115]

All analyses of primary and secondary outcomes will be pre-specified in detail in a Statistical Analysis Plan and signed off on by all investigators prior to data analysis.

Harms

Protocols to address particular adverse events (AEs) and SAEs are described in **Appendix 3.** We will implement REDCap alerts for AEs noted through the questionnaires. Additionally, participants will be asked if they have had any AEs at each study visit. All AEs will be assessed by a study physician. All SAEs will be reported to the DSMB. SAEs will be reported to the Research Ethics Board (REB) and Health Canada (HC) as per local regulations. For mild AEs, the patient may be reassured to continue taking the medication as per protocol. Previous studies and our clinical experience have suggested that LDN is generally well tolerated, and mild AEs will often ease with treatment continuation.[41,50,52–54,116]

Inspections and Auditing

The trial will be subject to inspections or audits by HC, REB, and the Canadian Institutes for Health Research (CIHR).

ETHICS AND DISSEMINATION

Research Ethics Approval

This study was approved by the UBC/C&W harmonized REB (#H21-02254); any protocol modifications will be reported to the REB.

Consent or Assent

Research staff will consent interested participants via the secure REDCap electronic consent framework. Participants will receive information regarding the trial electronically and will have the opportunity to discuss the trial specifics and meet with a research team member (virtually or in-person) before deciding on participation. The consent form is provided in **Appendix 4**.

Access to Data

The study principal investigator, co-investigators, clinical research coordinator, research assistants, and statistician will have access to the collected data.

Dissemination Policy

We will follow the Consolidated Standards of Reporting Trials (CONSORT) guidelines for reporting results of parallel arm trials.[117] We will submit manuscripts to peer review journals, give presentations at conferences, and media release will be organised. We will leverage our connections with PCC and ME/CFS networks to share our findings with patients, their caregivers, PCPs, and other care providers.

DISCUSSION

This report described the protocol for a 16-week, phase II randomised controlled trial to investigate the efficacy of LDN for treating fatigue severity in patients with PCFS, an illness we have defined as ME/CFS symptoms persisting at least 3 months following SARS-CoV-2 infection.

Our study will build on prior and ongoing evaluations of LDN. In our review of studies listed on ClinicalTrials.gov, we identified one upcoming trial (NCT05946551) which also investigates LDN in PCC. However, this trial is smaller (expected n=36) and focused on feasibility outcomes. There are no trials listed that investigate LDN in ME/CFS.

A positive outcome in our trial would inspire greater confidence in LDN as a treatment for the millions of patients with PCFS symptoms, and could prompt larger, multi-institutional phase III studies. Unlike other candidate PCC treatments such as Paxlovid, stellate ganglion blockade and hyperbaric oxygen,[118–124] LDN is widely available, relatively inexpensive, and generally safe. A negative outcome in

this trial would also be a valuable contribution to the literature and would directly impact clinician decisions regarding prescribing LDN.

The trial has limitations. It is limited to English speakers and is based in a single province. We do not have a restriction on how long a participant may have had their symptoms since COVID-19. This may limit the treatment effect if LDN efficacy is greater earlier in the disease course. The remote nature of the trial also limits the number of objective outcomes that can be collected in all participants.

However, our decentralized strategy for this trial has several advantages. First, it will allow individuals who live outside Vancouver to participate, including those in communities who may not have access to off-label or investigational treatments.[125] Second, it will permit inclusion of more symptomatic individuals. Some individuals with PCFS have reported symptom exacerbation from even minimal cognitive and physical exertion,[126] and remote participation may prevent flare-ups experienced from in-person visits. Third, it will encourage participation from patients who may be reluctant to attend in-person given the risks of COVID-19 re-infection. Lastly, it will expedite study completion by broadening the pool of eligible applicants and reducing logistical barriers associated with in-person recruitment and enrollment.

This trial has other strengths. By using the provincial PC-ICCN and REACH BC directories, we will be able to efficiently identify and contact hundreds of potential participants by email. Our focus specifically on individuals with the ME/CFS phenotype distinguishes this trial from others for PCC and increases the likelihood that participants will have a similar underlying pathophysiology. Lastly, our trial includes multiple secondary and exploratory outcome measures that may be valuable for further hypothesis generation.

This is one of the first trials in Canada investigating a pharmacological treatment for PCC and will have a direct impact on how this illness is treated. Our hope is that it will also promote engagement, good faith, and optimism among the PCC community- a group that has experienced stigma and one that has expressed frustration regarding the paucity of interventional studies for their illness.[22,127–131] Furthermore, the trial has implications beyond COVID-19; we expect that the results will have applicability to ME/CFS and other post-infection fatigue syndromes, including those that could emerge from future pandemics.[132]

Trial Status

This trial will start recruitment in January 2024 and aims to complete follow-up by the end of 2024.

Confidentiality

Following UBC REB guidelines, all study-related information will be stored in locked facilities at C&W, and all electronic material stored on secure network drives. Participants will be allocated study identification (ID) numbers and a master file linking the study ID and personal information will be saved separately.

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Supplementary Material

Study protocol for a double-blind, randomised trial of low-dose naltrexone for post-COVID fatigue syndrome

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Box S1: Withdrawal criteria

Withdrawal criteria
Participants can withdraw from the study at any time without giving reasons. If a participant withdraws from the study, they may still be asked to attend a final safety visit to ensure their safety. There will be no additional follow-up for a withdrawn participant, except in the event of an adverse event (AE) or serious adverse event (SAE), where they will be followed until the AE or SAE fully resolves. Participants should be withdrawn from the study if: 1. The study investigator judges it is not in the participant's best interest to continue. 2. The participant experiences serious side effects due to the study treatment. The study doctor will assess the participants symptoms from the AE/SAE and the participant will be withdrawn if determined appropriate by the investigator. If withdrawn for an AE/SAE, the participant will still be followed by the study team until the AE/SAE resolves. 3. The participant needs to begin take long-term opioid medication. Participants who take opioid medication over a short time during the study to manage acute pain, will be allowed to continue in the study. The protocol deviation will be recorded. The study doctor and team will reinforce guidance on taking alternative medication for pain and ensure the participant is aware that taking opioids for pain relief may not work well due to the naltrexone's opioid blocking effects. 4. The participant becomes pregnant during the study. The study treatment will be stopped, and they be withdrawn from the study. 5. The participant is unable to fulfill the requirements for the study. For example, if the participant misses or does not complete the 1) baseline assessments or 2) assessments at more than one consecutive time point (does not complete 6-week and 12-week assessments). Participants withdrawn from the study will not be replaced.
Data collection from participants who withdraw
No new data/information will be collected from participants after they withdraw, except for: 1. Reason for withdrawal (if by participant choice: reason will not be collected unless participant provides a reason for their withdrawal). 2. Any information that is required to be collected related to an AE/SAE that the withdrawn participant is being followed for. The withdrawn participant will be followed until the AE/SAE has fully resolved. All data that has been collected prior to withdrawal will be retained for analysis.

Box S2: Trial oversight

Trial Steering Committee
A Trial Steering Committee (TSC) will be formed with patient partners, the investigators, and other team members. The TSC will meet virtually at least monthly in the first two months and then every 2 months for the remaining study period. The goals of the Steering Committee will include: 1. Ensuring adherence to the study timeline. 2. Reviewing protocol, reporting, discussing, and resolving challenges. 3. Providing a regular environment for the presentation of study data (including recruitment and final trial data). 4. Fostering patient and knowledge user engagement. 5. Planning for knowledge translation activities.
Data Safety and Monitoring Board
The Data Safety and Monitoring Board (DSMB) will be comprised of peer researchers with expertise in clinical trials and ethics, who are once removed from the study team. The frequency of scheduled meetings depends on patient enrollment and safety event rates. For the DSMB, a research assistant from the study team will coordinate meetings, draft agendas, take meeting minutes and log discussions. In instances where closed DSMB meetings are called, a research assistant independent of the study team will perform the tasks noted above. Before the study starts, the DSMB will: 1. Review the study protocol, overall data collection methods and safety monitoring procedures. 2. Define safety and related parameters to be monitored, including methods for review and frequency of monitoring. 3. Provide letter of review/approval of study initiation to the PI/Trial Steering Committee. During the study period, the DSMB will: 1. Review data generated by the study, particularly adverse events, unexpected and serious adverse events on a periodic basis and as advised by the study team and recommend one of the following actions to the Trial Steering Committee – continue as is, continue with modification, discontinue part of study, or discontinue completely. 2. Provide letter of review/approval at pre-determined intervals.
Study Monitor
The role of the study monitor is to verify: 1. That the rights and wellbeing of the participants are protected. 2. That reported trial data is accurate, compete and verifiable from source documents. 3. The conduct of the trial is compliance with the currently approved protocol, good clinical practices (GCP) and any applicable regulatory requirements.

Table S1: Exploratory outcomes

Laboratory measures (peripheral blood serum)	
Inflammatory markers	Comprised of IL-6 (measured in pg/mL) and hsCRP (measured in mg/L).
Cytokine profile	Comprised of GM-CSF, IFNγ, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-12p40, IL-12p70, IL-13, MCP-1, TNFα. These are measured in pg/mL using the Human Cytokine Proinflammatory Focused 15-plex Discovery Assay® Array (Eve Technologies).
Creatine kinase (CK)[1]	An assessment of disease severity,[1] measured in units/L.
Thyroid profile[2]	An indirect measure of disease severity,[2] comprised of rT3 (measured in ng/dL), fT3 (measured in pmmol/L), fT4 (measured in pmmol/L) and TSH (measured in mIU/L).
AM cortisol*	Measured between 800h and 1000h, in nmol/L.
Adrenocorticotropic hormone (ACTH)*	Measured between 800h and 1000h, in pmol/L.
Patient reported outcome measures	
Fatigue VAS [3]	1 item, scored from 0 (least fatigue) to 10 (most fatigue).
Sleep Questionnaire (SQ-2)[4]	4 items, scored from 0 (no sleep problems) to 24 (most sleep problems)
Sleep VAS[4]	1 item Scored from 0 (no sleep problems) to 10 (worst quality of sleep imaginable)
Patient Health Questionnaire-9 (PHQ-9)[5]	Scored from 0 (no depression symptoms) to 27 (most depression symptoms).
Generalized Anxiety Disorder-2 (GAD-7)[6]	Scored from 0 (no anxiety symptoms) to 21 (most anxiety symptoms).
Self-reported health EQ-5D VAS[7]	Scored from 0 (worst imaginable health) to 100 (best imaginable health).
Post-COVID-19 Functional Status Scale[8]	Scored from 0 (no functional limitations) to 4 (severe functional limitations).
Physical measurements^	
Hand grip strength[9]	Measured in kg using a digital hand dynamometer. The highest value over 3 attempts will be for each time point will be used.
Sit to stand test[10]	To test leg strength and endurance, measured in number of repetitions in 30 seconds.
Postural blood pressure and heart rate	Measurements are taken in supine position and then three times over 5 minutes following passive standing (i.e. at 1, 3, and 5 minutes)

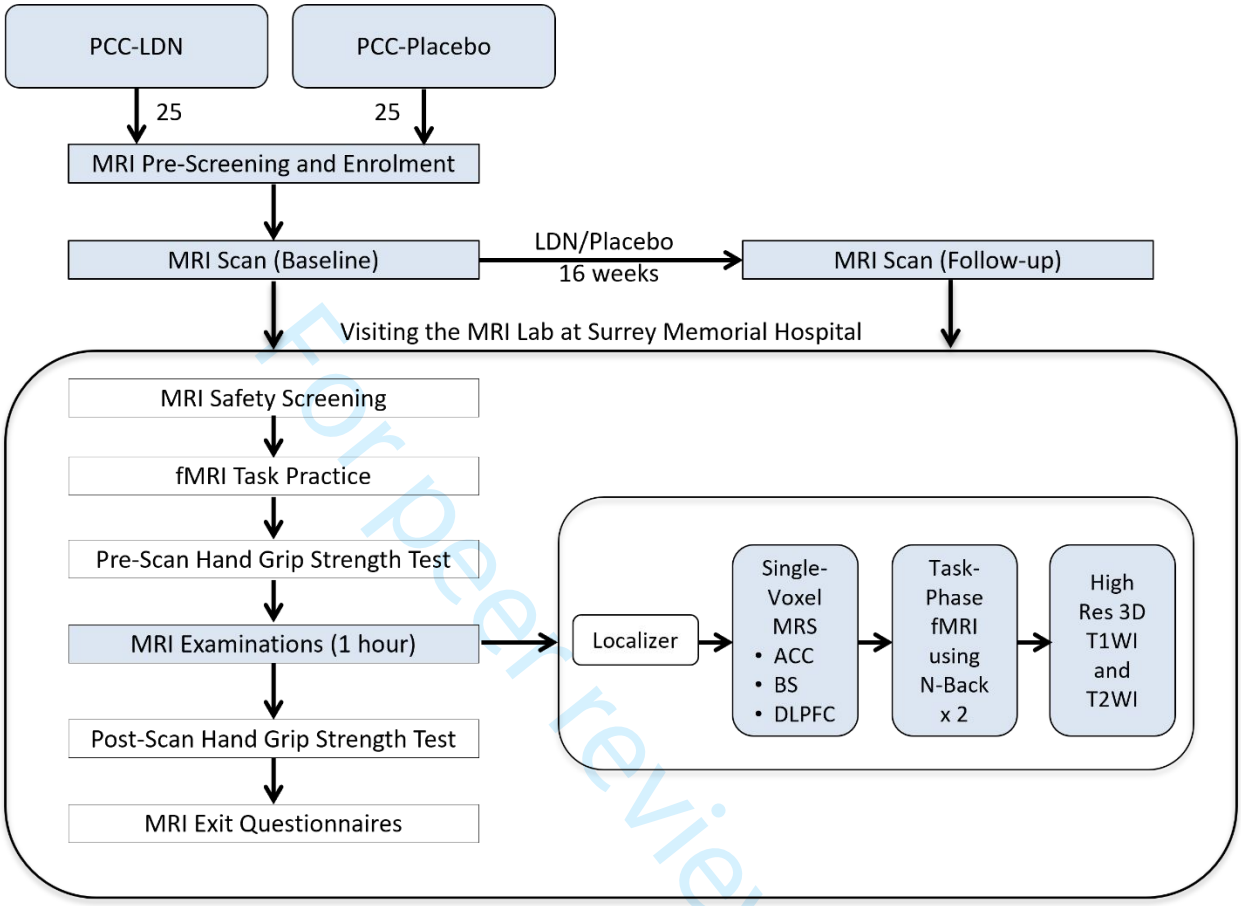
Legend. This table outlines the exploratory outcome measures. Details regarding primary and secondary outcomes are detailed in the main text. For all exploratory outcomes, we will evaluate changes in the measure between baseline and 16 weeks. Abbreviations: EQ-5D- Euroqol 5-dimensions; fT3- free triiodothyronine; fT4- free thyroxine; GM-CSF-granulocyte-macrophage colony-stimulating factor; hsCRP-high sensitivity CRP; IL-interleukin; MCP-1- monocyte chemoattractant protein 1; p40- subunit p40; POTS- postural orthostatic tachycardia syndrome; rT3- reverse triiodothyronine; TNF- tumor necrosis factor; TSH- thyroid stimulating hormone; VAS-visual analogue scale. *Optional for participants who choose to have bloodwork done in AM; ^optional for participants who choose in-person assessments.

Table S2: Laboratory tests

Test	Type	Purpose	Participants	Timing
ACTH	Serum	Exploratory outcome	Optional	Baseline, 16 weeks
Albumin	Serum	Eligibility, safety	All	Baseline, 16 weeks
ALP	Serum	Eligibility, safety	All	Baseline, 16 weeks
AM Cortisol	Serum	Exploratory outcome	Optional	Baseline, 16 weeks
ALT	Serum	Eligibility, safety	All	Baseline, 16 weeks
AST	Serum	Eligibility, safety	All	Baseline, 16 weeks
Bilirubin	Serum	Eligibility, safety	All	Baseline, 16 weeks
Calcium	Serum	Eligibility, safety	All	Baseline, 16 weeks
CBC with differential	Serum	Eligibility, safety	All	Baseline, 16 weeks
Creatinine	Serum	Eligibility, safety	All	Baseline, 16 weeks
CK	Serum	Eligibility, safety, exploratory outcome	All	Baseline, 16 weeks
fT3	Serum	Exploratory outcome	All	Baseline, 16 weeks
fT4	Serum	Exploratory outcome	All	Baseline, 16 weeks
GM-CSF	Serum	Exploratory outcome	All	Baseline, 16 weeks
hsCRP	Serum	Eligibility, safety, exploratory outcome	All	Baseline, 16 weeks
IFNγ	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-1β	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-1RA	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-2	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-4	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-5	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-6	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-8	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-10	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-12	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-12p40	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-13	Serum	Exploratory outcome	All	Baseline, 16 weeks
LDH	Serum	Eligibility, safety	All	Baseline, 16 weeks
MCP-1	Serum	Exploratory outcome	All	Baseline, 16 weeks
Potassium	Serum	Eligibility, safety	All	Baseline, 16 weeks
rT3	Serum	Exploratory outcome	All	Baseline, 16 weeks
Sodium	Serum	Eligibility, safety	All	Baseline, 16 weeks
TNFα	Serum	Exploratory outcome	All	Baseline, 16 weeks
TSH	Serum	Eligibility, safety, exploratory outcome	All	Baseline, 16 weeks
Urine opioid screen	Urine	Eligibility	First 16	Baseline

Legend. This is a complete list of laboratory tests completed during the study. Blood samples will be taken at a LifeLabs of the participants' choice, which will usually be local to participant location. Abbreviations: ACTH- adrenocorticotropic hormone; ALP- alkaline phosphatase; ALT- alanine aminotransferase; AST- aspartate aminotransferase; CBC- complete blood count; CK- creatine kinase; ; fT3- free triiodothyronine; fT4- free thyroxine; GM-CSF-granulocyte-macrophage colony-stimulating factor; hsCRP- high sensitivity c-reactive protein; IL-interleukin; LDH- lactate dehydrogenase; MCP-1- monocyte chemoattractant protein 1; p40- subunit p40; rT3- reverse triiodothyronine; TSH- thyroid stimulating hormone; TNFα- tumour necrosis factor alpha.

Figure S1: Protocol for MRI Study



Legend. As part of a sub-study of this clinical trial, 25 consenting participants in each study arm will undergo functional MRI testing. Participants will complete a hand grip assessment before and after imaging, and complete cognitive tasks during the fMRI testing. Abbreviations: ACC-agenesis of corpus collosum; BS-brainstem; DLPFC-dorsolateral pre-frontal cortex; fMRI- functional magnetic resonance imaging; MRI-magnetic resonance imaging; T1WI- T1 weighted imaging; T2WI- T2 weighted imaging.

Appendix 1: Batch testing protocol

The following plan is for batch testing the first batch of compounded study drug for the clinical trial, as requested by Heath Canada during the approval process. This study has Health Canada authorization. The batch testing must be completed, and the results reported to Health Canada, before dosing the first patient in the trial.

Naltrexone capsules in 1mg or 4.5mg size are to be analysed by ultra-high pressure liquid chromatograph-tandem mass spectrometer (UHPLC-MSMS) for confirmation of concentration in each capsule. This process will require a deuterium labelled standard (Sigma N-047 naltrexone-d3 solution. Certified reference material)

We will use a multiple reaction monitoring (MRM) approach for naltrexone with a transition of 342 -- 324 for unlabelled naltrexone and 345 – 327 for the deuterium labelled standard. The MRM approach uses a tandem mass spectrometer (two mass spectrometers in line one after the other) The first mass (342 for naltrexone) is isolated by the first mass spectrometer, the naltrexone will then be fragmented by collision with gas molecules and the fragment, or second ion (324 for naltrexone) will be isolated by the second mass spectrometer. The fragment ion will then be detected, and the intensity of the signal is equivalent to the concentration of the naltrexone.

Appropriate standard curves will be created using a 2.1 X 50mm Accucore phenyl-hexyl column (Thermo Scientific 17926-052130) and water/methanol as mobile phases. Standard curves will be created using dilutions of a European Pharmacopoeia standard from Sigma: Naltrexone Hydrochloride #Y0000400 with n=10 standards ranging in concentration from 10 nanogram per millilitre (ng/ml) through 500ng/ml. Standard curves will be included in the report to demonstrate linearity and range. The UHPLC-MSMS utilized is a Waters H-class UHPLC and Xevo TQS tandem mass spectrometer and will be run in positive ion mode.

Naltrexone impurity C (Sigma nomenclature or USP naltrexone related compound A), a European Pharmacopoeia reference standard from Sigma Y-0000410, will be analysed as a system suitability test as impurity C has the same molecular weight as Naltrexone and has the same MRM transitions of 342 – 327. Consequently, impurity C and naltrexone must be separated by UHPLC prior to entering the mass spectrometer. System suitability testing will be done to ensure the chromatographic separation of these two compounds prior to sample analysis.

