Impact of colchicine and low-dose naltrexone on COVID-19 disease progression and clinical course in hospitalized patients

Daniel Delaney, PharmD; Elie Gertner, MD; Sadia Ali, MD; Christine Behrendt, PharmD; Joseph Johnson, MD; Alison Knutson, PharmD; Marek Kokoszka, MD; Meghan O'Brien, MPH; Tawatchai Paisansinsup, MD; Rebecca Peglow, MD; Anne Schullo-Feulner, PharmD; Daniel Sandgren, PharmD; Michael Schnaus, MD; Paula Skarda, MD.

1. Summary

The purpose of this study is to explore the impact of two medications—colchicine and low-dose naltrexone (LDN)—relative to standard of care (SOC) on COVID-19 disease progression to severe/critical illness and/or intubation in patients hospitalized with moderate COVID-19. As we have learned, COVID-19's clinical course suggests that the hyperinflammatory response seen in severe/critical cases is involved in the pathogenesis of associated adverse sequelae such as acute respiratory distress syndrome (ARDS), thromboembolic disease, and acute cardiac injury. Given colchicine has demonstrated clinical utility in inflammatory syndromes within these systems (e.g. endothelial/vascular/myocardial), and LDN acts both to boost the immune system, and limit an excessive response; they may prove useful in minimizing the risk of disease progression and associated adverse sequelae.

2. Study Aims

Primary Outcome

Relative to standard supportive care, the primary aim of this study is to determine the impact of colchicine and LDN, administered alone or in combination, on the progression of moderate COVID-19 to more severe/critical disease. We also aim to determine whether the addition of LDN to colchicine changes the odds of progression towards more severe/critical COVID-19 versus colchicine or naltrexone alone. Moderate illness is defined as patients requiring hospitalization due to laboratory confirmed COVID-19, and a clinical score of 2 or 3 (see below). Additionally, to prevent enrollment of patients with incidental COVID-19 findings, they must meet one or more of the following criteria at some point in the 48 hours preceding enrollment:

- Dyspnea limiting usual activities on baseline O2 needs
- Respiratory rate ≥ 30/min at or above baseline O2 needs
- Blood oxygen saturations ≤94% on room air (or on baseline O2 needs if on home oxygen therapy prior to presentation at the hospital).
- Requiring supplemental O2 above baseline needs (i.e. new receipt of supplemental

- O2 or an increase over typical oxygen requirements in patients on existing O2 regimen)COVID-19 contributed to the current hospital admission, per attending provider's clinical assessment of the patient.

For the purposes of our primary outcome, progression of moderate COVID-19 (score of 2-3) to severe/critical illness (score of 4-6) is defined as an increase of two or more points on a 6-point ordinal scale. Patients enrolled in the study with a baseline score of 2 must progress to at least 4 in order to meet the primary outcome. Similarly, those enrolled in the study with a baseline score of 3 must progress to at least a 5 in order to meet the primary outcome. Patients who progress from a moderate score of 3 to a severe score of 4 will not be considered to have met the primary outcome. This will ensure comparability across severity groups, as both 2s and 3s will be required to move the same number of points on the ordinal scale to meet the primary outcome.

Clinical Score Scale

Severity	Score	Definition
N/A	1	Discharged or ready for discharge (i.e. not requiring further medical care or monitoring - afebrile, O2 sats >94% on room air or baseline O2 needs, respiratory rate < 24 per minute [all x 48 hours])
Hospitalized, moderate disease	2	Hospitalized, not requiring supplemental O2 (but requiring ongoing medical care or monitoring). If decreasing from a 3, must be at baseline O2 requirements for 24 hours.
	3	Hospitalized requiring supplemental oxygen via nasal cannula (for a minimum of 24 hours if score increasing from 2 or decreasing from a 4)
Hospitalized, severe disease	4	Hospitalized on high-flow nasal cannula (HFNC) or noninvasive positive-pressure ventilation (NIPPV) support, (for a minimum of 24 hours if score is increasing from 3 or decreasing from a 5). Patients with baseline use of NIPPV, e.g. nocturnal CPAP/BiPAP for obstructive sleep apnea must also require qualifying O2 support during the day to obtain a score of 4.
Hospitalized, critical disease	5	Mechanically ventilated, or a required transfer for ECMO
N/A	6	Death

Secondary Outcomes

To investigate the effects of colchicine, alone or in combination with LDN, relative to standard supportive care on the following:

