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Study protocol for a randomised, double-blinded, placebo-controlled phase III trial examining the add-on efficacy, cost-utility and neurobiological effects of low-dose naltrexone (LDN) in patients with fibromyalgia (INNOVA study)

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-055351
Article Type:	Protocol
Date Submitted by the Author:	09-Jul-2021
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Keywords:	PAIN MANAGEMENT, HEALTH ECONOMICS, Clinical trials < THERAPEUTICS



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3 **Study protocol for a randomised, double-blinded, placebo-controlled phase III trial**
4 **examining the add-on efficacy, cost-utility and neurobiological effects of low-dose**
5 **naltrexone (LDN) in patients with fibromyalgia (INNOVA study)**
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ABSTRACT

Introduction. There is evidence that low-dose naltrexone (LDN; < 5.0 mg/day) reduces pain and improves the quality of life of people with fibromyalgia syndrome (FMS). However, no randomised controlled trials with long-term follow-ups have been carried out. The INNOVA study will evaluate the add-on efficacy, safety, cost-utility, and neurobiological effects of LDN for reducing pain in FMS patients, with a one-year follow-up.

Methods and analysis. A single-site, prospective, randomised, double-blinded, placebo-controlled, parallel design phase III trial will be performed. Eligibility criteria include being adult, having a diagnosis of FMS and experiencing pain of 4 or higher on a 10-point numerical rating scale. Participants will be randomised to a LDN intervention group (4.5 mg/day) or to a placebo control group. Clinical assessments will be performed at baseline (T0), 3-months (T1), 6-months (T2), and 12-months (T3). The primary endpoint will be pain intensity. A sample size of 60 patients per study arm (120 in total), as calculated prior to recruitment for sufficient power, will be monitored between October 2021 and June 2024. Assessment will also include daily ecological momentary evaluations of FMS-related symptoms (e.g., pain intensity, fatigue, and sleep disturbance), and side effects via ecological momentary assessment (EMA) through the Pain Monitor app® during the first three months. Costs and quality-adjusted life years will be also calculated. Half of the participants in each arm will be scanned with magnetic resonance imaging (MRI) at T0 and T1 for changes in brain metabolites related to neuroinflammation and central sensitization. Immune-inflammatory biomarkers in serum will also be measured.

Ethics and dissemination. This study has been approved by the Ethics Committee of the Fundació Sant Joan de Déu. The results will be actively disseminated through peer-reviewed journals, conference presentations, social media, and community engagement activities.

Trial registration number: ClinicalTrials.gov (NCT04739995)

Strengths and limitations of this study

- This is thought to be the first randomised, double-blinded, placebo-controlled phase III trial to assess the efficacy, safety, cost-utility, and neurobiological effects of low-dose naltrexone (LDN) for reducing pain in patients with fibromyalgia syndrome.
- The INNOVA protocol combines mobile-technology-based ecological momentary assessment and assessment with classical legacy measures to obtain more precise information on the dynamics of the assessed primary and secondary outcomes.
- This study will include immune-inflammatory and neuroimaging biomarkers in order to explore the neurobiological underpinnings of LDN.
- This is an adequately powered exploratory trial in which the long-term effects of LDN will be systematically evaluated.

INTRODUCTION

Fibromyalgia: definition, prevalence, and pharmaceutical indications

Fibromyalgia syndrome (FMS) is a chronic condition of unknown origin that is characterised by generalised musculoskeletal pain, fatigue, stiffness, cognitive problems, sleep disturbances, and malaise.^{1,2} This syndrome is highly prevalent in the general population (2.7% worldwide).³ Around 6% of adult patients who visit their general practitioner, and between 10-20% of those who visit rheumatology services, have FMS.² In 2007, the U.S. Food and Drug Administration approved pregabalin as the first drug indicated for the treatment of FMS, and later approved duloxetine and milnacipran for this indication. However, the European regulatory authorities rejected the indication of these three drugs in the treatment of FMS given the small effect sizes in various studies and the associated adverse effects.⁴⁻⁶

Pathogenesis of FMS

Although the etiological factors of FMS are not known, the primary hypothesis of the pathogenesis of this syndrome highlights the role of the central nervous system in the amplification of pain perception as well as in the development of comorbid symptoms such as sleep-related problems, fatigue, cognitive difficulties, and emotional distress.^{1,7,8} Structural brain alterations have also been found in patients with FMS. For example, lower volumes of grey matter have been observed in areas associated with the processing of stress (e.g., parahippocampal gyrus) and pain (e.g., anterior cingulate cortex, insula, prefrontal cortex, and primary and secondary somatosensory cortices).⁷ Functional MRI alterations been associated with self-reported pain intensity. Brain activity exhibited greater connectivity between different pain-processing areas (e.g., insula and secondary somatosensory cortex), the default-model network in persons with FMS, as well as in the association between these areas and the pain levels reported by patients and the right executive attention network.⁹

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3 Altered functional connectivity has also been reported among various pain-inhibiting
4 areas.¹⁰ Some studies have reported reduced levels of neurotransmitters involved in the
5 regulation of the descending analgesic response (serotonin and noradrenaline) and increased
6 levels of glutamate (Glu) and substance P in people with FMS.¹¹ For example, high levels of
7 Glu have been reported in the posterior insula, posterior cingulate cortex and prefrontal
8 ventrolateral cortex of patients with FMS when compared to healthy controls¹⁵⁻¹⁸. Ultimately,
9 these abnormal levels of brain metabolites seem to be associated with increases in the pain
10 response, which may facilitate hyperalgesia and allodynia.¹¹⁻¹⁵

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22 Activation of the microglia could be a contributing factor to the alteration of glutamate
23 neurotransmission in FMS.^{20,21} The microglia is normally found in a state of rest but it is
24 activated by a wide range of stimuli such as cell death, peripheral inflammation, chronic stress
25 and infections.²² Once activated, microglia release pro-inflammatory agents such as cytokines,
26 excitatory amino acids and nitric oxide.²³ These inflammatory factors across multiple neural
27 pathways can induce hyperalgesia, fatigue, depression and other symptoms which are known
28 collectively as “cytokine induced sickness behavior”.^{24,25} Microglia activation might trigger a
29 series of actions that lead to an increase in Glu that ultimately results in synaptic dysfunction.²⁶
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41 A recent study using Positron Emission Tomography²⁷ of translocator protein revealed a
42 widespread cortical glial activation in patients with FMS, which gives support to the role of
43 neuroinflammation in the aetiology of FMS. In addition, there is evidence that chronic stress
44 facilitates the “priming” and exaggerated activation of the microglia.²⁸

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50 Although FMS is not considered a classic inflammatory disease, there is extensive
51 evidence that immune-inflammatory pathways play a significant role in the pathogenesis and
52 maintenance of the syndrome. Cytokines play a key role in immune-inflammatory response
53 and in boosting the nociceptive response due to their sensitization actions, both on a peripheral
54 and central level.³¹⁻³² Thus, there is evidence that FMS involves an imbalance in pro-

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3 inflammatory (e.g. IL-1, IL-6, IL-17A, and TNF- α) and anti-inflammatory (e.g. IL-4 and IL-
4 10) levels of cytokines that could lead to a low-intensity, chronic state of inflammation.^{29,30}
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6 Bäckryd and colleagues³⁰ identified both neuroinflammation and systemic inflammation by
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8 evaluating levels of a broad panel of cytokines and chemokines in cerebrospinal fluid and
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10 plasma.
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14 **Low-dose naltrexone (LDN): A promising treatment for FMS**

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16 Naltrexone is an opioid antagonist medication used to treat opioid and alcohol
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18 dependency. The drug blocks mu-opioid receptors, the delta-opioid receptors and, to a lesser
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20 extent, the kappa-opioid receptors. There is promising evidence to suggest that naltrexone
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22 administered in low doses (i.e., low-dose naltrexone; <5 mg/day) is effective in the
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24 management of some pathologies which present with altered immune-inflammatory pathways,
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26 such as Crohn's disease, multiple sclerosis, or FMS.^{33,34} The immune-regulatory effect of LDN
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28 seems to be driven through the inhibition of the Toll-like receptor 4 (TLR-4) activity expressed
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30 in the membrane of various immune system cells (e.g., microglia and macrophages).³³
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32 Moreover, due to a “rebound effect”, LDN could exert an analgesic effect that strengthens the
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34 endogenous opioid pain inhibitory system. According to this hypothesis, the low-intensity and
35
36 intermittent blockade of the opioid receptors generated by LDN induces a compensatory
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38 mechanism that facilitates an increase in the production of endogenous opioids and greater
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40 sensitivity of the system to their effects.^{33,34}
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49 To date, the effects of LDN in patients with FMS have only been evaluated through
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51 crossover pilot studies that have yielded preliminary results. In the first study conducted with
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53 LDN in FMS, significant reductions in pain, stress, and fatigue levels were observed.³⁵ In a
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55 subsequent study, significant improvements in daily pain, satisfaction with life and mood were
56
57 also observed.³⁶ In another crossover investigation, the pre and post changes in the levels of
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59 plasma cytokines were evaluated over eight weeks. Significant reductions in a wide range of
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3 immune-inflammatory markers were obtained (e.g., IL-1 β , sIL-1ra, IL-4, IL-6, IL-10, IL-17A,
4 and TNF- α), together with a reduction in the pain levels and the severity of FMS symptoms.³⁷
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8 While acknowledging the contribution of past studies into the field, these have included
9 small sample sizes (n= 8 to 31 participants) and crossover designs. Therefore, a single-site,
10 prospective, randomised, double-blinded, placebo-controlled study (RCT) with a sufficiently
11 powered sample is presented here to conduct a methodologically robust investigation into the
12 role of LDN in FMS. Specifically, the main objective of the INNOVA study is to evaluate the
13 efficacy, safety, and cost-utility, and neurobiological effects of LDN to reduce pain in FMS.
14 There is currently no gold standard pharmacological treatment for pain reduction in persons
15 with FMS. Therefore, in the present study, placebo will be used instead of another drug in the
16 control group.
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29 **METHODS AND ANALYSIS**

30 **Trial design**

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33 The randomised controlled trial (RCT) protocol has been developed following the
34 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)³⁸. In addition,
35 the RCT was approved by European Union Drug Regulating Authorities Clinical Trials
36 (EudraCT; 2021-002534-16). For reporting purposes, we will follow the guidelines of the
37 Consolidated Standards of Reporting Trials (CONSORT)³⁹ and the Consolidated Health
38 Economic Evaluation Reporting Standards (CHEERS) statement.⁴⁰ INNOVA is a 12-month
39 double-blind RCT with two arms: LDN vs. Placebo. LDN will be considered an add-on
40 treatment to the usual care provided in the Spanish National Health System for FMS. For
41 transparency and analytical reproducibility purposes, the dataset and data coding will be
42 deposited in the Open Science Framework.
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57 **Sample size**

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There are no previous RCTs about the efficacy of LDN for FMS, therefore, we estimated the sample size taking into account a previous LDN crossover study³⁶ that had used self-reported pain as main outcome (the effect size was $d = .99$). Thus, with a sample of 60 participants per arm, we aim to detect between-group differences with a significance level of 5% and a power of 80%. Allowing for a potential attrition rate of 20%, our final sample size is 60 participants per group. For the analysis of biomarkers (involving 50% of the sample), an initial sample size of 30 patients per arm is considered sufficient according to previous studies.^{37,41}

Eligibility criteria

General selection criteria. All participants will meet the following inclusion criteria: women between 18-70 years; diagnoses of FMS according to American College of Rheumatology 2016 criteria⁴² by a rheumatologist; pain intensity ranked ≥ 4 out of 10 on a 10-point numerical rating scale in the past week; fluent in Spanish; provision of written informed consent; stable pharmacological treatment in the last two months; and having a smartphone with android operating system for Ecological Momentary Assessment (EMA). Potential participants will be excluded according to the following exclusion criteria: treatment with naltrexone, opioids, anticoagulants, or central anti-hypertensives in the last 3 months; diagnosis of severe medical/psychiatric disorders (e.g., cancer, haematological diseases, abnormal hepatic/liver function, renal failure, suicide ideation, psychotic disorder); pregnant (or planning to become pregnant during the study period) or breastfeeding; known allergy to naltrexone, naloxone or excipients; currently participating in other RCTs; ongoing litigation related to FMS.

Additional selection criteria for the biomarkers and neuroimaging sub-study (50% of patients in each study arm). All participants will meet the following inclusion criteria: right-handed (for the neuroimaging tests); and no comorbid rheumatologic conditions (e.g.,

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3 rheumatoid arthritis, lupus). Potential participants will be excluded according to the following
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5 exclusion criteria: fever (> 38°C); infection in the last two weeks; vaccination in the last month;
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7 taking cortisone or anti-cytokine therapy; needle phobia or claustrophobia, metal implants or
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9 pacemakers; body mass index ≥ 36 kg/m²; smoking over 5 cigarettes/day; presence of acute
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11 pain (e.g, headache or back pain) unrelated to FMS on the day of the scan.
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14 15 **Recruitment strategy, procedure and randomisation**

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17 Patients diagnosed with FMS with an appointment at the Rheumatology Service of Parc
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19 Sanitari Sant Joan de Déu (St. Boi de Llobregat, Spain) will be invited to participate in the
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21 study and will be asked to attend a screening evaluation with a research assistant and a clinician.
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23 Once the informed consent is obtained, the clinician will review the study selection criteria to
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25 confirm eligibility. The week after, a face-to-face assessment (T0) including clinical history
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27 and variables related to the use of services will be conducted with those patients meeting all
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29 the eligibility criteria. Only the participants that are included in the biomarkers sub-study will
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31 require an additional blood extraction and neuroimaging scan, which will be performed in the
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33 following 3-5 days. Participants will be given a sealed envelope with an identifying code which
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35 they will have to take to the pharmacy service. There, they will be given the dose of the
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37 corresponding drug (according to the randomisation) for the first 3 months (90 tablets). As
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39 shown in the patients' flow chart (Figure 1), further in-person evaluations will be performed at
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41 3 months (T1), 6 months (T2), and 12 months (T3). Neurobiological variables will be obtained
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43 at T1 using the same protocol as in the baseline assessment (T0).
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49 Participants will be asked to abstain from taking any analgesic or anti-inflammatory
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51 drug in the 72h prior to the blood extractions/scans. All patients, including those who do not
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53 participate in the biomarker sub-study, shall be subjected to a blood test at baseline. Participants
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55 will return to the pharmacy service every 3 months and will be given the assigned amount of
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57 LDN/placebo for the following 3 months (approximately 90 tablets). Unconsumed tablets will
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3 be returned for treatment adherence monitoring. The randomisation to conditions will be
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5 conducted by a biostatistician from the Clinical Trials Unit of Fundació Sant Joan de Déu who
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7 has no involvement in the eligibility screening, enrolment, and treatment processes. The
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9 computer-generated randomisation will apply a permuted block design to ensure that the study
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11 arms are balanced taking the biomarkers sub-study eligibility criteria into account. As this is a
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13 double-blind RCT, neither the patient nor the evaluator or the clinician will know to which
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15 treatment arm each patient has been assigned. Only the clinical trial pharmacist who stores and
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17 delivers capsules, but is not involved in patient care, will know the allocation.
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22 Figure 1
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24 **Data management, central monitoring and audit**

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26 The clinical data entry, data management, and central monitoring will be performed
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28 with REDCap®. An independent Clinical Research Organization (CRO) will be responsible
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30 for overseeing the intra-study data sharing and storing process. Any modifications in the study
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32 protocol will be communicated to the CRO.
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35 **Treatments**

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38 **Low-dose naltrexone (LDN).** The intervention group will take one 4.5 mg naltrexone
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40 tablet (lactose-free) daily for 12 months before going to sleep.
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43 **Placebo.** The control group will take the placebo daily for 12 months (a film-coated
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45 tablet identical to the LDN and filled with a lactose-free filler). For the control arm, the same
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47 guidelines will be followed.
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50 In order to maintain the external validity of the study and for ethical reasons, the study
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52 participants' active treatments will be unchanged by this clinical trial. In Spain, chronic pain
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54 management is mainly managed by general practitioners in regular consultations. These
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56 generally consist of face-to-face appointments with a duration of 5-10 minutes in which the
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58 clinicians monitor the physical and, ideally, the emotional status of the patient. General
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3 practitioners usually provide advice prescribe pharmacotherapy (pain killers, hypnotics,
4 antidepressants, etc.) or refer patients to pain units in tertiary hospitals when more specialised
5 pain management procedures are required. The frequency of consultations is based on the type
6 of disease and its severity. In this study, usual care will be the same as in routine daily practice,
7 without any modifications. In addition, participants will be allowed to withdraw from this study
8 for any reason at any time without detriment to the provision or quality of their usual care. If a
9 severe adverse event occurs, unblinding will be possible and study participation will be
10 discontinued. If the adverse effects are tolerable, the treatment will be administered until the
11 end of the study. All these events will be recorded and reported at the end of the study.
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24 **Study measures**

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26 All participants will be assessed with a computer-administered battery of measures
27 using the REDCap® software (see Table 1).
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31 Table 1
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33 **Measures for sociodemographic characteristics, clinical features, and screening**

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35 A *sociodemographic questionnaire* will be used to obtain information about the
36 following variables: gender, date of birth, marital status, living arrangements, educational level,
37 income level, and employment status.
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42 The *Clinical data* interview will be used to collect information about history and
43 duration of FMS symptoms, as well as family history of medical/mental illness. Information
44 regarding comorbidity with other diagnosed physical-psychiatric conditions and the type and
45 dose of current drugs will be checked from medical records.
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51 The *Fibromyalgia Survey Diagnostic Criteria* (FSDC)^{43,44} is a 6-item self-report
52 measure of the core FMS symptoms according to the latest revision of the American College
53 of Rheumatology (ACR) 2016 criteria.⁴² It includes two subscales: the Widespread Pain Index,
54 which is used to identify the presence of pain in 19 body areas in the last week, and the
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3 Symptom Severity Scale, in which the three major FMS symptoms (fatigue, “fibrofog” and
4 waking up tired) are assessed along with three additional symptoms (pain in the lower stomach,
5 depression and headache). A total score is obtained by adding the two subscales. This total
6 score ranges from 0 to 31, where higher values indicate greater FMS severity.
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10 11 12 **Primary outcome measure**