Other known impurities of naltrexone will not be detected with a 345 -- 327 MRM as they have different molecular weights.

Analytical accuracy will be determined by spiking capsules with analytical standards of naltrexone. 1mg capsules will be cut open with a scalpel and the contents added to 1000milliliters (ml) water. 100microliters (ul) of this solution will be added to 900ul water for a dilution of 1/10 and an approximate concentration of 100 nanograms/millilitre (ng/ml).

4.5mg capsules will be treated in the same manner as the 1mg capsules except the final dilution will be 20ul into 980ul water providing an expected concentration of 90ng/ml.

5ul of deuterium labelled internal standard (naltrexone-d3 diluted to 1ug/ml concentration) will be added to all samples to allow for quantitation of the naltrexone. Results will be determined and expressed as a percentage of the expected dose.

To determine analytical accuracy, solutions from the 1mg and 4.5mg capsules (diluted to the equivalent of 100ng/ml and 90ng/ml) will be spiked and recovery determined.

Naltrexone standard will be spiked into the diluted capsule contents using the scheme in the following tables.

Placebo capsule:

	expected	measured	% recovery
no spike	0		
50ng/ml	50		
100ng/ml	100		
150ng/ml	150		
200ng/ml	200		

1mg capsule diluted to 100ng/ml:

	spiked	measured	expected	% recovery
no spike	0		100	
50ng/ml	50		150	
100ng/ml	100		200	
150ng/ml	150		250	
200ng/ml	200		300	

4.5 mg capsule diluted to 90ng/ml:

	spiked	measured	expected	% recovery
no spike	0		90	
50ng/ml	50		140	
100ng/ml	100		190	
150ng/ml	150		240	

Analytical precision will be determined using 8 capsules of 1mg and 4.5mg quantity. Capsules will be treated as described above.All 8 replicates at each concentration will be analysed by HPLC-MS and the mean, standard deviation and %RSD will be determined for each.

Confirmation of capsule dose and content uniformity will be combined.

A total of 10 capsules of 1mg and 4.5mg will be treated as described above. Samples will be analysed by HPLC-MS and results expressed as a percentage of the expected dose (1mg or 4.5mg). This will confirm the accuracy of the expected doses.

These results will also be used to calculate an acceptance criterion for content uniformity. If the results of the 10 capsules do not meet the USP definition of acceptance criteria, then an additional 20 capsules will be analysed and acceptance criteria calculated.

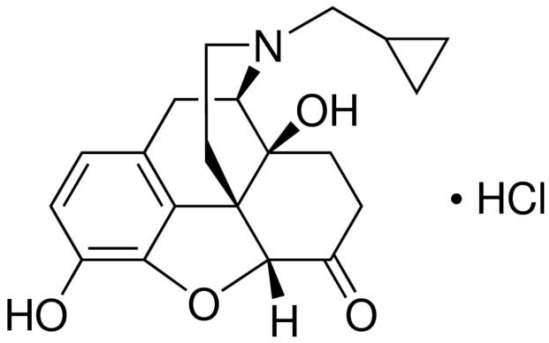
The compounding pharmacy has criteria for dose variability stating results should be between 90% and 110% of the expected value.

Purity determination will utilize aThermo Scientific Q-exactif Orbitrap. The Orbitrap has a mass accuracy of about 1part per million error. A 2.1 X 50mm C18 column will be used and all 10 of the final solutions for both 1mg and 4.5mg capsules, used for content uniformity will be analysed by Orbitrap mass spectrometry in positive ion mode.

The placebo capsule will also be analysed by Orbitrap.

We will actively search for each of the 10 known contaminants and breakdown products of naltrexone shown in the list below, based on accurate mass. Percent purity will be calculated based on area counts of naltrexone and any known contaminants/breakdown products found in each chromatogram.

Additionally, chromatograms of naltrexone capsules and placebo capsule will be compared to identify peaks found only in the naltrexone capsules. Any such peaks will have accurate mass recorded and be included in the percent purity calculation.



naltrexone		341.1627	C20H23NO4
impurity	a	315.1107	C17H17NO5
	b	287.1158	C16H17NO4
	c	341.1627	C20H23NO4
	d	680.3170	C40H44N2O8
	e	395.2097	C24H29NO4
	f	357.1576	C20H23NO5
	g	357.1576	C20H23NO5
	h	343.1784	C20H25NO4
	i	355.1420	C20H21NO5
	j	355.1784	C21H25NO4

Appendix 2: Sensitivity analysis for sample size calculation

For this trial, the sample size was calculated using data from the CCDP registry,[11] and based on effect size (d)=0.5.

As a sensitivity analysis, we also performed a sample size calculation through an alternative approach of using a minimal clinically important difference (MCID). As there are no published MCIDs for ME/CFS or PCC that we could identify in the literature, we used a published MCID ascertained from patients with systemic lupus erythematous (SLE).[12] This study included 80 participants and reported a MCID (95%CI) of 0.6 (0.3-0.9), or 5.4 (2.7-8.1) on the 7 to 54 scale for FSS.

After assuming a standard normal confidence interval was derived using all 80 patients, we calculated a standard deviation of 1.36, which corresponds to an effect size of 0.6/1.36 = 0.44. This results in a sample size of 81 per group, or 101 accounting for 20% loss to follow up.

Appendix 3: Side effects and adverse events

Trials with LDN for conditions other than PCFS have shown that LDN is safe and well-tolerated.[13–16] In our clinical experience, minor side effects were observed in <10% of patients. These include sleep dysfunction, in particular vivid dreams, and minor gastro-intestinal upset.

Hepatotoxicity

Naltrexone has been identified as a hepatotoxin. This was identified using doses five times the recommended dose and no cases of hepatic failure due to naltrexone have ever been reported. The doses in this study are significantly less (4.5 mg) than the recommended doses for opiate blockade or treatment for opioid or alcohol dependence (50 mg). Even though the risk of this is very low, participants will be informed of the potential, told to call the study team, and seek emergency medical attention if they experience any symptoms of hepatitis or liver dysfunction.

Other Adverse Events

Adverse reactions that have been reported both at baseline and during clinical trials with naltrexone are as follows (note the maximum dose used in this study is over 10 times lower than those used for listed uses)[17]:

- More than 10%: Difficulty sleeping, anxiety, nervousness, abdominal pain/cramps, nausea and/or vomiting, low energy, joint and muscle pain, and headache.
- Less than 10%: Loss of appetite, constipation, increased thirst, increased energy, feeling down, irritability, dizziness, skin rash, delayed ejaculation, decreased potency, and chills.
- Less than 1% of subjects:
    - Respiratory: nasal congestion, itching, rhinorrhea, sneezing, sore throat, excess mucus or phlegm, sinus trouble, heavy breathing, hoarseness, cough, shortness of breath;
    - Cardiovascular: nose bleeds, phlebitis, edema, increased blood pressure, non-specific EGG changes, palpitations, tachycardia;
    - Gastrointestinal: excessive gas, hemorrhoids, diarrhea, ulcer;
    - Musculoskeletal: painful shoulders, legs or knees, tremors, twitching;
    - Genitourinary: increased frequency of, or discomfort during urination, increased or decreased sexual interest;
    - Dermatologic: oily skin, pruritus, acne, athlete's foot, cold sore, alopecia;
    - Psychiatric: depression, paranoia, fatigue, restlessness, confusion, disorientation, hallucinations, nightmares, bad dreams;
    - Special Senses: eyes- blurred, burning, light sensitive, swollen, aching, strained; ears "clogged", aching, tinnitus;
    - General: increased appetite, weight loss, weight gain, yawning, somnolence, fever, dry mouth, head "pounding", inguinal pain, swollen glands, "side" pains, cold feet, "hot spells"

Data collected since the approval of naltrexone shows that most adverse events occur early in treatment and are transient in nature[29].

Adverse Events Reporting

We will implement REDCap alerts for adverse events noted through the questionnaires. Any adverse events (AE) will be assessed by the qualified investigator or their delegate as soon as possible upon becoming aware of the AE. All serious adverse events (SAE) will be reported as per local REB and Health Canada regulations. Adverse events determined to be serious, unexpected, and related to the study drug will be reported to Health Canada within:

- 15 days of becoming aware of the SAE, if it is not fatal or life-threatening.
- Immediately where possible, but within 7 days of becoming aware of the SAE, if it is fatal or life-threatening.

All SAEs that meet the criteria of an unanticipated problem will be reported to the REB. An unanticipated problem meets the following criteria: 1) unexpected; 2) related or possibly related to participation in the research; 3) suggests that the research places participants at greater risk than previously known.

SAE related to study treatment will be followed by the study team until it resolves.

AE reports will be submitted to the DSMB at intervals defined in the DSMB charter.

Appendix 4: Consent form

PARTICIPANT INFORMATION AND CONSENT FORM

A Double Blind Randomized Trial of Low-Dose Naltrexone for Post-COVID Fatigue Syndrome

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Protocol Number/ Trial ID: H21-02254

Short Title: LDN Trial

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BC Women's Hospital + Health Centre
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Research Fellow
University of British Columbia

Research Coordinator: Travis Boulter
Email: LDNtrial@phsa.ca
Phone: 236-990-9519

Sponsor/Funder: Canadian Institutes of Health Research (CIHR)

Emergency Contact Number: 604-786-2473

1. Introduction/Invitation
You are being invited to take part in this study because you are a current or former patient of the BC Post-COVID Recovery Clinics (PCRC), or you have indicated that you are interested to participate in our clinical trial through the REACH BC Platform and have previously had SARS-CoV-2 (COVID-19) virus, and are continuing to experience symptoms.

2. Your participation is voluntary
Participating in this study is voluntary. You have the right to refuse to participate in this study. If you decide to participate, you may still choose to withdraw from the study at any time without any negative consequences to the medical care, education, or other services to which you are entitled or are presently receiving.

You should be aware that there is a difference for both you and your study doctor between being a patient and being a research participant. As a patient, all medical procedures and treatments are carried out for your benefit only according to standard accepted practice. As a research participant, you and your study doctor also must take into account the requirements for the research study. These may include procedures and treatments that are not part of standard practice or are not yet proven. This consent form describes the treatment procedures that are being carried out for research purposes. Please review the consent document carefully when deciding whether or not you wish to be part of the research and sign this consent form only if you accept being a research participant.

Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

3. Who is conducting this study?
This study is being conducted by Dr. Luis Nacul at BC Women's Hospital (BCW) and is funded by the Canadian Institutes for Health Research. Dr. Nacul is the Research Director of the Complex Chronic Diseases Program (CCDP) at BCW. Dr. Nacul is a well-established researcher and doctor in the area of complex chronic diseases. Other members of the study team are: Dr. Jane McKay who is the Head of Division of Internal Medicine and Medicine Program Director at Providence Health BC, Co-chair of the Post-COVID Interdisciplinary Clinical Care Network (PC-ICCN), and is a myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) specialist, and the Physician Lead of the clinical coordination working group at the PCRC; Dr. Adeera Levin is the Medical Lead for the PC-ICCN and PCRC in BC, Head of the Division of Nephrology at University of British Columbia; Dr Xiaowei Song is a Clinical Neuroimaging Senior Scientist with Fraser Health; and Dr. Jeffery Bone is a Biostatistician with BC Children's Hospital Research Institute, Dr. Hiten Naik is a research fellow with the Post-COVID Interdisciplinary Clinical Care Network.

4. Background
There are individuals who do not recover to their previous level of health following infection by SARS-CoV-2 (COVID-19). Many of them have similar symptoms to those with a condition called post-viral fatigue syndrome. This is known as post-COVID-19 fatigue syndrome (PCFS). The main symptoms of PCFS are profound and persistent fatigue, sleep problems, and brain fog. There is currently no treatment for PCFS.

Naltrexone is a medication that attaches to opioid receptors and blocks the effects of opioid drugs. It is approved by Health Canada for the treatment of alcohol and opioid use disorders. It is believed that when naltrexone is used in low doses, about 1/10th of a normal dose, it has the effect of increasing the amount of opioid-like substances made within the body (met-enkephalin and beta-endorphin), which may help reduce pain and inflammation.

Low-dose naltrexone (LDN) has been used in individuals with a number of conditions, such as ME/CFS and fibromyalgia, which have similarities with PCFS. There have not been any clinical trials with LDN for ME/CFS or PCFS. However, there has been an observational study (treatment is part of routine medical care), for ME/CFS, and two small clinical trials for fibromyalgia. These studies have reported benefits of LDN treatment and have shown that LDN is safe and well-tolerated. The Complex Chronic Diseases Program at BC Women's Hospital has extensive experience prescribing LDN treatment for fibromyalgia and ME/CFS patients. Their studies suggest LDN treatment may improve energy, pain, and sleep in many patients, with only minor side effects.

This study aims to determine if treatment with LDN reduces fatigue and improves the related symptoms of PCFS, and thus improve quality of life and health of those with PCFS.

Health Canada, the regulatory body that oversees the use of drugs in Canada, has not approved the sale or use of naltrexone for PCFS. However, Health Canada has allowed naltrexone to be used in this study.

5. What is the purpose of the study?

This study aims to determine if low-dose naltrexone improves fatigue, pain, and related symptoms of individuals with PCFS. It will also determine if it reduces inflammation in the body, by measuring markers of inflammation in the blood.

This study is a double-blind randomized controlled trial. A randomized controlled trial is a study in which participants are randomly assigned to either the study drug (low-dose naltrexone) or placebo (an inactive substance). Randomization is like the flip of a coin so that there is an equal chance of being in either group. This is done to examine the potential benefits and side effects of the drug with a direct comparison group. A double-blind trial is where both participants and researchers directly involved in the trial will not know which treatment is being given (i.e. whether the active treatment or placebo).

6. Who can participate in this study?

You may be able to participate in this study if you:

- Are between the ages 19 to 69 years old
- Had COVID-19 at least three months ago, as confirmed by a physician or positive test result
- Meet the clinical diagnostic criteria for PCFS (this will be confirmed by a physician at your appointment)
- Agree to maintain any other regular medications at their current doses for the duration of the trial (essential need of new medication or dose change, as prescribed by physician, is permitted)

- Agree to use effective contraception while receiving the study treatment (if you could potentially become pregnant)
- You reside within the delivery area for the drug as determined by FedEx Clinical Trial Services

7. Who should not participate in this study?

You will not be eligible to participate in this study if you:

- Are pregnant, planning to become pregnant, or breastfeeding
- Have used opioid medications within the last 15 days or use of opioids during the trial^
- Have a positive urine test for opioids (test to be done after you sign the consent form)
- Have a history of alcohol, opioid or other substance misuse
- Participated in another interventional clinical trial in the last 30 days or plan to during the trial period
- Have a confirmed ME/CFS or fibromyalgia diagnosis existing prior to COVID-19.
- Are allergic to naltrexone or medication components (gelatin†; cellulose)
- Have acute hepatitis, liver failure, or severe kidney failure
- Are currently using or have used naltrexone in the last 30 days

^If you are taking any opioids and wish to stop them to become eligible for the trial, you can enter a washout period, where you will stop taking the opioids for 15 days before continuing with the screening process.

†The study treatment is prepared with gelatin, which is an animal-based product.

8. What does this study involve?

This study is a 16-week double-blinded randomized trial, meaning that neither you nor your study doctor will know which study medication you take. However, this information is available in case of an emergency, and will be available at the end of the trial to those analyzing the results. A total of 160 SARS-CoV-2 survivors are anticipated to be enrolled in this study with 80 randomized to the placebo and 80 randomized to the low-dose naltrexone treatment. Study treatment will be administered for 16 weeks and will start at 1 mg/day, increasing each week by 1 mg for a maximum of 4.5 mg/day at week 4. The latter dose will be continued until the end of the trial.

If you agree to take part in this study, the procedures and visits you can expect will include the following:

- Review and completion of the consent form
- Meet with a Study Physician virtually who will confirm a diagnosis of PCFS and confirm it is safe for you to take the study drug. To do this, the study doctor may need to review your medical record and/or call your family doctor for additional information. If you sign this form, you are agreeing to the study doctor reviewing your medical record and/or calling your family doctor. If you decide you do not want your study doctor to know about your participation in this study, the study doctor will not be able to call your family doctor and this may make it harder for the study doctor to determine if you are eligible.
- Completion of research questionnaires. These questionnaires will ask about items like fatigue, sleep, pain, other symptoms you may be experiencing, demographics, medical history, quality of life, mental health, medications & supplements you take, details about your COVID-19 infection, vaccination status, questions to check your daily use of the study drug, and if you have experienced any side effects. These questionnaires will be emailed to you to complete at home. If

you don't have access to a device with which you can complete the questionnaires on, you can arrange for the questionnaires to administered to you by telephone.

- Blood sample collection at LifeLabs (you can collect samples at any location that is convenient for you). Some of the blood collected will be analyzed at LifeLabs, and some will be sent to Eve Technologies in Alberta for analysis. The samples will be stored at the BC Children's Biobank until they are sent for cytokine profile testing. LifeLabs will use the study ID to collect, process, analyze and/or ship the samples for this study (your name will not be used to send the samples). When the sample arrives at the BC Children's Biobank, the samples will be stored until they are shipped to Eve Technologies in Alberta.
    - All of the study test results will be reported to the ordering physician (the study principal investigator Dr. Luis Nacul).
    - Samples taken at LifeLabs may be referred to a testing laboratory outside of BC (this may be in another province or in the USA).
- Hand grip strength assessment is a short test of the strength you have in your hands (if you don't have any in-person contact with the study team, this assessment will not be done). This involves squeezing a hand device with all your strength and is done three times with each hand.
- Completion of a short study drug diary daily for the first 4 weeks of taking the study treatment, and for a week after any change in the dose, if that happens (unlikely).
- Completion of study visits and check-ins with study personnel. The study visits can be done in-person, through phone call, or video call. The check-ins can be done by phone call, text, email, or video call.
- Wear a pedometer (step counter) for 7 days, while awake, at two time points during the study, and record the number of steps per day in the diary. Note: The pedometer does not collect any information besides your daily steps.
- Return the empty containers and unused study drug to the study team by mailing it back via Canada Post (pre-paid) or returning it in-person to the study site (BC Women's Hospital).

Study Timeline

Screening & Eligibility

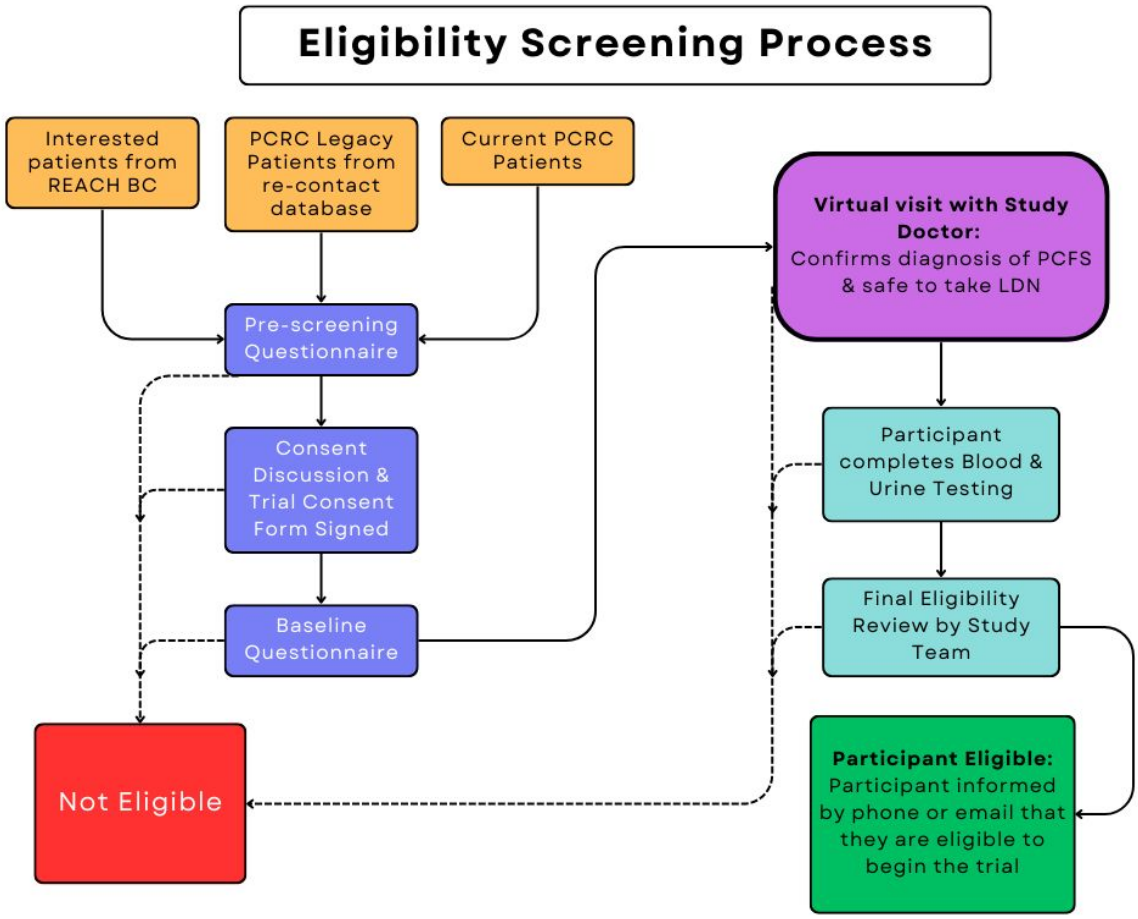
After consenting to the study, you will be asked to complete the following study procedures:

- Completion of questionnaires
- Have a scheduled phone, or telehealth visit with the study doctor in which they will confirm you meet the clinical diagnostic criteria for PCFS and that it is safe for you to take naltrexone.

