The effect of multi-modal minocycline and N-acetylcysteine for the treatment of fibromyalgia: a double-blind, randomized, crossover pilot study.

Study Protocol

Statistical Analysis Plan

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PROTOCOL TITLE:

The effect of multi-modal minocycline and N-acetylcysteine for the treatment of fibromyalgia: a double-blind, randomized, crossover pilot study.

PRINCIPAL INVESTIGATOR:

M. Gabriel Hillegass, III, M.D.

1.0 Objectives / Specific Aims

Our objective is to design and test treatments for chronic pain that target multiple non-neuronal cell types in the clinical setting.

The forefront of the science of pain inhibition, and the most fruitful area of research for drug development, is the complete understanding of the molecular mechanisms that govern pain sensation. Towards that end, gaining a better understanding of the physiological mechanisms linked to pain signaling, like inflammation,[1,2] are of the utmost importance. In the fields of cellular and molecular neuroscience, it has been demonstrated that the action and signaling of non-neuronal cells, astroglia and microglia, serve as potent regulators of neuronal communication.[3-5] Over the last decade, preclinical scientists have begun to incorporate bidirectional communication between neurons and neighboring non-neuronal cells into their working models of synaptic plasticity.[6,7] Recently, activation of microglial cells, and the release of pro-inflammatory cytokines like tumor necrosis factor alpha (TNFα) and Interleukin 1 (IL-1β) have been directly linked to the induction of pain sensation and the expression of pain-related phenotypes including hyperalgesia and allodynia.[8] Further, microglia have been linked to the mechanisms that govern chronic pain.[9-12] In addition, injury-related decreases in astrocyte-mediated glutamate clearance have also been shown to contribute to elevated pain responses following nerve injury.[13]

Our central hypothesis is that a multimodal approach, designed to inhibit microglial activation and promote healthy homeostatic regulation of glutamate by astrocytes, will provide a therapeutic advantage in treating pain and other symptoms related to fibromyalgia. Enticingly, two FDA approved compounds exist that can be used to implement this precise multi-modal approach, minocycline, a tetracycline antibiotic and inhibitor of microglia[14,15] and N-acetylcysteine (NAC), a powerful antioxidant shown to promote homeostatic glutamate regulation by astrocytes.[16,17] This proposal aims to address the current lack of evidence of both classes of non-neuronal cells to alleviate pain. We posit that our multimodal approach will lessen the severity of chronic pain and enhance efficacy and implementation of traditional methods for inhibiting pain in patients. This pilot study will assess the feasibility of this approach and provide important data for refining this research.

2.0 Background

Microglia, pain signaling and pain sensitivity

Microglia are a subset of neuroglia or non-neuronal cells, located throughout the central and peripheral nervous system. Microglia act as the resident macrophages of nervous tissue, recognizing and responding to external pathogens by engaging the neuroinflammatory response. When microglia transition from a quiescent or resting state, to a state associated with the release of pro-inflammatory neuromodulators, they are said to be ‘active’, or ‘activated’. Remarkably, microglia are incredibly structurally plastic and

their activation states are associated with specific alterations in cellular architecture and the dimensions of particular cellular compartments.[18-20]

Recent discoveries have illuminated that microglia and microglia activation are central in engaging the cellular signaling responsible for the sensation of pain.[8,21] Specifically, spinal microglia are strongly activated after peripheral nerve injury,[22,23] resulting in elevated pro-inflammatory cytokine release in the central and peripheral nervous system.[24] Microglia are activated in response to pain,[22,25] and are directly implicated in pain, as microglia respond to injury by becoming more sensitive to future insults by upregulating cell surface receptors and secreted pro-inflammatory molecules,[26] including TNFα.[27] In rodent models, inhibition of microglia activation following nerve injury with the antibiotic minocycline[28] has been shown to reduce the severity of nociception-related phenotypes.[29-32] Mechanistically, minocycline inhibits or attenuates microglial activation by altering the program of cytokine release,[33-35] countering pro-inflammatory signaling.[36] Outside of microglia, minocycline also reduces expression of receptors for TNFα in nearby neurons, limiting the ability of microglia to engage TNFα-mediated neuroinflammatory signaling and synaptic scaling.[37] Importantly, minocycline treatment also reverses the reorganization of microglia ultrastructure occurring concomitantly with activation.[15,38-40] These data support the efficacy of minocycline in attenuating microglia activation and expression of pain-related phenotypes, while underscoring the well-defined structure-function relationship for this cell type.[41-43]