- Obtained via Epic
    - Composite in-hospital mortality

- Total duration of hospitalization (hours) from first dose of any study medication (or when first dose would be for standard of care arm) to hospital discharge (or when ready for discharge but remains hospitalized for non-medical reasons [e.g. transitional care unit placement delays])
- Total duration of ICU admission (hours)
- Total duration of intubation (hours)
- Total duration of HFNC or NIPPV (hours), (excluding baseline NIPPV use, as above) from first dose of study drug (or anticipated first dose, if in standard of care arm) to discharge
- Total time above baseline oxygen requirements (hours) from first dose of study drug (or anticipated study drug, if in SOC arm) to discharge (i.e. patients not on supplemental oxygen prior to presentation at the hospital = time on any supplemental oxygen after first dose/anticipated dose of study drug to discharge); for patients on home oxygen therapy of two liters per minute via nasal cannula = time on oxygen >2 liters per minute via nasal cannula or any other oxygen delivery device from first drug dose/anticipated dose to discharge).
- In-hospital days with a fever ≥38 degrees Celsius (00:00 to 23:59:59) from start of first dose of study drug (or anticipated first dose, if in standard of care arm) to discharge.
- Adverse events related to colchicine or LDN
- Incidence of significant adverse outcomes associated with/attributable to COVID-19; including ARDS, thromboembolic disease, myocardial injury, and acute kidney injury. Must be documented in patient's problem list during their hospitalization, but not present or clinically suspected prior to receiving the first dose of any study medication (or when a dose would be due if randomized to standard of care).
- Lab values (captured and recorded per the Schedule of Events below) obtained during hospital admission and coded as low, normal, high per hospital reference range
- Cumulative dose of corticosteroids received in ED/hospital (excluding prior to admission/pre-existing use of steroids for an alternative indication); converted to a single equivalent unit.
- Cumulative dose of remdesivir received
- Discharge anticoagulation needs (i.e. number of patients discharged on a direct oral anticoagulant specifically prescribed for prophylaxis of increased venous thromboembolism risk due to COVID-19)

3. Background and Rationale

In December 2019, a novel coronavirus caused a cluster of pneumonia cases in Wuhan, China. The identified virus was officially named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated illness named coronavirus disease (COVID-19) (WHO). In the months since its discovery, the spread of SARS-CoV-2 has led to tens of millions of cases worldwide. In the United States alone, there have been over 11.6 million cases of COVID-19, with over 340,000 attributed deaths reported as of December 31, 2020 (CDC Cases in the US). As is common with emerging infectious diseases, the magnitude of their novelty is inverse to the quality of accessible treatment

options. COVID-19 is no exception to this, and despite the recent availability of a preventative vaccine, there are limited pharmacotherapeutic treatment options for those with an active infection, the majority of which remain investigational.

Common symptoms among patients with mild disease include fever, altered senses of smell or taste, fatigue, or cough. More severe cases of COVID-19 may lead to hypoxemia and pneumonia. When compared to those with less-severe disease, severe/critical COVID-19 pneumonia generally results in an increase in circulating proinflammatory chemokines and cytokines. These changes have been implicated in the pathogenesis of a hyperinflammatory response, and disease severity has been shown to correlate with the level of these markers (1-7). Hyperinflammation is apparent with evidence of increased circulating levels of IL-1b, IL-6, IL-8, IL-12, IL-17, IL-18, TNF-α, and IFNγ (1-2, 4-5, 8), and this "cytokine storm" plays a role in the development of adverse sequelae seen with COVID-19, such as ARDS (8-10), thromboembolic disease (9, 11-14), and myocardial injury (15-20).

The SARS-CoV-2 virus has specific structural components (e.g. viroporin E and viroporins 3a and 8A) that have been shown to activate NLRP3 inflammasome (21-22). NLRP3 has been found to be a major pathophysiologic component in developing ARDS (23-25); and inflammasome activation along with subsequent cytokine production is seen with myocardial injury, which may provide mechanistic insight to SARS-CoV-2's ability to cause cardiac insult (26-27). Additionally, aberrant activation of neutrophils and formation of excessive neutrophil extracellular traps (NETs) were found to be associated with ARDS, and recently to be the potential central cause of severe/critical COVID-19 (10).

Treatments that influence neutrophil recruitment to sites of inflammation and hyper-inflammatory response may prove beneficial for reduction in disease progression, adverse sequelae, and mortality associated with COVID-19 infections. Agents under investigation to mitigate this detrimental host response include the IL-6 receptor antagonists (28). However, their extreme expense, scarce availability, and high-risk for severe adverse effects relegate the use of these injectable agents to cases that have already progressed to critical illness.