After the study doctor confirms you have PCFS, blood and urine samples will be collected to confirm your eligibility to the study and as study data that will help with the analysis of the trial results.

Eligibility Process

The flowchart below outlines the eligibility screening process from introduction to the study, to final eligibility review before acceptance and enrollment into the clinical trial. You will be informed of your acceptance into the clinical trial by email or phone call from our clinical trial staff.



Baseline and Treatment Initiation (Week 1)

During the screening period, after completing our Baseline Questionnaires, we will mail you a pedometer with diary sheets and the Participant Instructions. This will be before confirming you are eligible to participate in the study, so there is the possibility you may not be eligible to participate after receiving the pedometer. You can then keep the pedometer and do not need to return it to the study team.

- You will wear a pedometer for 7 days and record the number of steps each day in the diary.

After passing the screening period, you will be asked to complete the following study procedures:

- Have a scheduled in-person, phone, or telehealth visit with study personnel in which they will explain the study procedures and confirm your eligibility to receive the study treatment.
    - If having in-person study visits, additional assessments include: weight, height, blood pressure and pulse at laying down and over 5 minutes following passive standing, sit and stand test, and hand grip strength test.

Once the study team has confirmed you are eligible for the trial, you will be randomized into one of the two treatment groups (low-dose naltrexone or placebo).

The study treatment for the first 6 weeks will then be delivered to you, or you can pick it up in-person at the study site (BC Women's Hospital). You will be asked to take the study treatment once daily at the same time each day.

You will start the study treatment at 1 mg per day; this will be one 1 mg capsule daily. You will also record your dose and any side effects that you experience on the paper diary every day.

Week 2

Towards the end of the first week or after the first week of study treatment, you will meet with the study team via a scheduled telephone or video call. You will be asked about your use of the study treatment, report of any potential side effects, and have the opportunity to ask any questions you may have.

In week 2, you will start at 2 mg per day; this will be two 1 mg capsules daily. You will continue to record your dose and any side effects on the paper diary daily.

If you experience any side effects, such as problems with sleep or gastrointestinal (digestive system) upset, it is suggested that you stay at your current dose, as they will usually decrease as the treatment continues. You will have instructions that outline how and when to change your dose, and can contact the study team at any time if you have questions. Severe side effects to the treatment are very rare. However, if you experience any side effects that persist and/or you find difficult to tolerate, you can return to the dose you were taking in the previous week. If you go back to a lower dose, you will then use this dose for the remainder of the study. You will also be asked to complete the diary for 7 days after you make this change to the dose.

Week 3

In week 3, you will start at 3 mg per day (if you were at the 2 mg dose at the end of the previous week); this will be three 1 mg capsules daily. You will continue to record your dose and any side effects on the paper diary daily.

Week 4

In week 4, you will start at 4.5 mg per day (if you were at the 3 mg dose at the end of the week), this will be three 1 mg capsules and one 1.5 mg capsule daily. This capsule will have a slightly different colour. You will continue to record your dose and any side effects on the paper diary daily.

Weeks 5-6

You will continue taking the study treatment based on the last dose you took in week 4. If you haven't needed to go back to a lower dose, it will stay at 4.5 mg daily.

During week 5, you will have a brief check-in with the study team by email, text, phone or video call, to confirm the study treatment dose you are taking and will be using for the rest of the study duration. At this check-in, the study team may also ask about your experience taking the treatment and you will have the opportunity to ask any questions.

During week 5 or 6, you will receive your second package of study treatment (delivered or picked up at BCW). You will be asked to return any unused study drug, empty containers, and your completed diary sheets from the first package via Canada Post using the prepaid return label provided or in-person to the study site.

In week 6, you will be expected to complete another round of study questionnaires. There is an optional study visit available if you wish to meet with the study team.

Week 7
You will continue taking the study treatment based on the last dose you took in week 4. The only difference is that the whole dose will be in one capsule instead of multiple, and you will only need to take one capsule daily for the rest of the treatment period.

Week 12
You will continue taking the study treatment daily.

At this time, you will be expected to complete another round of study questionnaires. There is a study visit to meet with the study team and check-in with you.

Week 16
You will continue taking the study treatment until the end of week 16.

You will be asked to provide a blood sample at LifeLabs.

You will be asked to wear the pedometer again for 7 days after, while awake, from the beginning of week 16 (you will receive an email reminder) and record the number of steps each day on a paper form. You can then keep the pedometer and do not need to return it to the study team.

After completion of treatment at 16 weeks, you will meet with the study personnel one last time via a scheduled telephone, video call, or in-person, according to your preference.

You will be asked to return any unused study drug, empty containers, and your completed diary sheets from the second package via Canada Post using the prepaid return label provided or in-person to the study site.

You will be expected to complete the final round of study questionnaires.

Study Team Contact during the study
The study team is available to answer any questions you may have at any time during the study. You can contact Dr. Luis Nacul (email: luis.nacul@cw.bc.ca; phone: 604-875-2174), or the study team (email: LDNtrial@phsa.ca; phone: 236-990-9519). The study team email will be monitored daily by multiple staff.

Text messages, phone calls and/or emails will be used to contact you while you are in the study. These may be for items like to remind you of tasks to complete, to thank you for completing

task, scheduling study visits, completing study check-ins, or following up on questionnaire items the study team may have questions about.

Summary of Study Procedures:

Timeline	Participant Procedures	Anticipated Time
Screening	Screening Visit with Study Physician	15-60 min
	Complete study questionnaires	25-60 min
	Collection of blood and urine sample at LifeLabs	30 min (time will vary)
	Wear a pedometer (7 days), record number of steps each day	2-5 min/day
Baseline & Week 1	Study Visit – Study procedures & instructions	30-60 min
	Hand grip strength assessment (in-person only)	3 min
	In person assessments (for those agreeing to in person visits)	15 min
	Receive first treatment package for weeks 1-4	n/a
	Take study treatment (1 mg daily) & fill out diary daily	2 min or less/day
Week 2	Study Check-in - Side effects & questions	5-10 min
	Take study treatment (2 mg daily) & fill out diary daily	2 min or less/day
Week 3	Take study treatment (3 mg daily) & fill out diary daily	2 min or less/day
Week 4	Take study treatment (4.5 mg daily) & fill out diary daily	2 min or less/day
Week 5-6	Take study treatment (4.5 mg daily)	2 min or less/day
	Study Check-in – Confirm final dose & side effects	5-10 min
	Complete study questionnaires	15-45 min
	Receive second treatment package for weeks 7-16	n/a
	Return unused study drug & paper documents (post office or in-person to BCW)	10-30 min*
Week 7-11	Take study treatment (4.5 mg daily)	2 min or less/day
Week 12	Study Visit – Check-in & reminder to book blood draw	15 min
	Take study treatment (4.5 mg daily)	2 min or less/day
	Complete study questionnaires	15-45 minutes
Week 16	Wear a pedometer (7 days), record number of steps each day	2-5 min/day
	Collection of blood sample at LifeLabs	30 min (time will vary)
	Hand grip strength assessment (in-person only)	3 min
	Study Visit – Check-in, side effects, questions & instructions for end of study	30-60 min
	In person assessments (for those agreeing to in person visits)	15 min
	Complete study questionnaires	15-45 min
	Return unused study drug & paper documents (post office or in-person to BCW)	10-30 min*
Total Duration: 16 weeks	**Total Anticipated Time:** 5 hours & 4 min - 9 hours & 38 min	

* timing may vary depending on wait times at the post office.

Study Drug Shipment

The drug will be shipped via the FedEx Clinical Care Service which specializes in shipment solutions for clinical trials or delivered by a local courier to those who live in the Lower Mainland. The exterior of the box has no indication of the contents, and will have your name, address, and the BC Women's Hospital return address. You will be asked how you would like your package delivered. You will be asked if you will allow the package to be handed to an adult within your household, or you can specify that your package will only be handed directly to you. The package will only be handed off by FedEx upon a valid ID check and signature.

- If you receive your study drug from FedEx, you will need to be available for the delivery window (8 am -12 pm). The driver will wait as you remove the study package and reseal the outer shipping box for return. Instructions and tape will be provided inside the outer shipping box.

9. What are the possible harms and discomforts?

Naltrexone in the low doses used in this study are considered safe. Although it is not anticipated with the doses of naltrexone being given in this study, you may experience side effects. It is not known what the impact of Post-COVID fatigue syndrome may have in terms of potential side effects with LDN. In the very unlikely event you experience any serious side effects, you should contact Emergency Services immediately and inform the study doctor.

If you need to go to Emergency or be admitted to the hospital for any reason, please let the study team know via the 24 hour phone number (604-786-2473). If you experience any serious side effects due to the study treatment, the study doctor will assess your symptoms and withdraw you from the study if necessary. Even if withdrawn from the study, the study team will follow your recovery progress until the side effects have fully resolved.

Side effects of taking low-dose naltrexone were seen in less than 10% of patients (from the experience of the CCDP at BCW) and they include:

- Sleep dysfunction
- Gastrointestinal upset
- Skin rash
- Vivid Dreams

If you experience severe or persistent side effects after going back down to a lower dose of the study drug, you can stop the study treatment without tapering. You may withdraw from the study or you can continue even after you have stopped the study treatment.

Other symptoms & possible harms:

If other symptoms, such as shortness of breath, oral swelling, faintness, or any other severe symptom, you should seek emergency medical attention.

Naltrexone has been identified as a hepatotoxin, which is a toxic chemical that damages the liver. This was found using doses five times the recommended dose for opioid blockade or the treatment of opioid/alcohol dependence. No cases of liver failure due to naltrexone have ever been reported. The doses in this study are significantly less (4.5 mg) than the recommended doses (50 mg). Even though the risk of this is very low, if you experience any symptoms of hepatitis (inflammation of the liver) or liver problems, such as feeling very unwell, with jaundice (your skin becomes yellow), fever, vomiting and/or

drowsiness (reduction of level of consciousness), please seek emergency medical attention and advise the study team.

Side effects that have been reported during clinical trials with naltrexone at the recommended doses (50mg tablets) are outlined in the table below. To note, study participants will be receiving less than 1/10th of the recommended dose.

More than 10% of participants experienced the following:
Difficulty sleeping, anxiety, nervousness, abdominal pain/cramps, nausea and/or vomiting, low energy, joint and muscle pain, headache
Less than 10% of participants experienced the following:
Loss of appetite, constipation, increased thirst, increased energy, feeling down, irritability, dizziness, skin rash, delayed ejaculation, decreased potency, chills
Less than 1% of participants experienced the following:
Respiratory: nasal congestion, itching, rhinorrhea (runny nose), sneezing, sore throat, excess mucus or phlegm, sinus trouble, heavy breathing, cough, shortness of breath Cardiovascular: nose bleeds, phlebitis (inflammation of the vein), edema, increased blood pressure, non-specific EGG changes, palpitations, tachycardia Gastrointestinal: excessive gas, haemorrhoids, diarrhea, ulcer Musculoskeletal: painful shoulders, legs or knees, tremors, twitching Genitourinary: increased frequency of, or discomfort during urination, increased or decreased sexual interest Dermatologic: oily skin, pruritus (itchy skin), acne, athlete's foot, cold sore, alopecia (hair loss) Psychiatric: depression, paranoia, fatigue, restlessness, confusion, disorientation, hallucinations, nightmares, bad dreams Special Senses: eyes – blurred, burning, light sensitive, swollen, aching, strained; ears – "clogged", arching, tinnitus General: increased appetite, weight loss, weight gain, yawning, somnolence (drowsy), fever, dry mouth, head "pounding", inguinal pain (groin pain), swollen glands, "side" pains, cold feet, "hot spells"

Data collected since the approval of naltrexone shows that most side effects occur early during treatment and only last for a short time.

Pregnancy and Breastfeeding

It is not known what effect naltrexone may have on a fetus or if it is secreted in breastmilk. You cannot participate if you are pregnant, may become pregnant, or are currently breastfeeding. If you are a female participant who can become pregnant, you can participate if you agree to use an effective method of birth control during the study. The approved methods are the following:

- Hormonal birth control (for at least 1 month before study drug start)
- Intra-uterine device (IUD)
- Condom with spermicidal foam/gel/film/cream/suppository
- Male partner who has had a vasectomy

- Abstinence

If you get pregnant during the study, inform the study doctor immediately. The study treatment will need to be stopped and you will be withdrawn from the study.

Questionnaires
Completing the questionnaires may or may not make you feel tired. We encourage you to complete the surveys at your own pace.

10. What are the potential benefits of participating?
Participants may experience improvements in fatigue, pain, other symptoms, and well-being. However, there may not be direct benefit to you from taking part in this study.

We hope that the information learned from this study can be used in the future to benefit other people with a similar disease.

11. What are the alternatives to study treatment?
There are no alternatives to the study treatment at this time. You can discuss available options with your study doctor on how to treat your symptoms.

12. After the study is finished
You may not be able to receive the study treatment after your participation in the study is completed. There are several possible reasons for this, some of which are:

- The treatment may not turn out to be effective.
- The treatment may not be approved for use in Canada to treat PCFS.
- You may not feel it is the best option for you.
- You may decide it is too expensive and insurance coverage may not be available.
- The treatment, even if approved in Canada, may not be available free of charge.

The study treatment (naltrexone) is available in Canada but it is not approved for treating PCFS. It can be used off label if a physician prescribes it for you. LDN treatment needs to be compounded by a compounding pharmacy and these costs may not be covered by most insurance plans.

After study completion, we ask that you return any unused study drug, empty containers, and completed paper forms in-person to the study team or via Canada Post.

The leftover biological samples will be stored for up to 10 years after the completion of the study. These samples will only be used for analysis related to this protocol. Samples for this specific research question will not be shared with outside researchers. You will not get the results of any of the research blood test for this study.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time. We would also be happy to provide a summary of the study results via your preferred method of contact.

13. What if new information becomes available that may affect my decision to participate?
If you choose to enter this study and at a later date, a more effective treatment becomes available, it will be discussed with you. You will also be advised of any new information that becomes available that may affect your willingness to remain in this study. You may be invited to sign an amended consent form to indicate your continued consent to participate in the study.

14. What happens if I decide to withdraw my consent to participate?
You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, all information about you collected up to the point of your withdrawal (including information obtained from your biological samples) will be retained for analysis in order to protect the integrity of the research, which may benefit future research participants and patients. However, no further information will be collected. If you decide to withdraw, you may still be asked to come in for a final safety visit to ensure your safety.

If samples have been collected before you withdraw, they will be destroyed or returned to the facility from which they were obtained. There may be exceptions where the samples will not be able to be withdrawn, for example, where the sample is no longer identifiable (meaning it cannot be linked in any way back to your identity).

15. Can I be asked to leave the study?
You may be asked to leave the study if the study doctor judges it is not in your best interest to continue, you experience serious side effects due to the study treatment, you become pregnant during the study, if you are unable to fulfill the requirements for the study, or for any other reason. If you are asked to leave the study, the reasons for this will be explained to you and you will have the opportunity to ask questions about this decision.

If you decide to take opioid medication over a short time during the study to manage acute pain, you will be allowed to continue in the study, and your study doctor and team will reinforce guidance on taking alternative medication. Please let the study team know if you take any opioid medication or if you start any new medications during the study. You should be aware that naltrexone is an opioid antagonist, which is medication that blocks the effects of opioids. If you do take opioids for pain relief during the study, they may not work well due to the naltrexone.

In the event that you need to take long-term opioid medication, you will be asked to withdraw from the study. All information about you collected up to the point of your withdrawal (including information obtained from your biological samples) will be retained for analysis in order to protect the integrity of the research.

The study doctor will arrange for you to continue your care outside of the study. The study may also be stopped at any time by the sponsor, the C&W Research Ethics Board or Health Canada if new information rises about the safety of the study treatment. The reasons for study stoppage will be explained to you by the study doctor.

16. How will my taking part in this study be kept confidential?

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator or his/her designate by representatives of Health Canada and the C&W Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc.). Only this number will be used on any research-related information collected about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected. You also have the legal right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

The study doctor may need to review your medical record or call your family doctor to help determine if you are eligible for the study and that it is safe for you to take naltrexone. Only the study doctors will access your medical record, the other members of the study team (research coordinator, research assist, etc.) will not be accessing your medical record. If you sign this form you are agreeing to the study doctor reviewing your medical record and/or calling your family doctor.

You should be aware that all of the study test results will be reported to the ordering physician (the study principal investigator Dr. Nacul).

The samples taken at LifeLabs may be referred to a testing laboratory outside of BC to be analyzed (this may be in another province or in the USA). This is because not all tests are performed at LifeLabs and they use other laboratories in these cases.

Studies involving humans now routinely collect information on race and ethnic origin as well as other characteristics of individuals because these characteristics may influence how people respond to different medications. You should be aware that providing this information is not mandatory.

We are asking you to provide a personal email address and phone number (for phone calls and texts, if you prefer) so that we can communicate with you about the study, your participation, send you an electronic copy of your consent form and other study documents, and send reminders.

Before you provide your consent, please carefully consider whether this email account is secure, whether other people have access to it, or whether you have concerns about the security of any information sent to this account. We will only send your personal information to the email address and/or phone number you have provided to us, and all of the information which you provide to us will be kept confidential by the research team. However, you should be aware of the fact that some webmail services (e.g. Gmail, Hotmail, etc.) may store the contents of your email account outside of Canada (for example, in the United States), where privacy and data security standards may be different than they are in Canada. Due to the fact that future emails may contain some personal information about you, including your name, the Freedom of Information and Protection of Privacy Act (British Columbia) requires that we obtain your consent. Providing your email address means that you voluntarily agree and give your consent for the study team to email your consent form, containing personal information, to you.

Your de-identified research data (which means your name, birth date, and other identifiers have been removed) may/will be deposited into a publicly accessible location at the time of publication. This can enhance the transparency of the research data and allows for external validation and fraud control, but it also allows others to access the data for re-analysis of this study, or to do other kinds of analyses in the future beyond those you are consenting to in this study. Also, this future use of your data may not be subject to oversight by a research ethics board, and thus the data may be publicly shared and used in currently unknown ways. Once the data is made publicly available, you will not be able to withdraw your data. Even though the identifying information will be removed from the data, it is possible that others may be able to find out who you are. The chance of this is currently thought to be quite low.

17. What happens if something goes wrong?

By signing this form, you do not give up any of your legal rights and you do not release the study doctor, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan.

In case of a serious medical event, please report to an emergency room and inform them that you are participating in a clinical study and show them your study card. They can contact the study team and/or Dr. Luis Nacul for further information with the phone numbers listed on the study card. The Emergency Contact Number is 604-786-2473.

18. What will this study cost me?

All research-related medical care and treatment, and any related tests that you will receive during your participation in this study will be provided at no cost to you.

To thank you for your participation you will receive $25 gift cards or honoraria (cheque issued by UBC; need to provide personal information (contact details) to UBC for cheques to be issued) twice - one after 6 weeks of treatment and one at the end of study at 16 weeks for a total of $50.

If you agree to have the first and last visit in person, you will receive and additional $25 gift cards or honoraria (cheque) for each visit for a total of $50 for both in person visits.

19. If I have questions about the study procedures during my participation, who should I speak to?
If you have any questions or desire further information about this study before or during participation, or if you experience any adverse effects, you can contact the Study Team at LDNtrial@phsa.ca or 236-990-9519.

20. Who do I contact if I have any questions or concerns about my rights as a participant?
If you have any concerns or complaints about your rights as a research participant and/or your experiences while participating in this study, contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics by e-mail at RSIL@ors.ubc.ca or by phone at 604-822-8598 (Toll Free: 1-877-822-8598.) Please reference the study number *(H21-02254)* when calling so the Complaint Line staff can better assist you.