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15 The *Numeric Rating Scale* (NRS)⁴⁵ is a unidimensional measure of pain intensity
16 mainly used for adults. The most frequently used version is an 11-point numeric scale (a
17 horizontal bar or line) scored from 0 (“no pain”) to 10 (“worst pain imaginable”). Time frames
18 vary between studies. In the present study, respondents will be asked to report average pain
19 intensity over the last week.
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26 27 **Secondary outcome measures**

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29 The *Revised Fibromyalgia Impact Questionnaire* (FIQR)⁴⁶ includes 21 items that are
30 answered on a 0 to 10 numerical scale in which higher scores indicate greater functional
31 impairment. The questionnaire asks about the previous seven days. The items are distributed
32 into three domains: physical impairment, overall impact, and severity of symptoms (i.e., pain,
33 energy, stiffness, sleep quality, depression, memory issues, anxiety, pain to the touch, balance
34 problems and increased sensitivity to noises, lights, smells, or temperatures). A total score is
35 obtained by summing the three subscale scores. This can range from 0 to 100. Higher scores
36 indicate greater impairment. The Spanish version of the FIQR and has obtained high internal
37 consistency estimates ($\alpha = .91 - .95$), adequate test-retest reliability indices ($r = .82$), and good
38 construct validity.⁴⁷
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52 The *Depression Anxiety Stress Scales-21* (DASS-21)⁴⁸ is a self-report scale developed
53 to discriminate between features of depression (anhedonia/low positive affect), anxiety
54 (physical arousal) and stress (psychological tension/agitation) in clinical and non-clinical
55 samples. The DASS has been validated in patients with FMS.⁴⁹ Responders are required to
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3 indicate the presence of a symptom over the previous week. Each item is scored from 0 (“did
4 not apply to me at all over the last week”) to 3 (“applied to me very much or most of the time
5 over the past week”). There are seven items on each of the three subscales: depression, anxiety,
6 and stress. Therefore, total scores in each scale can range from 0 to 21. Higher scores indicate
7 more severe levels of depression, anxiety and stress. The Spanish version showed adequate
8 internal consistency for depression ($\alpha = .84$), anxiety ($\alpha = .70$) and stress ($\alpha = .82$).⁵⁰
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12 The *Multidimensional Inventory of Subjective Cognitive Impairment* (MISCI)⁵¹ is a
13 10-item self-report measure of subjective cognitive dysfunction (i.e., “fibrofog”) in FMS.
14 Each item is scored from 1 (“never”) to 5 (“very often”) and the total score ranges from 10 to
15 50. Lower scores indicate higher cognitive dysfunction. The MISCI showed excellent internal
16 reliability, low ceiling/floor effects and good convergent validity with a similar measure. The
17 Spanish version of the MISCI had sound psychometric properties ($\alpha = .91$ and ICC = .88).⁵²
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22 The *World Health Organization Disability Assessment Schedule 2.0* (WHODAS
23 2.0)⁵³ is a 12-item self-report measure of the level of difficulty experienced taking into
24 consideration how they usually do the activity. This includes the use of any devices to assist
25 them and/or the help of a person. In each item, individuals estimate the magnitude of the
26 disability during the previous 30 days using a five-point scale scored from 1 (none) to 5
27 (extreme/cannot do). The total score ranges from 0 to 100. Higher scores reflect greater
28 disability. The 12-item WHODAS 2.0 has sound psychometric properties in patients with
29 FMS.⁵⁴
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33 The *Generalized Anxiety Disorder 7-item scale* (GAD-7)⁵⁵ is a 7-item self-report
34 measure of pathological worry. Each item is scored from 0 (“not at all”) to 3 (“nearly every
35 day”). The total score ranges from 0 to 21, where higher scores reflecting greater anxiety
36 symptoms. The GAD-7 has sound psychometric properties ($\alpha = .92$ and ICC = .83) in patients
37 with FMS in previous studies.⁵⁶
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Other measures

The *ACTION AE* is a *reporting checklist* used to measure safety and benefit-risk of a clinical trial.⁵⁷ The Safety and Benefit-Risk Reporting and Evaluation (SABRRE) Working Group of the Analgesic, Anaesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks (ACTION; <http://www.action.org>) public-private partnership with the FDA developed an adverse events (AE) reporting checklist that will be used in the present study.

The *EuroQoL* (version EQ-5D-5L)⁵⁸ is a health-related quality of life questionnaire that consists of two parts. In the first one, the individual's difficulties concerning mobility, self-care, pain/discomfort and anxiety/depression are evaluated. In the second part, the perceived health is assessed by means of a Visual Analogue Scale ranging from 0 to 100. The EQ-5D-5L scores will be used to calculate the Quality-Adjusted Life Years (QALYs) during the follow-up period by adjusting the duration of time affected by the health outcome by the value of the utility.

The *Client Service Receipt Inventory* (CSRI)⁵⁹ is a self-report tool used to collect retrospective data on medication consumption and service receipt. Patients are asked to bring their daily medication prescriptions and information about pain-related drugs (analgesics, anti-inflammatories, opioids, muscle relaxants, antidepressants, etc.) is recorded. This includes the name of the drug, the dosage, total number of prescription days and daily dosage consumed. Concerning service receipt, patients are asked about the total appointments for accident and emergency services, total number of general inpatient hospital admissions, number of diagnostic tests administered and total appointments with healthcare professionals for pain management (family physicians, nurses, social workers, psychologists, psychiatrists, group psychotherapy and other community healthcare professionals). The CSRI will be administered on two occasions: at baseline and at 12-month follow-up, both referring to the

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2
3 previous 12 months. Medical records will be checked to verify the accuracy of the collected
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5 data.
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8 The *Patient Global Impression of Change* (PGIC) measures meaningful change in
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10 overall status and the *Pain Specific Impression of Change* (PSIC)⁶⁰ measures the perception
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12 of pain improvement. The most frequently used scale is a 7-point numerical scale scored from
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14 1= “much better”) to 7= “much worse”).
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17 **Ecological momentary assessment (EMA)**

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19 Pain intensity and other pain-related variables (e.g., depressive-anxious symptoms and
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21 activity level) can fluctuate during the day and across days depending on personal and
22
23 environmental factors. Collecting self-reported data prospectively and closer in time to its
24
25 occurrence substantially improves the accuracy, reliability and quality of data. EMA has been
26
27 successfully performed in patients with a variety of physical and mental problems.^{61,62} There
28
29 is growing evidence indicating that well-designed smartphone apps can be easy to use and well-
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31 tolerated even in relatively old pain populations, with compliance rates as high as 85%.⁶¹ In
32
33 this RCT, we will use the Pain Monitor® (Monitor de Dolor, by its Spanish name) app⁶³ to
34
35 assess a wide range of variables (see items in Table 2) twice a day (once in the morning and
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37 once in the evening, at convenient times along the week) during 120 days. The app and the data
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39 will be stored on different servers with different domain names and connected locally only (the
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41 server containing the data does not have Internet access). According to a recent meta-analysis,⁶⁴
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43 EMA-completion rates are higher among elderly patients compared to younger patients.
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45 Considering that the majority of FMS patients in our study are not expected to be young and
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47 that the EMA item battery does not require a long response time (< 1 min.), it is expected not
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49 to place an excessive burden on participants.
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56 Table 2

57 **Immune-inflammatory markers**

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3 After obtaining the blood sample, it will be allowed to coagulate for a minimum of 30
4 minutes at room temperature. It will then be centrifuged for 10 minutes at 1000g. The resulting
5 serum will be stored at -80° C during the same morning of extraction until it is ready to be
6 analysed. All samples (at T0 and T1) will be analysed in a single analytical batch to reduce
7 inter-assay variability (approx. 15%). The serum levels of IL-1 β , sIL-1ra, IL-4, IL-6, sIL-6r,
8 sgp130, CXCL-8, IL-10, IL-17, TNF- α , and high-sensitivity C-reactive protein (hs-CRP) will
9 be evaluated.²⁹ For the quantification of the cytokines, the Milliplex® reagents from the
10 company MerckMillipore® will be used and analysed using a Luminex® platform. The high
11 sensitivity multiplex kit will be used: Human High Sensitivity T Cell, catalogue number:
12 HSTCMAG28SPMX11, adapted to the aforementioned cytokines. The hs-PCR will be
13 quantified using turbidimetry in an Olympus AU5400 auto-analyser. These biomarkers will
14 only be evaluated at baseline (T0) and 3-months (T1) for the following reasons: (a) there is
15 evidence of significant inflammatory changes at 8 weeks with LDN;³⁷ (b) this results in lower
16 risk of dropout (vs. evaluating them at 6 or 12 months); (c) conducting at least two measures
17 allows to use the change between baseline and 3-months as a mediator of long-term clinical
18 changes; and (d) budget constraints.

40 Neuroimaging

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42 The scans (protocol duration: approximately 30 minutes) will be performed in a
43 Phillips Ingenia 3T MRI scanner with a 32-channel head coil at Hospital Sant Joan de Déu
44 (Esplugues de Llobregat, Spain). To examine cingulate, insular, amygdalar, occipital, angular,
45 parahippocampal, and prefrontal gray matter volume, we will use voxel-based morphometry
46 (VBM). We will also use surface-based morphometry (with FreeSurfer calculation of cortical
47 thickness, surface area, and local gyrification index) for examining cortical abnormalities.
48 Additionally, glutamate, glutamine, myo-inositol, N-acetylaspartate, choline, and creatine
49 (and creatinine ratios) levels will be analysed using magnetic resonance spectroscopy.
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3 Specifically, we will conduct the following processing for the regions of interest according to
4 the corresponding hypotheses. For VBM, we will apply a bias field correction, tissue
5 segmentation with SPM12, normalization with DARTEL, modulation, and smoothing. We
6 will use both unmodulated and modulated grey matter images to convey complementary
7 volumetric information. We will use FreeSurfer ENIGMA pipelines to perform the VBM. In
8 addition, we will quantify metabolites concentrations using LCModel (v6.3-1J). We will only
9 include high-quality spectra, defined as signal-to-noise ratio > 15 , Cramer-Rao lower bounds
10 $< 15\%$, and full width at half maximum of metabolites $< .07$. The spectroscopy analysis will
11 account for the effects of cerebrospinal fluid and grey matter within the voxel, and inter-
12 individual differences in cortical grey matter.
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25 26 **Statistical analysis**

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28 The main analysis will compare the effect of LDN vs. placebo on the primary outcome
29 (pain intensity at T1). Data analyses will be performed following an intention-to-treat (ITT)
30 plan. Then, we will compute analysis of the primary outcome (at T2 and T3) and analysis of
31 the secondary outcomes at T1, T2 and T3. Linear mixed models will be created using the
32 restricted maximum likelihood method for the estimation of parameters. The effect sizes will
33 be calculated according to Cohen's *d*. An interim analysis is planned at T1 once 50% of the
34 total sample has been evaluated. A 5% significance level will be used in all two-tailed tests,
35 applying the Benjamini-Hochberg correction for multiple comparisons. Additionally, to make
36 the findings from our study clinically meaningful, the number needed to treat will be reported.
37 For this analysis, we will dichotomise participants into responders or non-responders using two
38 different cut-off criteria in compliance with the IMMPACT recommendations:⁴⁵ At least 50%
39 pain relief over baseline (substantial benefit) or 30% or more pain relief (moderate benefit).
40 For these analyses, we will use SPSS v26 (IBM Corp, Armonk, NY, USA).
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3 Regarding EMA, a recent recommended approach is "network analysis". There has
4 been burgeoning interest in conceptualizing chronic pain as a network of interacting symptoms
5 and psychobiological processes.⁶⁵ Network analysis will offer us a good chance to quantify and
6 visualize relationships between pain intensity and pain-related variables (e.g., depression,
7 anxiety, fatigue, sleep disturbance). We will estimate temporal networks by means of vector
8 autoregression techniques;⁶⁶ These "temporal networks", would indicate potential causality
9 with one or more variables preceding one or more variables in time. Network analysis will be
10 performed with the free statistical software JASP.⁶⁷
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22 In economic evaluation, it is important to calculate the relationship between the costs
23 of each treatment and its consequences in the form of QALYs, an index measure designed to
24 assess both quantity of life (years) and health-related quality of life. A year lived with the
25 maximum quality of life would be transformed into 1 QALY; a year lived with half the
26 maximum quality of life would be transformed into 1/2 QALY. This relative value is called the
27 incremental cost-utility ratio (ICUR) and it expresses the relationship between the costs and
28 the effects of one option compared to another. The QALYs obtained in the 12 months after the
29 treatment onset will be calculated by the area under the curve. The direct costs will be
30 calculated by adding together the costs derived from the medication and the use of the health
31 services. The cost of medications will be calculated by multiplying the price per milligram by
32 the total daily dose consumed (in milligrams) and the number of days that the treatment is
33 received. The cost arising from the use of the health services (primary care, specialist and
34 accident and emergency consultations, and hospital admissions) will be obtained from the
35 clinical electronic records (<http://www.oblikue.com/en/esalud.html>). The indirect costs will be
36 calculated based on the days off work, which will be multiplied by the official minimum wage
37 during the study period. The effect of the treatments will be estimated using ordinary least
38 squares multivariate regression, adjusting for the baseline differences between groups. In order
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3 to manage uncertainty in the sampling distribution of the ICUR, non-parametric bootstrapping
4 will be applied, with 1000 replications in each comparison. Cost-utility analyses will be
5 conducted with STATA v16.0 (StataCorp, College Station, TX, USA).
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10 **Patient and public involvement**

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12 Patients and the public will not be involved in the design, conduct, reporting, or
13 dissemination of our research.
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16 **ETHICS AND DISSEMINATION**

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18 All procedures performed in this study will be in accordance with the 1964 Helsinki
19 declaration and its last amendments (7th revision, adopted by the 64th World Medical
20 Association General Assembly, Fortaleza, Brazil). Signed informed consent will be obtained
21 from all patients once they have been informed of the study procedures, potential risks, and
22 their right to withdraw at any time from the RCT. The FSJD Ethics Committee Board evaluated
23 and approved the study protocol in June 2021 (PIC-178-19). Only the principal investigators
24 (ARS and JVL) will have full access to the final trial dataset. Modifications in the study
25 protocol will be reported to the FSJD Ethics Committee Board as well as the independent CRO.
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38 Once the RCT is completed, we will publish our results in international peer-reviewed
39 biomedical journals and present them at national and international conferences. Authorship
40 will be assigned in accordance with the International Committee of Medical Journal Editors
41 guidance. In addition, we will send participating patients a short report of our findings. A copy
42 of the report will also be sent to Institute of Health Carlos III (main funding body). The
43 principal investigators will organize an end-of-study seminar. The main objective of this
44 activity will be to share the study findings with stakeholders to discuss how to maximize uptake
45 of the findings in patient treatment and clinical practice, and to determine future research
46 directions.
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59 **DISCUSSION**

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3 As far as we know, no RCT has been published about the efficacy, safety, cost-utility,
4 and neurobiological underpinnings of LDN in patients with FMS. This manuscript presents the
5 design and rationale of a randomised, double-blinded, placebo-controlled phase III study,
6 which is a powerful design to assess the efficacy of LDN. We have decided to administer 4.5
7 mg/day of LDN in this RCT because this dose seems to provide an optimal balance between
8 significant analgesic efficacy and minimal side effects (nausea, sleep disturbance, nightmares,
9 etc.) according to a recent study.⁶⁸

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19 Our findings using this design in conjunction with those that will be obtained in another
20 ongoing RCT that is being carried out in Denmark (The FINAL study)⁶⁹ may facilitate the
21 approval of the first drug indicated for the treatment of FMS in Europe. The FINAL study is
22 an ongoing single-centre, randomised, double-blinded, placebo-controlled trial that is being
23 carried out in Odense (Denmark). A total of 100 women between 18–64 years-old with FMS
24 will take either LDN or placebo for three months. Besides self-report measures, Danish
25 researchers will also examine the levels of pro- and anti-inflammatory cytokines. If our
26 respective findings strongly differ in efficacy or safety, we might analyse which factors can
27 account for the divergence and plan a multi-country confirmatory trial with an agreed design
28 and methodology. As pointed out by Kim and Fishman⁷⁰, a common problem with a generic,
29 compounded medication is the lack of commercial support for research. To begin studies such
30 as INNOVA or FINAL, it is crucial to have the synergistic support from public funding bodies,
31 private entities, and commercial companies. This has been the case in the present study, with
32 different public and private organizations providing economic and logistic support.