Coordinated non-neuronal cell regulation of the cellular signaling underlying chronic pain

Microglia and astroglia serve as potent regulators of neuronal communication.[3-5] However, the concomitant bidirectional chemical communication between these three cell types has only recently begun to be been incorporated into working models of chronic pain.[6,7,44]
The non-neuronal and neuronal signaling mechanisms in chronic pain are illustrated in **Figure 1.**

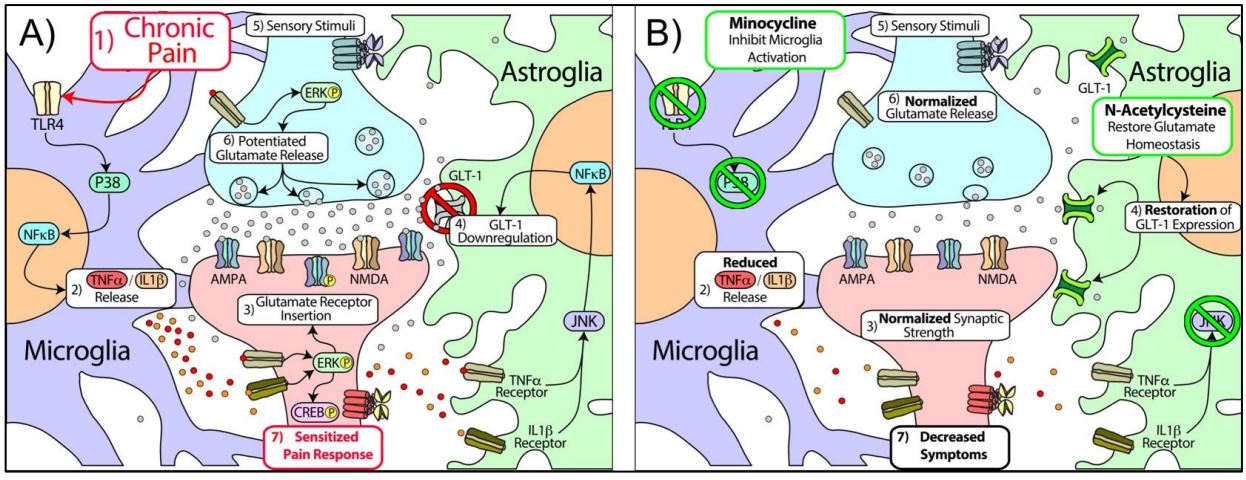

Figure 1: Illustration of microglia and astroglial components of chronic pain, and minocycline and N-Aceytlcysteine treatment**. A)** Disease state at the dorsal horn spinal cord synapse. 1) chronic pain causes activation of microglia. 2) Activation of microglia engages TNFα (red spheres) and IL1β (orange sphere) release. 3) TNFα and IL1β engages postsynaptic glutamate receptor insertion in neurons. 4) TNFα and IL1β engage GLT-1 downregulation in astroctyes. 5) Sensory stimuli then evoke, 6) a potentiated glutamate release (grey spheres) due to the presynaptic action of TNFα. 7) Glutamatergic communication, and pain sensation is evoked and enhanced due to strengthening of this synapse. **B)** The dorsal horn spinal cord synapse following treatment. 1) Treatment. Minocycline inhibits persistent microglia activation. 2) this limits TNFα and IL1β release. 3) Reduced TNFα and IL1β release limits postsynaptic strengthening, and the negative regulation of GLT-1 expression. 4) The NAC component of the treatment restores GLT-1 function and expression. Allowing for removal of glutamate from the cleft and termination of signaling. 5) Following treatment, sensory stimuli should no longer provoke an exaggerated glutamatergic response due to the reduced levels of TNFα. 7) Finally, following treatment, allodynia, hyperalgesia and other pain related phenotypes will be reduced / eliminated.

During nerve injury or chronic pain, microglial activation engages a TNFα-dependent enhancement of glutamatergic synaptic communication with a multimodal molecular mechanism.[45,46] In neurons, TNFα engages TNFα receptors, resulting in enhanced glutamate release and insertion of glutamate receptors, respectively.[47,48] Within the astrocyte itself, TNFα receptor activation acts to down-regulate the astroglial glutamate transporter, required for the termination of sensory synaptic signaling.[48] Our overarching hypothesis is that our combined non-neuronal cell targeted treatment will reverse this maladaptive plasticity by both suppressing microglial activation, and restoring astroglial glutamate homeostasis, thus providing analgesia and potentially other symptoms of fibromyalgia.