If you are a patient from the Fraser Health and you have any concerns about your rights as a research participant, you can also contact Fraser Health Research Ethics Board Co-Chair by calling 604-587-4681.

21. Primary Care Physician(s)/Specialist(s) Notification
Please indicate, by checking the applicable box, whether you want us to notify your primary care physician(s) or specialist(s) of your participation in this study. This is not a consent to release medical information.

☐ Yes, I want the study investigator to advise my primary care physician(s) or specialist(s) of my participation in this study. My primary care physician(s) and/or specialist(s) name(s) is/are:

_____

The name of the medical clinic I attend is: _____

Participant Initials: _____

☐ No, I do not want the study investigator to advise my primary care physician(s) or specialist(s) of my participation in this study.

Participant Initials: _____

☐ I do not have a primary care physician or specialist.

Participant Initials: _____

☐ The study investigator is my primary care physician/specialist.

Participant Initials: _____

I understand that if I choose not to advise my primary care physician(s) or specialist(s) of my participation in this study, there may be potential medical consequences, which may affect my

comprehensive medical care or treatment. I understand that the study investigator may not be responsible for these consequences.

You may wish to discuss the consequences of your decision with the study staff.

22. Optional MRI Sub-Study
There is an optional sub-study that will available for some participants. This sub-study will involve MRI scans of the brain at study start and towards the end of the 12-week study period

If you are interested in learning more about the sub-study, check the box below and the research team will provide you further information and connect you with the MRI study personnel.

I am interested in learning more about the MRI sub-study
☐ Yes
☐ No

23. Optional Morning Blood Sampling at LifeLabs
The optional morning blood sampling is for 50 participants who agree to have their study samples drawn at LifeLabs between 8-10 am on the days they have their study bloodwork done. There are two tests, cortisol and adrenocorticotropic hormone, that require that the blood sample is taken in the morning. These tests are expected to be predictors of treatment response and we expect that treatment will be associated with an increase of these concentrations back to normal values.

If you participate in the morning blood sampling, you are agreeing to go to LifeLabs in the morning (8-10am) for your study bloodwork for both time points bloodwork is collected.

I agree to participate in the optional morning blood sampling and will go to LifeLabs between 8-10am for my bloodwork for this study.
☐ Yes
☐ No

24. Optional In Person Study Visits at BCW
Participants who agree to have their first and last study visits (baseline & 16 weeks) will have additional in person assessments completed which will include weight and height, blood pressure and pulse at laying down and over 5 minutes following passive standing, sit and stand test, and hand grip strength test. We are aiming to enroll 50 participants minimum into this sub-group.

If you agree to have the in person study visits, you will receive an additional $25 gift card or honoraria (cheque) for each visit, for a total of $50.

I agree to participate in the optional in person study visits and will attend in person visits at BCW for the baseline and 16 week visits for this study.
☐ Yes
☐ No

25. Participant Consent

A Double Blind Randomized Trial of Low-Dose Naltrexone for Post-COVID Fatigue Syndrome (LDN Trial)

My signature on this consent form means:

- I have read and understood the information in this consent form.
- I have had enough time to think about the information provided.
- I have been able to ask for advice if needed.
- I have been able to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the results will only be used for scientific purposes.
- I understand that my de-identified research data may/will be deposited into a publicly accessible location at the time of publication.
- I understand that my participation in this study is voluntary.
- I understand that I am completely free at any time to refuse to participate or to withdraw from this study at any time, and that this will not change the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me.
- I authorize access to my health records and samples as described in this consent form.
- I understand that the study doctor will review my medical record and/or call my family doctor to help determine if I am eligible for the study.

I will receive a signed and dated copy of this consent form for my own records.

I consent to participate in this study.

_____	_____	_____
Participant's Signature	Printed name	Date

_____	_____	_____	_____
Signature of Person Obtaining Consent	Printed name	Study Role	Date

Please provide your contact details:

Phone: _____

Email: _____

References

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym *Page 1*
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry *Page 4*
	2b	All items from the World Health Organization Trial Registration Data Set *Pages 1-2; 6-22*
Protocol version	3	Date and version identifier *Page 4*
Funding	4	Sources and types of financial, material, and other support *Page 2*
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors *Page 2*
	5b	Name and contact information for the trial sponsor *Page 2*
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities *Page 2*

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) *Pages 18-19*
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention *Pages 6-7*
	6b	Explanation for choice of comparators *Page 7*
Objectives	7	Specific objectives or hypotheses *Pages 7-8*
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) *Page 9*
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained *Page 9*
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) *Pages 9-10*
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered *Pages 11-12*

	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
		Page 12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
		Page 12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
		Page 12
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
		Pages 12-13
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
		Pages 14-15
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
		Page 16
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size
		Page 16

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions *Pages 16-17*
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned *Pages 16-17*
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions *Pages 16-17*
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how *Page 17*
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial *Page 17*

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol *Page 17*
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols *Page 17*

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
		Page 17
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
		Pages 18-19
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
		Pages 18-19
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
		Pages 18-19
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
		Page 18
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
		Not applicable
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
		Page 19

Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor *Page 18*

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval *Page 19*
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) *Page 19*
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) *Pages 19-20*
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable *Pages 19-20*
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial *Pages 22*
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site *Page 2*
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators *Page 20*

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation *Not applicable*
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions *Page 20*
	31b	Authorship eligibility guidelines and any intended use of professional writers *Not applicable*
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code *Page 2*
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates *Supplementary material, page 13*
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable *Page 17*

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Study protocol for a double-blind, randomised trial of low-dose naltrexone for post-COVID fatigue syndrome in British Columbia

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Study protocol for a double-blind, randomised trial of low-dose naltrexone for post-COVID fatigue syndrome in British Columbia

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Abbreviations

ACTH	Adrenocorticotropic hormone
AE	Adverse events
BC	British Columbia
CCDP	Complex Chronic Diseases Program
CK	Creatinine kinase
CRP	C-reactive protein
C&W	Children's and Women's Hospital and Health Centre of British Columbia
DSMB	Data Safety and Monitoring Board
EQ-5D	EuroQol 5 dimensions
FDA	Food and drug administration
FM	Fibromyalgia
FSS	Fatigue severity scale
GAD-7	Generalized anxiety disorder-7
HC	Health Canada
HRQOL	Health-related quality of life
IL-6	Interleukin-6
IFNγ	Interferon-gamma
IOM	Institute of Medicine
LDN	Low dose naltrexone
MCID	Minimal clinically important difference
ME/CFS	Myalgic encephalomyelitis/ chronic fatigue syndrome
MHRA	Medicines and Healthcare products Regulatory Agency
NINDS CDE	National Institute of Neurological Disorders and Stroke Common Data Elements
PCC	Post-COVID-19 condition
PC-COS	Post-COVID-19 core outcome set
PCFS	Post-COVID fatigue syndrome
PC-ICCN	Post-COVID-19 Interdisciplinary Clinical Care Network
PCP	Primary care provider
PCR	Polymerase chain reaction
PHQ-9	Patient health questionnaire-9
POTS	Postural orthostatic tachycardia syndrome
PQSymp-12	Patient phenotyping questionnaire short form
PROMIS	Patient Records and Outcome Management Information System
PROMs	Patient reported outcome measures
PVFS	Post-viral fatigue syndrome
RAT	Rapid antigen test
RCT	Randomized controlled trial
REB	Research ethics board
rT3	Reverse triiodothyronine
SAE	Serious adverse events
SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials
SQ-2	Sleep questionnaire-2
T3	Triiodothyronine
T4	Thyroxine
TNF	Tumour necrosis factor
TSC	Trial Steering Committee
TSH	Thyroid stimulating hormone
UBC	University of British Columbia
VAS	Visual analogue scale
WHRI	Women's Health Research Institute

ABSTRACT

Introduction: A significant proportion of individuals suffering from post-COVID-19 condition (PCC, also known as *long COVID*) can present with persistent, disabling fatigue similar to myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS) and post-viral fatigue syndromes. There remains no clear pharmacologic therapy for patients with this subtype of PCC, which can be referred to as post-COVID fatigue syndrome (PCFS). A low dose of the opioid antagonist naltrexone (i.e., low dose naltrexone, LDN) has emerged as an off-label treatment for treating fatigue and other symptoms in PCC. However, only small, non-controlled studies have assessed LDN in PCC, so randomised trials are urgently required.

Methods and analysis: A prospective, randomised, double-blind, parallel-arm, placebo-controlled phase II trial will be performed to assess the efficacy of LDN for improving fatigue in PCFS. The trial will be decentralized and open to eligible individuals throughout the Canadian province of British Columbia (BC). Participants will be recruited through the province-wide Post-COVID-19 Interdisciplinary Clinical Care Network (PC-ICCN) and research volunteer platform (REACH BC). Eligible participants will be 19-69 years-old, have had a confirmed or physician-suspected SARS-CoV-2 infection at least 3 months prior, and meet clinical criteria for PCFS adapted from the Institute of Medicine ME/CFS criteria. Individuals who are taking opioid medications, have a history of ME/CFS prior to COVID-19 or history of significant liver disease will be excluded. Participants will be randomised to an LDN intervention arm (n=80) or placebo arm (n=80). Participants in each arm will be prescribed identical capsules starting at 1mg daily and follow a prespecified schedule for up titration to 4.5mg daily or the maximum tolerated dose. The trial will be conducted over 16 weeks, with assessments at baseline, 6, 12, and 16 weeks. The primary outcome will be fatigue severity at 16 weeks evaluated by the fatigue severity scale. Secondary outcomes will include pain visual analogue scale score, overall symptom severity as measured by the Patient Phenotyping Questionnaire Short Form, 7-day step count, and health-related-quality of life measured by the EuroQol 5-dimension questionnaire.

Ethics and dissemination: The trial has been authorized by Health Canada and approved by The University of British Columbia / Children's and Women's Health Centre of British Columbia Research Ethics Board. Upon completion, findings will be disseminated to patients, caregivers, and clinicians through engagement activities within existing PCC and ME/CFS networks. Results will be published in academic journals and presented at conferences.

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Strengths and limitations of this study

- The trial will be decentralized and will recruit participants from throughout the geographically large and ethnically diverse Canadian province of British Columbia (BC); this will permit inclusion of patients from communities that do not typically have access to investigational treatments, and patients who may be too symptomatic to attend in-person assessments.
- In addition to evaluating fatigue severity as the primary outcome, the study will capture several secondary outcome measures known to be important to PCC patients, including pain, overall symptom burden, health-related quality of life, and activity levels (as measured by step count).
- The study does not have a restriction on how long a participant may have had their symptoms since COVID-19; this may limit the treatment effect if LDN efficacy is greater earlier in the disease course.
- As in-person evaluation is optional, this limits the ability to assess potentially important objective outcomes in some participants.

INTRODUCTION

Background and Rationale

Approximately 15-20% of adults with a confirmed or suspected SARS-CoV-2 infection experience long-term symptoms lasting over 3 months.[1–4] The presence of new or persistent symptoms following acute COVID-19 disease is now referred to as post-COVID-19 condition (PCC), or 'long-COVID'.[5–11] Among the hundreds of symptoms reported by PCC patients, fatigue is one of the most common and may have the greatest impact on functioning.[12–21] Given that millions of individuals may be currently affected by PCC worldwide, it has become a priority to investigate potential treatments in randomized controlled trials (RCTs).[5,22,23]

However, it has been challenging to identify candidate treatments for PCC as it is a heterogeneous illness, and the underlying pathobiology is poorly understood. It is suspected that different groups of PCC patients may have distinct underlying disease processes, such that the ideal pharmacologic therapy may not be the same for all.[5,22] Increasingly, studies have suggested that PCC may not represent a single disease, but rather a collection of different conditions or subtypes.[24–26]

For example, clinical experience and patient-centred studies have indicated that a proportion of PCC patients present with a symptom profile indistinguishable from myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS).[27–32] ME/CFS is characterized by persistent disabling fatigue accompanied by other symptoms including nonrestorative sleep and post-exertional malaise.[33–35] While the precise pathogenesis of ME/CFS also remains unresolved, it usually follows acute infections.[36] When provoked by a viral infection, ME/CFS is often referred to as a post-viral fatigue syndrome (PVFS).[36–39] It is believed that some PCC patients have developed a PVFS from SARS-CoV-2, and we will refer to this subset of PCC patients as having 'post-COVID fatigue syndrome' (PCFS).[37–40]

A low dose of the medication naltrexone is a potential treatment for PCFS.[41,42] Naltrexone is an opiate antagonist that is approved for treatment for alcohol and opiate use disorders.[43] For these indications, it is typically prescribed at 25-50 mg.[44] At lower doses (≤5 mg) it has been used off-label for chronic pain, multiple sclerosis, Crohn's disease, recurrent depression, fibromyalgia (FM) and ME/CFS.[45–59] Although evidence supporting the use of low dose naltrexone (LDN) in ME/CFS has

been limited to case series and chart reviews,[48,56] it has been investigated in clinical trials for related conditions such as fibromyalgia.[52,53,59,60] In these and other studies, LDN has been shown to be safe with a limited side-effect profile.[51,53–55,59]

Based on its hypothesized mechanism of action, it is plausible that LDN could be efficacious for ME/CFS and PCFS. LDN increases circulation of the endogenously produced opiate-like molecule beta-endorphin, which is reduced in ME/CFS.[61,62] Furthermore, LDN has been found to antagonize toll-like receptors on neuroglia and peripheral blood mononuclear cells, resulting in reduced production of inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor (TNF).[63–66] Increased IL-6 and TNF signalling have been implicated in PCC,[67] and studies have implicated increased neuroinflammation in ME/CFS and PCC pathogenesis.[18,68–71]

There is ongoing public interest in investigating LDN for PCC. Media outlets including *Rolling Stone, National Geographic*, *Reuters,* and *The New York Times Magazine* have all touted LDN as a potential PCC treatment, citing the anecdotal experiences of PCC patients and physicians who have used LDN.[72–79] However, published evidence for LDN in the post-COVID-19 context remains limited. In a single-centre study, 52 patients treated with LDN had, on average, overall improvement in activities of daily living, energy levels, pain, concentration, and sleep disturbance.[42] In a retrospective study, 37 of 59 (62.7%) patients treated with LDN reported improvement in at least one symptom.[80]

RCTs are required to determine whether LDN is an effective treatment for post-COVID-19 symptoms. Since there is no widely accepted pharmacologic treatment for PCFS, the ideal comparator group is a placebo. Accordingly, we have designed a double-blinded placebo-controlled trial of daily LDN vs. placebo for the treatment of fatigue severity in PCFS.

Objectives

Study objectives are outlined in **Table 1**. The primary objective is to determine whether LDN can reduce fatigue severity associated with PCFS, as measured by the fatigue severity scale (FSS). The secondary objectives are to determine whether it can reduce pain, reduce symptom severity, improve health-related quality of life (HRQOL) and increase activity levels. We have developed additional

exploratory objectives that examine other patient reported outcome measures (PROMs), laboratory outcomes, and physical measurements.

Table 1: Summary of study objectives and associated outcomes

Primary objective		***Primary outcome***
To determine if LDN, administered at 1-4.5 mg/day to individuals with PCFS, reduces fatigue severity.		FSS score at 16 weeks.
Secondary objectives		***Secondary outcomes***
To determine if LDN, administered at 1-4.5 mg/day to individuals with PCFS:	Reduces pain.	Pain VAS score at 16 weeks.
	Improves severity of symptoms associated with PCFS.	PQSymp-12 score at 16 weeks.
	Increases activity levels.	Average number of steps over 7 days at 16 weeks.
	Improves self-reported quality of life.	EQ-5D-5L health utility score at 16 weeks.
Exploratory objectives		***Exploratory outcomes***
To determine if LDN, administered at 1-4.5 mg/day to individuals with PCFS:	Reduces inflammatory marker values in peripheral blood.	IL-6, IFNγ, and CRP values at 16 weeks. Cytokine profile values using Human Cytokine Proinflammatory Focused 15-plex Discovery Assay® Array at 16 weeks.
	Improves disease severity associated with PCFS.[81]	CK level at 16 weeks.
	Improves rT3 as an indirect marker of disease severity.[82]	rT3 in conjunction with TSH, free T3 and free T4 at 16 weeks.
	Improves AM blood cortisol and improves ACTH.	AM blood cortisol level at 16 weeks. ACTH level at 16 weeks.
	Reduces fatigue VAS score.	Fatigue VAS score at 16 weeks.
	Improves sleep.	SQ-2 at 16 weeks. Seep VAS score at 16 weeks.
	Improves depression symptoms.	PHQ-9 score at 16 weeks.
	Improves anxiety symptoms.	GAD-7 score at 16 weeks.
	Improves self-reported health.	Self-reported health VAS score at 16 weeks.
	Improves functional status.	Post-COVID-19 Functional Status Scale at 16 weeks.
	Reduces prevalence markers of POTS or postural hypotension*	Prevalence of POTS or postural hypotension symptoms based on serial blood pressure and heart rate measurements at 16 weeks.
	Improves clinical endurance/ strength parameters in subjects with PCFS*	Hand grip strength at 16 weeks.[83] Sit and stand test results at 16 weeks.

Legend. Abbreviations: ACTH- adrenocorticotropic hormone; CRP- c-reactive protein; EQ-5D-5L EuroQol 5 Dimension 5-level; FSS- fatigue severity scale; LDN- low dose naltrexone; PQSymp-12- Patient Phenotyping Questionnaire Short Form-12; PCFS- post-COVID fatigue syndrome; rT3- reverse triiodothyronine; SQ-2- sleep questionnaire-2; T3- triiodothyronine; T4-thyroxine; TSH- thyroid stimulating hormone; VAS- visual analogue scale. *Optional in person visits.

Trial Design

The development of this trial protocol followed the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines.[84] This trial is designed as a randomised, controlled, double-blind prospective trial with two parallel groups and primary end point of fatigue severity at 16 weeks. The intervention group will receive LDN capsules dosed at 1.0mg to 4.5mg daily and the control group will receive placebo capsules. Randomisation will be stratified by sex and performed as permuted block randomisation with a 1:1 allocation. The trial will be conducted in British Columbia (BC), Canada.

METHODS AND ANALYSIS

Study Setting

The trial will involve a collaboration between BC's Post-COVID-19 Interdisciplinary Clinical Care Network (PC-ICCN) and the Complex Chronic Diseases Program (CCDP) at BC Children's and Women's Hospital and Health Centre (C&W) located in Vancouver. The PC-ICCN was founded as a learning health system for post-COVID-19 care and research in BC.[85–88] The network previously comprised of 5 physical Post-COVID Recovery Clinics (PCRCs) but has now consolidated to a single virtual program. Adults throughout BC may be referred to this program by their primary care provider (PCP) if they have had COVID-19 and meet criteria for PCC. The CCDP is an interdisciplinary program that supports patients with ME/CFS and related conditions.[89]

Eligible participants will be recruited from throughout BC. Participants will have virtual or in-person study visits, may have their study product and pedometer delivered to them, complete questionnaires electronically, and have blood tests done at their local laboratories. Collection of exploratory data during in-person visits will be optional.

Eligibility Criteria

Inclusion and exclusion criteria are listed in **Table 2**. To be included, participants must be aged 19 to 69 years, have significant fatigue and related symptoms that started after a SARS-CoV-2 infection and meet the criteria we have developed for PCFS. These criteria are adapted from the Institute of Medicine (IOM) ME/CFS standard clinical criteria,[35] but with duration of symptoms of 3 months rather

than 6 months to be consistent with PCC definitions (**Box 1**).[11] Diagnosis for eligibility purposes will be determined from clinical assessment by a study physician and supported by laboratory data and responses to the screening and baseline questionnaires. Participants who do not have a documented positive polymerase chain reaction (PCR) test will be eligible if they are determined by a physician through medical history to have had a positive rapid antigen test (RAT) or compatible symptoms following close contact with individuals who tested positive. Individuals will be excluded if they have a history of ME/CFS prior to SARS-CoV-2 infection, have significant liver disease, have taken naltrexone within 30 days, or if they have taken opioids within 15 days.