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52 The inclusion of brain and blood immune-inflammatory biomarkers will allow us to
53 determine whether LDN modulates neuro-inflammatory processes involving inflammatory
54 cells such as glial cells. These markers will also allow us to explore the “hormetic” effects of
55 the drug, that is, if a low dose of an antagonist (naltrexone) may paradoxically act as an agonist
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3 of the endogenous opiate system. As explained above, it is posited that LDN mainly acts as an
4 immunomodulatory drug via blockade of TLR-4, which provides a therapeutic pathway to
5 reduce activation of the inflammatory cascade and the nociceptive system.⁷¹
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10 Obtaining empirical evidence for cost-utility of treatments or interventions is required
11 by the Ministry of Health in Spain for reimbursement. In Spain, a threshold of €22,000–25,000
12 per QALY gained is found to be consistent with decisions of adopting new technologies by the
13 National Health Service.⁷² To our knowledge, there is an absence of economic evaluations for
14 LDN; therefore, an important feature of the present study is the cost-utility assessment of the
15 drug.
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24 FMS remains a chronic, debilitating, and difficult to manage condition for many
25 individuals around the world. After three decades of intensive research, the clinical benefits of
26 pharmacological treatments remain unclear and limited. This study will evaluate the analgesic
27 efficacy, safety, and cost-utility of LDN using a rigorous and powered design. If efficacious
28 and cost-effective, LDN might be the first drug approved for FMS in Europe.
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35 **Trial status**

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38 This study is currently in the recruitment phase. The first patient will be enrolled in
39 October 2021, and the study is expected to end in June 2024.
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42 **Confidentially**

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45 Personal data will be stored in accordance with the Spanish regulation guidelines for
46 clinical research. Participants will be allocated a unique identification (ID) number at entry.
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48 The master list linking participant personal information and ID number will be maintained in
49 a password-protected hard drive at the PSSJD. Data will be stored for 10 years after study
50 completion.
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56 **Contributors**

JVL, ARS, and AFS conceived the study and revised the manuscript. JVL drafted the manuscript. ACC, JPSM, HHN, XB, CSR, AGP, JM, JM, FDA, MM, JWY, AFS and ARS provided feedback on the manuscript, and all authors reviewed and approved the final version of the manuscript.

Funding

This study has been funded by the Institute of Health Carlos III (ISCIII; ICI20/00080; CPII19/00003) and has been co-financed with European Union ERDF funds. JPS-M has a PFIS predoctoral contract from the ISCIII (FI20/00034). AC-C has a FI predoctoral contract from AGAUR (FI_B/00216). AF-S and JVL acknowledge the funding from the Serra Húnter program (UAB-LE-8015 and UAB-LE-120014, respectively). The ISCIII did not have any role in the analysis and interpretation of data, in the writing of the manuscript, or in the decision to submit the paper for publication.

Competing interests

None declared.

Patient consent for publication

Not required.

Provenance and peer review

Not commissioned; externally peer reviewed.

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Table 1. Time points for data collection.

Measures	T0 (baseline)	T1 (3-m)	T2 (6-m)	T3 (12-m)
<i>Sociodemographic, clinical, and screening measures</i>				
Sociodemographic data	X			
Clinical data (years of evolution, comorbidities, etc.)	X			
FSDC screening and secondary outcome measure (fibromyalginess)	X	X	X	X
<i>Primary outcome measure</i>				
NRS (pain intensity)	X	X	X	X
<i>Secondary outcome measures</i>				
FIQR (functional impairment)	X	X	X	X
DASS-21 (anxiety, depression, and stress)	X	X	X	X
MISCI (subjective cognitive impairment)	X	X	X	X
WHODAS 2.0 (disability)	X	X	X	X
GAD-7 (general anxiety / worry)	X	X	X	X
<i>Other measures</i>				
EQ-5D-5L (quality of life)	X			X
CSRI (medication consumption and service receipt)	X			X
PGIC and PSIC (impression of change)		X	X	X
ACTTION checklist (adverse events throughout the trial)	X	X	X	X
Pain Monitor® app (EMA)	X	X		
Physiological variables				
Immune-inflammatory markers	X	X		
Neuroimaging	X	X		

ACTTION checklist: Analgesic, Anesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks; CSRI: Client Service Receipt Inventory; DASS-21: Depression Anxiety Stress Scales-21; EQ-5D-5L: EuroQoL; EMA: Ecological Momentary Assessment; FIQR: Fibromyalgia Impact Questionnaire Revised; FSDC: Fibromyalgia Survey Diagnostic Criteria; GAD-7: Generalized Anxiety Disorder 7-item scale; NRS: Numerical Pain Rating Scale; PGIC and PSIC= Patient Global Impression of Change and Pain Specific Impression of Change; WHODAS 2.0: 12-item interviewer administered version of the World Health Organization Disability Assessment Schedule 2.0

Table 2. List of items administered via Pain Monitor® app.

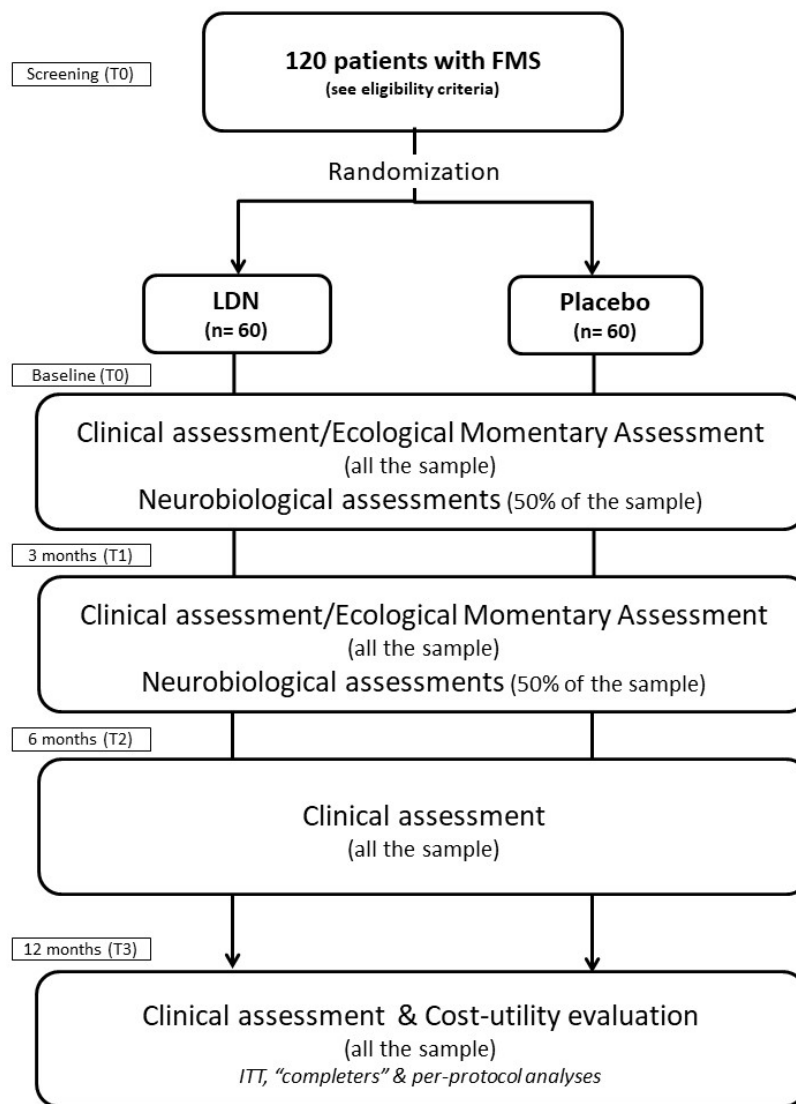
Items	Morning	Evening
Pain intensity	X	X
Fatigue	X	X
Perceived control over pain	X	X
Depression	X	X
Anxiety	X	X
Stress	X	X
Sleep disturbance	X	
Activity level		X
Interference with leisure activities		X
Interference with work-related activities		X
Adverse effects		X
Rescue medications		X

The Pain Monitor app automatically informs patients when to respond (by default, at 11 AM and 7 PM) using a push notification system, but patients can respond with a margin of 2 hours from given times. Collected data are stored on a secure server at the Jaume I University, Spain.

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Figure 1. Flowchart of the INNOVA study based on the CONSORT guidelines.

For peer review only



190x275mm (96 x 96 DPI)



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 N°	Description	Pages
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration	2b	All items from the World Health Organization Trial Registration Data Set	n.a
	3	Date and version identifier	n.a
Protocol version	3	Date and version identifier	n.a
Funding	4	Sources and types of financial, material, and other support	23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,23
	5b	Name and contact information for the trial sponsor	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	23
	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)	11
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	5-8
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	8

1 2 3 4 5 6 7	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)	8
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Methods: Participants, interventions, and outcomes

8 9 10 11 12 13	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	10
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14 15 16 17 18	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)	9
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19 20 21	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11
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22 23 24 25	Interventions	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)	12
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26 27 28 29	Interventions	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
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30 31 32 33	Interventions	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
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34 35 36 37 38 39 40 41	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	12-18 Table 1 Table 2
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42 43 44 45 46	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)	Fig. 1
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47 48 49 50	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	9
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51 52 53	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10
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Methods: Assignment of interventions (for controlled trials)

Allocation:

1				
2			Method of generating the allocation sequence (eg, computer-	
3			generated random numbers), and list of any factors for	
4	Sequence		stratification. To reduce predictability of a random sequence,	
5	generation	16a	details of any planned restriction (eg, blocking) should be	10
6			provided in a separate document that is unavailable to those	
7			who enrol participants or assign interventions	
8				
9				
10	Allocation		Mechanism of implementing the allocation sequence (eg, central	
11	concealment		telephone; sequentially numbered, opaque, sealed envelopes),	
12	mechanism	16b	describing any steps to conceal the sequence until interventions	10
13			are assigned	
14				
15	Implementation	16c	Who will generate the allocation sequence, who will enrol	10
16			participants, and who will assign participants to interventions	
17				
18				
19			Who will be blinded after assignment to interventions (eg, trial	
20		17a	participants, care providers, outcome assessors, data analysts),	10-11
21			and how	
22	Blinding (masking)			
23			If blinded, circumstances under which unblinding is permissible,	
24		17b	and procedure for revealing a participant's allocated intervention	11
25			during the trial	
26				
27				
28	Methods: Data collection, management, and analysis			
29				
30			Plans for assessment and collection of outcome, baseline, and	
31			other trial data, including any related processes to promote data	
32			quality (eg, duplicate measurements, training of assessors) and	
33		18a	a description of study instruments (eg, questionnaires,	12-18
34			laboratory tests) along with their reliability and validity, if known.	
35	Data collection		Reference to where data collection forms can be found, if not in	
36	methods		the protocol	
37				
38				
39			Plans to promote participant retention and complete follow-up,	
40		18b	including list of any outcome data to be collected for participants	10
41			who discontinue or deviate from intervention protocols	
42				
43				
44			Plans for data entry, coding, security, and storage, including any	
45			related processes to promote data quality (eg, double data entry;	
46	Data management	19	range checks for data values). Reference to where details of	11
47			data management procedures can be found, if not in the	
48			protocol	
49				
50				
51			Statistical methods for analysing primary and secondary	
52		20a	outcomes. Reference to where other details of the statistical	18-19
53	Statistical		analysis plan can be found, if not in the protocol	
54	methods			
55				
56		20b	Methods for any additional analyses (eg, subgroup and adjusted	19-20
57			analyses)	
58				
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60				

		Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
		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	
	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	18
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	14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	11
		Ethics and dissemination	
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21
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)	21
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n.a
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	23
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
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	21

1				
2	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care, and for	n.a
3	trial care		compensation to those who suffer harm from trial participation	
4				
5			Plans for investigators and sponsor to communicate trial results	
6			to participants, healthcare professionals, the public, and other	
7		31a	relevant groups (eg, via publication, reporting in results	21
8			databases, or other data sharing arrangements), including any	
9			publication restrictions	
10	Dissemination			
11	policy			
12		31b	Authorship eligibility guidelines and any intended use of	24
13			professional writers	
14				
15		31c	Plans, if any, for granting public access to the full protocol,	8
16			participant-level dataset, and statistical code	
17				
18				
19	Appendices			
20				
21	Informed consent	32	Model consent form and other related documentation given to	Attached
22	materials		participants and authorised surrogates	
23				
24			Plans for collection, laboratory evaluation, and storage of	
25	Biological	33	biological specimens for genetic or molecular analysis in the	17
26	specimens		current trial and for future use in ancillary studies, if applicable	
27				

*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

BMJ Open

Study protocol for a randomised, double-blinded, placebo-controlled phase III trial examining the add-on efficacy, cost-utility and neurobiological effects of low-dose naltrexone (LDN) in patients with fibromyalgia (INNOVA study)

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-055351.R1
Article Type:	Protocol
Date Submitted by the Author:	27-Oct-2021
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Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Health economics, Pharmacology and therapeutics, Public health
Keywords:	PAIN MANAGEMENT, HEALTH ECONOMICS, Clinical trials <

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Manuscripts

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3 **Study protocol for a randomised, double-blinded, placebo-controlled phase III trial**
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5 **examining the add-on efficacy, cost-utility and neurobiological effects of low-dose**
6
7 **naltrexone (LDN) in patients with fibromyalgia (INNOVA study)**
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ABSTRACT

Introduction. There is evidence that low-dose naltrexone (LDN; < 5.0 mg/day) reduces pain and improves the quality of life of people with fibromyalgia syndrome (FMS). However, no randomised controlled trials with long-term follow-ups have been carried out. The INNOVA study will evaluate the add-on efficacy, safety, cost-utility, and neurobiological effects of LDN for reducing pain in FMS patients, with a one-year follow-up.

Methods and analysis. A single-site, prospective, randomised, double-blinded, placebo-controlled, parallel design phase III trial will be performed. Eligibility criteria include being adult, having a diagnosis of FMS and experiencing pain of 4 or higher on a 10-point numerical rating scale. Participants will be randomised to a LDN intervention group (4.5 mg/day) or to a placebo control group. Clinical assessments will be performed at baseline (T0), 3-months (T1), 6-months (T2), and 12-months (T3). The primary endpoint will be pain intensity. A sample size of 60 patients per study arm (120 in total), as calculated prior to recruitment for sufficient power, will be monitored between January 2022 and August 2024. Assessment will also include daily ecological momentary evaluations of FMS-related symptoms (e.g., pain intensity, fatigue, and sleep disturbance), and side effects via ecological momentary assessment (EMA) through the Pain Monitor app® during the first three months. Costs and quality-adjusted life years will be also calculated. Half of the participants in each arm will be scanned with magnetic resonance imaging (MRI) at T0 and T1 for changes in brain metabolites related to neuroinflammation and central sensitization. Inflammatory biomarkers in serum will also be measured.

Ethics and dissemination. This study has been approved by the Ethics Committee of the Fundació Sant Joan de Déu. The results will be actively disseminated through peer-reviewed journals, conference presentations, social media, and community engagement activities.

Trial registration number: ClinicalTrials.gov (NCT04739995)

Strengths and limitations of this study

- This is thought to be the first randomised, double-blinded, placebo-controlled phase III trial to assess the efficacy, safety, cost-utility, and neurobiological effects of low-dose naltrexone (LDN) for reducing pain in patients with fibromyalgia syndrome.
- The INNOVA protocol combines mobile-technology-based ecological momentary assessment and assessment with classical legacy measures to obtain more precise information on the dynamics of the assessed primary and secondary outcomes.
- This study will include immune and neuroimaging biomarkers in order to explore the neurobiological underpinnings of LDN.
- Challenges of the RCT include the long follow-up period (one year) and potential measurement burden that takes the risk of high dropout rate.

INTRODUCTION

Fibromyalgia: definition, prevalence, and pharmaceutical indications

Fibromyalgia syndrome (FMS) is a chronic condition of unknown origin that is characterised by generalised musculoskeletal pain, fatigue, stiffness, cognitive problems, sleep disturbances, and malaise.^{1,2} This syndrome is highly prevalent in the general population (2.7% worldwide).³ Around 6% of adult patients who visit their general practitioner, and between 10-20% of those who visit rheumatology services, have FMS.² In 2007, the U.S. Food and Drug Administration approved pregabalin as the first drug indicated for the treatment of FMS, and later approved duloxetine and milnacipran for this indication. However, the European regulatory authorities rejected the indication of these three drugs in the treatment of FMS given the small effect sizes in various studies and the associated adverse effects.⁴⁻⁶

Pathogenesis of FMS

Although the etiological factors of FMS are not known, the primary hypothesis of the pathogenesis of this syndrome highlights the role of the central nervous system in the amplification of pain perception as well as in the development of comorbid symptoms such as sleep-related problems, fatigue, cognitive difficulties, and emotional distress.^{1,7,8} Structural brain alterations have also been found in patients with FMS. For example, lower volumes of grey matter have been observed in areas associated with the processing of stress (e.g., parahippocampal gyrus) and pain (e.g., anterior cingulate cortex, insula, prefrontal cortex, and primary and secondary somatosensory cortices).⁷ Functional MRI alterations been associated with self-reported pain intensity. Brain activity exhibited greater connectivity between different pain-processing areas (e.g., insula and secondary somatosensory cortex), the default-model network in persons with FMS, as well as in the association between these areas and the pain levels reported by patients and the right executive attention network.⁹

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3 Altered functional connectivity has also been reported among various pain-inhibiting
4 areas.¹⁰ Some studies have reported reduced levels of neurotransmitters involved in the
5 regulation of the descending analgesic response (serotonin and noradrenaline) and increased
6 levels of glutamate (Glu) and substance P in people with FMS.¹¹⁻¹⁴ For example, high levels of
7 Glu have been reported in the posterior insula, posterior cingulate cortex and prefrontal
8 ventrolateral cortex of patients with FMS when compared to healthy controls¹⁵⁻¹⁹.