3.0 Intervention to be studied

Recent research demonstrates microglia are crucial mediators of neuroinflammation and central sensitization, which are both mechanisms that underlie the altered synaptic plasticity responsible for chronic widespread pain.[49] Another microglial inhibitor, naltrexone, has been found to reduce fibromyalgia associated symptoms.[50] While N-acetylcysteine has not been studied clinically in patients suffering from chronic pain, it has been successfully used to treat types of addiction and compulsivity.[51]

- Minocycline is FDA approved. Typical dosing is 100 mg every 12 hours. It is prescribed chronically for certain latent bacterial infections as well as for adjunctive therapy in severe acne vulgaris. Tetracyclines may render oral contraceptives less effective. Like other tetracycline antibiotics, minocycline crosses the placenta and can adversely affect skeletal development and may have other embryotoxic effects. It is also excreted in breast milk and can permanently discolor developing teeth. Photosensitivity may result in exaggerated sunburn reactions, although minocycline is less likely to produce this reaction than other tetracyclines. Sunscreen is not protective from this reaction. CNS side effects may include light-headedness, dizziness, or vertigo, which may dissipate during therapy. These symptoms could affect driving or the operation of heavy machinery. Pseudotumor cerebri (benign intracranial hypertension) has been associated with tetracyclines. Alteration of the normal gut flora may cause diarrhea or even predispose for the development of *Clostridium difficile* associate diarrhea and related complications, which could be mild or as severe as causing fatal colitis if not treated. This potential complication has been reported with nearly all antibiotics. It may also result in the overgrowth of fungi. If minocycline becomes stuck in the esophagus it could cause esophageal irritation and ulceration. Hypersensitivity reactions that included, but were not limited to anaphylaxis, angioedema, urticaria, rash, swelling of the face, and pruritus have been reported. Postmarketing cases of anaphylaxis and serious rash such as exfoliative dermatitis, Stevens-Johnson syndrome, and erythema multiforme have been reported with minocycline. Postmarketing reports have included cases of severe hypersensitivity reactions and autoimmune syndromes including a lupus-like syndrome. Drug Rash with Eosinophilia and Systemic Symptoms (DRESS), including fatal cases, have been reported with minocycline use. Hepatotoxicity has been reported with minocycline; therefore, minocycline should be used with caution in patients with hepatic

disease and in conjunction with other hepatotoxic drugs. Hepatitis, including autoimmune hepatitis, and liver failure have been reported. The antianabolic action of the tetracyclines may cause an increase in BUN. While this is not a problem in those with normal renal function, in patients with significantly impaired function, higher serum concentrations of minocycline or other tetracyclines may lead to azotemia, hyperphosphatemia, and acidosis.

- N-acetylcysteine (NAC) is FDA approved in some formulations and considered a nutritional supplement in others. We will be utilizing pure powder of NAC that will have a Certificate of Analysis. The CoA confirms the chemical powder has the potency labeled, the pH, whether or not it contains heavy metals, the particle size and density, that is it free from bacteria, mold or fungus, and the physical description and appearance of the product. An Investigation New Drug (IND) application will be submitted to the FDA and approval will be required prior to initiation of this study. NAC was introduced in the 1960s as a mucolytic drug for chronic respiratory diseases (e.g., COPD, tuberculosis, bronchiectasis, etc.). It is also used for nephrotoxicity prophylaxis against radiographic-contrast-induced reactions in patients with preexisting renal insufficiency. It has a well-established safety profile and is still commonly used orally at doses of 600 mg/day as a mucolytic. However, at higher doses (≥1200mg), acetylcysteine also acts as an antioxidant through complex mechanisms which can combat conditions of oxidative stress. Acetylcysteine is a derivative of the natural amino acid cysteine, which serves as a substrate for the synthesis of glutathione (GSH) in the body which an antioxidant effect. This reduces the formation of proinflammatory cytokines, such as IL-9 and TNF-α and also has vasodilator properties by increasing cyclic GMP levels and by contributing to the regeneration of endothelial-derived relaxing factor.
- The control/placebo will contain only microcrystalline cellulose, which is refined wood pulp. Chemically, it is an inert substance, is not degraded during digestion and has no appreciable absorption. This has been used by our collaborating pharmacist in previous NAC studies, with no issues or problems reported, however large quantities provide dietary bulk and may lead to a laxative effect. This adverse effect may be seen with a daily dose of 30 grams of M-cellulose. The placebo for the NAC capsule will contain about 300 mg of M-cellulose per capsule, and the placebo for the minocycline capsule will contain about 100 mg of M-cellulose per capsule. The empty placebo capsules will be exposed to a “NAC bomb” prior to being filled so that they smell similar to the active drug. A “NAC bomb” is essentially powdered NAC inside of a panty hose that prevents the medication from escaping but allows the empty placebo capsules to take up the rotten egg smell.
- There will be two different sized capsules used: 600 mg NAC (or placebo) will be in a #1 size capsule and 100 mg of minocycline (or placebo) will fit into a smaller #3 size capsule. Blister packs will be utilized to improve compliance. These will fit both a #1 and #3 capsule in a single blister pack and be labeled “Take both capsules every 12 hours”.