Table 2: Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
1. Male and female patients ages 19 to less than 70 years. 2. Case of SARS-CoV-2 over 3 months previously, confirmed by a positive test result or clinical confirmation by a physician. 3. Meet the clinical diagnostic criteria for PCFS (**Table 3**). 4. Agree to maintain any other regular medications at current doses for the duration of the trial (except for essential need of new medication or dose change, as prescribed by a physician). 5. Agree to use effective contraception for the trial duration, as appropriate (if female). 6. The participant resides within the delivery area for the drug as determined by FedEx Clinical Trial Services.	1. Pregnant, planning to become pregnant, or breastfeeding. 2. Use of opioid medications within last 15 days, as reported by the patient or during the trial. 3. A positive urine test for opioids (only for the first 16 participants; see below). 4. History of alcohol, opioid or other substance misuse. 5. Participation in another interventional clinical trial in the last 30 days or planned during the trial period. 6. Confirmed ME/CFS or FM existing prior to SARS-CoV-2 infection. 7. Allergy to naltrexone or medication components. 8. Acute hepatitis, liver failure, or severe kidney failure. 9. Current or recent use of naltrexone within 30 days.

Legend. Abbreviations: PCFS- post-COVID fatigue syndrome; ME/CFS- myalgic encephalomyelitis/ chronic fatigue syndrome; FM- fibromyalgia.

Box 1: Study diagnostic criteria of post-COVID fatigue syndrome

Diagnosis requires that the patient have the following 3 symptoms following a SARS-CoV-2 infection:
1. **A substantial reduction or impairment in the ability to engage in pre-illness levels of activity** (occupational, educational, social, or personal life) that: 1. lasts for more than 3 months. 2. is accompanied by fatigue that is: 1. often profound 2. of new onset (not life-long) 3. not the result of ongoing or unusual excessive exertion 4. not substantially alleviated by rest 2. **Post-exertional malaise** 3. **Unrefreshing sleep**
At least 1 of the 2 manifestations must be present:
1. **Cognitive impairment** 2. **Orthostatic intolerance**
AND
Absence of other diseases or conditions that explain symptoms, based on differential diagnosis

Legend. For this study, we have developed criteria for post-COVID fatigue syndrome, a form of post-COVID-19 condition which presents with symptoms similar to an myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS) following a SARS-CoV-2 infection. The criteria are based on the IOM for ME/CFS but requires 3 months of symptoms as opposed to 6.[35]

Interventions

Eligible participants will be randomized at a ratio of 1:1 (n=80 each) into either active treatment group with LDN or placebo. The treatment duration is 16 weeks. The LDN and placebo will be compounded by Macdonald's Prescriptions Labs Ltd. (Vancouver, BC) and dispensed at the C&W Pharmacy where the blinding will occur.

Macdonald's Prescriptions Labs Ltd. will compound the required doses of LDN from Naltrexone Hydrochloride USP supplied by MEDISCA in empty gelatin CONI-SNAP capsules.[90] The compounded LDN capsules will be filled with CELLULOSE, NF/EP (Microcrystalline) (Flocel® 101).[91] Placebo capsules will look identical to the compounded LDN capsules and filled with the same diluent and food colouring. We will complete batch testing of the LDN and placebo compounds (**Appendix 1**).

The dose-titration schedule from 1 mg to 4.5 mg is outlined in **Table 4**. The drug will be dispensed to participants by certified courier, temperature-controlled shipping, in-person pick-up, or delivered by staff. Participants will be able to adjust treatment doses by reverting to the previous well-tolerated dose if they experience persistent but minor side effects following any increase in dose. If a participant has reverted to a previous dose, that dosage will be maintained for the remainder of the study

period. Changes in doses will be documented by the participant by completing a daily dosing diary, completed for the first 4 weeks and for 7 days after any change in dose.

By allowing participants to reduce doses if experiencing any potential side effects, we expect low rates of medication use interruption. In addition to diaries, participants may also have visits or contact with the study team where adherence can be discussed. Furthermore, there are treatment compliance questions asked with each series of questionnaires. The participants will be asked to return the unused study drug, empty containers, and study drug diary sheet(s).

Participants will be asked to maintain any other regular medications at their current doses for the duration of the trial, unless there is an essential need of a new medication or dose change. Participants can withdraw from the study at any time without giving reasons. Withdrawal criteria are described in **Box S1.**

Table 3: Product supply timeline and titration schedule

Week (s)	Supply	Dose	Capsules
1	First	1 mg/day	*1 mg cap*
2	First	2 mg/day	*Two 1 mg capsule*
3	First	3 mg/day	*Three 1mg capsule*
4-6	First	4.5 mg/day	*Three 1 mg capsule, plus one 1.5 mg capsule*
7-16	Second	4.5 mg/day *	*One 4.5 mg capsule**

Legend. There will be two dispensing time points when participants will be supplied with the study product. The first supply will be for weeks 1-6 and the second supply will be for weeks 7-16 of the study. For the first supply, participants will receive 1mg and 1.5mg capsules of the study product (low dose naltrexone or placebo). They will be asked to up-titrate the dosage as tolerated and keep a diary of their dosage. In the second dispensing period, they will be supplied with capsules of their maximum tolerated dose. *Or maximum tolerated dosage (i.e. one 1mg capsule, one 2mg capsule or one 3mg capsule).

Outcome Measures

The primary outcome measure is fatigue severity, as measured by the FSS. The FSS is a 9-item PROM scored from 9 (least fatigue) to 63 (most fatigue).[92] A score of >36 is consistent with clinically significant fatigue.[93,94] The FSS has been has been validated in multiple diseases, and has been used in randomised trials for ME/CFS.[93,95–97] The FSS received the highest level of recommendation of any subjective fatigue measure for ME/CFS by the National Institute of Neurological Disorders and Stroke Common Data Elements (NINDS CDE) Project and was a recommended measure by the Post-COVID-19 Core Outcome Set (PC-COS) initiative.[19,98–102] We have previously investigated the FSS in patients

with PCC in BC, and demonstrated that the instrument has strong acceptability, internal consistency, and construct validity in this population.[100]

Secondary PROMs will include pain severity as measured by the pain visual analogue scale (VAS); total symptom score on the Patient Phenotyping Questionnaire Short Form (PQSymp-12) and HRQOL captured by the EQ-5D-5L instrument. Pain is a common symptom in PCC and studies have suggested that LDN may be an effective analgesic.[16,18,45,54,55,57,103] The pain VAS is a single-item tool that has been shown to have strong psychometric properties among patients with chronic pain.[104] The PQSymp-12 is a 12-item questionnaire which covers seven clusters of symptoms derived from the Canadian Consensus Criteria for ME/CFS; it has been recommended as a core assessment measure for ME/CFS by the European Network on ME/CFS (EUROMENE)[105] and is included in the UK ME/CFS biobank.[106] The EQ-5D-5L is a generic HRQOL instrument that was recommended by PC-COS.[102] By applying Canadian preference weights, responses to the EQ-5D will be used to derive a health utility (HU) score from 0 (dead) to 1 (perfect health).[107]

An additional secondary outcome is average step count. We will ask participants to wear a pedometer and document daily step counts for 7 days prior to starting the study drug, and again in week 16. All participants will use the same brand and type of pedometer (OZO Fitness CS1 Easy Walk Pedometer). Step counts have been used previously in randomised trials to measure change in activity levels among patients with ME/CFS.[97,108–110]

There will be several exploratory outcomes (**Table S1)** including PROMs (fatigue VAS, sleep, depression symptoms, anxiety symptoms, self-reported health, and functional status), laboratory-based (inflammatory markers, CK, thyroid profile, AM cortisol and ACTH level) and based on physical measurements (grip strength, sit and stand test, and orthostatic changes in vitals). Physical measurements will be limited to participants who choose to attend in-person visits.

MRI Study

As a sub-study of the RCT, 25 participants of each study arm are planned to have brain magnetic resonance imaging (MRI) scans at baseline prior to the intervention/placebo and at 16 weeks. A multimodal functional and spectroscopy (fMRI/MRS) protocol piloted in a ME/CFS study (REB# H20-

01804, unpublished) will be employed (**Figure S1**). MRI findings will be linked to the primary and other outcome measures.

Participant Timeline

The participant timeline is detailed in **Table 4** and **Figure 1**.

Table 4: Participant timeline and schedule of study procedures

TIMEPOINT	Screening and Baseline	Week 1	Weeks 4-5	Week 6	Week 12 weeks	Week 16 weeks
ENROLLMENT:						
Eligibility screening by research staff and study physician	X					
Informed consent	X					
Allocation	X					
INTERVENTIONS:						
Study drug (LDN or placebo)	X	X	X	X	X	X
Study drug diary (daily for first 4 weeks and for 7 days after any change in dose)	X	X	X			
VISITS:						
Adverse effects check		X	X	X*	X*	X
Monitor study drug use		X	X	X*	X*	X
ASSESSMENTS:						
Questionnaires^	X			X	X	X
Laboratory investigations	X					X
Pedometer (Number of steps per day)	X					X
Hand grip (Muscle Strength) ‡	X					X
Blood pressure and heart rate‡	X					X
Sit and stand test‡	X					X

Legend. This table outlines the schedule of study procedures. See Figure 1 for timeline of recruitment, eligibility screening and baseline assessments. Abbreviations: LDN- low dose naltrexone. *Occurs as part of questionnaires if optional visit does not occur; ^Short answer questionnaire for demographic and clinical information is done at baseline only; ‡Only for those agreeing to have in person visits.

Figure 1: Flowchart of initial study procedures

[Insert Figure 1 here]

Legend. This flowchart outlines the process for study recruitment, eligibility assessment and baseline assessments. A full study timeline is outlined in Table 4. Abbreviations: BC- British Columbia; LDN- low dose naltrexone; PCC- post-COVID-19 condition; PC-ICCN- Post-COVID-19 Interdisciplinary Clinical Care Network; SAQ- short answer questionnaire.

Sample Size

The sample size was calculated based on the primary hypothesis of reduction in fatigue severity with treatment. To detect a 4.7-point difference (effect size [d]=0.5) in the Fatigue Severity Scale (FSS) (9-63) between arms, we estimate a sample size of 64 participants per arm assuming 80% power, 5% significance and a pooled standard deviation of 9.4 (estimated from the CCDP Data Registry).[89] To account for possible loss-to-follow up of 20%, we estimate a final sample size of 80 per arm, for a total target sample size of 160 participants. We chose this method for sample size estimation (as opposed to use of a minimal clinically important difference (MCID)) because we believed this to be a realistic treatment effect and there were no published MCID values available for the FSS in ME/CFS or PCC.[111,112] In a sensitivity analysis, we calculated sample size using a published MCID for systemic lupus erythematosus and this yielded a similar estimate (**Appendix 2**).[113]

Recruitment and Screening

New PC-ICCN patients will be contacted regarding study participation, and the PC-ICCN directory will be used to identify other candidate PCC patients to contact. Additionally, the trial will be accessible to patients through REACH BC, a provincial online platform that facilitates connections between research studies and participants. All potential participants will be asked to complete an online pre-screening questionnaire, and those potentially eligible will meet with research staff to provide consent. Consented participants will complete baseline questionnaires and be assessed by a physician to confirm eligibility. Baseline laboratory studies for all participants will be done prior to initiation of study drug and abnormal results will be reviewed by a study physician.

Allocation

Participants will be randomised into either the LDN treatment group or placebo at a ratio of 1:1 (n=80 each), as per a computer-generated randomisation schedule stratified by sex and using permuted blocks varying between 2, 4, and 6 participants. A statistician who is not part of the study team will generate a randomisation sequence and corresponding randomization codes. The randomisation codes will be used to maintain the blinding and will be uploaded to REDCap. The randomization sequence will

be provided to the unblinded CWH Research Pharmacy. After confirmation of eligibility, research staff will randomise participants by REDCap, which will provide the randomisation code. CWH Pharmacy staff will then dispense the study drug based on the randomisation allocation sequence.

Blinding

All participants will be blinded to their treatment regimen. The placebo and intervention capsules will appear identical, and the C&W Pharmacy will distribute study drug to study staff in identical containers. Participants, their healthcare providers, and all study staff including research assistants, coordinators, statisticians, trial physicians, and investigators will be blind to allocation. Unblinding will only occur when knowledge of the actual treatment is essential for further management of the patient or investigation of serious adverse events (SAEs). If unblinding is deemed necessary by the DSMB or investigator, the C&W Pharmacy will be contacted for release of treatment allocation.

Data Collection and Management

We will use the secure REDCap platform for storage of study data.[114,115] Participants will complete questionnaires electronically, with the links provided to the participant via email. Data from other sources will be entered manually, and will include study physician assessments, laboratory results, dose diary information, step counts, physical assessment measures, and adverse events (AEs). REDCap field validation tools will be used where possible to optimise data accuracy (e.g. dates that are out of range and data that are missing). No new data will be collected from participants who withdraw, except for reason for withdrawal and details regarding AEs and SAEs.

Biological Specimens

Leftover plasma will be stored at -80C at the BC Children's Hospital Biobank for up to 10 years to allow for additional sample testing related to this protocol that may be identified from the results of this study.

Monitoring and Oversight

A Trial Steering Committee (TSC) will be formed with patient partners, investigators, and other research team members. Additionally, we have formed a Data Safety and Monitoring Board (DSMB) that is comprised of peer researchers with expertise in clinical trials and ethics and independent from the study team. Lastly, an independent study monitor from the CWH Quality Assurance Office has been hired to verify participant rights and wellbeing, data collection and compliance with regulatory requirements. Roles of the TSC, DSMB and study monitor are outlined in **Box S2.**

Statistical Methods

Primary and secondary outcomes will be analyzed by intention-to-treat. The primary outcome (FSS score at 16 weeks) will be analyzed using a linear mixed effects model adjusting for baseline level, sex (stratification factor) and other relevant prognostic factors identified *a priori*. The model will include interaction between treatment arm and time, and treatment arm and baseline level and include all post-randomization timepoints at which the FSS is captured. To assess the FSS at 16 weeks, we will calculate an estimated marginal mean difference between arms with corresponding 95% confidence interval, with statistical significance set at 0.05. Similar contrasts at each interim time point will be provided. Effect modification by baseline FSS level will be demonstrated graphically. Participants who are lost to follow up will be compared descriptively to those who remain in the trial. If selection bias occurs, we will consider inverse probability weights for censored individuals.

For secondary and exploratory outcomes, questionnaire, laboratory, and physical measure data will be analyzed similarly with generalised linear models, adjusting for baseline level, other relevant prognostic factors and using link function based on the variable type from questionnaires (e.g., logit for binary outcomes).

Effect modification by baseline factors will be considered by the inclusion of interaction terms with treatment arm in the above models. Possible effect modifiers include baseline fatigue severity, sex, gender, age, severity of and time of acute SARS-CoV-2 infection, pre-existing co-morbidities, COVID-19 vaccination, final dose, and side effects. Significance of effect modification will be based on the likelihood ratio test comparing models with and without the interaction term.

Dose response analyses will involve comparison of various dosing levels as a covariate versus control in the primary linear model. Secondary analysis will look only at dose comparisons within the intervention arm Dose will be included in these models as a nonlinear effect via restricted cubic splines.

We will conduct a per-protocol analysis to assess the expected effect of adhering to the trial protocol using G-methods, which allow for adjustment for post-randomization confounding.[116]

All analyses of primary and secondary outcomes will be pre-specified in detail in a Statistical Analysis Plan and signed off on by all investigators prior to data analysis.

Harms

Protocols to address particular adverse events (AEs) and SAEs are described in **Appendix 3.** We will implement REDCap alerts for AEs noted through the questionnaires. Additionally, participants will be asked if they have had any AEs at each study visit. All AEs will be assessed by a study physician. All SAEs will be reported to the DSMB. SAEs will be reported to the Research Ethics Board (REB) and Health Canada (HC) as per local regulations. For mild AEs, the patient may be reassured to continue taking the medication as per protocol. Previous studies and our clinical experience have suggested that LDN is generally well tolerated, and mild AEs will often ease with treatment continuation.[42,51,53–55,117]

Inspections and Auditing

The trial will be subject to inspections or audits by HC, REB, and the Canadian Institutes for Health Research (CIHR).

Patient and Public Involvement

Patient partners will be included as part of the Trial Steering Committee.

Trial Dates

This trial has started recruitment in January 2024 and aims to complete follow-up by the end of 2024.

ETHICS AND DISSEMINATION

Research Ethics Approval

This study was approved by the UBC/C&W harmonized REB (#H21-02254); any protocol modifications will be reported to the REB.

Consent or Assent

Research staff will consent interested participants via the secure REDCap electronic consent framework. Participants will receive information regarding the trial electronically and will have the opportunity to discuss the trial specifics and meet with a research team member (virtually or in-person) before deciding on participation. The consent form is provided in **Appendix 4**.

Access to Data

The study principal investigator, co-investigators, clinical research coordinator, research assistants, and statistician will have access to the collected data.

Dissemination Policy

We will follow the Consolidated Standards of Reporting Trials (CONSORT) guidelines for reporting results of parallel arm trials.[118] We will submit manuscripts to peer review journals, give presentations at conferences, and media release will be organised. We will leverage our connections with PCC and ME/CFS networks to share our findings with patients, their caregivers, PCPs, and other care providers.

DISCUSSION

This report described the protocol for a 16-week, phase II randomised controlled trial to investigate the efficacy of LDN for treating fatigue severity in patients with PCFS, an illness we have defined as ME/CFS symptoms persisting at least 3 months following SARS-CoV-2 infection.

Our study will build on prior and ongoing evaluations of LDN. In our review of studies listed on ClinicalTrials.gov, we identified one upcoming trial (NCT05946551) which also investigates LDN in PCC.

However, this trial is smaller (expected n=36) and focused on feasibility outcomes. There are no trials listed that investigate LDN in ME/CFS.

A positive outcome in our trial would inspire greater confidence in LDN as a treatment for the millions of patients with PCFS symptoms, and could prompt larger, multi-institutional phase III studies. Unlike other candidate PCC treatments such as Paxlovid, stellate ganglion blockade and hyperbaric oxygen,[119–125] LDN is widely available, relatively inexpensive, and generally safe. A negative outcome in this trial would also be a valuable contribution to the literature and would directly impact clinician decisions regarding prescribing LDN.

The trial has limitations. It is limited to English speakers and is based in a single province. We do not have a restriction on how long a participant may have had their symptoms since COVID-19. This may limit the treatment effect if LDN efficacy is greater earlier in the disease course. The remote nature of the trial also limits the number of objective outcomes that can be collected in all participants.

However, our decentralized strategy for this trial has several advantages. First, it will allow individuals who live outside Vancouver to participate, including those in communities who may not have access to off-label or investigational treatments.[126] Second, it will permit inclusion of more symptomatic individuals. Some individuals with PCFS have reported symptom exacerbation from even minimal cognitive and physical exertion,[127] and remote participation may prevent flare-ups experienced from in-person visits. Third, it will encourage participation from patients who may be reluctant to attend in-person given the risks of COVID-19 re-infection. Lastly, it will expedite study completion by broadening the pool of eligible applicants and reducing logistical barriers associated with in-person recruitment and enrollment.

This trial has other strengths. By using the provincial PC-ICCN and REACH BC directories, we will be able to efficiently identify and contact hundreds of potential participants by email. Our focus specifically on individuals with the ME/CFS phenotype distinguishes this trial from others for PCC and increases the likelihood that participants will have a similar underlying pathophysiology. Lastly, our trial includes multiple secondary and exploratory outcome measures that may be valuable for further hypothesis generation.

This is one of the first trials in Canada investigating a pharmacological treatment for PCC and will have a direct impact on how this illness is treated. Our hope is that it will also promote engagement, good faith, and optimism among the PCC community- a group that has experienced stigma and one that has expressed frustration regarding the paucity of interventional studies for their illness.[22,128–132] Furthermore, the trial has implications beyond COVID-19; we expect that the results will have applicability to ME/CFS and other post-infection fatigue syndromes, including those that could emerge from future pandemics.[133]

Confidentiality

Following UBC REB guidelines, all study-related information will be stored in locked facilities at C&W, and all electronic material stored on secure network drives. Participants will be allocated study identification (ID) numbers and a master file linking the study ID and personal information will be saved separately.

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This flowchart outlines the process for study recruitment, eligibility assessment and baseline assessments. A full study timeline is outlined in Table 4. Abbreviations: BC- British Columbia; LDN- low dose naltrexone; PCC- post-COVID-19 condition; PC-ICCN- Post-COVID-19 Interdisciplinary Clinical Care Network; SAQ- short answer questionnaire.