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18 Activation of the microglia could be a contributing factor to the alteration of glutamate
19 neurotransmission in FMS.^{20,21} The microglia is normally found in a state of rest but it is
20 activated by a wide range of stimuli such as cell death, peripheral inflammation, chronic stress
21 and infections.²² Once activated, microglia release pro-inflammatory agents such as cytokines,
22 excitatory amino acids and nitric oxide.²³ These inflammatory factors across multiple neural
23 pathways can induce hyperalgesia, fatigue, depression and other symptoms which are known
24 collectively as “cytokine induced sickness behavior”.^{24,25} Microglia activation might trigger a
25 series of actions that lead to an increase in Glu that ultimately results in synaptic dysfunction.²⁶
26 A recent study using Positron Emission Tomography²⁷ of translocator protein revealed a
27 widespread cortical glial activation in patients with FMS, which gives support to the role of
28 neuroinflammation in the aetiology of FMS. In addition, there is evidence that chronic stress
29 facilitates the “priming” and exaggerated activation of the microglia.²⁸

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36 Although FMS is not considered a classic inflammatory disease, there is extensive
37 evidence that immune pathways play a significant role in the pathogenesis and maintenance of
38 the syndrome. Cytokines play a key role in inflammatory response and in boosting the
39 nociceptive response due to their sensitization actions, both on a peripheral and central level.²⁹⁻
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Although FMS is not considered a classic inflammatory disease, there is extensive evidence that immune pathways play a significant role in the pathogenesis and maintenance of the syndrome. Cytokines play a key role in inflammatory response and in boosting the nociceptive response due to their sensitization actions, both on a peripheral and central level.²⁹⁻
³² Thus, there is evidence that FMS involves an imbalance in pro-inflammatory (e.g. IL-1, IL-6, IL-17A, and TNF- α) and anti-inflammatory (e.g. IL-4 and IL-10) levels of cytokines that could lead to a low-intensity, chronic state of inflammation. Bäckryd and colleagues³⁰

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3 identified both neuroinflammation and systemic inflammation by evaluating levels of a broad
4 panel of cytokines and chemokines in cerebrospinal fluid and plasma.
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8 **Low-dose naltrexone (LDN): A promising treatment for FMS**

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11 Naltrexone is an opioid antagonist medication used to treat opioid and alcohol
12 dependency. The drug blocks mu-opioid receptors, the delta-opioid receptors and, to a lesser
13 extent, the kappa-opioid receptors. There is promising evidence to suggest that naltrexone
14 administered in low doses (i.e., low-dose naltrexone; < 5 mg/day) is effective in the
15 management of some pathologies which present with altered immune pathways, such as
16 Crohn's disease, multiple sclerosis, or FMS.^{33,34} The immune-regulatory effect of LDN seems
17 to be driven through the inhibition of the Toll-like receptor 4 (TLR-4) activity expressed in the
18 membrane of various immune system cells (e.g., microglia and macrophages).³³ Moreover, due
19 to a “rebound effect”, LDN could exert an analgesic effect that strengthens the endogenous
20 opioid pain inhibitory system. According to this hypothesis, the low-intensity and intermittent
21 blockade of the opioid receptors generated by LDN induces a compensatory mechanism that
22 facilitates an increase in the production of endogenous opioids and greater sensitivity of the
23 system to their effects.^{33,34}
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41 To date, the effects of LDN in patients with FMS have only been evaluated through
42 crossover pilot studies that have yielded preliminary results. In the first study conducted with
43 LDN in FMS, significant reductions in pain, stress, and fatigue levels were observed.³⁵ In a
44 subsequent study, significant improvements in daily pain, satisfaction with life and mood were
45 also observed.³⁶ In another crossover investigation, the pre and post changes in the levels of
46 plasma cytokines were evaluated over eight weeks. Significant reductions in a wide range of
47 immune biomarkers were obtained (e.g., IL-1 β , sIL-1ra, IL-4, IL-6, IL-10, IL-17A, and TNF-
48 α), together with a reduction in the pain levels and the severity of FMS symptoms.³⁷
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3 While acknowledging the contribution of past studies into the field, these have included
4 small sample sizes (n= 8 to 31 participants) and crossover designs. Therefore, a single-site,
5 prospective, randomised, double-blinded, placebo-controlled study (RCT) with a sufficiently
6 powered sample is presented here to conduct a methodologically robust investigation into the
7 role of LDN in FMS. Specifically, the main objective of the INNOVA study is to evaluate the
8 efficacy, safety, and cost-utility, and neurobiological effects of LDN to reduce pain in FMS.
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10 There is currently no gold standard pharmacological treatment for pain reduction in persons
11 with FMS. Therefore, in the present study, placebo will be used instead of another drug in the
12 control group.
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24 **METHODS AND ANALYSIS**

25 **Trial design**

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27 The randomised controlled trial (RCT) protocol has been developed following the
28 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)³⁸. In addition,
29 the RCT was approved by European Union Drug Regulating Authorities Clinical Trials
30 (EudraCT; 2021-002534-16). For reporting purposes, we will follow the guidelines of the
31 Consolidated Standards of Reporting Trials (CONSORT)³⁹ and the Consolidated Health
32 Economic Evaluation Reporting Standards (CHEERS) statement.⁴⁰ INNOVA is a 12-month
33 double-blind RCT with two arms: LDN vs. Placebo. LDN will be considered an add-on
34 treatment to the usual care provided in the Spanish National Health System for FMS. For
35 transparency and analytical reproducibility purposes, the dataset and data coding will be
36 deposited in the Open Science Framework.
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52 **Sample size**

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54 There are no previous RCTs about the efficacy of LDN for FMS, therefore, we
55 estimated the sample size taking into account a previous LDN crossover study³⁶ that had used
56 self-reported pain as main outcome (the effect size was $d = .99$). Thus, with a sample of 60
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3 participants per arm, we aim to detect between-group differences with a significance level of
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5 5% and a power of 80%. Allowing for a potential attrition rate of 20%, our final sample size is
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7 60 participants per group. For the analysis of biomarkers (involving 50% of the sample), an
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9 initial sample size of 30 patients per arm is considered sufficient according to previous
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11 studies.^{37,41}

14 Eligibility criteria

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17 *General selection criteria.* All participants will meet the following inclusion criteria:
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19 women between 18-70 years; diagnoses of FMS according to American College of
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21 Rheumatology 2016 criteria⁴² by a rheumatologist; pain intensity ranked ≥ 4 out of 10 on a 10-
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23 point numerical rating scale in the past week; fluent in Spanish; provision of written informed
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25 consent; stable pharmacological treatment in the last two months; and having a smartphone
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27 with android operating system for Ecological Momentary Assessment (EMA). Potential
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29 participants will be excluded according to the following exclusion criteria: treatment with
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31 naltrexone, opioids, anticoagulants, or central anti-hypertensives in the last 3 months; diagnosis
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33 of severe medical/psychiatric disorders (e.g., cancer, haematological diseases, abnormal
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35 hepatic/liver function, renal failure, suicide ideation, psychotic disorder); pregnant (or planning
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37 to become pregnant during the study period) or breastfeeding; known allergy to naltrexone,
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39 naloxone or excipients; currently participating in other RCTs; ongoing litigation related to
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41 FMS.
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48 *Additional selection criteria for the biomarkers and neuroimaging sub-study (50% of*
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50 *patients in each study arm).* All participants will meet the following inclusion criteria:
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52 right-handed (for the neuroimaging tests); and no comorbid rheumatologic conditions (e.g.,
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54 rheumatoid arthritis, lupus). Potential participants will be excluded according to the following
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56 exclusion criteria: fever ($> 38^{\circ}\text{C}$); infection in the last two weeks; vaccination in the last month;
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58 taking cortisone or anti-cytokine therapy; needle phobia or claustrophobia, metal implants or
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3 pacemakers; body mass index ≥ 36 kg/m²; smoking over 5 cigarettes/day; presence of acute
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5 pain (e.g, headache or back pain) unrelated to FMS on the day of the scan.
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8 **Recruitment strategy, procedure and randomisation**

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10 Patients diagnosed with FMS with an appointment at the Rheumatology Service of Parc
11 Sanitari Sant Joan de Déu (St. Boi de Llobregat, Spain) will be invited to participate in the
12 study and will be asked to attend a screening evaluation with a research assistant and a clinician.
13
14 Once the informed consent is obtained, the clinician will review the study selection criteria to
15 confirm eligibility. The week after, a face-to-face assessment (T0) including clinical history
16 and variables related to the use of services will be conducted with those patients meeting all
17 the eligibility criteria. Only the participants that are included in the biomarkers sub-study will
18 require an additional blood extraction and neuroimaging scan, which will be performed in the
19 following 3-5 days. Participants will be given a sealed envelope with an identifying code which
20 they will have to take to the pharmacy service. There, they will be given the dose of the
21 corresponding drug (according to the randomisation) for the first 3 months (90 tablets). As
22 shown in the patients' flow chart (Figure 1), further in-person evaluations will be performed at
23 3 months (T1), 6 months (T2), and 12 months (T3). Neurobiological variables will be obtained
24 at T1 using the same protocol as in the baseline assessment (T0).
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42 Participants will be asked to abstain from taking any analgesic or anti-inflammatory
43 drug in the 72h prior to the blood extractions/scans. All patients, including those who do not
44 participate in the biomarker sub-study, shall be subjected to a blood test at baseline. Participants
45 will return to the pharmacy service every 3 months and will be given the assigned amount of
46 LDN/placebo for the following 3 months (approximately 90 tablets). Unconsumed tablets will
47 be returned for treatment adherence monitoring. The randomisation to conditions will be
48 conducted by a biostatistician from the Clinical Trials Unit of Fundació Sant Joan de Déu who
49 has no involvement in the eligibility screening, enrolment, and treatment processes. The
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3 computer-generated randomisation will apply a permuted block design to ensure that the study
4 arms are balanced taking the biomarkers sub-study eligibility criteria into account. As this is a
5 double-blind RCT, neither the patient nor the evaluator or the clinician will know to which
6 treatment arm each patient has been assigned. Only the clinical trial pharmacist who stores and
7 delivers capsules, but is not involved in patient care, will know the allocation.
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15 Figure 1

16 17 **Data management, central monitoring and audit**

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19 The clinical data entry, data management, and central monitoring will be performed
20 with REDCap®. An independent Clinical Research Organization (CRO) will be responsible
21 for overseeing the intra-study data sharing and storing process. Any modifications in the study
22 protocol will be communicated to the CRO.
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28 29 **Treatments**

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31 **Low-dose naltrexone (LDN).** The intervention group will take one 4.5 mg naltrexone
32 tablet (lactose-free) daily for 12 months before going to sleep.
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36 **Placebo.** The control group will take the placebo daily for 12 months (a film-coated
37 tablet identical to the LDN and filled with a lactose-free filler). For the control arm, the same
38 guidelines will be followed.
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43 In order to maintain the external validity of the study and for ethical reasons, the study
44 participants' active treatments will be unchanged by this clinical trial. In Spain, chronic pain
45 management is mainly managed by general practitioners in regular consultations. These
46 generally consist of face-to-face appointments with a duration of 5-10 minutes in which the
47 clinicians monitor the physical and, ideally, the emotional status of the patient. General
48 practitioners usually provide advice prescribe pharmacotherapy (pain killers, hypnotics,
49 antidepressants, etc.) or refer patients to pain units in tertiary hospitals when more specialised
50 pain management procedures are required. The frequency of consultations is based on the type
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3 of disease and its severity. In this study, usual care will be the same as in routine daily practice,
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5 without any modifications. In addition, participants will be allowed to withdraw from this study
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7 for any reason at any time without detriment to the provision or quality of their usual care. If a
8
9 severe adverse event occurs, unblinding will be possible and study participation will be
10
11 discontinued. If the adverse effects are tolerable, the treatment will be administered until the
12
13 end of the study. All these events will be recorded and reported at the end of the study.
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16 17 **Study measures**

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19 All participants will be assessed with a computer-administered battery of measures
20
21 using the REDCap® software (see Table 1).
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24
25 Table 1

26 27 **Measures for sociodemographic characteristics, clinical features, and screening**

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29 A *sociodemographic questionnaire* will be used to obtain information about the
30
31 following variables: gender, date of birth, marital status, living arrangements, educational level,
32
33 income level, and employment status.
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37 The *Clinical data* interview will be used to collect information about history and
38
39 duration of FMS symptoms, as well as family history of medical/mental illness. Information
40
41 regarding comorbidity with other diagnosed physical-psychiatric conditions and the type and
42
43 dose of current drugs will be checked from medical records.
44

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46 The *Fibromyalgia Survey Diagnostic Criteria* (FSDC)^{43,44} is a 6-item self-report
47
48 measure of the core FMS symptoms according to the latest revision of the American College
49
50 of Rheumatology (ACR) 2016 criteria.⁴² It includes two subscales: the Widespread Pain Index,
51
52 which is used to identify the presence of pain in 19 body areas in the last week, and the
53
54 Symptom Severity Scale, in which the three major FMS symptoms (fatigue, “fibrofog” and
55
56 waking up tired) are assessed along with three additional symptoms (pain in the lower stomach,
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3 depression and headache). A total score is obtained by adding the two subscales. This total
4
5 score ranges from 0 to 31, where higher values indicate greater FMS severity.
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8 **Primary outcome measure**

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10 The *Numeric Rating Scale* (NRS)⁴⁵ is a unidimensional measure of pain intensity
11
12 mainly used for adults. The most frequently used version is an 11-point numeric scale (a
13
14 horizontal bar or line) scored from 0 (“no pain”) to 10 (“worst pain imaginable”). Time frames
15
16 vary between studies. In the present study, respondents will be asked to report average pain
17
18 intensity over the last week.
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22 **Secondary outcome measures**

23

24 The *Revised Fibromyalgia Impact Questionnaire* (FIQR)⁴⁶ includes 21 items that are
25
26 answered on a 0 to 10 numerical scale in which higher scores indicate greater functional
27
28 impairment. The questionnaire asks about the previous seven days. The items are distributed
29
30 into three domains: physical impairment, overall impact, and severity of symptoms (i.e., pain,
31
32 energy, stiffness, sleep quality, depression, memory issues, anxiety, pain to the touch, balance
33
34 problems and increased sensitivity to noises, lights, smells, or temperatures). A total score is
35
36 obtained by summing the three subscale scores. This can range from 0 to 100. Higher scores
37
38 indicate greater impairment. The Spanish version of the FIQR and has obtained high internal
39
40 consistency estimates ($\alpha = .91 - .95$), adequate test-retest reliability indices ($r = .82$), and good
41
42 construct validity.⁴⁷
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47 The *Depression Anxiety Stress Scales-21* (DASS-21)⁴⁸ is a self-report scale developed
48
49 to discriminate between features of depression (anhedonia/low positive affect), anxiety
50
51 (physical arousal) and stress (psychological tension/agitation) in clinical and non-clinical
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53 samples. The DASS has been validated in patients with FMS.⁴⁹ Responders are required to
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55 indicate the presence of a symptom over the previous week. Each item is scored from 0 (“did
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57 not apply to me at all over the last week”) to 3 (“applied to me very much or most of the time
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3 over the past week”). There are seven items on each of the three subscales: depression, anxiety,
4 and stress. Therefore, total scores in each scale can range from 0 to 21. Higher scores indicate
5 more severe levels of depression, anxiety and stress. The Spanish version showed adequate
6 internal consistency for depression ($\alpha = .84$), anxiety ($\alpha = .70$) and stress ($\alpha = .82$).⁵⁰
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12 The *Multidimensional Inventory of Subjective Cognitive Impairment* (MISCI)⁵¹ is a
13 10-item self-report measure of subjective cognitive dysfunction (i.e., “fibrofog”) in FMS.
14 Each item is scored from 1 (“never”) to 5 (“very often”) and the total score ranges from 10 to
15 50. Lower scores indicate higher cognitive dysfunction. The MISCI showed excellent internal
16 reliability, low ceiling/floor effects and good convergent validity with a similar measure. The
17 Spanish version of the MISCI had sound psychometric properties ($\alpha = .91$ and ICC = .88).⁵²
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26 The *World Health Organization Disability Assessment Schedule 2.0* (WHODAS
27 2.0)⁵³ is a 12-item self-report measure of the level of difficulty experienced taking into
28 consideration how they usually do the activity. This includes the use of any devices to assist
29 them and/or the help of a person. In each item, individuals estimate the magnitude of the
30 disability during the previous 30 days using a five-point scale scored from 1 (none) to 5
31 (extreme/cannot do). The total score ranges from 0 to 100. Higher scores reflect greater
32 disability. The 12-item WHODAS 2.0 has sound psychometric properties in patients with
33 FMS.⁵⁴
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45 The *Generalized Anxiety Disorder 7-item scale* (GAD-7)⁵⁵ is a 7-item self-report
46 measure of pathological worry. Each item is scored from 0 (“not at all”) to 3 (“nearly every
47 day”). The total score ranges from 0 to 21, where higher scores reflecting greater anxiety
48 symptoms. The GAD-7 has sound psychometric properties ($\alpha = .92$ and ICC = .83) in patients
49 with FMS in previous studies.⁵⁶
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56 **Other measures**

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3 The *ACTION AE* is a *reporting checklist* used to measure safety and benefit-risk of
4 a clinical trial.⁵⁷ The Safety and Benefit-Risk Reporting and Evaluation (SABRRE) Working
5 Group of the Analgesic, Anaesthetic, and Addiction Clinical Trial Translations, Innovations,
6 Opportunities, and Networks (ACTION; <http://www.action.org>) public-private partnership
7 with the FDA developed an adverse events (AE) reporting checklist that will be used in the
8 present study.
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12 The *EuroQoL* (version EQ-5D-5L)⁵⁸ is a health-related quality of life questionnaire
13 that consists of two parts. In the first one, the individual's difficulties concerning mobility,
14 self-care, pain/discomfort and anxiety/depression are evaluated. In the second part, the
15 perceived health is assessed by means of a Visual Analogue Scale ranging from 0 to 100. The
16 EQ-5D-5L scores will be used to calculate the Quality-Adjusted Life Years (QALYs) during
17 the follow-up period by adjusting the duration of time affected by the health outcome by the
18 value of the utility.
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33 The *Client Service Receipt Inventory* (CSRI)⁵⁹ is a self-report tool used to collect
34 retrospective data on medication consumption and service receipt. Patients are asked to bring
35 their daily medication prescriptions and information about pain-related drugs (analgesics,
36 anti-inflammatories, opioids, muscle relaxants, antidepressants, etc.) is recorded. This
37 includes the name of the drug, the dosage, total number of prescription days and daily dosage
38 consumed. Concerning service receipt, patients are asked about the total appointments for
39 accident and emergency services, total number of general inpatient hospital admissions,
40 number of diagnostic tests administered and total appointments with healthcare professionals
41 for pain management (family physicians, nurses, social workers, psychologists, psychiatrists,
42 group psychotherapy and other community healthcare professionals). The CSRI will be
43 administered on two occasions: at baseline and at 12-month follow-up, both referring to the
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3 previous 12 months. Medical records will be checked to verify the accuracy of the collected
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5 data.
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8 The *Patient Global Impression of Change* (PGIC) measures meaningful change in
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10 overall status and the *Pain Specific Impression of Change* (PSIC)⁶⁰ measures the perception
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12 of pain improvement. The most frequently used scale is a 7-point numerical scale scored from
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14 1= “much better”) to 7= “much worse”).
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16