4.0 Study Endpoints

- Primary Outcome: Change in the Revised Fibromyalgia Impact Questionnaire (FIQR) scores. 21 questions; 3 domains = function, overall impact, symptoms; 11-point numeric rating scale; questions framed with the context of the past 7 days. Measured weekly (48-hour response time with reminders every 12 hours until lockout occurs at 48 hours past due).

Secondary Outcomes: (1) Patient Global Impression of Change (PGIC)[56]: Likert scale of patient's rating of overall improvement. The PGIC and the modified 2010 American College of Rheumatology Preliminary Diagnostic Criteria for Fibromyalgia (modified ACR 2010)[57] questionnaire will be utilized at baseline and conclusion of each treatment period.

Other Data Collected: (2) "Pain on average", "interference with Enjoyment of life", "interference with General activity" (PEG)[53]; (3) Patient Health Questionnaire-2 (PHQ-2)[54]: 2 question depression severity measure; (4) Generalized Anxiety Disorder scale (GAD-2)[55]: 2 question anxiety severity measure; The PEG will be measured at baseline and weekly. The PHQ-2 and GAD-2 will be measured at baseline & biweekly during treatment phases & washout periods.

5.0 Inclusion and Exclusion Criteria/ Study Population

The PI and coinvestigators will screen patients based on their pre-existing diagnosis of fibromyalgia. A chart review will also be performed to ensure the subject meets the inclusion and exclusion criteria.

Inclusion Criteria

- Female
- Age 18 years or older
- Formal diagnosis of fibromyalgia and at least 6 months of widespread pain (confirmed by the modified 2010 ACR Diagnostic Criteria for Fibromyalgia Widespread Pain Inventory and Symptom Severity Scale)
- Currently on birth control or unable to become pregnant
- Willingness to avoid taking opioid and opiate medications during the duration of the study (5-6 months)

Exclusion Criteria

- Known hypersensitivity to minocycline or tetracycline antibiotics or to N-acetylcysteine
- Unwillingness to pause opioid therapy thirty days prior to starting the study and during the duration of the study
- Active pregnancy, lactation or plans to become pregnant in the next 6 months
- Significant hepatic disease as indicated by an AST or ALT greater than twice the upper limits of normal or bilirubin greater than twice the upper limits of normal at baseline
- Significant renal disease as indicated by an estimated glomerular filtration rate less than 60 mL/min/1.73m^2 at baseline or during the first washout period
- History of autoimmune syndromes (systemic lupus erythematosus, myasthenia gravis, rheumatoid arthritis)
- History of intracranial hypertension or pseudotumor cerebri
- History of IBD (Crohns disease, ulcerative colitis), *Clostridium difficile* infection