384x526mm (130 x 130 DPI)

Supplementary Material

Study protocol for a double-blind, randomised trial of low-dose naltrexone for post-COVID fatigue syndrome

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Box S1: Withdrawal criteria

Withdrawal criteria
Participants can withdraw from the study at any time without giving reasons. If a participant withdraws from the study, they may still be asked to attend a final safety visit to ensure their safety. There will be no additional follow-up for a withdrawn participant, except in the event of an adverse event (AE) or serious adverse event (SAE), where they will be followed until the AE or SAE fully resolves. Participants should be withdrawn from the study if: 1. The study investigator judges it is not in the participant's best interest to continue. 2. The participant experiences serious side effects due to the study treatment. The study doctor will assess the participants symptoms from the AE/SAE and the participant will be withdrawn if determined appropriate by the investigator. If withdrawn for an AE/SAE, the participant will still be followed by the study team until the AE/SAE resolves. 3. The participant needs to begin take long-term opioid medication. Participants who take opioid medication over a short time during the study to manage acute pain, will be allowed to continue in the study. The protocol deviation will be recorded. The study doctor and team will reinforce guidance on taking alternative medication for pain and ensure the participant is aware that taking opioids for pain relief may not work well due to the naltrexone's opioid blocking effects. 4. The participant becomes pregnant during the study. The study treatment will be stopped, and they be withdrawn from the study. 5. The participant is unable to fulfill the requirements for the study. For example, if the participant misses or does not complete the 1) baseline assessments or 2) assessments at more than one consecutive time point (does not complete 6-week and 12-week assessments). Participants withdrawn from the study will not be replaced.
Data collection from participants who withdraw
No new data/information will be collected from participants after they withdraw, except for: 1. Reason for withdrawal (if by participant choice: reason will not be collected unless participant provides a reason for their withdrawal). 2. Any information that is required to be collected related to an AE/SAE that the withdrawn participant is being followed for. The withdrawn participant will be followed until the AE/SAE has fully resolved. All data that has been collected prior to withdrawal will be retained for analysis.

Box S2: Trial oversight

Trial Steering Committee
A Trial Steering Committee (TSC) will be formed with patient partners, the investigators, and other team members. The TSC will meet virtually at least monthly in the first two months and then every 2 months for the remaining study period. The goals of the Steering Committee will include: 1. Ensuring adherence to the study timeline. 2. Reviewing protocol, reporting, discussing, and resolving challenges. 3. Providing a regular environment for the presentation of study data (including recruitment and final trial data). 4. Fostering patient and knowledge user engagement. 5. Planning for knowledge translation activities.
Data Safety and Monitoring Board
The Data Safety and Monitoring Board (DSMB) will be comprised of peer researchers with expertise in clinical trials and ethics, who are once removed from the study team. The frequency of scheduled meetings depends on patient enrollment and safety event rates. For the DSMB, a research assistant from the study team will coordinate meetings, draft agendas, take meeting minutes and log discussions. In instances where closed DSMB meetings are called, a research assistant independent of the study team will perform the tasks noted above. Before the study starts, the DSMB will: 1. Review the study protocol, overall data collection methods and safety monitoring procedures. 2. Define safety and related parameters to be monitored, including methods for review and frequency of monitoring. 3. Provide letter of review/approval of study initiation to the PI/Trial Steering Committee. During the study period, the DSMB will: 1. Review data generated by the study, particularly adverse events, unexpected and serious adverse events on a periodic basis and as advised by the study team and recommend one of the following actions to the Trial Steering Committee – continue as is, continue with modification, discontinue part of study, or discontinue completely. 2. Provide letter of review/approval at pre-determined intervals.
Study Monitor
The role of the study monitor is to verify: 1. That the rights and wellbeing of the participants are protected. 2. That reported trial data is accurate, compete and verifiable from source documents. 3. The conduct of the trial is compliance with the currently approved protocol, good clinical practices (GCP) and any applicable regulatory requirements.

Table S1: Exploratory outcomes

Laboratory measures (peripheral blood serum)	
Inflammatory markers	Comprised of IL-6 (measured in pg/mL) and hsCRP (measured in mg/L).
Cytokine profile	Comprised of GM-CSF, IFNγ, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-12p40, IL-12p70, IL-13, MCP-1, TNFα. These are measured in pg/mL using the Human Cytokine Proinflammatory Focused 15-plex Discovery Assay® Array (Eve Technologies).
Creatine kinase (CK)[1]	An assessment of disease severity,[1] measured in units/L.
Thyroid profile[2]	An indirect measure of disease severity,[2] comprised of rT3 (measured in ng/dL), fT3 (measured in pmmol/L), fT4 (measured in pmmol/L) and TSH (measured in mIU/L).
AM cortisol*	Measured between 800h and 1000h, in nmol/L.
Adrenocorticotropic hormone (ACTH)*	Measured between 800h and 1000h, in pmol/L.
Patient reported outcome measures	
Fatigue VAS [3]	1 item, scored from 0 (least fatigue) to 10 (most fatigue).
Sleep Questionnaire (SQ-2)[4]	4 items, scored from 0 (no sleep problems) to 24 (most sleep problems)
Sleep VAS[4]	1 item Scored from 0 (no sleep problems) to 10 (worst quality of sleep imaginable)
Patient Health Questionnaire-9 (PHQ-9)[5]	Scored from 0 (no depression symptoms) to 27 (most depression symptoms).
Generalized Anxiety Disorder-2 (GAD-7)[6]	Scored from 0 (no anxiety symptoms) to 21 (most anxiety symptoms).
Self-reported health EQ-5D VAS[7]	Scored from 0 (worst imaginable health) to 100 (best imaginable health).
Post-COVID-19 Functional Status Scale[8]	Scored from 0 (no functional limitations) to 4 (severe functional limitations).
Physical measurements^	
Hand grip strength[9]	Measured in kg using a digital hand dynamometer. The highest value over 3 attempts will be for each time point will be used.
Sit to stand test[10]	To test leg strength and endurance, measured in number of repetitions in 30 seconds.
Postural blood pressure and heart rate	Measurements are taken in supine position and then three times over 5 minutes following passive standing (i.e. at 1, 3, and 5 minutes)

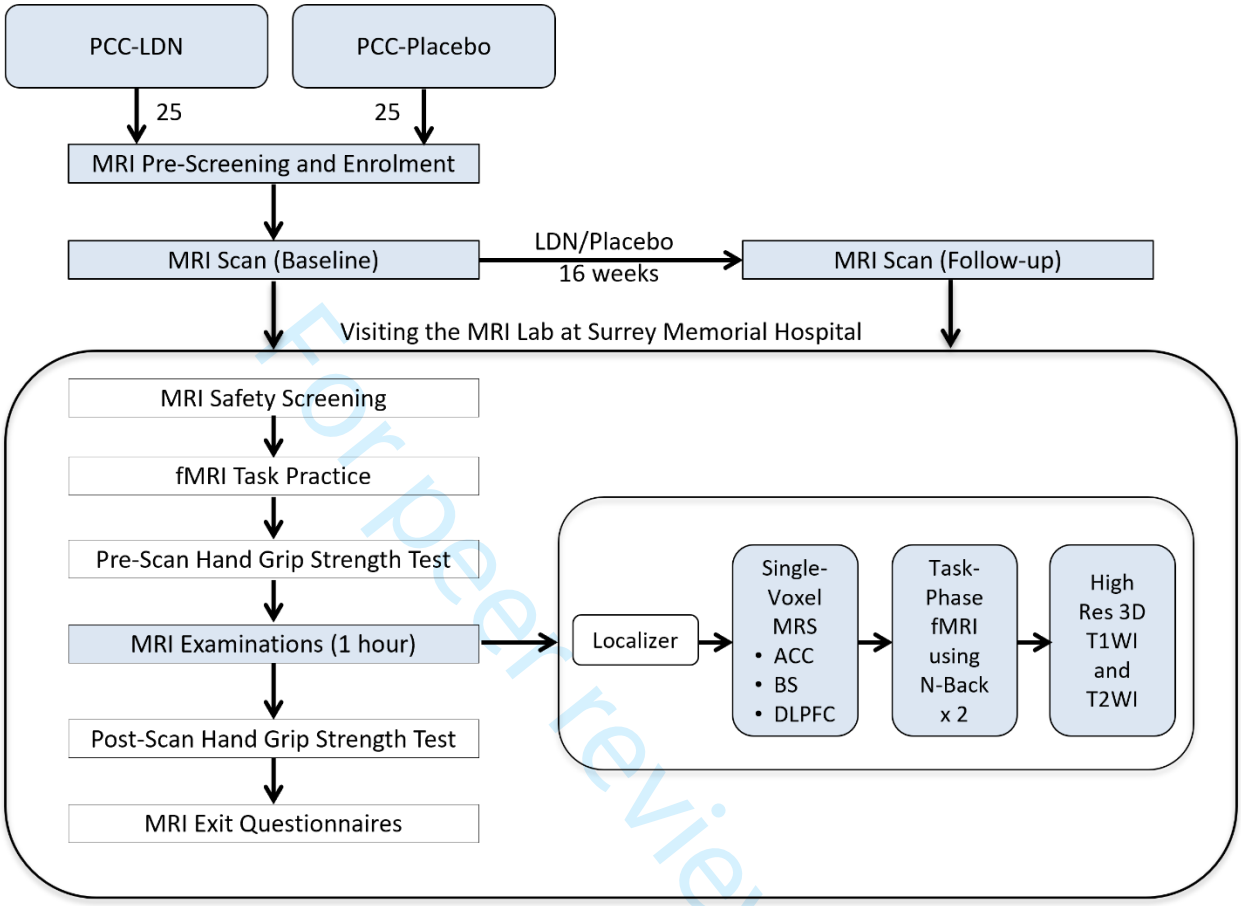
Legend. This table outlines the exploratory outcome measures. Details regarding primary and secondary outcomes are detailed in the main text. For all exploratory outcomes, we will evaluate changes in the measure between baseline and 16 weeks. Abbreviations: EQ-5D- Euroqol 5-dimensions; fT3- free triiodothyronine; fT4- free thyroxine; GM-CSF-granulocyte-macrophage colony-stimulating factor; hsCRP-high sensitivity CRP; IL-interleukin; MCP-1- monocyte chemoattractant protein 1; p40- subunit p40; POTS- postural orthostatic tachycardia syndrome; rT3- reverse triiodothyronine; TNF- tumor necrosis factor; TSH- thyroid stimulating hormone; VAS-visual analogue scale. *Optional for participants who choose to have bloodwork done in AM; ^optional for participants who choose in-person assessments.

Table S2: Laboratory tests

Test	Type	Purpose	Participants	Timing
ACTH	Serum	Exploratory outcome	Optional	Baseline, 16 weeks
Albumin	Serum	Eligibility, safety	All	Baseline, 16 weeks
ALP	Serum	Eligibility, safety	All	Baseline, 16 weeks
AM Cortisol	Serum	Exploratory outcome	Optional	Baseline, 16 weeks
ALT	Serum	Eligibility, safety	All	Baseline, 16 weeks
AST	Serum	Eligibility, safety	All	Baseline, 16 weeks
Bilirubin	Serum	Eligibility, safety	All	Baseline, 16 weeks
Calcium	Serum	Eligibility, safety	All	Baseline, 16 weeks
CBC with differential	Serum	Eligibility, safety	All	Baseline, 16 weeks
Creatinine	Serum	Eligibility, safety	All	Baseline, 16 weeks
CK	Serum	Eligibility, safety, exploratory outcome	All	Baseline, 16 weeks
fT3	Serum	Exploratory outcome	All	Baseline, 16 weeks
fT4	Serum	Exploratory outcome	All	Baseline, 16 weeks
GM-CSF	Serum	Exploratory outcome	All	Baseline, 16 weeks
hsCRP	Serum	Eligibility, safety, exploratory outcome	All	Baseline, 16 weeks
IFNγ	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-1β	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-1RA	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-2	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-4	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-5	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-6	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-8	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-10	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-12	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-12p40	Serum	Exploratory outcome	All	Baseline, 16 weeks
IL-13	Serum	Exploratory outcome	All	Baseline, 16 weeks
LDH	Serum	Eligibility, safety	All	Baseline, 16 weeks
MCP-1	Serum	Exploratory outcome	All	Baseline, 16 weeks
Potassium	Serum	Eligibility, safety	All	Baseline, 16 weeks
rT3	Serum	Exploratory outcome	All	Baseline, 16 weeks
Sodium	Serum	Eligibility, safety	All	Baseline, 16 weeks
TNFα	Serum	Exploratory outcome	All	Baseline, 16 weeks
TSH	Serum	Eligibility, safety, exploratory outcome	All	Baseline, 16 weeks
Urine opioid screen	Urine	Eligibility	First 16	Baseline

Legend. This is a complete list of laboratory tests completed during the study. Blood samples will be taken at a LifeLabs of the participants' choice, which will usually be local to participant location. Abbreviations: ACTH- adrenocorticotropic hormone; ALP- alkaline phosphatase; ALT- alanine aminotransferase; AST- aspartate aminotransferase; CBC- complete blood count; CK- creatine kinase; ; fT3- free triiodothyronine; fT4- free thyroxine; GM-CSF-granulocyte-macrophage colony-stimulating factor; hsCRP- high sensitivity c-reactive protein; IL-interleukin; LDH- lactate dehydrogenase; MCP-1- monocyte chemoattractant protein 1; p40- subunit p40; rT3- reverse triiodothyronine; TSH- thyroid stimulating hormone; TNFα- tumour necrosis factor alpha.

Figure S1: Protocol for MRI Study



Legend. As part of a sub-study of this clinical trial, 25 consenting participants in each study arm will undergo functional MRI testing. Participants will complete a hand grip assessment before and after imaging, and complete cognitive tasks during the fMRI testing. Abbreviations: ACC-agenesis of corpus collosum; BS-brainstem; DLPFC-dorsolateral pre-frontal cortex; fMRI- functional magnetic resonance imaging; MRI-magnetic resonance imaging; T1WI- T1 weighted imaging; T2WI- T2 weighted imaging.

Appendix 1: Batch testing protocol

The following plan is for batch testing the first batch of compounded study drug for the clinical trial, as requested by Heath Canada during the approval process. This study has Health Canada authorization. The batch testing must be completed, and the results reported to Health Canada, before dosing the first patient in the trial.

Naltrexone capsules in 1mg or 4.5mg size are to be analysed by ultra-high pressure liquid chromatograph-tandem mass spectrometer (UHPLC-MSMS) for confirmation of concentration in each capsule. This process will require a deuterium labelled standard (Sigma N-047 naltrexone-d3 solution. Certified reference material)

We will use a multiple reaction monitoring (MRM) approach for naltrexone with a transition of 342 -- 324 for unlabelled naltrexone and 345 – 327 for the deuterium labelled standard. The MRM approach uses a tandem mass spectrometer (two mass spectrometers in line one after the other) The first mass (342 for naltrexone) is isolated by the first mass spectrometer, the naltrexone will then be fragmented by collision with gas molecules and the fragment, or second ion (324 for naltrexone) will be isolated by the second mass spectrometer. The fragment ion will then be detected, and the intensity of the signal is equivalent to the concentration of the naltrexone.

Appropriate standard curves will be created using a 2.1 X 50mm Accucore phenyl-hexyl column (Thermo Scientific 17926-052130) and water/methanol as mobile phases. Standard curves will be created using dilutions of a European Pharmacopoeia standard from Sigma: Naltrexone Hydrochloride #Y0000400 with n=10 standards ranging in concentration from 10 nanogram per millilitre (ng/ml) through 500ng/ml. Standard curves will be included in the report to demonstrate linearity and range. The UHPLC-MSMS utilized is a Waters H-class UHPLC and Xevo TQS tandem mass spectrometer and will be run in positive ion mode.

Naltrexone impurity C (Sigma nomenclature or USP naltrexone related compound A), a European Pharmacopoeia reference standard from Sigma Y-0000410, will be analysed as a system suitability test as impurity C has the same molecular weight as Naltrexone and has the same MRM transitions of 342 – 327. Consequently, impurity C and naltrexone must be separated by UHPLC prior to entering the mass spectrometer. System suitability testing will be done to ensure the chromatographic separation of these two compounds prior to sample analysis.

Other known impurities of naltrexone will not be detected with a 345 -- 327 MRM as they have different molecular weights.

Analytical accuracy will be determined by spiking capsules with analytical standards of naltrexone. 1mg capsules will be cut open with a scalpel and the contents added to 1000milliliters (ml) water. 100microliters (ul) of this solution will be added to 900ul water for a dilution of 1/10 and an approximate concentration of 100 nanograms/millilitre (ng/ml).

4.5mg capsules will be treated in the same manner as the 1mg capsules except the final dilution will be 20ul into 980ul water providing an expected concentration of 90ng/ml.

5ul of deuterium labelled internal standard (naltrexone-d3 diluted to 1ug/ml concentration) will be added to all samples to allow for quantitation of the naltrexone. Results will be determined and expressed as a percentage of the expected dose.

To determine analytical accuracy, solutions from the 1mg and 4.5mg capsules (diluted to the equivalent of 100ng/ml and 90ng/ml) will be spiked and recovery determined.

Naltrexone standard will be spiked into the diluted capsule contents using the scheme in the following tables.

Placebo capsule:

	expected	measured	% recovery
no spike	0		
50ng/ml	50		
100ng/ml	100		
150ng/ml	150		
200ng/ml	200		

1mg capsule diluted to 100ng/ml:

	spiked	measured	expected	% recovery
no spike	0		100	
50ng/ml	50		150	
100ng/ml	100		200	
150ng/ml	150		250	
200ng/ml	200		300	

4.5 mg capsule diluted to 90ng/ml:

	spiked	measured	expected	% recovery
no spike	0		90	
50ng/ml	50		140	
100ng/ml	100		190	
150ng/ml	150		240	

Analytical precision will be determined using 8 capsules of 1mg and 4.5mg quantity. Capsules will be treated as described above.All 8 replicates at each concentration will be analysed by HPLC-MS and the mean, standard deviation and %RSD will be determined for each.

Confirmation of capsule dose and content uniformity will be combined.

A total of 10 capsules of 1mg and 4.5mg will be treated as described above. Samples will be analysed by HPLC-MS and results expressed as a percentage of the expected dose (1mg or 4.5mg). This will confirm the accuracy of the expected doses.

These results will also be used to calculate an acceptance criterion for content uniformity. If the results of the 10 capsules do not meet the USP definition of acceptance criteria, then an additional 20 capsules will be analysed and acceptance criteria calculated.

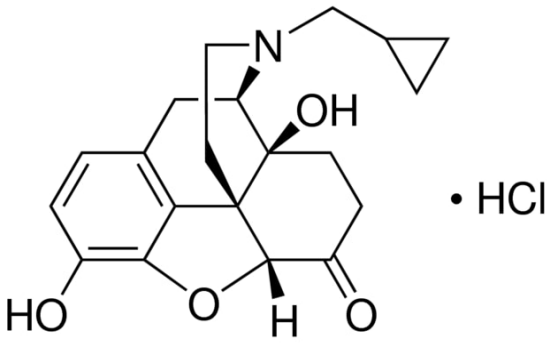
The compounding pharmacy has criteria for dose variability stating results should be between 90% and 110% of the expected value.

Purity determination will utilize aThermo Scientific Q-exactif Orbitrap. The Orbitrap has a mass accuracy of about 1part per million error. A 2.1 X 50mm C18 column will be used and all 10 of the final solutions for both 1mg and 4.5mg capsules, used for content uniformity will be analysed by Orbitrap mass spectrometry in positive ion mode.

The placebo capsule will also be analysed by Orbitrap.

We will actively search for each of the 10 known contaminants and breakdown products of naltrexone shown in the list below, based on accurate mass. Percent purity will be calculated based on area counts of naltrexone and any known contaminants/breakdown products found in each chromatogram.

Additionally, chromatograms of naltrexone capsules and placebo capsule will be compared to identify peaks found only in the naltrexone capsules. Any such peaks will have accurate mass recorded and be included in the percent purity calculation.



naltrexone		341.1627	C20H23NO4
impurity	a	315.1107	C17H17NO5
	b	287.1158	C16H17NO4
	c	341.1627	C20H23NO4
	d	680.3170	C40H44N2O8
	e	395.2097	C24H29NO4
	f	357.1576	C20H23NO5
	g	357.1576	C20H23NO5
	h	343.1784	C20H25NO4
	i	355.1420	C20H21NO5
	j	355.1784	C21H25NO4

Appendix 2: Sensitivity analysis for sample size calculation

For this trial, the sample size was calculated using data from the CCDP registry,[11] and based on effect size (d)=0.5.

As a sensitivity analysis, we also performed a sample size calculation through an alternative approach of using a minimal clinically important difference (MCID). As there are no published MCIDs for ME/CFS or PCC that we could identify in the literature, we used a published MCID ascertained from patients with systemic lupus erythematous (SLE).[12] This study included 80 participants and reported a MCID (95%CI) of 0.6 (0.3-0.9), or 5.4 (2.7-8.1) on the 7 to 54 scale for FSS.

After assuming a standard normal confidence interval was derived using all 80 patients, we calculated a standard deviation of 1.36, which corresponds to an effect size of 0.6/1.36 = 0.44. This results in a sample size of 81 per group, or 101 accounting for 20% loss to follow up.