17 **Ecological momentary assessment (EMA)**

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19 Pain intensity and other pain-related variables (e.g., depressive-anxious symptoms and
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21 activity level) can fluctuate during the day and across days depending on personal and
22
23 environmental factors. Collecting self-reported data prospectively and closer in time to its
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25 occurrence substantially improves the accuracy, reliability and quality of data. EMA has been
26
27 successfully performed in patients with a variety of physical and mental problems.^{61,62} There
28
29 is growing evidence indicating that well-designed smartphone apps can be easy to use and well-
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31 tolerated even in relatively old pain populations, with compliance rates as high as 85%.⁶¹ In
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33 this RCT, we will use the Pain Monitor® (Monitor de Dolor, by its Spanish name) app⁶³ to
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35 assess a wide range of variables (see items in Table 2) twice a day (once in the morning and
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37 once in the evening, at convenient times along the week) during 120 days. The app and the data
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39 will be stored on different servers with different domain names and connected locally only (the
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41 server containing the data does not have Internet access). According to a recent meta-analysis,⁶⁴
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43 EMA-completion rates are higher among elderly patients compared to younger patients.
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45 Considering that the majority of FMS patients in our study are not expected to be young and
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47 that the EMA item battery does not require a long response time (< 1 min.), it is expected not
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49 to place an excessive burden on participants.
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56 Table 2
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Inflammatory biomarkers

After obtaining the blood sample, it will be allowed to coagulate for a minimum of 30 minutes at room temperature. It will then be centrifuged for 10 minutes at 1000g. The resulting serum will be stored at -80° C during the same morning of extraction until it is ready to be analysed. All samples (at T0 and T1) will be analysed in a single analytical batch to reduce inter-assay variability (approx. 15%). The serum levels of IL-1 β , sIL-1ra, IL-4, IL-6, sIL-6r, sgp130, CXCL-8, IL-10, IL-17, TNF- α , and high-sensitivity C-reactive protein (hs-CRP) will be evaluated.²⁹ For the quantification of the cytokines, the Milliplex® reagents from the company MerckMillipore® will be used and analysed using a Luminex® platform. The high sensitivity multiplex kit will be used: Human High Sensitivity T Cell, catalogue number: HSTCMAG28SPMX11, adapted to the aforementioned cytokines. The hs-PCR will be quantified using turbidimetry in an Olympus AU5400 auto-analyser. These biomarkers will only be evaluated at baseline (T0) and 3-months (T1) for the following reasons: (a) there is evidence of significant inflammatory changes at 8 weeks with LDN;³⁷ (b) this results in lower risk of dropout (vs. evaluating them at 6 or 12 months); (c) conducting at least two measures allows to use the change between baseline and 3-months as a mediator of long-term clinical changes; and (d) budget constraints.

Neuroimaging

The scans (protocol duration: approximately 30 minutes) will be performed in a Phillips Ingenia 3T MRI scanner with a 32-channel head coil at Hospital Sant Joan de Déu (Esplugues de Llobregat, Spain). To examine cingulate, insular, amygdalar, occipital, angular, parahippocampal, and prefrontal gray matter volume, we will use voxel-based morphometry (VBM). We will also use surface-based morphometry (with FreeSurfer calculation of cortical thickness, surface area, and local gyrification index) for examining cortical abnormalities. Additionally, glutamate, glutamine, myo-inositol, N-acetylaspartate, choline, and creatine

(and creatinine ratios) levels will be analysed using magnetic resonance spectroscopy. Specifically, we will conduct the following processing for the regions of interest according to the corresponding hypotheses. For VBM, we will apply a bias field correction, tissue segmentation with SPM12, normalization with DARTEL, modulation, and smoothing. We will use both unmodulated and modulated grey matter images to convey complementary volumetric information. We will use FreeSurfer ENIGMA pipelines to perform the VBM. In addition, we will quantify metabolites concentrations using LCModel (v6.3-1J). We will only include high-quality spectra, defined as signal-to-noise ratio > 15, Cramer-Rao lower bounds < 15%, and full width at half maximum of metabolites < .07. The spectroscopy analysis will account for the effects of cerebrospinal fluid and grey matter within the voxel, and inter-individual differences in cortical grey matter.

Statistical analysis

The main analysis will compare the effect of LDN vs. placebo on the primary outcome (pain intensity at T1). Data analyses will be performed following an intention-to-treat (ITT) plan. Then, we will compute analysis of the primary outcome (at T2 and T3) and analysis of the secondary outcomes at T1, T2 and T3. Linear mixed models will be created using the restricted maximum likelihood method for the estimation of parameters. The effect sizes will be calculated according to Cohen's *d*. An interim analysis is planned at T1 once 50% of the total sample has been evaluated. A 5% significance level will be used in all two-tailed tests, applying the Benjamini-Hochberg correction for multiple comparisons. Additionally, to make the findings from our study clinically meaningful, the number needed to treat will be reported. For this analysis, we will dichotomise participants into responders or non-responders using two different cut-off criteria in compliance with the IMMPACT recommendations:⁴⁵ At least 50% pain relief over baseline (substantial benefit) or 30% or more pain relief (moderate benefit). For these analyses, we will use SPSS v26 (IBM Corp, Armonk, NY, USA).

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Regarding EMA, a recent recommended approach is "network analysis". There has been burgeoning interest in conceptualizing chronic pain as a network of interacting symptoms and psychobiological processes.⁶⁵ Network analysis will offer us a good chance to quantify and visualize relationships between pain intensity and pain-related variables (e.g., depression, anxiety, fatigue, sleep disturbance). We will estimate temporal networks by means of vector autoregression techniques;⁶⁶ These "temporal networks", would indicate potential causality with one or more variables preceding one or more variables in time. Network analysis will be performed with the free statistical software JASP.⁶⁷

In economic evaluation, it is important to calculate the relationship between the costs of each treatment and its consequences in the form of QALYs, an index measure designed to assess both quantity of life (years) and health-related quality of life. A year lived with the maximum quality of life would be transformed into 1 QALY; a year lived with half the maximum quality of life would be transformed into 1/2 QALY. This relative value is called the incremental cost-utility ratio (ICUR) and it expresses the relationship between the costs and the effects of one option compared to another. The QALYs obtained in the 12 months after the treatment onset will be calculated by the area under the curve. The direct costs will be calculated by adding together the costs derived from the medication and the use of the health services. The cost of medications will be calculated by multiplying the price per milligram by the total daily dose consumed (in milligrams) and the number of days that the treatment is received. The cost arising from the use of the health services (primary care, specialist and accident and emergency consultations, and hospital admissions) will be obtained from the clinical electronic records (<http://www.oblikue.com/en/esalud.html>). The indirect costs will be calculated based on the days off work, which will be multiplied by the official minimum wage during the study period. The effect of the treatments will be estimated using ordinary least squares multivariate regression, adjusting for the baseline differences between groups. In order

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3 to manage uncertainty in the sampling distribution of the ICUR, non-parametric bootstrapping
4 will be applied, with 1000 replications in each comparison. Cost-utility analyses will be
5 conducted with STATA v16.0 (StataCorp, College Station, TX, USA).
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10 **Patient and public involvement**

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12 Patients and the public will not be involved in the design, conduct, reporting, or
13 dissemination of our research.
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17 **ETHICS AND DISSEMINATION**

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19 All procedures performed in this study will be in accordance with the 1964 Helsinki
20 declaration and its last amendments (7th revision, adopted by the 64th World Medical
21 Association General Assembly, Fortaleza, Brazil). Signed informed consent will be obtained
22 from all patients once they have been informed of the study procedures, potential risks, and
23 their right to withdraw at any time from the RCT. The FSJD Ethics Committee Board evaluated
24 and approved the study protocol in June 2021 (PIC-178-19). Only the principal investigators
25 (ARS and JVL) will have full access to the final trial dataset. Modifications in the study
26 protocol will be reported to the FSJD Ethics Committee Board as well as the independent CRO.
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39 Once the RCT is completed, we will publish our results in international peer-reviewed
40 biomedical journals and present them at national and international conferences. Authorship
41 will be assigned in accordance with the International Committee of Medical Journal Editors
42 guidance. In addition, we will send participating patients a short report of our findings. A copy
43 of the report will also be sent to Institute of Health Carlos III (main funding body). The
44 principal investigators will organize an end-of-study seminar. The main objective of this
45 activity will be to share the study findings with stakeholders to discuss how to maximize uptake
46 of the findings in patient treatment and clinical practice, and to determine future research
47 directions.
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DISCUSSION

As far as we know, no RCT has been published about the efficacy, safety, cost-utility, and neurobiological underpinnings of LDN in patients with FMS. This manuscript presents the design and rationale of a randomised, double-blinded, placebo-controlled phase III study, which is a powerful design to assess the efficacy of LDN. We have decided to administer 4.5 mg/day of LDN in this RCT because this dose seems to provide an optimal balance between significant analgesic efficacy and minimal side effects (nausea, sleep disturbance, nightmares, etc.) according to a recent study.⁶⁸

Our findings using this design in conjunction with those that will be obtained in another ongoing RCT that is being carried out in Denmark (The FINAL study)⁶⁹ may facilitate the approval of the first drug indicated for the treatment of FMS in Europe. The FINAL study is an ongoing single-centre, randomised, double-blinded, placebo-controlled trial that is being carried out in Odense (Denmark). A total of 100 women between 18–64 years-old with FMS will take either LDN or placebo for three months. Besides self-report measures, Danish researchers will also examine the levels of pro- and anti-inflammatory cytokines. If our respective findings strongly differ in efficacy or safety, we might analyse which factors can account for the divergence and plan a multi-country confirmatory trial with an agreed design and methodology. As pointed out by Kim and Fishman⁷⁰, a common problem with a generic, compounded medication is the lack of commercial support for research. To begin studies such as INNOVA or FINAL, it is crucial to have the synergistic support from public funding bodies, private entities, and commercial companies. This has been the case in the present study, with different public and private organizations providing economic and logistic support.

The inclusion of brain and blood immune biomarkers will allow us to determine whether LDN modulates neuro-inflammatory processes involving inflammatory cells such as glial cells. These markers will also allow us to explore the “hormetic” effects of the drug, that

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3 is, if a low dose of an antagonist (naltrexone) may paradoxically act as an agonist of the
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5 endogenous opiate system. As explained above, it is posited that LDN mainly acts as an
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7 immunomodulatory drug via blockade of TLR-4, which provides a therapeutic pathway to
8
9 reduce activation of the inflammatory cascade and the nociceptive system.⁷¹ In a recent pilot
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11 study⁷², patients with opioid induced hyperalgesia and patients with FMS were treated with
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13 LDN for 3 months. Via different mechanisms of action, LDN improved pain tolerance
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15 (measured with the cold pressor test) in both groups of patients, being the effect even stronger
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17 in those participants with opioid induced hyperalgesia. According to the authors, the
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19 neuroimmunological component seems to play an important role in the explanation of the
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21 beneficial effects of LDN in the case of FMS.
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26 Obtaining empirical evidence for cost-utility of treatments or interventions is required
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28 by the Ministry of Health in Spain for reimbursement. In Spain, a threshold of €22,000–25,000
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30 per QALY gained is found to be consistent with decisions of adopting new technologies by the
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32 National Health Service.⁷³ To our knowledge, there is an absence of economic evaluations for
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34 LDN; therefore, an important feature of the present study is the cost-utility assessment of the
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36 drug.
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41 FMS remains a chronic, debilitating, and difficult to manage condition for many
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43 individuals around the world. After three decades of intensive research, the clinical benefits of
44
45 pharmacological treatments remain unclear and limited. This study will evaluate the analgesic
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47 efficacy, safety, and cost-utility of LDN using a rigorous and powered design. If efficacious
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49 and cost-effective, LDN might be the first drug approved for FMS in Europe.
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51 **Trial status**

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54 This study is currently in the recruitment phase. The first patient will be enrolled in
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56 January 2022, and the study is expected to end in August 2024.
57

58 **Confidentially**

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3 Personal data will be stored in accordance with the Spanish regulation guidelines for
4 clinical research. Participants will be allocated a unique identification (ID) number at entry.
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6 The master list linking participant personal information and ID number will be maintained in
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8 a password-protected hard drive at the PSSJD. Data will be stored for 10 years after study
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10 completion.
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14 **Contributors**

15
16 JVL, ARS, and AFS conceived the study and revised the manuscript. JVL drafted the
17 manuscript. ACC, JPSM, HHN, XB, CSR, AGP, JM, JM, FDA, MM, JWY, AFS and ARS
18 provided feedback on the manuscript, and all authors reviewed and approved the final version
19 of the manuscript.
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26 **Funding**

27
28 This study has been funded by the Institute of Health Carlos III (ISCIII; ICI20/00080;
29 CPII19/00003) and has been co-financed with European Union ERDF funds. JPS-M has a PFIS
30 predoctoral contract from the ISCIII (FI20/00034). AC-C has a FI predoctoral contract from
31 AGAUR (FI_B/00216). AF-S acknowledges the funding from the Serra Húnter program
32 (UAB-LE-8015). The ISCIII did not have any role in the analysis and interpretation of data, in
33 the writing of the manuscript, or in the decision to submit the paper for publication.
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43 **Competing interests**

44
45 None declared.
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47 **Patient consent for publication**

48
49 Not required.
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51 **Provenance and peer review**

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53 Not commissioned; externally peer reviewed.
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Table 1. Time points for data collection.

Measures	T0 (baseline)	T1 (3-m)	T2 (6-m)	T3 (12-m)
<i>Sociodemographic, clinical, and screening measures</i>				
Sociodemographic data	X			
Clinical data (years of evolution, comorbidities, etc.)	X			
FSDC screening and secondary outcome measure (fibromyalginess)	X	X	X	X
<i>Primary outcome measure</i>				
NRS (pain intensity)	X	X	X	X
<i>Secondary outcome measures</i>				
FIQR (functional impairment)	X	X	X	X
DASS-21 (anxiety, depression, and stress)	X	X	X	X
MISCI (subjective cognitive impairment)	X	X	X	X
WHODAS 2.0 (disability)	X	X	X	X
GAD-7 (general anxiety / worry)	X	X	X	X
<i>Other measures</i>				
EQ-5D-5L (quality of life)	X			X
CSRI (medication consumption and service receipt)	X			X
PGIC and PSIC (impression of change)		X	X	X
ACTTION checklist (adverse events throughout the trial)	X	X	X	X
Pain Monitor® app (EMA)	X	X		
Physiological variables				
Immune biomarkers	X	X		
Neuroimaging	X	X		

ACTTION checklist: Analgesic, Anesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks; CSRI: Client Service Receipt Inventory; DASS-21: Depression Anxiety Stress Scales-21; EQ-5D-5L: EuroQoL; EMA: Ecological Momentary Assessment; FIQR: Fibromyalgia Impact Questionnaire Revised; FSDC: Fibromyalgia Survey Diagnostic Criteria; GAD-7: Generalized Anxiety Disorder 7-item scale; NRS: Numerical Pain Rating Scale; PGIC and PSIC= Patient Global Impression of Change and Pain Specific Impression of Change; WHODAS 2.0: 12-item interviewer administered version of the World Health Organization Disability Assessment Schedule 2.0

Table 2. List of items administered via Pain Monitor® app.

Items	Morning	Evening
Pain intensity	X	X
Fatigue	X	X
Perceived control over pain	X	X
Depression	X	X
Anxiety	X	X
Stress	X	X
Sleep disturbance	X	
Activity level		X
Interference with leisure activities		X
Interference with work-related activities		X
Adverse effects		X
Rescue medications		X

The Pain Monitor app automatically informs patients when to respond (by default, at 11 AM and 7 PM) using a push notification system, but patients can respond with a margin of 2 hours from given times. Collected data are stored on a secure server at the Jaume I University, Spain.

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Figure 1. Flowchart of the INNOVA study based on the CONSORT guidelines.

For peer review only

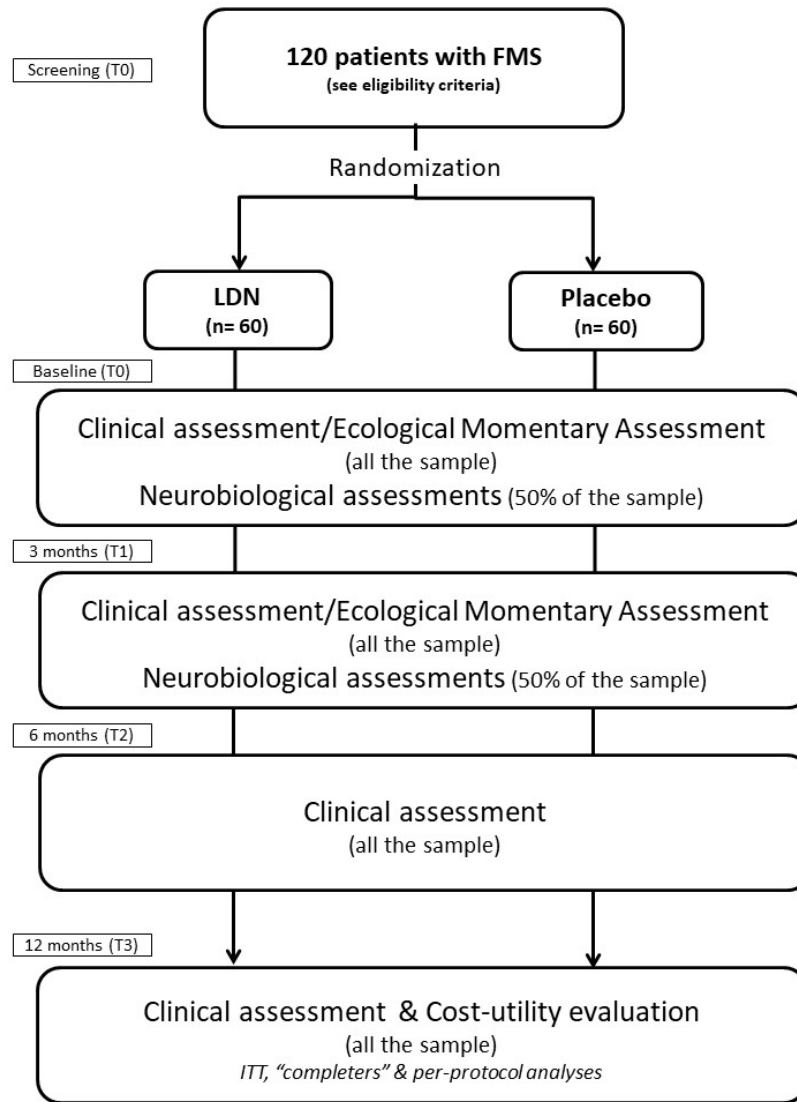


Figure 1. Flowchart of the INNOVA study based on the CONSORT guidelines.