- History of esophagitis, esophageal obstruction, achalasia or esophageal dysmotility
- History of GI hemorrhage or known risk factors for GI hemorrhage, such as esophageal varices, peptic ulcer disease, etc.
- Unwillingness to limit intake of divalent and trivalent cations such as oral iron supplements, certain dietary supplements (multivitamins) that contain manganese or zinc, or antacids that contain aluminum, calcium, or magnesium, for at least three hours before and after taking study drugs, which would decrease minocycline absorption
- Subjects taking anticoagulant medication since minocycline can decrease plasma prothrombin activity
- Subjects taking isotretinoin
- Subject taking ergot alkaloids for migraines
- Subjects taking penicillin antibiotics
- Subjects who work outdoors or otherwise have prolonged exposure to UV light and sunlight
- Lack of access to reliable technology to be able to complete emailed REDCap questionnaires
- Cognitive deficits that may make it difficult to adhere to the medication regimen or provide consistent and timely completion of questionnaires
- Inability or unwillingness to give informed consent
- Unwillingness to use two forms of birth control for the entire duration of participation in the study (if capable of becoming pregnant)
- Unwillingness to complete home pregnancy tests throughout the study (if capable of becoming pregnant)
- Inability to swallow large pills

6.0 Number of Subjects

14 subjects recruited with the goal of 12 patients completing the study

7.0 Setting

The protocol will mostly be conducted remotely (telephone, video conference, text or email), however three in-person lab visits will be required to obtain blood draws for a comprehensive metabolic panel and a urine sample for pregnancy testing (if applicable) at baseline and during each washout period. The baseline study visit appointment will require an in person visit with study personnel for consenting, which will be done electronically using the REDCap eConsent platform, and enrollment, distribution of study meds and administration of study instruments. Subjects will receive all 8 weeks of study medication at the start of each treatment period. Subjects will complete all measures via REDCap surveys that will be delivered electronically to them via email with a secure link to REDCap or the REDCap application.

8.0 Recruitment Methods

Subjects will be recruited through the MUSC Pain Clinic by the PI and coinvestigators, but some patients may be referred to the study from MUSC colleagues who routinely collaborate with the Pain Clinic staff.

The flyer will also be mailed, emailed and sent via mychart message to potential participants diagnosed with fibromyalgia utilizing MUSC's patient outreach recruitment strategies. The communications will include the required direct contact information letter. Doing this will allow us to be inclusive of all potential subjects diagnosed with fibromyalgia since they are seen in various clinics that we may not have access to.

Placement in flyers in community areas such as pharmacies, MUSC clinical areas (waiting rooms and bulletin boards).

Those who express interest in participation will be provided a phone number and email to contact our research support staff who will then provide additional information and a pre-screening survey. A chart review of each potential participant who completes the screening survey will be conducted to ensure that no exclusionary criteria are indeed present.

9.0 Consent Process

Informed consent will occur in person during the initial screening visit. At this visit, participants will give consent electronically by using the REDCap eConsent platform. Potential participants will be given time to read the consent. Adequate time will be taken to review the consent and HIPAA documents with the subject and answer all questions.

10.0 Study Design / Methods

- A double-blind, randomized, crossover design will be used. Fourteen female fibromyalgia patients will be recruited with a goal of 12 patients completing the study. This pilot study will only include women, which reflects the affected population with most diagnosed cases being in women. Population based studies have shown lifetime prevalence to be 2:1 for women compared to men. This study seeks to assess the multimodal effects of the intervention in the most at-risk population.
- Prior to enrollment, a urine sample for a pregnancy test (if appropriate) and a blood draw for a comprehensive metabolic panel (CMP) will be assessed. This lab work will be completed at a MUSC lab and will not be billed to the patient. If the potential participant is pregnant or has evidence of significant kidney or liver dysfunction they will not be enrolled.
- A simple randomization method will be used to assign the initial treatment and then the opposite treatment will ensue for the second treatment period. Patients will be randomized to (i) 8 weeks of placebo minocycline and placebo NAC, minimum 2 week washout period, and then 8 weeks of a combination minocycline (200 mg daily) and NAC (1200 mg daily) followed by a final 2 week washout or (ii) 8 weeks of a combination minocycline (200 mg daily) and NAC (1200 mg daily), minimum 2 week washout period, and then 8 weeks of placebo minocycline and placebo NAC followed by a final 2 week washout. The washout period is intended to capture any post-treatment changes in the primary and secondary outcomes following the conclusion of each active treatment phase. Treatment will be in addition to standard of care. Our primary outcome is change in the Revised Fibromyalgia Impact Questionnaire (FIQR) score[52]. Secondary clinical outcomes include subjective measurements of pain and interference (PEG)[53], depression (PHQ-2)[54], anxiety (GAD-2)[55], patient global impression of change (PGIC)[56]. The modified 2010 American College of

Rheumatology Preliminary Diagnostic Criteria for Fibromyalgia (modified ACR 2010)[57] questionnaire will be utilized at baseline and conclusion of each treatment period.