Colchicine

Colchicine is an oral anti-inflammatory agent that is relatively inexpensive, readily available, and has been used for generations. Approved for treatment and prophylaxis of gout flares and Mediterranean fever, it is also used in a variety of other inflammatory conditions (e.g. pericarditis and diffuse vascular inflammation such as Behcet syndrome) (29-31). Colchicine binds to tubulin causing depolymerization, which interferes with neutrophil chemotaxis, adhesion, and mobilization to sites of inflammation, and contributes to reduction in superoxide production; through interference of the NLRP3 inflammasome protein complex, colchicine inhibits IL-1b, IL-6, and IL-18 production. As described, these are recognized as playing an important role in acute coronary syndrome (32-33), pericarditis, and ARDS. In addition to these anti-fibrotic and cardio-protective features, colchicine has an anti-apoptotic action on endothelial cells (34, 35) that may provide benefit in minimizing extravasation, capillary leak (36), and therefore progression or development of ARDS. Lastly, coronavirus replication, virion particle assembly, and subsequent exocytosis from the host cell, have been shown to rely on cytoplasmic structural proteins (microtubules) for trafficking during its lifecycle

(37). Perhaps most importantly, this includes the constitutive exocytic pathway responsible for releasing newly synthesized virions (38). Disrupting microtubule trafficking has the potential to interfere with these key viral replication steps, and therefore introduction of a microtubule depolymerizing agent may help treat a coronavirus infection through decreased viral replication (37-40).

Considered together, colchicine's anti-inflammatory benefits, well-known and minimal toxicity profile, in conjunction with the pharmacologic properties detailed above, justify the investigation of colchicine as a treatment option to minimize disease progression for patients hospitalized with moderate COVID-19.

The use of colchicine in patients hospitalized with COVID-19 has been studied in several small proof-of-concept, uncontrolled case series, and comparative cohort studies, as well as small, randomized controlled trials (41-46). Results have been unanimously favorable thus far, though they have substantial limitations, and two remain unpublished pre-prints (43, 46). Some were performed during local COVID-19 surges, rates of intubation and mortality were likely inflated as a result, and therefore the benefits of colchicine may be overestimated.

Low-Dose Naltrexone

Most well known as an opioid antagonist, or a treatment for alcohol dependence, naltrexone also possesses immunomodulatory effects. Seen exclusively at low doses, this attribute is being employed in the pain community as a novel anti-inflammatory agent that has been shown to reduce symptom severity in fibromyalgia, Crohn's disease, multiple sclerosis, and complex regional pain syndrome (47-49). Naltrexone is a 50:50 racemic mixture of both L and R isomers - in theory, these isomers allow it to act in a dualistic fashion, both boosting the immune system yet limiting an excessive immune response. The L isomer is a competitive inhibitor of the Mu and Kappa opioid receptor, resulting in elevated levels of endogenous endorphins and enkephalins, which in turn, functions as an immune enhancer. The R isomer blocks the toll like receptor and modulates T and B cell activity; the downstream effects limit the release of inflammatory cytokines including IL-6, IL-12, TNF alpha, and NF-KB (47). A study in 2017 demonstrated a significant reduction in cytokines after eight weeks of therapy in eight female patients (50).

While preliminary LDN studies demonstrate success in Crohn's disease, multiple sclerosis, and fibromyalgia, there is still a need for follow-up trials to establish definitive evidence of benefit (47-49). The lack of proprietary value in a generic medication with no industry backing has hampered widespread adoption and FDA approval. Thus, clinical efficacy data is slowly forthcoming on LDN. At the same time, the safety profile of naltrexone is well established and very well tolerated in alcohol use disorder. Considering that is a high-risk population, and the dose used is significantly higher (25-100mg versus 1-4.5mg for LDN), it stands to reason that LDN should have little to no toxicity. Hepatic function has to be monitored at higher doses, and the drug should be stopped if necessary. However, with LDN the majority of adverse drug effects are constitutional (headache, dizziness, nausea etc.), with no end organ damage and no evidence for opportunistic infections.