190x275mm (96 x 96 DPI)



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 N°	Description	Pages
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration	2b	All items from the World Health Organization Trial Registration Data Set	n.a
	3	Date and version identifier	n.a
Protocol version	3	Date and version identifier	n.a
Funding	4	Sources and types of financial, material, and other support	23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,23
	5b	Name and contact information for the trial sponsor	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	23
	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)	11
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	5-8
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	8

1 2 3 4 5 6 7	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)	8
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Methods: Participants, interventions, and outcomes

8 9 10 11 12 13	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	10
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14 15 16 17 18	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)	9
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19 20 21	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11
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22 23 24 25	Interventions	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)	12
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26 27 28 29	Interventions	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
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30 31 32 33	Interventions	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
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34 35 36 37 38 39 40 41	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	12-18 Table 1 Table 2
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42 43 44 45 46	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)	Fig. 1
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47 48 49 50	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	9
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51 52 53	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10
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Methods: Assignment of interventions (for controlled trials)

Allocation:

1				
2			Method of generating the allocation sequence (eg, computer-	
3			generated random numbers), and list of any factors for	
4	Sequence		stratification. To reduce predictability of a random sequence,	
5	generation	16a	details of any planned restriction (eg, blocking) should be	10
6			provided in a separate document that is unavailable to those	
7			who enrol participants or assign interventions	
8				
9				
10	Allocation		Mechanism of implementing the allocation sequence (eg, central	
11	concealment		telephone; sequentially numbered, opaque, sealed envelopes),	
12	mechanism	16b	describing any steps to conceal the sequence until interventions	10
13			are assigned	
14				
15	Implementation	16c	Who will generate the allocation sequence, who will enrol	10
16			participants, and who will assign participants to interventions	
17				
18				
19			Who will be blinded after assignment to interventions (eg, trial	
20		17a	participants, care providers, outcome assessors, data analysts),	10-11
21			and how	
22	Blinding (masking)			
23			If blinded, circumstances under which unblinding is permissible,	
24		17b	and procedure for revealing a participant's allocated intervention	11
25			during the trial	
26				
27				
28	Methods: Data collection, management, and analysis			
29				
30			Plans for assessment and collection of outcome, baseline, and	
31			other trial data, including any related processes to promote data	
32			quality (eg, duplicate measurements, training of assessors) and	
33		18a	a description of study instruments (eg, questionnaires,	12-18
34			laboratory tests) along with their reliability and validity, if known.	
35	Data collection		Reference to where data collection forms can be found, if not in	
36	methods		the protocol	
37				
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39			Plans to promote participant retention and complete follow-up,	
40		18b	including list of any outcome data to be collected for participants	10
41			who discontinue or deviate from intervention protocols	
42				
43				
44			Plans for data entry, coding, security, and storage, including any	
45			related processes to promote data quality (eg, double data entry;	
46	Data management	19	range checks for data values). Reference to where details of	11
47			data management procedures can be found, if not in the	
48			protocol	
49				
50				
51			Statistical methods for analysing primary and secondary	
52		20a	outcomes. Reference to where other details of the statistical	18-19
53	Statistical		analysis plan can be found, if not in the protocol	
54	methods			
55				
56		20b	Methods for any additional analyses (eg, subgroup and adjusted	19-20
57			analyses)	
58				
59				
60				

		Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
		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	
	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	18
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	14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	11
		Ethics and dissemination	
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21
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)	21
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n.a
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	23
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
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	21

1				
2	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care, and for	n.a
3	trial care		compensation to those who suffer harm from trial participation	
4				
5			Plans for investigators and sponsor to communicate trial results	
6			to participants, healthcare professionals, the public, and other	
7		31a	relevant groups (eg, via publication, reporting in results	21
8			databases, or other data sharing arrangements), including any	
9			publication restrictions	
10	Dissemination			
11	policy			
12		31b	Authorship eligibility guidelines and any intended use of	24
13			professional writers	
14				
15		31c	Plans, if any, for granting public access to the full protocol,	8
16			participant-level dataset, and statistical code	
17				
18				
19	Appendices			
20				
21	Informed consent	32	Model consent form and other related documentation given to	Attached
22	materials		participants and authorised surrogates	
23				
24			Plans for collection, laboratory evaluation, and storage of	
25	Biological	33	biological specimens for genetic or molecular analysis in the	17
26	specimens		current trial and for future use in ancillary studies, if applicable	
27				

*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

BMJ Open

Study protocol for a randomised, double-blinded, placebo-controlled phase III trial examining the add-on efficacy, cost-utility and neurobiological effects of low-dose naltrexone (LDN) in patients with fibromyalgia (INNOVA study)

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-055351.R2
Article Type:	Protocol
Date Submitted by the Author:	13-Nov-2021
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Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Health economics, Pharmacology and therapeutics, Public health
Keywords:	PAIN MANAGEMENT, HEALTH ECONOMICS, Clinical trials <

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	THERAPEUTICS

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Manuscripts

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3 **Study protocol for a randomised, double-blinded, placebo-controlled phase III trial**
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5 **examining the add-on efficacy, cost-utility and neurobiological effects of low-dose**
6
7 **naltrexone (LDN) in patients with fibromyalgia (INNOVA study)**
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ABSTRACT

Introduction. There is evidence that low-dose naltrexone (LDN; < 5.0 mg/day) reduces pain and improves the quality of life of people with fibromyalgia syndrome (FMS). However, no randomised controlled trials with long-term follow-ups have been carried out. The INNOVA study will evaluate the add-on efficacy, safety, cost-utility, and neurobiological effects of LDN for reducing pain in FMS patients, with a one-year follow-up.

Methods and analysis. A single-site, prospective, randomised, double-blinded, placebo-controlled, parallel design phase III trial will be performed. Eligibility criteria include being adult, having a diagnosis of FMS and experiencing pain of 4 or higher on a 10-point numerical rating scale. Participants will be randomised to a LDN intervention group (4.5 mg/day) or to a placebo control group. Clinical assessments will be performed at baseline (T0), 3-months (T1), 6-months (T2), and 12-months (T3). The primary endpoint will be pain intensity. A sample size of 60 patients per study arm (120 in total), as calculated prior to recruitment for sufficient power, will be monitored between January 2022 and August 2024. Assessment will also include daily ecological momentary evaluations of FMS-related symptoms (e.g., pain intensity, fatigue, and sleep disturbance), and side effects via ecological momentary assessment (EMA) through the Pain Monitor app® during the first three months. Costs and quality-adjusted life years will be also calculated. Half of the participants in each arm will be scanned with magnetic resonance imaging (MRI) at T0 and T1 for changes in brain metabolites related to neuroinflammation and central sensitization. Inflammatory biomarkers in serum will also be measured.

Ethics and dissemination. This study has been approved by the Ethics Committee of the Fundació Sant Joan de Déu. The results will be actively disseminated through peer-reviewed journals, conference presentations, social media, and community engagement activities.

Trial registration number: ClinicalTrials.gov (NCT04739995)

Strengths and limitations of this study

- This is thought to be the first randomised, double-blinded, placebo-controlled phase III trial to assess the efficacy, safety, cost-utility, and neurobiological effects of low-dose naltrexone (LDN) for reducing pain in patients with fibromyalgia syndrome.
- The INNOVA protocol combines mobile-technology-based ecological momentary assessment and assessment with classical legacy measures to obtain more precise information on the dynamics of the assessed primary and secondary outcomes.
- This study will include immune and neuroimaging biomarkers in order to explore the neurobiological underpinnings of LDN.
- Challenges of the RCT include the long follow-up period (one year) and potential measurement burden that takes the risk of high dropout rate.

INTRODUCTION

Fibromyalgia: definition, prevalence, and pharmaceutical indications

Fibromyalgia syndrome (FMS) is a chronic condition of unknown origin that is characterised by generalised musculoskeletal pain, fatigue, stiffness, cognitive problems, sleep disturbances, and malaise.^{1,2} This syndrome is highly prevalent in the general population (2.7% worldwide).³ Around 6% of adult patients who visit their general practitioner, and between 10-20% of those who visit rheumatology services, have FMS.² In 2007, the U.S. Food and Drug Administration approved pregabalin as the first drug indicated for the treatment of FMS, and later approved duloxetine and milnacipran for this indication. However, the European regulatory authorities rejected the indication of these three drugs in the treatment of FMS given the small effect sizes in various studies and the associated adverse effects.⁴⁻⁶

Pathogenesis of FMS

Although the etiological factors of FMS are not known, the primary hypothesis of the pathogenesis of this syndrome highlights the role of the central nervous system in the amplification of pain perception as well as in the development of comorbid symptoms such as sleep-related problems, fatigue, cognitive difficulties, and emotional distress.^{1,7,8} Structural brain alterations have also been found in patients with FMS. For example, lower volumes of grey matter have been observed in areas associated with the processing of stress (e.g., parahippocampal gyrus) and pain (e.g., anterior cingulate cortex, insula, prefrontal cortex, and primary and secondary somatosensory cortices).⁷ Functional MRI alterations been associated with self-reported pain intensity. Brain activity exhibited greater connectivity between different pain-processing areas (e.g., insula and secondary somatosensory cortex), the default-model network in persons with FMS, as well as in the association between these areas and the pain levels reported by patients and the right executive attention network.⁹

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3 Altered functional connectivity has also been reported among various pain-inhibiting
4 areas.¹⁰ Some studies have reported reduced levels of neurotransmitters involved in the
5 regulation of the descending analgesic response (serotonin and noradrenaline) and increased
6 levels of glutamate (Glu) and substance P in people with FMS.¹¹⁻¹⁴ For example, high levels of
7 Glu have been reported in the posterior insula, posterior cingulate cortex and prefrontal
8 ventrolateral cortex of patients with FMS when compared to healthy controls¹⁵⁻¹⁹.

17
18 Activation of the microglia could be a contributing factor to the alteration of glutamate
19 neurotransmission in FMS.^{20,21} The microglia is normally found in a state of rest but it is
20 activated by a wide range of stimuli such as cell death, peripheral inflammation, chronic stress
21 and infections.²² Once activated, microglia release pro-inflammatory agents such as cytokines,
22 excitatory amino acids and nitric oxide.²³ These inflammatory factors across multiple neural
23 pathways can induce hyperalgesia, fatigue, depression and other symptoms which are known
24 collectively as “cytokine induced sickness behavior”.^{24,25} Microglia activation might trigger a
25 series of actions that lead to an increase in Glu that ultimately results in synaptic dysfunction.²⁶
26 A recent study using Positron Emission Tomography²⁷ of translocator protein revealed a
27 widespread cortical glial activation in patients with FMS, which gives support to the role of
28 neuroinflammation in the aetiology of FMS. In addition, there is evidence that chronic stress
29 facilitates the “priming” and exaggerated activation of the microglia.²⁸

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36 Although FMS is not considered a classic inflammatory disease, there is extensive
37 evidence that immune pathways play a significant role in the pathogenesis and maintenance of
38 the syndrome. Cytokines play a key role in inflammatory response and in boosting the
39 nociceptive response due to their sensitization actions, both on a peripheral and central level.²⁹⁻
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Although FMS is not considered a classic inflammatory disease, there is extensive evidence that immune pathways play a significant role in the pathogenesis and maintenance of the syndrome. Cytokines play a key role in inflammatory response and in boosting the nociceptive response due to their sensitization actions, both on a peripheral and central level.²⁹⁻
³² Thus, there is evidence that FMS involves an imbalance in pro-inflammatory (e.g. IL-1, IL-6, IL-17A, and TNF- α) and anti-inflammatory (e.g. IL-4 and IL-10) levels of cytokines that could lead to a low-intensity, chronic state of inflammation. Bäckryd and colleagues³⁰

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2
3 identified both neuroinflammation and systemic inflammation by evaluating levels of a broad
4 panel of cytokines and chemokines in cerebrospinal fluid and plasma.
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8 **Low-dose naltrexone (LDN): A promising treatment for FMS**

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11 Naltrexone is an opioid antagonist medication used to treat opioid and alcohol
12 dependency. The drug blocks mu-opioid receptors, the delta-opioid receptors and, to a lesser
13 extent, the kappa-opioid receptors. There is promising evidence to suggest that naltrexone
14 administered in low doses (i.e., low-dose naltrexone; < 5 mg/day) is effective in the
15 management of some pathologies which present with altered immune pathways, such as
16 Crohn's disease, multiple sclerosis, or FMS.^{33,34} The immune-regulatory effect of LDN seems
17 to be driven through the inhibition of the Toll-like receptor 4 (TLR-4) activity expressed in the
18 membrane of various immune system cells (e.g., microglia and macrophages).³³ Moreover, due
19 to a “rebound effect”, LDN could exert an analgesic effect that strengthens the endogenous
20 opioid pain inhibitory system. According to this hypothesis, the low-intensity and intermittent
21 blockade of the opioid receptors generated by LDN induces a compensatory mechanism that
22 facilitates an increase in the production of endogenous opioids and greater sensitivity of the
23 system to their effects.^{33,34}
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41 To date, the effects of LDN in patients with FMS have only been evaluated through
42 crossover pilot studies that have yielded preliminary results. In the first study conducted with
43 LDN in FMS, significant reductions in pain, stress, and fatigue levels were observed.³⁵ In a
44 subsequent study, significant improvements in daily pain, satisfaction with life and mood were
45 also observed.³⁶ In another crossover investigation, the pre and post changes in the levels of
46 plasma cytokines were evaluated over eight weeks. Significant reductions in a wide range of
47 immune biomarkers were obtained (e.g., IL-1 β , sIL-1ra, IL-4, IL-6, IL-10, IL-17A, and TNF-
48 α), together with a reduction in the pain levels and the severity of FMS symptoms.³⁷
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3 While acknowledging the contribution of past studies into the field, these have included
4 small sample sizes (n= 8 to 31 participants) and crossover designs. Therefore, a single-site,
5 prospective, randomised, double-blinded, placebo-controlled study (RCT) with a sufficiently
6 powered sample is presented here to conduct a methodologically robust investigation into the
7 role of LDN in FMS. Specifically, the main objective of the INNOVA study is to evaluate the
8 efficacy, safety, and cost-utility, and neurobiological effects of LDN to reduce pain in FMS.
9
10 There is currently no gold standard pharmacological treatment for pain reduction in persons
11 with FMS. Therefore, in the present study, placebo will be used instead of another drug in the
12 control group.
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24 **METHODS AND ANALYSIS**

25 **Trial design**

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27 The randomised controlled trial (RCT) protocol has been developed following the
28 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)³⁸. In addition,
29 the RCT was approved by European Union Drug Regulating Authorities Clinical Trials
30 (EudraCT; 2021-002534-16). For reporting purposes, we will follow the guidelines of the
31 Consolidated Standards of Reporting Trials (CONSORT)³⁹ and the Consolidated Health
32 Economic Evaluation Reporting Standards (CHEERS) statement.⁴⁰ INNOVA is a 12-month
33 double-blind RCT with two arms: LDN vs. Placebo. LDN will be considered an add-on
34 treatment to the usual care provided in the Spanish National Health System for FMS. For
35 transparency and analytical reproducibility purposes, the dataset and data coding will be
36 deposited in the Open Science Framework.
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52 **Sample size**

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54 There are no previous RCTs about the efficacy of LDN for FMS, therefore, we
55 estimated the sample size taking into account a previous LDN crossover study³⁶ that had used
56 self-reported pain as main outcome (the effect size was $d = .99$). Thus, with a sample of 60
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3 participants per arm, we aim to detect between-group differences with a significance level of
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5 5% and a power of 80%. Allowing for a potential attrition rate of 20%, our final sample size is
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7 60 participants per group. For the analysis of biomarkers (involving 50% of the sample), an
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9 initial sample size of 30 patients per arm is considered sufficient according to previous
10
11 studies.^{37,41}