Appendix 3: Side effects and adverse events

Trials with LDN for conditions other than PCFS have shown that LDN is safe and well-tolerated.[13–16] In our clinical experience, minor side effects were observed in <10% of patients. These include sleep dysfunction, in particular vivid dreams, and minor gastro-intestinal upset.

Hepatotoxicity

Naltrexone has been identified as a hepatotoxin. This was identified using doses five times the recommended dose and no cases of hepatic failure due to naltrexone have ever been reported. The doses in this study are significantly less (4.5 mg) than the recommended doses for opiate blockade or treatment for opioid or alcohol dependence (50 mg). Even though the risk of this is very low, participants will be informed of the potential, told to call the study team, and seek emergency medical attention if they experience any symptoms of hepatitis or liver dysfunction.

Other Adverse Events

Adverse reactions that have been reported both at baseline and during clinical trials with naltrexone are as follows (note the maximum dose used in this study is over 10 times lower than those used for listed uses)[17]:

- More than 10%: Difficulty sleeping, anxiety, nervousness, abdominal pain/cramps, nausea and/or vomiting, low energy, joint and muscle pain, and headache.
- Less than 10%: Loss of appetite, constipation, increased thirst, increased energy, feeling down, irritability, dizziness, skin rash, delayed ejaculation, decreased potency, and chills.
- Less than 1% of subjects:
    - Respiratory: nasal congestion, itching, rhinorrhea, sneezing, sore throat, excess mucus or phlegm, sinus trouble, heavy breathing, hoarseness, cough, shortness of breath;
    - Cardiovascular: nose bleeds, phlebitis, edema, increased blood pressure, non-specific EGG changes, palpitations, tachycardia;
    - Gastrointestinal: excessive gas, hemorrhoids, diarrhea, ulcer;
    - Musculoskeletal: painful shoulders, legs or knees, tremors, twitching;
    - Genitourinary: increased frequency of, or discomfort during urination, increased or decreased sexual interest;
    - Dermatologic: oily skin, pruritus, acne, athlete's foot, cold sore, alopecia;
    - Psychiatric: depression, paranoia, fatigue, restlessness, confusion, disorientation, hallucinations, nightmares, bad dreams;
    - Special Senses: eyes- blurred, burning, light sensitive, swollen, aching, strained; ears "clogged", aching, tinnitus;
    - General: increased appetite, weight loss, weight gain, yawning, somnolence, fever, dry mouth, head "pounding", inguinal pain, swollen glands, "side" pains, cold feet, "hot spells"

Data collected since the approval of naltrexone shows that most adverse events occur early in treatment and are transient in nature[29].

Adverse Events Reporting

We will implement REDCap alerts for adverse events noted through the questionnaires. Any adverse events (AE) will be assessed by the qualified investigator or their delegate as soon as possible upon becoming aware of the AE. All serious adverse events (SAE) will be reported as per local REB and Health Canada regulations. Adverse events determined to be serious, unexpected, and related to the study drug will be reported to Health Canada within:

- 15 days of becoming aware of the SAE, if it is not fatal or life-threatening.
- Immediately where possible, but within 7 days of becoming aware of the SAE, if it is fatal or life-threatening.

All SAEs that meet the criteria of an unanticipated problem will be reported to the REB. An unanticipated problem meets the following criteria: 1) unexpected; 2) related or possibly related to participation in the research; 3) suggests that the research places participants at greater risk than previously known.

SAE related to study treatment will be followed by the study team until it resolves.

AE reports will be submitted to the DSMB at intervals defined in the DSMB charter.

Appendix 4: Consent form

PARTICIPANT INFORMATION AND CONSENT FORM

A Double Blind Randomized Trial of Low-Dose Naltrexone for Post-COVID Fatigue Syndrome

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Protocol Number/ Trial ID: H21-02254

Short Title: LDN Trial

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Phone: 236-990-9519

Sponsor/Funder: Canadian Institutes of Health Research (CIHR)

Emergency Contact Number: 604-786-2473

1. Introduction/Invitation
You are being invited to take part in this study because you are a current or former patient of the BC Post-COVID Recovery Clinics (PCRC), or you have indicated that you are interested to participate in our clinical trial through the REACH BC Platform and have previously had SARS-CoV-2 (COVID-19) virus, and are continuing to experience symptoms.

2. Your participation is voluntary
Participating in this study is voluntary. You have the right to refuse to participate in this study. If you decide to participate, you may still choose to withdraw from the study at any time without any negative consequences to the medical care, education, or other services to which you are entitled or are presently receiving.

You should be aware that there is a difference for both you and your study doctor between being a patient and being a research participant. As a patient, all medical procedures and treatments are carried out for your benefit only according to standard accepted practice. As a research participant, you and your study doctor also must take into account the requirements for the research study. These may include procedures and treatments that are not part of standard practice or are not yet proven. This consent form describes the treatment procedures that are being carried out for research purposes. Please review the consent document carefully when deciding whether or not you wish to be part of the research and sign this consent form only if you accept being a research participant.

Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

3. Who is conducting this study?
This study is being conducted by Dr. Luis Nacul at BC Women's Hospital (BCW) and is funded by the Canadian Institutes for Health Research. Dr. Nacul is the Research Director of the Complex Chronic Diseases Program (CCDP) at BCW. Dr. Nacul is a well-established researcher and doctor in the area of complex chronic diseases. Other members of the study team are: Dr. Jane McKay who is the Head of Division of Internal Medicine and Medicine Program Director at Providence Health BC, Co-chair of the Post-COVID Interdisciplinary Clinical Care Network (PC-ICCN), and is a myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) specialist, and the Physician Lead of the clinical coordination working group at the PCRC; Dr. Adeera Levin is the Medical Lead for the PC-ICCN and PCRC in BC, Head of the Division of Nephrology at University of British Columbia; Dr Xiaowei Song is a Clinical Neuroimaging Senior Scientist with Fraser Health; and Dr. Jeffery Bone is a Biostatistician with BC Children's Hospital Research Institute, Dr. Hiten Naik is a research fellow with the Post-COVID Interdisciplinary Clinical Care Network.

4. Background
There are individuals who do not recover to their previous level of health following infection by SARS-CoV-2 (COVID-19). Many of them have similar symptoms to those with a condition called post-viral fatigue syndrome. This is known as post-COVID-19 fatigue syndrome (PCFS). The main symptoms of PCFS are profound and persistent fatigue, sleep problems, and brain fog. There is currently no treatment for PCFS.

Naltrexone is a medication that attaches to opioid receptors and blocks the effects of opioid drugs. It is approved by Health Canada for the treatment of alcohol and opioid use disorders. It is believed that when naltrexone is used in low doses, about 1/10th of a normal dose, it has the effect of increasing the amount of opioid-like substances made within the body (met-enkephalin and beta-endorphin), which may help reduce pain and inflammation.

Low-dose naltrexone (LDN) has been used in individuals with a number of conditions, such as ME/CFS and fibromyalgia, which have similarities with PCFS. There have not been any clinical trials with LDN for ME/CFS or PCFS. However, there has been an observational study (treatment is part of routine medical care), for ME/CFS, and two small clinical trials for fibromyalgia. These studies have reported benefits of LDN treatment and have shown that LDN is safe and well-tolerated. The Complex Chronic Diseases Program at BC Women's Hospital has extensive experience prescribing LDN treatment for fibromyalgia and ME/CFS patients. Their studies suggest LDN treatment may improve energy, pain, and sleep in many patients, with only minor side effects.

This study aims to determine if treatment with LDN reduces fatigue and improves the related symptoms of PCFS, and thus improve quality of life and health of those with PCFS.

Health Canada, the regulatory body that oversees the use of drugs in Canada, has not approved the sale or use of naltrexone for PCFS. However, Health Canada has allowed naltrexone to be used in this study.

5. What is the purpose of the study?

This study aims to determine if low-dose naltrexone improves fatigue, pain, and related symptoms of individuals with PCFS. It will also determine if it reduces inflammation in the body, by measuring markers of inflammation in the blood.

This study is a double-blind randomized controlled trial. A randomized controlled trial is a study in which participants are randomly assigned to either the study drug (low-dose naltrexone) or placebo (an inactive substance). Randomization is like the flip of a coin so that there is an equal chance of being in either group. This is done to examine the potential benefits and side effects of the drug with a direct comparison group. A double-blind trial is where both participants and researchers directly involved in the trial will not know which treatment is being given (i.e. whether the active treatment or placebo).

6. Who can participate in this study?

You may be able to participate in this study if you:

- Are between the ages 19 to 69 years old
- Had COVID-19 at least three months ago, as confirmed by a physician or positive test result
- Meet the clinical diagnostic criteria for PCFS (this will be confirmed by a physician at your appointment)
- Agree to maintain any other regular medications at their current doses for the duration of the trial (essential need of new medication or dose change, as prescribed by physician, is permitted)

- Agree to use effective contraception while receiving the study treatment (if you could potentially become pregnant)
- You reside within the delivery area for the drug as determined by FedEx Clinical Trial Services

7. Who should not participate in this study?

You will not be eligible to participate in this study if you:

- Are pregnant, planning to become pregnant, or breastfeeding
- Have used opioid medications within the last 15 days or use of opioids during the trial^
- Have a positive urine test for opioids (test to be done after you sign the consent form)
- Have a history of alcohol, opioid or other substance misuse
- Participated in another interventional clinical trial in the last 30 days or plan to during the trial period
- Have a confirmed ME/CFS or fibromyalgia diagnosis existing prior to COVID-19.
- Are allergic to naltrexone or medication components (gelatin†; cellulose)
- Have acute hepatitis, liver failure, or severe kidney failure
- Are currently using or have used naltrexone in the last 30 days

^If you are taking any opioids and wish to stop them to become eligible for the trial, you can enter a washout period, where you will stop taking the opioids for 15 days before continuing with the screening process.

†The study treatment is prepared with gelatin, which is an animal-based product.

8. What does this study involve?

This study is a 16-week double-blinded randomized trial, meaning that neither you nor your study doctor will know which study medication you take. However, this information is available in case of an emergency, and will be available at the end of the trial to those analyzing the results. A total of 160 SARS-CoV-2 survivors are anticipated to be enrolled in this study with 80 randomized to the placebo and 80 randomized to the low-dose naltrexone treatment. Study treatment will be administered for 16 weeks and will start at 1 mg/day, increasing each week by 1 mg for a maximum of 4.5 mg/day at week 4. The latter dose will be continued until the end of the trial.

If you agree to take part in this study, the procedures and visits you can expect will include the following:

- Review and completion of the consent form
- Meet with a Study Physician virtually who will confirm a diagnosis of PCFS and confirm it is safe for you to take the study drug. To do this, the study doctor may need to review your medical record and/or call your family doctor for additional information. If you sign this form, you are agreeing to the study doctor reviewing your medical record and/or calling your family doctor. If you decide you do not want your study doctor to know about your participation in this study, the study doctor will not be able to call your family doctor and this may make it harder for the study doctor to determine if you are eligible.
- Completion of research questionnaires. These questionnaires will ask about items like fatigue, sleep, pain, other symptoms you may be experiencing, demographics, medical history, quality of life, mental health, medications & supplements you take, details about your COVID-19 infection, vaccination status, questions to check your daily use of the study drug, and if you have experienced any side effects. These questionnaires will be emailed to you to complete at home. If

you don't have access to a device with which you can complete the questionnaires on, you can arrange for the questionnaires to administered to you by telephone.

- Blood sample collection at LifeLabs (you can collect samples at any location that is convenient for you). Some of the blood collected will be analyzed at LifeLabs, and some will be sent to Eve Technologies in Alberta for analysis. The samples will be stored at the BC Children's Biobank until they are sent for cytokine profile testing. LifeLabs will use the study ID to collect, process, analyze and/or ship the samples for this study (your name will not be used to send the samples). When the sample arrives at the BC Children's Biobank, the samples will be stored until they are shipped to Eve Technologies in Alberta.
    - All of the study test results will be reported to the ordering physician (the study principal investigator Dr. Luis Nacul).
    - Samples taken at LifeLabs may be referred to a testing laboratory outside of BC (this may be in another province or in the USA).
- Hand grip strength assessment is a short test of the strength you have in your hands (if you don't have any in-person contact with the study team, this assessment will not be done). This involves squeezing a hand device with all your strength and is done three times with each hand.
- Completion of a short study drug diary daily for the first 4 weeks of taking the study treatment, and for a week after any change in the dose, if that happens (unlikely).
- Completion of study visits and check-ins with study personnel. The study visits can be done in-person, through phone call, or video call. The check-ins can be done by phone call, text, email, or video call.
- Wear a pedometer (step counter) for 7 days, while awake, at two time points during the study, and record the number of steps per day in the diary. Note: The pedometer does not collect any information besides your daily steps.
- Return the empty containers and unused study drug to the study team by mailing it back via Canada Post (pre-paid) or returning it in-person to the study site (BC Women's Hospital).

Study Timeline

Screening & Eligibility

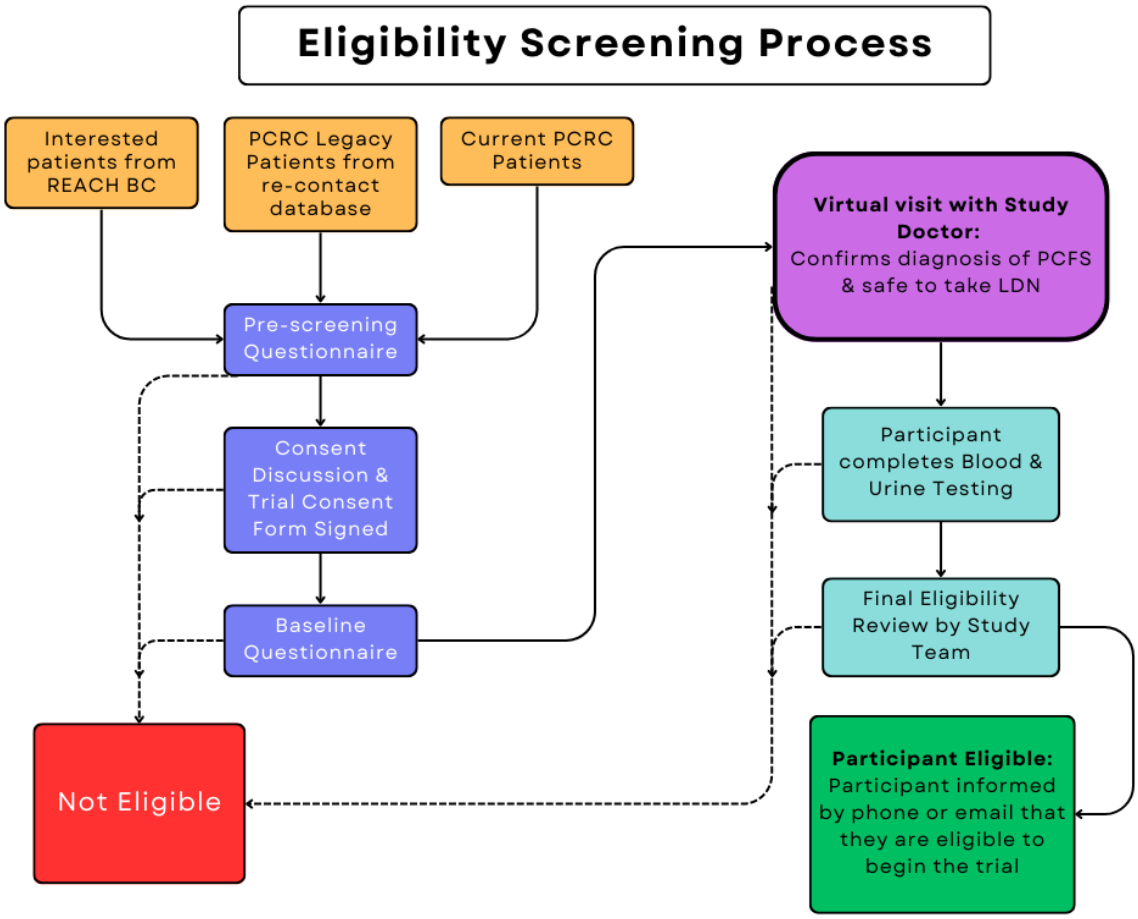
After consenting to the study, you will be asked to complete the following study procedures:

- Completion of questionnaires
- Have a scheduled phone, or telehealth visit with the study doctor in which they will confirm you meet the clinical diagnostic criteria for PCFS and that it is safe for you to take naltrexone.

After the study doctor confirms you have PCFS, blood and urine samples will be collected to confirm your eligibility to the study and as study data that will help with the analysis of the trial results.

Eligibility Process

The flowchart below outlines the eligibility screening process from introduction to the study, to final eligibility review before acceptance and enrollment into the clinical trial. You will be informed of your acceptance into the clinical trial by email or phone call from our clinical trial staff.



Baseline and Treatment Initiation (Week 1)

During the screening period, after completing our Baseline Questionnaires, we will mail you a pedometer with diary sheets and the Participant Instructions. This will be before confirming you are eligible to participate in the study, so there is the possibility you may not be eligible to participate after receiving the pedometer. You can then keep the pedometer and do not need to return it to the study team.

- You will wear a pedometer for 7 days and record the number of steps each day in the diary.

After passing the screening period, you will be asked to complete the following study procedures:

- Have a scheduled in-person, phone, or telehealth visit with study personnel in which they will explain the study procedures and confirm your eligibility to receive the study treatment.
    - If having in-person study visits, additional assessments include: weight, height, blood pressure and pulse at laying down and over 5 minutes following passive standing, sit and stand test, and hand grip strength test.

Once the study team has confirmed you are eligible for the trial, you will be randomized into one of the two treatment groups (low-dose naltrexone or placebo).

The study treatment for the first 6 weeks will then be delivered to you, or you can pick it up in-person at the study site (BC Women's Hospital). You will be asked to take the study treatment once daily at the same time each day.

You will start the study treatment at 1 mg per day; this will be one 1 mg capsule daily. You will also record your dose and any side effects that you experience on the paper diary every day.

Week 2

Towards the end of the first week or after the first week of study treatment, you will meet with the study team via a scheduled telephone or video call. You will be asked about your use of the study treatment, report of any potential side effects, and have the opportunity to ask any questions you may have.

In week 2, you will start at 2 mg per day; this will be two 1 mg capsules daily. You will continue to record your dose and any side effects on the paper diary daily.

If you experience any side effects, such as problems with sleep or gastrointestinal (digestive system) upset, it is suggested that you stay at your current dose, as they will usually decrease as the treatment continues. You will have instructions that outline how and when to change your dose, and can contact the study team at any time if you have questions. Severe side effects to the treatment are very rare. However, if you experience any side effects that persist and/or you find difficult to tolerate, you can return to the dose you were taking in the previous week. If you go back to a lower dose, you will then use this dose for the remainder of the study. You will also be asked to complete the diary for 7 days after you make this change to the dose.

Week 3

In week 3, you will start at 3 mg per day (if you were at the 2 mg dose at the end of the previous week); this will be three 1 mg capsules daily. You will continue to record your dose and any side effects on the paper diary daily.

Week 4

In week 4, you will start at 4.5 mg per day (if you were at the 3 mg dose at the end of the week), this will be three 1 mg capsules and one 1.5 mg capsule daily. This capsule will have a slightly different colour. You will continue to record your dose and any side effects on the paper diary daily.

Weeks 5-6

You will continue taking the study treatment based on the last dose you took in week 4. If you haven't needed to go back to a lower dose, it will stay at 4.5 mg daily.

During week 5, you will have a brief check-in with the study team by email, text, phone or video call, to confirm the study treatment dose you are taking and will be using for the rest of the study duration. At this check-in, the study team may also ask about your experience taking the treatment and you will have the opportunity to ask any questions.

During week 5 or 6, you will receive your second package of study treatment (delivered or picked up at BCW). You will be asked to return any unused study drug, empty containers, and your completed diary sheets from the first package via Canada Post using the prepaid return label provided or in-person to the study site.

In week 6, you will be expected to complete another round of study questionnaires. There is an optional study visit available if you wish to meet with the study team.

Week 7
You will continue taking the study treatment based on the last dose you took in week 4. The only difference is that the whole dose will be in one capsule instead of multiple, and you will only need to take one capsule daily for the rest of the treatment period.

Week 12
You will continue taking the study treatment daily.

At this time, you will be expected to complete another round of study questionnaires. There is a study visit to meet with the study team and check-in with you.

Week 16
You will continue taking the study treatment until the end of week 16.

You will be asked to provide a blood sample at LifeLabs.

You will be asked to wear the pedometer again for 7 days after, while awake, from the beginning of week 16 (you will receive an email reminder) and record the number of steps each day on a paper form. You can then keep the pedometer and do not need to return it to the study team.

After completion of treatment at 16 weeks, you will meet with the study personnel one last time via a scheduled telephone, video call, or in-person, according to your preference.