From a neurological standpoint, LDN has an affinity for microglial cells, which has been shown to reduce neuroinflammation (47). A study of 214 CoVID-19 patients in Wuhan China found that 45.5% (40/88) of severe patients had neurological symptoms (51). This was defined in three categories as (A) central: headache, dizziness, impaired consciousness, ataxia, acute cerebrovascular disease, and epilepsy; or (B) peripheral: hypogeusia, hyposmia, hypopsia, and neuralgia, and (C) skeletal muscular symptoms. The authors did note possible confounding factors such as hypoxia or hypercarbia (51). Interestingly, during the SARS-CoV outbreak in 2002-2004, post-mortem pathological evaluation in animals and patients demonstrated a high viral presence in the brainstem, demonstrating invasion into the medullary cardiorespiratory center (52). Whether or not SARS-CoV2 carries the same capabilities remains to be seen, but early evidence indicates 30-50% of patients hospitalized with COVID-19 will have neurological manifestations (53).

As previously outlined, available evidence demonstrates that excessive immune response in the form of a “cytokine storm” plays a key role in the pathophysiology of COVID-19 offering this process as a possible target for drug therapy. Known immunomodulatory effects of LDN suggest that this drug could be used to reduce this exaggerated immune response. Additionally, the frequency of neurologic symptoms associated with COVID-19 implies that there may be a treatment role for a drug with neuro-anti-inflammatory properties, such as LDN.

In summary, the proposed actions of LDN in the treatment of SARS-CoV-2include its potential to act as an immune enhancer via the upregulation of the endogenous opioid system, with increased endorphins and enkephalins, decrease multiple inflammatory cytokines, including IL-6, IL12, TNF alpha, and NF- KB. Combined, this may blunt the hyperinflammatory response and subsequent injury that can lead to, and has been observed in, critically ill COVID-19 patients

While the efficacy of LDN is being studied in COVID-19 (NCT04604704, NCT04604678, NCT04365985) in combination with a nutritional supplement, metformin, and ketamine (respectively), only the latter trial is recruiting at this time, and none includes colchicine.

Summary

An important step in mitigating the morbidity and mortality of COVID-19, both from an individual and community standpoint, is minimizing the duration of hospitalization to extend healthcare capacity, frequency of disease progression to more severe illness, and need for more invasive, finite, respiratory support (mechanical ventilation). Treatment modalities aimed to reduce severe, resource-intense cases of COVID-19 should be prioritized to alleviate an overburdened healthcare infrastructure.

To that end, this work will specifically determine if there is clinical utility of colchicine and low dose naltrexone (alone or in combination), relative to SOC in decreasing the incidence of both 1) progression of moderate COVID-19 to severe/critical illness, and 2) its subsequent complications.

The above rationale for the use of these two agents specifically notes that combining them is not yet being investigated elsewhere. The combination is worthy of inclusion in this research for multiple reasons. Chiefly: there is no evidence to suggest that combining these agents would be harmful,

there are no known drug interactions, and we have decades of clinical experience and safety data available for both of these well-tolerated, low-toxicity agents. Outside of its novelty, their pharmacologic actions in blunting a hyperinflammatory response act independently of each other, with similar downstream effects (e.g. decreased cytokine production). Their complementary mechanisms may therefore have an additive, potentially synergistic, impact. Further, their mechanisms work independently of the anti-inflammatory agents used in COVID-19 that come with significant potential for toxicity, namely glucocorticoids. This may provide further anti-inflammatory benefit, and potentially generate additional hypotheses for research regarding ways to minimize the need/use of higher-risk anti-inflammatory agents.

As described, the available evidence and rationale to assess colchicine and LDN for use in COVID-19 are currently found within distinct areas of clinical and pharmacologic knowledge. To ethically test this novel intervention and bridge this siloed information, a clinical trial that measures their potential impact on COVID-19's clinical course is required.

4. Approach

a. Study Design

This will be a four arm, prospective, randomized, open label trial in patients hospitalized with moderate COVID-19.

b. Population

i. Inclusion/Exclusion Criteria

To be eligible for this study, patients must meet the following Inclusion criteria at enrollment:

1) Male and (non-pregnant, non-breastfeeding) females aged 18 years or older
2) Requiring admission to Methodist or Regions Hospital due to laboratory-confirmed COVID-19
3) Meets criteria of only up to moderate COVID-19 disease as defined by a clinical score of 2 or 3 at the time of enrollment, and one or more of the following noted at some point in the 48 hours preceding enrollment:
    a) Dyspnea limiting usual activities on baseline O2 needs
    b) Respiratory rate ≥30/min on O2 or room air
    c) Blood oxygen saturations ≤94% on room air (or on baseline O2 needs if on supplemental oxygen prior to presentation at the hospital for a condition unrelated to COVID-19).
    d) Requiring supplemental 02 above baseline needs (i.e. prior to presentation at hospital)
    e) COVID-19 contributed to the current hospital admission, per attending provider's clinical assessment of the patient.