14 Eligibility criteria

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17 *General selection criteria.* All participants will meet the following inclusion criteria:
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19 women between 18-70 years; diagnoses of FMS according to American College of
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21 Rheumatology 2016 criteria⁴² by a rheumatologist; pain intensity ranked ≥ 4 out of 10 on a 10-
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23 point numerical rating scale in the past week; fluent in Spanish; provision of written informed
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25 consent; stable pharmacological treatment in the last two months; and having a smartphone
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27 with android operating system for Ecological Momentary Assessment (EMA). Potential
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29 participants will be excluded according to the following exclusion criteria: treatment with
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31 naltrexone, opioids, anticoagulants, or central anti-hypertensives in the last 3 months; diagnosis
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33 of severe medical/psychiatric disorders (e.g., cancer, haematological diseases, abnormal
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35 hepatic/liver function, renal failure, suicide ideation, psychotic disorder); pregnant (or planning
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37 to become pregnant during the study period) or breastfeeding; known allergy to naltrexone,
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39 naloxone or excipients; currently participating in other RCTs; ongoing litigation related to
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41 FMS.
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48 *Additional selection criteria for the biomarkers and neuroimaging sub-study (50% of*
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50 *patients in each study arm).* All participants will meet the following inclusion criteria:
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52 right-handed (for the neuroimaging tests); and no comorbid rheumatologic conditions (e.g.,
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54 rheumatoid arthritis, lupus). Potential participants will be excluded according to the following
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56 exclusion criteria: fever ($> 38^{\circ}\text{C}$); infection in the last two weeks; vaccination in the last month;
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58 taking cortisone or anti-cytokine therapy; needle phobia or claustrophobia, metal implants or
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3 pacemakers; body mass index ≥ 36 kg/m²; smoking over 5 cigarettes/day; presence of acute
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5 pain (e.g, headache or back pain) unrelated to FMS on the day of the scan.
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8 **Recruitment strategy, procedure and randomisation**

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10 Patients diagnosed with FMS with an appointment at the Rheumatology Service of Parc
11 Sanitari Sant Joan de Déu (St. Boi de Llobregat, Spain) will be invited to participate in the
12 study and will be asked to attend a screening evaluation with a research assistant and a clinician.
13
14 Once the informed consent is obtained, the clinician will review the study selection criteria to
15 confirm eligibility. The week after, a face-to-face assessment (T0) including clinical history
16 and variables related to the use of services will be conducted with those patients meeting all
17 the eligibility criteria. Only the participants that are included in the biomarkers sub-study will
18 require an additional blood extraction and neuroimaging scan, which will be performed in the
19 following 3-5 days. Participants will be given a sealed envelope with an identifying code which
20 they will have to take to the pharmacy service. There, they will be given the dose of the
21 corresponding drug (according to the randomisation) for the first 3 months (90 tablets). As
22 shown in the patients' flow chart (Figure 1), further in-person evaluations will be performed at
23 3 months (T1), 6 months (T2), and 12 months (T3). Neurobiological variables will be obtained
24 at T1 using the same protocol as in the baseline assessment (T0).
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42 Participants will be asked to abstain from taking any analgesic or anti-inflammatory
43 drug in the 72h prior to the blood extractions/scans. All patients, including those who do not
44 participate in the biomarker sub-study, shall be subjected to a blood test at baseline. Participants
45 will return to the pharmacy service every 3 months and will be given the assigned amount of
46 LDN/placebo for the following 3 months (approximately 90 tablets). Unconsumed tablets will
47 be returned for treatment adherence monitoring. The randomisation to conditions will be
48 conducted by a biostatistician from the Clinical Trials Unit of Fundació Sant Joan de Déu who
49 has no involvement in the eligibility screening, enrolment, and treatment processes. The
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3 computer-generated randomisation will apply a permuted block design to ensure that the study
4 arms are balanced taking the biomarkers sub-study eligibility criteria into account. As this is a
5 double-blind RCT, neither the patient nor the evaluator or the clinician will know to which
6 treatment arm each patient has been assigned. Only the clinical trial pharmacist who stores and
7 delivers capsules, but is not involved in patient care, will know the allocation.
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15 Figure 1

16 17 **Data management, central monitoring and audit**

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19 The clinical data entry, data management, and central monitoring will be performed
20 with REDCap®. An independent Clinical Research Organization (CRO) will be responsible
21 for overseeing the intra-study data sharing and storing process. Any modifications in the study
22 protocol will be communicated to the CRO.
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28 29 **Treatments**

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31 **Low-dose naltrexone (LDN).** The intervention group will take one 4.5 mg naltrexone
32 tablet (lactose-free) daily for 12 months before going to sleep.
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36 **Placebo.** The control group will take the placebo daily for 12 months (a film-coated
37 tablet identical to the LDN and filled with a lactose-free filler). For the control arm, the same
38 guidelines will be followed.
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43 In order to maintain the external validity of the study and for ethical reasons, the study
44 participants' active treatments will be unchanged by this clinical trial. In Spain, chronic pain
45 management is mainly managed by general practitioners in regular consultations. These
46 generally consist of face-to-face appointments with a duration of 5-10 minutes in which the
47 clinicians monitor the physical and, ideally, the emotional status of the patient. General
48 practitioners usually provide advice prescribe pharmacotherapy (pain killers, hypnotics,
49 antidepressants, etc.) or refer patients to pain units in tertiary hospitals when more specialised
50 pain management procedures are required. The frequency of consultations is based on the type
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3 of disease and its severity. In this study, usual care will be the same as in routine daily practice,
4 without any modifications. In addition, participants will be allowed to withdraw from this study
5 for any reason at any time without detriment to the provision or quality of their usual care. If a
6 severe adverse event occurs, unblinding will be possible and study participation will be
7 discontinued. If the adverse effects are tolerable, the treatment will be administered until the
8 end of the study. All these events will be recorded and reported at the end of the study.
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16 **Study measures**

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18 All participants will be assessed with a computer-administered battery of measures
19 using the REDCap® software (see Table 1).
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24 Table 1

25 **Measures for sociodemographic characteristics, clinical features, and screening**

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27 A *sociodemographic questionnaire* will be used to obtain information about the
28 following variables: gender, date of birth, marital status, living arrangements, educational level,
29 income level, and employment status.
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34 The *Clinical data* interview will be used to collect information about history and
35 duration of FMS symptoms, as well as family history of medical/mental illness. Information
36 regarding comorbidity with other diagnosed physical-psychiatric conditions and the type and
37 dose of current drugs will be checked from medical records.
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45 The *Fibromyalgia Survey Diagnostic Criteria* (FSDC)^{43,44} is a 6-item self-report
46 measure of the core FMS symptoms according to the latest revision of the American College
47 of Rheumatology (ACR) 2016 criteria.⁴² It includes two subscales: the Widespread Pain Index,
48 which is used to identify the presence of pain in 19 body areas in the last week, and the
49 Symptom Severity Scale, in which the three major FMS symptoms (fatigue, “fibrofog” and
50 waking up tired) are assessed along with three additional symptoms (pain in the lower stomach,
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3 depression and headache). A total score is obtained by adding the two subscales. This total
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5 score ranges from 0 to 31, where higher values indicate greater FMS severity.
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8 **Primary outcome measure**

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10 The *Numeric Rating Scale* (NRS)⁴⁵ is a unidimensional measure of pain intensity
11
12 mainly used for adults. The most frequently used version is an 11-point numeric scale (a
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14 horizontal bar or line) scored from 0 (“no pain”) to 10 (“worst pain imaginable”). Time frames
15
16 vary between studies. In the present study, respondents will be asked to report average pain
17
18 intensity over the last week.
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22 **Secondary outcome measures**

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24 The *Revised Fibromyalgia Impact Questionnaire* (FIQR)⁴⁶ includes 21 items that are
25
26 answered on a 0 to 10 numerical scale in which higher scores indicate greater functional
27
28 impairment. The questionnaire asks about the previous seven days. The items are distributed
29
30 into three domains: physical impairment, overall impact, and severity of symptoms (i.e., pain,
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32 energy, stiffness, sleep quality, depression, memory issues, anxiety, pain to the touch, balance
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34 problems and increased sensitivity to noises, lights, smells, or temperatures). A total score is
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36 obtained by summing the three subscale scores. This can range from 0 to 100. Higher scores
37
38 indicate greater impairment. The Spanish version of the FIQR and has obtained high internal
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40 consistency estimates ($\alpha = .91 - .95$), adequate test-retest reliability indices ($r = .82$), and good
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42 construct validity.⁴⁷
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47 The *Depression Anxiety Stress Scales-21* (DASS-21)⁴⁸ is a self-report scale developed
48
49 to discriminate between features of depression (anhedonia/low positive affect), anxiety
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51 (physical arousal) and stress (psychological tension/agitation) in clinical and non-clinical
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53 samples. The DASS has been validated in patients with FMS.⁴⁹ Responders are required to
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55 indicate the presence of a symptom over the previous week. Each item is scored from 0 (“did
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57 not apply to me at all over the last week”) to 3 (“applied to me very much or most of the time
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3 over the past week”). There are seven items on each of the three subscales: depression, anxiety,
4 and stress. Therefore, total scores in each scale can range from 0 to 21. Higher scores indicate
5 more severe levels of depression, anxiety and stress. The Spanish version showed adequate
6 internal consistency for depression ($\alpha = .84$), anxiety ($\alpha = .70$) and stress ($\alpha = .82$).⁵⁰
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12 The *Multidimensional Inventory of Subjective Cognitive Impairment* (MISCI)⁵¹ is a
13 10-item self-report measure of subjective cognitive dysfunction (i.e., “fibrofog”) in FMS.
14 Each item is scored from 1 (“never”) to 5 (“very often”) and the total score ranges from 10 to
15 50. Lower scores indicate higher cognitive dysfunction. The MISCI showed excellent internal
16 reliability, low ceiling/floor effects and good convergent validity with a similar measure. The
17 Spanish version of the MISCI had sound psychometric properties ($\alpha = .91$ and ICC = .88).⁵²
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26 The *World Health Organization Disability Assessment Schedule 2.0* (WHODAS
27 2.0)⁵³ is a 12-item self-report measure of the level of difficulty experienced taking into
28 consideration how they usually do the activity. This includes the use of any devices to assist
29 them and/or the help of a person. In each item, individuals estimate the magnitude of the
30 disability during the previous 30 days using a five-point scale scored from 1 (none) to 5
31 (extreme/cannot do). The total score ranges from 0 to 100. Higher scores reflect greater
32 disability. The 12-item WHODAS 2.0 has sound psychometric properties in patients with
33 FMS.⁵⁴
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45 The *Generalized Anxiety Disorder 7-item scale* (GAD-7)⁵⁵ is a 7-item self-report
46 measure of pathological worry. Each item is scored from 0 (“not at all”) to 3 (“nearly every
47 day”). The total score ranges from 0 to 21, where higher scores reflecting greater anxiety
48 symptoms. The GAD-7 has sound psychometric properties ($\alpha = .92$ and ICC = .83) in patients
49 with FMS in previous studies.⁵⁶
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56 **Other measures**

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3 The *ACTION AE* is a *reporting checklist* used to measure safety and benefit-risk of
4 a clinical trial.⁵⁷ The Safety and Benefit-Risk Reporting and Evaluation (SABRRE) Working
5 Group of the Analgesic, Anaesthetic, and Addiction Clinical Trial Translations, Innovations,
6 Opportunities, and Networks (ACTION; <http://www.action.org>) public-private partnership
7 with the FDA developed an adverse events (AE) reporting checklist that will be used in the
8 present study.
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12 The *EuroQoL* (version EQ-5D-5L)⁵⁸ is a health-related quality of life questionnaire
13 that consists of two parts. In the first one, the individual's difficulties concerning mobility,
14 self-care, pain/discomfort and anxiety/depression are evaluated. In the second part, the
15 perceived health is assessed by means of a Visual Analogue Scale ranging from 0 to 100. The
16 EQ-5D-5L scores will be used to calculate the Quality-Adjusted Life Years (QALYs) during
17 the follow-up period by adjusting the duration of time affected by the health outcome by the
18 value of the utility.
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33 The *Client Service Receipt Inventory* (CSRI)⁵⁹ is a self-report tool used to collect
34 retrospective data on medication consumption and service receipt. Patients are asked to bring
35 their daily medication prescriptions and information about pain-related drugs (analgesics,
36 anti-inflammatories, opioids, muscle relaxants, antidepressants, etc.) is recorded. This
37 includes the name of the drug, the dosage, total number of prescription days and daily dosage
38 consumed. Concerning service receipt, patients are asked about the total appointments for
39 accident and emergency services, total number of general inpatient hospital admissions,
40 number of diagnostic tests administered and total appointments with healthcare professionals
41 for pain management (family physicians, nurses, social workers, psychologists, psychiatrists,
42 group psychotherapy and other community healthcare professionals). The CSRI will be
43 administered on two occasions: at baseline and at 12-month follow-up, both referring to the
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3 previous 12 months. Medical records will be checked to verify the accuracy of the collected
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5 data.
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8 The *Patient Global Impression of Change* (PGIC) measures meaningful change in
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10 overall status and the *Pain Specific Impression of Change* (PSIC)⁶⁰ measures the perception
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12 of pain improvement. The most frequently used scale is a 7-point numerical scale scored from
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14 1= “much better”) to 7= “much worse”).
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16

17 **Ecological momentary assessment (EMA)**

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19 Pain intensity and other pain-related variables (e.g., depressive-anxious symptoms and
20
21 activity level) can fluctuate during the day and across days depending on personal and
22
23 environmental factors. Collecting self-reported data prospectively and closer in time to its
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25 occurrence substantially improves the accuracy, reliability and quality of data. EMA has been
26
27 successfully performed in patients with a variety of physical and mental problems.^{61,62} There
28
29 is growing evidence indicating that well-designed smartphone apps can be easy to use and well-
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31 tolerated even in relatively old pain populations, with compliance rates as high as 85%.⁶¹ In
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33 this RCT, we will use the Pain Monitor® (Monitor de Dolor, by its Spanish name) app⁶³ to
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35 assess a wide range of variables (see items in Table 2) twice a day (once in the morning and
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37 once in the evening, at convenient times along the week) during 120 days. The app and the data
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39 will be stored on different servers with different domain names and connected locally only (the
40
41 server containing the data does not have Internet access). According to a recent meta-analysis,⁶⁴
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43 EMA-completion rates are higher among elderly patients compared to younger patients.
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45 Considering that the majority of FMS patients in our study are not expected to be young and
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47 that the EMA item battery does not require a long response time (< 1 min.), it is expected not
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49 to place an excessive burden on participants.
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56 Table 2
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Inflammatory biomarkers

After obtaining the blood sample, it will be allowed to coagulate for a minimum of 30 minutes at room temperature. It will then be centrifuged for 10 minutes at 1000g. The resulting serum will be stored at -80° C during the same morning of extraction until it is ready to be analysed. All samples (at T0 and T1) will be analysed in a single analytical batch to reduce inter-assay variability (approx. 15%). The serum levels of IL-1 β , sIL-1ra, IL-4, IL-6, sIL-6r, sgp130, CXCL-8, IL-10, IL-17, TNF- α , and high-sensitivity C-reactive protein (hs-CRP) will be evaluated.²⁹ For the quantification of the cytokines, the Milliplex® reagents from the company MerckMillipore® will be used and analysed using a Luminex® platform. The high sensitivity multiplex kit will be used: Human High Sensitivity T Cell, catalogue number: HSTCMAG28SPMX11, adapted to the aforementioned cytokines. The hs-PCR will be quantified using turbidimetry in an Olympus AU5400 auto-analyser. These biomarkers will only be evaluated at baseline (T0) and 3-months (T1) for the following reasons: (a) there is evidence of significant inflammatory changes at 8 weeks with LDN;³⁷ (b) this results in lower risk of dropout (vs. evaluating them at 6 or 12 months); (c) conducting at least two measures allows to use the change between baseline and 3-months as a mediator of long-term clinical changes; and (d) budget constraints.

Neuroimaging

The scans (protocol duration: approximately 30 minutes) will be performed in a Phillips Ingenia 3T MRI scanner with a 32-channel head coil at Hospital Sant Joan de Déu (Esplugues de Llobregat, Spain). To examine cingulate, insular, amygdalar, occipital, angular, parahippocampal, and prefrontal gray matter volume, we will use voxel-based morphometry (VBM). We will also use surface-based morphometry (with FreeSurfer calculation of cortical thickness, surface area, and local gyrification index) for examining cortical abnormalities. Additionally, glutamate, glutamine, myo-inositol, N-acetylaspartate, choline, and creatine

(and creatinine ratios) levels will be analysed using magnetic resonance spectroscopy. Specifically, we will conduct the following processing for the regions of interest according to the corresponding hypotheses. For VBM, we will apply a bias field correction, tissue segmentation with SPM12, normalization with DARTEL, modulation, and smoothing. We will use both unmodulated and modulated grey matter images to convey complementary volumetric information. We will use FreeSurfer ENIGMA pipelines to perform the VBM. In addition, we will quantify metabolites concentrations using LCModel (v6.3-1J). We will only include high-quality spectra, defined as signal-to-noise ratio > 15, Cramer-Rao lower bounds < 15%, and full width at half maximum of metabolites < .07. The spectroscopy analysis will account for the effects of cerebrospinal fluid and grey matter within the voxel, and inter-individual differences in cortical grey matter.

Statistical analysis

The main analysis will compare the effect of LDN vs. placebo on the primary outcome (pain intensity at T1). Data analyses will be performed following an intention-to-treat (ITT) plan. Then, we will compute analysis of the primary outcome (at T2 and T3) and analysis of the secondary outcomes at T1, T2 and T3. Linear mixed models will be created using the restricted maximum likelihood method for the estimation of parameters. The effect sizes will be calculated according to Cohen's *d*. An interim analysis is planned at T1 once 50% of the total sample has been evaluated. A 5% significance level will be used in all two-tailed tests, applying the Benjamini-Hochberg correction for multiple comparisons. Additionally, to make the findings from our study clinically meaningful, the number needed to treat will be reported. For this analysis, we will dichotomise participants into responders or non-responders using two different cut-off criteria in compliance with the IMMPACT recommendations:⁴⁵ At least 50% pain relief over baseline (substantial benefit) or 30% or more pain relief (moderate benefit). For these analyses, we will use SPSS v26 (IBM Corp, Armonk, NY, USA).