You will be asked to return any unused study drug, empty containers, and your completed diary sheets from the second package via Canada Post using the prepaid return label provided or in-person to the study site.

You will be expected to complete the final round of study questionnaires.

Study Team Contact during the study
The study team is available to answer any questions you may have at any time during the study. You can contact Dr. Luis Nacul (email: luis.nacul@cw.bc.ca; phone: 604-875-2174), or the study team (email: LDNtrial@phsa.ca; phone: 236-990-9519). The study team email will be monitored daily by multiple staff.

Text messages, phone calls and/or emails will be used to contact you while you are in the study. These may be for items like to remind you of tasks to complete, to thank you for completing

task, scheduling study visits, completing study check-ins, or following up on questionnaire items the study team may have questions about.

Summary of Study Procedures:

Timeline	Participant Procedures	Anticipated Time
Screening	Screening Visit with Study Physician	15-60 min
	Complete study questionnaires	25-60 min
	Collection of blood and urine sample at LifeLabs	30 min (time will vary)
	Wear a pedometer (7 days), record number of steps each day	2-5 min/day
Baseline & Week 1	Study Visit – Study procedures & instructions	30-60 min
	Hand grip strength assessment (in-person only)	3 min
	In person assessments (for those agreeing to in person visits)	15 min
	Receive first treatment package for weeks 1-4	n/a
	Take study treatment (1 mg daily) & fill out diary daily	2 min or less/day
Week 2	Study Check-in - Side effects & questions	5-10 min
	Take study treatment (2 mg daily) & fill out diary daily	2 min or less/day
Week 3	Take study treatment (3 mg daily) & fill out diary daily	2 min or less/day
Week 4	Take study treatment (4.5 mg daily) & fill out diary daily	2 min or less/day
Week 5-6	Take study treatment (4.5 mg daily)	2 min or less/day
	Study Check-in – Confirm final dose & side effects	5-10 min
	Complete study questionnaires	15-45 min
	Receive second treatment package for weeks 7-16	n/a
	Return unused study drug & paper documents (post office or in-person to BCW)	10-30 min*
Week 7-11	Take study treatment (4.5 mg daily)	2 min or less/day
Week 12	Study Visit – Check-in & reminder to book blood draw	15 min
	Take study treatment (4.5 mg daily)	2 min or less/day
	Complete study questionnaires	15-45 minutes
Week 16	Wear a pedometer (7 days), record number of steps each day	2-5 min/day
	Collection of blood sample at LifeLabs	30 min (time will vary)
	Hand grip strength assessment (in-person only)	3 min
	Study Visit – Check-in, side effects, questions & instructions for end of study	30-60 min
	In person assessments (for those agreeing to in person visits)	15 min
	Complete study questionnaires	15-45 min
	Return unused study drug & paper documents (post office or in-person to BCW)	10-30 min*
Total Duration: 16 weeks	**Total Anticipated Time:** 5 hours & 4 min - 9 hours & 38 min	

* timing may vary depending on wait times at the post office.

Study Drug Shipment

The drug will be shipped via the FedEx Clinical Care Service which specializes in shipment solutions for clinical trials or delivered by a local courier to those who live in the Lower Mainland. The exterior of the box has no indication of the contents, and will have your name, address, and the BC Women's Hospital return address. You will be asked how you would like your package delivered. You will be asked if you will allow the package to be handed to an adult within your household, or you can specify that your package will only be handed directly to you. The package will only be handed off by FedEx upon a valid ID check and signature.

- If you receive your study drug from FedEx, you will need to be available for the delivery window (8 am -12 pm). The driver will wait as you remove the study package and reseal the outer shipping box for return. Instructions and tape will be provided inside the outer shipping box.

9. What are the possible harms and discomforts?
Naltrexone in the low doses used in this study are considered safe. Although it is not anticipated with the doses of naltrexone being given in this study, you may experience side effects. It is not known what the impact of Post-COVID fatigue syndrome may have in terms of potential side effects with LDN. In the very unlikely event you experience any serious side effects, you should contact Emergency Services immediately and inform the study doctor.

If you need to go to Emergency or be admitted to the hospital for any reason, please let the study team know via the 24 hour phone number (604-786-2473). If you experience any serious side effects due to the study treatment, the study doctor will assess your symptoms and withdraw you from the study if necessary. Even if withdrawn from the study, the study team will follow your recovery progress until the side effects have fully resolved.

Side effects of taking low-dose naltrexone were seen in less than 10% of patients (from the experience of the CCDP at BCW) and they include:

- Sleep dysfunction
- Gastrointestinal upset
- Skin rash
- Vivid Dreams

If you experience severe or persistent side effects after going back down to a lower dose of the study drug, you can stop the study treatment without tapering. You may withdraw from the study or you can continue even after you have stopped the study treatment.

Other symptoms & possible harms:
If other symptoms, such as shortness of breath, oral swelling, faintness, or any other severe symptom, you should seek emergency medical attention.

Naltrexone has been identified as a hepatotoxin, which is a toxic chemical that damages the liver. This was found using doses five times the recommended dose for opioid blockade or the treatment of opioid/alcohol dependence. No cases of liver failure due to naltrexone have ever been reported. The doses in this study are significantly less (4.5 mg) than the recommended doses (50 mg). Even though the risk of this is very low, if you experience any symptoms of hepatitis (inflammation of the liver) or liver problems, such as feeling very unwell, with jaundice (your skin becomes yellow), fever, vomiting and/or

drowsiness (reduction of level of consciousness), please seek emergency medical attention and advise the study team.

Side effects that have been reported during clinical trials with naltrexone at the recommended doses (50mg tablets) are outlined in the table below. To note, study participants will be receiving less than 1/10th of the recommended dose.

More than 10% of participants experienced the following:
Difficulty sleeping, anxiety, nervousness, abdominal pain/cramps, nausea and/or vomiting, low energy, joint and muscle pain, headache
Less than 10% of participants experienced the following:
Loss of appetite, constipation, increased thirst, increased energy, feeling down, irritability, dizziness, skin rash, delayed ejaculation, decreased potency, chills
Less than 1% of participants experienced the following:
Respiratory: nasal congestion, itching, rhinorrhea (runny nose), sneezing, sore throat, excess mucus or phlegm, sinus trouble, heavy breathing, cough, shortness of breath Cardiovascular: nose bleeds, phlebitis (inflammation of the vein), edema, increased blood pressure, non-specific EGG changes, palpitations, tachycardia Gastrointestinal: excessive gas, haemorrhoids, diarrhea, ulcer Musculoskeletal: painful shoulders, legs or knees, tremors, twitching Genitourinary: increased frequency of, or discomfort during urination, increased or decreased sexual interest Dermatologic: oily skin, pruritus (itchy skin), acne, athlete's foot, cold sore, alopecia (hair loss) Psychiatric: depression, paranoia, fatigue, restlessness, confusion, disorientation, hallucinations, nightmares, bad dreams Special Senses: eyes – blurred, burning, light sensitive, swollen, aching, strained; ears – "clogged", arching, tinnitus General: increased appetite, weight loss, weight gain, yawning, somnolence (drowsy), fever, dry mouth, head "pounding", inguinal pain (groin pain), swollen glands, "side" pains, cold feet, "hot spells"

Data collected since the approval of naltrexone shows that most side effects occur early during treatment and only last for a short time.

Pregnancy and Breastfeeding

It is not known what effect naltrexone may have on a fetus or if it is secreted in breastmilk. You cannot participate if you are pregnant, may become pregnant, or are currently breastfeeding. If you are a female participant who can become pregnant, you can participate if you agree to use an effective method of birth control during the study. The approved methods are the following:

- Hormonal birth control (for at least 1 month before study drug start)
- Intra-uterine device (IUD)
- Condom with spermicidal foam/gel/film/cream/suppository
- Male partner who has had a vasectomy

- Abstinence

If you get pregnant during the study, inform the study doctor immediately. The study treatment will need to be stopped and you will be withdrawn from the study.

Questionnaires
Completing the questionnaires may or may not make you feel tired. We encourage you to complete the surveys at your own pace.

10. What are the potential benefits of participating?
Participants may experience improvements in fatigue, pain, other symptoms, and well-being. However, there may not be direct benefit to you from taking part in this study.

We hope that the information learned from this study can be used in the future to benefit other people with a similar disease.

11. What are the alternatives to study treatment?
There are no alternatives to the study treatment at this time. You can discuss available options with your study doctor on how to treat your symptoms.

12. After the study is finished
You may not be able to receive the study treatment after your participation in the study is completed. There are several possible reasons for this, some of which are:

- The treatment may not turn out to be effective.
- The treatment may not be approved for use in Canada to treat PCFS.
- You may not feel it is the best option for you.
- You may decide it is too expensive and insurance coverage may not be available.
- The treatment, even if approved in Canada, may not be available free of charge.

The study treatment (naltrexone) is available in Canada but it is not approved for treating PCFS. It can be used off label if a physician prescribes it for you. LDN treatment needs to be compounded by a compounding pharmacy and these costs may not be covered by most insurance plans.

After study completion, we ask that you return any unused study drug, empty containers, and completed paper forms in-person to the study team or via Canada Post.

The leftover biological samples will be stored for up to 10 years after the completion of the study. These samples will only be used for analysis related to this protocol. Samples for this specific research question will not be shared with outside researchers. You will not get the results of any of the research blood test for this study.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time. We would also be happy to provide a summary of the study results via your preferred method of contact.

13. What if new information becomes available that may affect my decision to participate?
If you choose to enter this study and at a later date, a more effective treatment becomes available, it will be discussed with you. You will also be advised of any new information that becomes available that may affect your willingness to remain in this study. You may be invited to sign an amended consent form to indicate your continued consent to participate in the study.

14. What happens if I decide to withdraw my consent to participate?
You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, all information about you collected up to the point of your withdrawal (including information obtained from your biological samples) will be retained for analysis in order to protect the integrity of the research, which may benefit future research participants and patients. However, no further information will be collected. If you decide to withdraw, you may still be asked to come in for a final safety visit to ensure your safety.

If samples have been collected before you withdraw, they will be destroyed or returned to the facility from which they were obtained. There may be exceptions where the samples will not be able to be withdrawn, for example, where the sample is no longer identifiable (meaning it cannot be linked in any way back to your identity).

15. Can I be asked to leave the study?
You may be asked to leave the study if the study doctor judges it is not in your best interest to continue, you experience serious side effects due to the study treatment, you become pregnant during the study, if you are unable to fulfill the requirements for the study, or for any other reason. If you are asked to leave the study, the reasons for this will be explained to you and you will have the opportunity to ask questions about this decision.

If you decide to take opioid medication over a short time during the study to manage acute pain, you will be allowed to continue in the study, and your study doctor and team will reinforce guidance on taking alternative medication. Please let the study team know if you take any opioid medication or if you start any new medications during the study. You should be aware that naltrexone is an opioid antagonist, which is medication that blocks the effects of opioids. If you do take opioids for pain relief during the study, they may not work well due to the naltrexone.

In the event that you need to take long-term opioid medication, you will be asked to withdraw from the study. All information about you collected up to the point of your withdrawal (including information obtained from your biological samples) will be retained for analysis in order to protect the integrity of the research.

The study doctor will arrange for you to continue your care outside of the study. The study may also be stopped at any time by the sponsor, the C&W Research Ethics Board or Health Canada if new information rises about the safety of the study treatment. The reasons for study stoppage will be explained to you by the study doctor.

16. How will my taking part in this study be kept confidential?

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator or his/her designate by representatives of Health Canada and the C&W Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc.). Only this number will be used on any research-related information collected about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected. You also have the legal right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

The study doctor may need to review your medical record or call your family doctor to help determine if you are eligible for the study and that it is safe for you to take naltrexone. Only the study doctors will access your medical record, the other members of the study team (research coordinator, research assist, etc.) will not be accessing your medical record. If you sign this form you are agreeing to the study doctor reviewing your medical record and/or calling your family doctor.

You should be aware that all of the study test results will be reported to the ordering physician (the study principal investigator Dr. Nacul).

The samples taken at LifeLabs may be referred to a testing laboratory outside of BC to be analyzed (this may be in another province or in the USA). This is because not all tests are performed at LifeLabs and they use other laboratories in these cases.

Studies involving humans now routinely collect information on race and ethnic origin as well as other characteristics of individuals because these characteristics may influence how people respond to different medications. You should be aware that providing this information is not mandatory.

We are asking you to provide a personal email address and phone number (for phone calls and texts, if you prefer) so that we can communicate with you about the study, your participation, send you an electronic copy of your consent form and other study documents, and send reminders.

Before you provide your consent, please carefully consider whether this email account is secure, whether other people have access to it, or whether you have concerns about the security of any information sent to this account. We will only send your personal information to the email address and/or phone number you have provided to us, and all of the information which you provide to us will be kept confidential by the research team. However, you should be aware of the fact that some webmail services (e.g. Gmail, Hotmail, etc.) may store the contents of your email account outside of Canada (for example, in the United States), where privacy and data security standards may be different than they are in Canada. Due to the fact that future emails may contain some personal information about you, including your name, the Freedom of Information and Protection of Privacy Act (British Columbia) requires that we obtain your consent. Providing your email address means that you voluntarily agree and give your consent for the study team to email your consent form, containing personal information, to you.

Your de-identified research data (which means your name, birth date, and other identifiers have been removed) may/will be deposited into a publicly accessible location at the time of publication. This can enhance the transparency of the research data and allows for external validation and fraud control, but it also allows others to access the data for re-analysis of this study, or to do other kinds of analyses in the future beyond those you are consenting to in this study. Also, this future use of your data may not be subject to oversight by a research ethics board, and thus the data may be publicly shared and used in currently unknown ways. Once the data is made publicly available, you will not be able to withdraw your data. Even though the identifying information will be removed from the data, it is possible that others may be able to find out who you are. The chance of this is currently thought to be quite low.

17. What happens if something goes wrong?

By signing this form, you do not give up any of your legal rights and you do not release the study doctor, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan.

In case of a serious medical event, please report to an emergency room and inform them that you are participating in a clinical study and show them your study card. They can contact the study team and/or Dr. Luis Nacul for further information with the phone numbers listed on the study card. The Emergency Contact Number is 604-786-2473.

18. What will this study cost me?

All research-related medical care and treatment, and any related tests that you will receive during your participation in this study will be provided at no cost to you.

To thank you for your participation you will receive $25 gift cards or honoraria (cheque issued by UBC; need to provide personal information (contact details) to UBC for cheques to be issued) twice - one after 6 weeks of treatment and one at the end of study at 16 weeks for a total of $50.

If you agree to have the first and last visit in person, you will receive and additional $25 gift cards or honoraria (cheque) for each visit for a total of $50 for both in person visits.

19. If I have questions about the study procedures during my participation, who should I speak to?

If you have any questions or desire further information about this study before or during participation, or if you experience any adverse effects, you can contact the Study Team at LDNtrial@phsa.ca or 236-990-9519.

20. Who do I contact if I have any questions or concerns about my rights as a participant?

If you have any concerns or complaints about your rights as a research participant and/or your experiences while participating in this study, contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics by e-mail at RSIL@ors.ubc.ca or by phone at 604-822-8598 (Toll Free: 1-877-822-8598.) Please reference the study number *(H21-02254)* when calling so the Complaint Line staff can better assist you.

If you are a patient from the Fraser Health and you have any concerns about your rights as a research participant, you can also contact Fraser Health Research Ethics Board Co-Chair by calling 604-587-4681.

21. Primary Care Physician(s)/Specialist(s) Notification

Please indicate, by checking the applicable box, whether you want us to notify your primary care physician(s) or specialist(s) of your participation in this study. This is not a consent to release medical information.

☐ Yes, I want the study investigator to advise my primary care physician(s) or specialist(s) of my participation in this study. My primary care physician(s) and/or specialist(s) name(s) is/are:

_____

The name of the medical clinic I attend is: _____

Participant Initials: _____

☐ No, I do not want the study investigator to advise my primary care physician(s) or specialist(s) of my participation in this study.

Participant Initials: _____

☐ I do not have a primary care physician or specialist.

Participant Initials: _____

☐ The study investigator is my primary care physician/specialist.

Participant Initials: _____

I understand that if I choose not to advise my primary care physician(s) or specialist(s) of my participation in this study, there may be potential medical consequences, which may affect my

comprehensive medical care or treatment. I understand that the study investigator may not be responsible for these consequences.

You may wish to discuss the consequences of your decision with the study staff.

22. Optional MRI Sub-Study
There is an optional sub-study that will available for some participants. This sub-study will involve MRI scans of the brain at study start and towards the end of the 12-week study period

If you are interested in learning more about the sub-study, check the box below and the research team will provide you further information and connect you with the MRI study personnel.

I am interested in learning more about the MRI sub-study
☐ Yes
☐ No

23. Optional Morning Blood Sampling at LifeLabs
The optional morning blood sampling is for 50 participants who agree to have their study samples drawn at LifeLabs between 8-10 am on the days they have their study bloodwork done. There are two tests, cortisol and adrenocorticotropic hormone, that require that the blood sample is taken in the morning. These tests are expected to be predictors of treatment response and we expect that treatment will be associated with an increase of these concentrations back to normal values.

If you participate in the morning blood sampling, you are agreeing to go to LifeLabs in the morning (8-10am) for your study bloodwork for both time points bloodwork is collected.

I agree to participate in the optional morning blood sampling and will go to LifeLabs between 8-10am for my bloodwork for this study.
☐ Yes
☐ No

24. Optional In Person Study Visits at BCW
Participants who agree to have their first and last study visits (baseline & 16 weeks) will have additional in person assessments completed which will include weight and height, blood pressure and pulse at laying down and over 5 minutes following passive standing, sit and stand test, and hand grip strength test. We are aiming to enroll 50 participants minimum into this sub-group.

If you agree to have the in person study visits, you will receive an additional $25 gift card or honoraria (cheque) for each visit, for a total of $50.

I agree to participate in the optional in person study visits and will attend in person visits at BCW for the baseline and 16 week visits for this study.
☐ Yes
☐ No

25. Participant Consent

A Double Blind Randomized Trial of Low-Dose Naltrexone for Post-COVID Fatigue Syndrome (LDN Trial)

My signature on this consent form means:

- I have read and understood the information in this consent form.
- I have had enough time to think about the information provided.
- I have been able to ask for advice if needed.
- I have been able to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the results will only be used for scientific purposes.
- I understand that my de-identified research data may/will be deposited into a publicly accessible location at the time of publication.
- I understand that my participation in this study is voluntary.
- I understand that I am completely free at any time to refuse to participate or to withdraw from this study at any time, and that this will not change the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me.
- I authorize access to my health records and samples as described in this consent form.
- I understand that the study doctor will review my medical record and/or call my family doctor to help determine if I am eligible for the study.

I will receive a signed and dated copy of this consent form for my own records.

I consent to participate in this study.

_____	_____	_____
Participant's Signature	Printed name	Date

_____	_____	_____	_____
Signature of Person Obtaining Consent	Printed name	Study Role	Date

Please provide your contact details:

Phone: _____

Email: _____

References

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SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym *Page 1*
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry *Page 4*
	2b	All items from the World Health Organization Trial Registration Data Set *Pages 1-2; 6-22*
Protocol version	3	Date and version identifier *Page 4*
Funding	4	Sources and types of financial, material, and other support *Page 2*
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors *Page 2*
	5b	Name and contact information for the trial sponsor *Page 2*
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities *Page 2*

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) *Pages 18-19*
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention *Pages 6-7*
	6b	Explanation for choice of comparators *Page 7*
Objectives	7	Specific objectives or hypotheses *Pages 7-8*
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) *Page 9*
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained *Page 9*
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) *Pages 9-10*
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered *Pages 11-12*

	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) *Page 12*
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) *Page 12*
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial *Page 12*
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended *Pages 12-13*
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) *Pages 14-15*
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations *Page 16*
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size *Page 16*

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions *Pages 16-17*
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned *Pages 16-17*
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions *Pages 16-17*
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how *Page 17*
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial *Page 17*

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol *Page 17*
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols *Page 17*

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol *Page 17*
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol *Pages 18-19*
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) *Pages 18-19*
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) *Pages 18-19*
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed *Page 18*
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial *Not applicable*
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct *Page 19*

Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor *Page 18*
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval *Page 19*
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) *Page 19*
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) *Pages 19-20*
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable *Pages 19-20*
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial *Pages 22*
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site *Page 2*
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators *Page 20*

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation *Not applicable*
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions *Page 20*
	31b	Authorship eligibility guidelines and any intended use of professional writers *Not applicable*
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code *Page 2*
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates *Supplementary material, page 13*
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable *Page 17*

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.