- Subjective pain measurements: Validated psychometric assessments will be utilized and delivered directly to the patient's email for response via the REDcap survey instrument.
  - Primary Outcome: Change in the Revised Fibromyalgia Impact Questionnaire (FIQR). 21 questions; 3 domains = function, overall impact, symptoms; 11-point numeric rating scale; questions framed with the context of the past 7 days. Measured weekly (48-hour response time with **reminders every 12** hours **until lockout occurs at 48 hours** past due).
  - Secondary Outcomes: Impression of Change (PGIC)[56]: Likert scale of patient's rating of overall improvement.
  - Other Collected Date: (1) "Pain on average", "interference with Enjoyment of life", "interference with General activity" (PEG)[53]; (2) Patient Health Questionnaire-2 (PHQ-2)[54]: 2 question depression severity measure; (3) Generalized Anxiety Disorder scale (GAD-2)[55]: 2 question anxiety severity measure; (4) Patient Global The PEG will be measured at baseline and weekly. The PHQ-2 and GAD-2 will be measured at baseline & biweekly during treatment phases & washout periods. The PGIC and modified ACR 2010 will be measured at the end of each treatment phase.

Clinical Procedure	Virtual/Phone Screening Visit	Pre-enrollment Labs	In-person Baseline Visit & Enrollment	Treatment Period 1 (8 weeks)	1st Washout (2 weeks) In-person Visit	Treatment Period 2 (8 weeks)	2nd Washout In-person Visit
Screening Inclusion/Exclusion	X						
Medical History/Chart Review	X						
Informed Consent			X				
Fibromyalgia Diagnosis (ACR)	X emailed to potential participant						
Randomization			X				
Study drug administration			Provided by study staff according to randomization		Provided by study staff according to randomization		
CMP		X			X		X
Urine HCG		X			X		X
FIQR & PEG			X	Weekly	Weekly	Weekly	Weekly
PHQ-2 & GAD-2			X	Biweekly	Biweekly	Biweekly	Biweekly
PGIC & ACR				End of treatment		End of treatment	
Telephone call to assess medication side effects				End of 1st week		End of 1st week	
Adverse event assessment & recording				Weekly	Weekly	Weekly	Weekly

Analysis Plan: The primary goal is to estimate the effect of multimodal treatment of fibromyalgia patients (standard of care + minocycline + NAC) relative to standard of care on subjective pain measurement (FIQR). All outcomes will be evaluated using a linear mixed model (LMM) approach. Models will include fixed effects for treatment regimen, time, and a treatment by time interaction as well as a random subject effect to account for repeated measures on subjects. Differences within and between treatment regimens will be estimated using a series of linear contrasts from the LMM. We will enroll 14 subjects to allow for up to 14% loss to follow-up. A sample size of 12 subjects provides a two-sided 95% confidence interval with a distance from the mean paired difference of ± 0.64 standard deviations. The analysis will be conducted as an intent-to-treat analysis.

11.0 Data Management

- Study-related records will be kept in the research office of the Department of Anesthesia & Perioperative Medicine, which is a physically secure location (locked office door and locked file cabinets). The REDCap survey instruments and associated data will be stored within the secure MUSC network. All study personnel will conduct themselves with integrity and work to maintain confidentiality at all times. All personnel will have current CITI certifications.
- Quality control will be ensured as all data will be collected utilizing the same REDCap survey instruments.