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3 Regarding EMA, a recent recommended approach is "network analysis". There has
4 been burgeoning interest in conceptualizing chronic pain as a network of interacting symptoms
5 and psychobiological processes.⁶⁵ Network analysis will offer us a good chance to quantify and
6 visualize relationships between pain intensity and pain-related variables (e.g., depression,
7 anxiety, fatigue, sleep disturbance). We will estimate temporal networks by means of vector
8 autoregression techniques;⁶⁶ These "temporal networks", would indicate potential causality
9 with one or more variables preceding one or more variables in time. Network analysis will be
10 performed with the free statistical software JASP.⁶⁷
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22 In economic evaluation, it is important to calculate the relationship between the costs
23 of each treatment and its consequences in the form of QALYs, an index measure designed to
24 assess both quantity of life (years) and health-related quality of life. A year lived with the
25 maximum quality of life would be transformed into 1 QALY; a year lived with half the
26 maximum quality of life would be transformed into 1/2 QALY. This relative value is called the
27 incremental cost-utility ratio (ICUR) and it expresses the relationship between the costs and
28 the effects of one option compared to another. The QALYs obtained in the 12 months after the
29 treatment onset will be calculated by the area under the curve. The direct costs will be
30 calculated by adding together the costs derived from the medication and the use of the health
31 services. The cost of medications will be calculated by multiplying the price per milligram by
32 the total daily dose consumed (in milligrams) and the number of days that the treatment is
33 received. The cost arising from the use of the health services (primary care, specialist and
34 accident and emergency consultations, and hospital admissions) will be obtained from the
35 clinical electronic records (<http://www.oblikue.com/en/esalud.html>). The indirect costs will be
36 calculated based on the days off work, which will be multiplied by the official minimum wage
37 during the study period. The effect of the treatments will be estimated using ordinary least
38 squares multivariate regression, adjusting for the baseline differences between groups. In order
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3 to manage uncertainty in the sampling distribution of the ICUR, non-parametric bootstrapping
4 will be applied, with 1000 replications in each comparison. Cost-utility analyses will be
5 conducted with STATA v16.0 (StataCorp, College Station, TX, USA).
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10 **Patient and public involvement**

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12 Patients and the public will not be involved in the design, conduct, reporting, or
13 dissemination of our research.
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17 **ETHICS AND DISSEMINATION**

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19 All procedures performed in this study will be in accordance with the 1964 Helsinki
20 declaration and its last amendments (7th revision, adopted by the 64th World Medical
21 Association General Assembly, Fortaleza, Brazil). Signed informed consent will be obtained
22 from all patients once they have been informed of the study procedures, potential risks, and
23 their right to withdraw at any time from the RCT. The FSJD Ethics Committee Board evaluated
24 and approved the study protocol in June 2021 (PIC-178-19). Only the principal investigators
25 (ARS and JVL) will have full access to the final trial dataset. Modifications in the study
26 protocol will be reported to the FSJD Ethics Committee Board as well as the independent CRO.
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39 Once the RCT is completed, we will publish our results in international peer-reviewed
40 biomedical journals and present them at national and international conferences. Authorship
41 will be assigned in accordance with the International Committee of Medical Journal Editors
42 guidance. In addition, we will send participating patients a short report of our findings. A copy
43 of the report will also be sent to Institute of Health Carlos III (main funding body). The
44 principal investigators will organize an end-of-study seminar. The main objective of this
45 activity will be to share the study findings with stakeholders to discuss how to maximize uptake
46 of the findings in patient treatment and clinical practice, and to determine future research
47 directions.
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DISCUSSION

As far as we know, no RCT has been published about the efficacy, safety, cost-utility, and neurobiological underpinnings of LDN in patients with FMS. This manuscript presents the design and rationale of a randomised, double-blinded, placebo-controlled phase III study, which is a powerful design to assess the efficacy of LDN. We have decided to administer 4.5 mg/day of LDN in this RCT because this dose seems to provide an optimal balance between significant analgesic efficacy and minimal side effects (nausea, sleep disturbance, nightmares, etc.) according to a recent study.⁶⁸

Our findings using this design in conjunction with those that will be obtained in another ongoing RCT that is being carried out in Denmark (The FINAL study)⁶⁹ may facilitate the approval of the first drug indicated for the treatment of FMS in Europe. The FINAL study is an ongoing single-centre, randomised, double-blinded, placebo-controlled trial that is being carried out in Odense (Denmark). A total of 100 women between 18–64 years-old with FMS will take either LDN or placebo for three months. Besides self-report measures, Danish researchers will also examine the levels of pro- and anti-inflammatory cytokines. If our respective findings strongly differ in efficacy or safety, we might analyse which factors can account for the divergence and plan a multi-country confirmatory trial with an agreed design and methodology. As pointed out by Kim and Fishman⁷⁰, a common problem with a generic, compounded medication is the lack of commercial support for research. To begin studies such as INNOVA or FINAL, it is crucial to have the synergistic support from public funding bodies, private entities, and commercial companies. This has been the case in the present study, with different public and private organizations providing economic and logistic support.

The inclusion of brain and blood immune biomarkers will allow us to determine whether LDN modulates neuro-inflammatory processes involving inflammatory cells such as glial cells. These markers will also allow us to explore the “hormetic” effects of the drug, that

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3 is, if a low dose of an antagonist (naltrexone) may paradoxically act as an agonist of the
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5 endogenous opiate system. As explained above, it is posited that LDN mainly acts as an
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7 immunomodulatory drug via blockade of TLR-4, which provides a therapeutic pathway to
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9 reduce activation of the inflammatory cascade and the nociceptive system.⁷¹ In a recent pilot
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11 study⁷², patients with opioid induced hyperalgesia and patients with FMS were treated with
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13 LDN for 3 months. Via different mechanisms of action, LDN improved pain tolerance
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15 (measured with the cold pressor test) in both groups of patients, being the effect even stronger
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17 in those participants with opioid induced hyperalgesia. According to the authors, the
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19 mechanism of action that would explain the beneficial effects of LDN for FMS may be transient
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21 blockade of the opioid growth factor receptor.
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27 Obtaining empirical evidence for cost-utility of treatments or interventions is required
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29 by the Ministry of Health in Spain for reimbursement. In Spain, a threshold of €22,000–25,000
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31 per QALY gained is found to be consistent with decisions of adopting new technologies by the
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33 National Health Service.⁷³ To our knowledge, there is an absence of economic evaluations for
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35 LDN; therefore, an important feature of the present study is the cost-utility assessment of the
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37 drug.
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41 FMS remains a chronic, debilitating, and difficult to manage condition for many
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43 individuals around the world. After three decades of intensive research, the clinical benefits of
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45 pharmacological treatments remain unclear and limited. This study will evaluate the analgesic
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47 efficacy, safety, and cost-utility of LDN using a rigorous and powered design. If efficacious
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49 and cost-effective, LDN might be the first drug approved for FMS in Europe.
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51 **Trial status**

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54 This study is currently in the recruitment phase. The first patient will be enrolled in
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56 January 2022, and the study is expected to end in August 2024.
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58 **Confidentially**

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3 Personal data will be stored in accordance with the Spanish regulation guidelines for
4 clinical research. Participants will be allocated a unique identification (ID) number at entry.
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6 The master list linking participant personal information and ID number will be maintained in
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8 a password-protected hard drive at the PSSJD. Data will be stored for 10 years after study
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10 completion.
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14 **Contributors**

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16 JVL, ARS, and AFS conceived the study and revised the manuscript. JVL drafted the
17 manuscript. ACC, JPSM, HHN, XB, CSR, AGP, JM, JM, FDA, MM, JWY, AFS and ARS
18 provided feedback on the manuscript, and all authors reviewed and approved the final version
19
20 of the manuscript.
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26 **Funding**

27
28 This study has been funded by the Institute of Health Carlos III (ISCIII; ICI20/00080;
29 CPII19/00003) and has been co-financed with European Union ERDF funds. JPS-M has a PFIS
30 predoctoral contract from the ISCIII (FI20/00034). AC-C has a FI predoctoral contract from
31 AGAUR (FI_B/00216). AF-S acknowledges the funding from the Serra Húnter program
32 (UAB-LE-8015). The ISCIII did not have any role in the analysis and interpretation of data, in
33 the writing of the manuscript, or in the decision to submit the paper for publication.
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43 **Competing interests**

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45 None declared.
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47 **Patient consent for publication**

48
49 Not required.
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51 **Provenance and peer review**

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53 Not commissioned; externally peer reviewed.
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Table 1. Time points for data collection.

Measures	T0 (baseline)	T1 (3-m)	T2 (6-m)	T3 (12-m)
<i>Sociodemographic, clinical, and screening measures</i>				
Sociodemographic data	X			
Clinical data (years of evolution, comorbidities, etc.)	X			
FSDC screening and secondary outcome measure (fibromyalginess)	X	X	X	X
<i>Primary outcome measure</i>				
NRS (pain intensity)	X	X	X	X
<i>Secondary outcome measures</i>				
FIQR (functional impairment)	X	X	X	X
DASS-21 (anxiety, depression, and stress)	X	X	X	X
MISCI (subjective cognitive impairment)	X	X	X	X
WHODAS 2.0 (disability)	X	X	X	X
GAD-7 (general anxiety / worry)	X	X	X	X
<i>Other measures</i>				
EQ-5D-5L (quality of life)	X			X
CSRI (medication consumption and service receipt)	X			X
PGIC and PSIC (impression of change)		X	X	X
ACTTION checklist (adverse events throughout the trial)	X	X	X	X
Pain Monitor® app (EMA)	X	X		
Physiological variables				
Immune biomarkers	X	X		
Neuroimaging	X	X		

ACTTION checklist: Analgesic, Anesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks; CSRI: Client Service Receipt Inventory; DASS-21: Depression Anxiety Stress Scales-21; EQ-5D-5L: EuroQoL; EMA: Ecological Momentary Assessment; FIQR: Fibromyalgia Impact Questionnaire Revised; FSDC: Fibromyalgia Survey Diagnostic Criteria; GAD-7: Generalized Anxiety Disorder 7-item scale; NRS: Numerical Pain Rating Scale; PGIC and PSIC= Patient Global Impression of Change and Pain Specific Impression of Change; WHODAS 2.0: 12-item interviewer administered version of the World Health Organization Disability Assessment Schedule 2.0

Table 2. List of items administered via Pain Monitor® app.

Items	Morning	Evening
Pain intensity	X	X
Fatigue	X	X
Perceived control over pain	X	X
Depression	X	X
Anxiety	X	X
Stress	X	X
Sleep disturbance	X	
Activity level		X
Interference with leisure activities		X
Interference with work-related activities		X
Adverse effects		X
Rescue medications		X

The Pain Monitor app automatically informs patients when to respond (by default, at 11 AM and 7 PM) using a push notification system, but patients can respond with a margin of 2 hours from given times. Collected data are stored on a secure server at the Jaume I University, Spain.

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Figure 1. Flowchart of the INNOVA study based on the CONSORT guidelines.

For peer review only

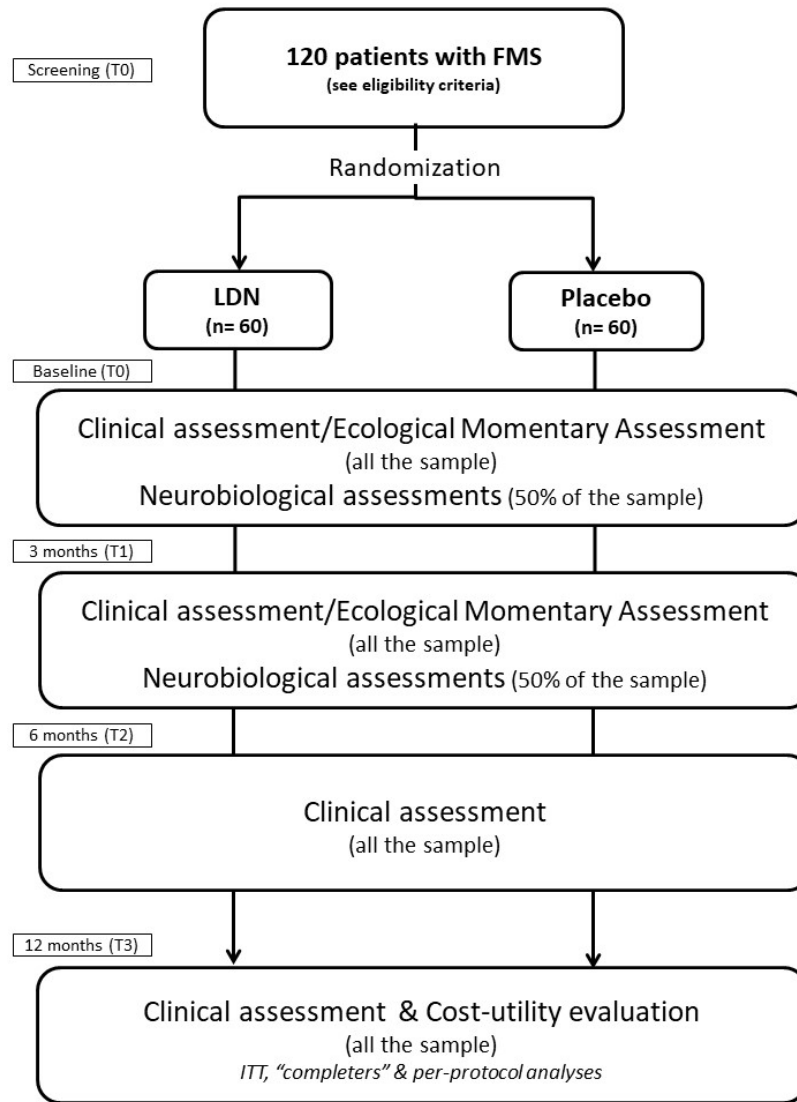


Figure 1. Flowchart of the INNOVA study based on the CONSORT guidelines.

190x275mm (96 x 96 DPI)



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 N°	Description	Pages
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration	2b	All items from the World Health Organization Trial Registration Data Set	n.a
	3	Date and version identifier	n.a
Protocol version	3	Date and version identifier	n.a
Funding	4	Sources and types of financial, material, and other support	23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,23
	5b	Name and contact information for the trial sponsor	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	23
	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)	11
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	5-8
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	8

1 2 3 4 5 6 7	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)	8
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Methods: Participants, interventions, and outcomes

8 9 10 11 12 13	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	10
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14 15 16 17 18	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)	9
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19 20 21	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11
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22 23 24 25	Interventions	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)	12
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26 27 28 29	Interventions	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
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30 31 32 33	Interventions	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
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34 35 36 37 38 39 40 41	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	12-18 Table 1 Table 2
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42 43 44 45 46	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)	Fig. 1
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47 48 49 50	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	9
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51 52 53	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10
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Methods: Assignment of interventions (for controlled trials)

Allocation:

1				
2			Method of generating the allocation sequence (eg, computer-	
3			generated random numbers), and list of any factors for	
4	Sequence		stratification. To reduce predictability of a random sequence,	
5	generation	16a	details of any planned restriction (eg, blocking) should be	10
6			provided in a separate document that is unavailable to those	
7			who enrol participants or assign interventions	
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9				
10	Allocation		Mechanism of implementing the allocation sequence (eg, central	
11	concealment		telephone; sequentially numbered, opaque, sealed envelopes),	
12	mechanism	16b	describing any steps to conceal the sequence until interventions	10
13			are assigned	
14				
15	Implementation	16c	Who will generate the allocation sequence, who will enrol	10
16			participants, and who will assign participants to interventions	
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18				
19			Who will be blinded after assignment to interventions (eg, trial	
20		17a	participants, care providers, outcome assessors, data analysts),	10-11
21			and how	
22	Blinding (masking)			
23			If blinded, circumstances under which unblinding is permissible,	
24		17b	and procedure for revealing a participant's allocated intervention	11
25			during the trial	
26				
27				
28	Methods: Data collection, management, and analysis			
29				
30			Plans for assessment and collection of outcome, baseline, and	
31			other trial data, including any related processes to promote data	
32			quality (eg, duplicate measurements, training of assessors) and	
33		18a	a description of study instruments (eg, questionnaires,	12-18
34			laboratory tests) along with their reliability and validity, if known.	
35	Data collection		Reference to where data collection forms can be found, if not in	
36	methods		the protocol	
37				
38				
39			Plans to promote participant retention and complete follow-up,	
40		18b	including list of any outcome data to be collected for participants	10
41			who discontinue or deviate from intervention protocols	
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44			Plans for data entry, coding, security, and storage, including any	
45			related processes to promote data quality (eg, double data entry;	
46	Data management	19	range checks for data values). Reference to where details of	11
47			data management procedures can be found, if not in the	
48			protocol	
49				
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51			Statistical methods for analysing primary and secondary	
52		20a	outcomes. Reference to where other details of the statistical	18-19
53	Statistical		analysis plan can be found, if not in the protocol	
54	methods			
55				
56		20b	Methods for any additional analyses (eg, subgroup and adjusted	19-20
57			analyses)	
58				
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60				

		Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
		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	
	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	18
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	14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	11
		Ethics and dissemination	
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21
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)	21
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n.a
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	23
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
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	21

1				
2	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care, and for	n.a
3	trial care		compensation to those who suffer harm from trial participation	
4				
5			Plans for investigators and sponsor to communicate trial results	
6			to participants, healthcare professionals, the public, and other	
7		31a	relevant groups (eg, via publication, reporting in results	21
8			databases, or other data sharing arrangements), including any	
9			publication restrictions	
10	Dissemination			
11	policy			
12		31b	Authorship eligibility guidelines and any intended use of	24
13			professional writers	
14				
15		31c	Plans, if any, for granting public access to the full protocol,	8
16			participant-level dataset, and statistical code	
17				
18				
19	Appendices			
20				
21	Informed consent	32	Model consent form and other related documentation given to	Attached
22	materials		participants and authorised surrogates	
23				
24			Plans for collection, laboratory evaluation, and storage of	
25	Biological	33	biological specimens for genetic or molecular analysis in the	17
26	specimens		current trial and for future use in ancillary studies, if applicable	
27				

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