12.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

- The study will be reviewed annually by the Department of Anesthesia's Data Safety Monitoring Board (DSMB). Minutes and outcomes from these meetings will be reported to the IRB as required.
- Adverse events will be recorded, reviewed and reported to the Department of Anesthesia's DSMB and the IRB per policy.
- PHI will be managed in a manner that complies with institutional rules and regulations. There will be an enrollment log that links the study ID number to the patient. This log will be kept on a MUSC password protected server that can only be accessed by IRB approved study personnel.
- Any side effects from the study will be evaluated by the principal investigator or co-investigators. The research nurse will unblind investigators if necessary, to further assess side effects or other study-related adverse events.
- In the event that a woman becomes pregnant during her study participation she will be followed until resolution of the pregnancy. Any fetal anomalies will need to be submitted to the IRB (and FDA) as SAEs.
- The weekly electronic surveys are very detailed with respect to assessing for adverse drug reactions and worsening symptoms. Should the patient report "NEW or WORSENING symptoms and/or medication side effects over the past week" in the "mild" range, a member of the research team will contact the patient to get more information. A virtual office visit with the PI or one of the co-I's will be offered if desired by the participant. If the severity is rated as "moderate" or "severe", a member of the research team will contact the patient to get more information and to coordinate a virtual or in-person office visit with the PI or one of the Co-I's.
- Regarding depressed patients, we anticipate that comorbid depression will be present in some participants as this is commonly associated with FMS. If a participant's

depression score (PHQ-2) is worsening a member of the research team will contact the patient to get more information and to coordinate a virtual office visit with the PI or one of the co-I's. The investigators will assess the patient and determine the appropriate treatment plan and whether or not the participant should remain in the study.

13.0 Withdrawal of Subjects

- The following circumstances could result in subject withdrawal:
  - Development of any significant adverse drug effects
  - Positive test for COVID-19
- Subjects may be terminated by the investigator for the following:
  - Failure to submit two consecutive weekly questionnaires or more than three weekly questionnaires over an 8-week treatment phase
  - Poor adherence to taking the study medication as instructed
- Subjects who voluntarily withdraw will no longer receive their weekly REDCap assessments and be instructed to stop taking the study medication and discard it immediately. Their data will remain for analysis as part of our intention-to-treat.

14.0 Risks to Subjects

- Adverse medication effects could cause subjects to stop the study prematurely. These are listed above. All subjects will be monitored for these events as they will be asked weekly about any new or worsening symptoms and side effects. Common adverse drug effects are generally mild/low risk.
- Relying on email-generated survey instruments for data collection could be a pitfall if subjects experience local internet or power outages or other barriers that prevent timely completion of the assessments. This could also be psychologically stressful for subjects if there are technical problems with completing the questionnaires.
- The study drug minocycline may have risks to an embryo or fetus should the subject be or become pregnant. We will require that subjects who have the capability of becoming pregnant take a pregnancy test at baseline, at their appointment during the first washout period and at the end of the study
- Loss of confidentiality is a risk with any clinical research.
- The study may have risks to the subjects that are currently unforeseeable.
- The study is highly unlikely to put others at risk who are not study subjects.

15.0 Potential Benefits to Subjects or Others

- Subjects may experience decreased pain and improvement in symptoms related to their fibromyalgia (fatigue, mood, cognition, etc.)
- There may be no direct benefit during the placebo phase or treatment phase of the study.

16.0 Sharing of Results with Subjects

We do not intend to share the results of the study with subjects outside of allowing them to read the abstract and manuscript if desired. Should a subject request their FIQR scores or any secondary outcome results, we'll supply that information on a case-by-case basis. In order to maintain blinding we would not provide this information until the conclusion of the study or completion of subject disenrollment.

17.0 Drugs

Dispensing of all study-related medication (both active drugs and placebo) will occur by our study personnel during the baseline visit for the first treatment phase and initial washout visit for the second treatment phase. Pitt Street Pharmacy will appropriately compound our subjects' medication for each treatment period according the randomization allocation provided by our statistician. The PI, study personnel distributing the medication nor the subjects will know if they are receiving active drug or placebo in order to maintain blinding. Social distancing, face coverings, and other standard precautions in accordance to MUSC Policy and CDC guidance for COVID-19 will be employed. Proper storage instructions for the medication will be included with receipt of the medication.

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Statistical Analysis Plan: The primary goal is to estimate the effect of combinatorial treatment of fibromyalgia patients (standard of care + minocycline + NAC) relative to standard of care on subjective pain measurement (FIQR). All outcomes will be evaluated using a linear mixed model (LMM) approach. Models will include fixed effects for treatment regimen, time, and a treatment by time interaction as well as a random subject effect to account for repeated measures on subjects. Differences within and between treatment regimens will be estimated using a series of linear contrasts from the LMM. We will enroll 16 subjects to allow for up to 25% loss to follow-up. A sample size of 12 subjects provides a two-sided 95% confidence interval with a distance from the mean paired difference of ± 0.64 standard deviations. The analysis will be conducted as an intent to treat analysis.