4) Ability to provide written informed consent, or has identifiable LAR that is able to do so on the patient's behalf as defined by study protocol, prior to performing study procedures.

<u>They cannot meet any of the following exclusion criteria at enrollment:</u>

1) Patients meeting criteria for severe/critical COVID-19 as defined by study protocol or requiring O2 supplementation ≥10L nasal cannula at screening
2) Patients currently in shock as defined by hemodynamic instability requiring vasopressors
3) Patients with a current hospitalization for COVID-19 that is >/=7 days at the time of screening.
4) Clinical estimation of attending physician that the patient will require mechanical respiratory support within 48 hours of enrollment
5) Patients in which EITHER symptom onset OR a positive COVID-19 laboratory test occurred ≥14 days prior to enrollment.
6) Patients with concomitant influenza A or B at time of hospitalization if tested as part of ED/hospital admission.
7) Female patients who are pregnant or breastfeeding at time of hospital admission
8) Diagnosis of Chronic Kidney Disease stage ≥4 as documented in the patient's problem list (not based on CrCl calculations alone)
9) CrCl < 30 mL/min or requiring renal replacement therapy (e.g. intermittent hemodialysis, continuous renal replacement therapy, peritoneal dialysis) at screening
10) History of cirrhosis or advanced liver disease, or active hepatic viral infection
11) Transplant of kidney, lung, heart, or liver in the past 2 years
12) Uncontrolled severe gastrointestinal disorders, Crohn's disease, ulcerative colitis, chronic diarrhea, diarrhea predominant irritable bowel syndrome, active stomach or intestinal ulcer, or one that was treated within the last 6 months
13) Patients currently receiving agents that are p-glycoprotein **AND *strong*** CYP3A4 inhibitors with CrCl < 60 mL/min, or any combination of drug interactions that is not amenable to dosage adjustment (refer to list of medications with potential Colchicine and Naltrexone interactions).
14) Patients actively undergoing chemotherapy for an active malignancy, or history of a hematologic malignancies
15) Chronic or current use of colchicine or any mu-opioid antagonist.
16) Chronic, scheduled opioid therapy (i.e. not intermittent as needed use), or, prior to enrollment, an acute condition requiring continued pain control that is unattainable without ongoing opioid therapy.
17) Pre-existing condition that is being treated with tocilizumab, anakinra, sarilumab, other interleukin-antagonists, TNF-inhibitors, or JAK inhibitors.
18) NOTE: Patients treated with tocilizumab will be permitted to enroll if their care team is us prescribing it for COVID-19. Use of tocilizumab at baseline for another indication will continue to be excluded.

19) Participation in any other clinical trial of an experimental treatment for COVID-19, **note:**
    a) While convalescent plasma is no longer recommended within HP, it can be given if deemed appropriate by the medical team once ≥ 24 hours has elapsed since enrollment
    b) Patients previously enrolled in the C3PO study can enroll in this study, as any convalescent plasma received would have been outpatient
    c) Remdesivir is allowed per standard protocol
    d) Dexamethasone is allowed per standard protocol
20) Patients actively enrolled in hospice or on palliative care
21) History of hypersensitivity reaction to colchicine or its inactive ingredients
22) History of hypersensitivity reaction to naltrexone or its inactive ingredients
23) Incarcerated or a ward of the state
24) Any patient considered an unsuitable candidate, for any reason, by study investigators.

ii. Sample Size

We will enroll up to 180 (136--168 estimated) patients between both sites (Methodist and Regions hospitals). Once enrollment has reached ~25% of anticipated sample size in each arm, an informal interim assessment will be performed to assess intervention futility. Due to current strains on staffing, we do not want to add any unnecessary burden for hospital staff including enrolling and consenting patients in the proposed study if the intervention doesn't have a noticeable positive effect. If this initial look shows no obvious difference in rate of severe/critical disease progression, the research team and clinicians providing care will be involved in deciding whether to terminate the study and will promptly alert the IRB if this determination is made. See Power Analysis section for more information. If we do move forward, another interim assessment may be recommended when enrollment reaches ~50% in each arm. We will update the IRB either way.

c. Data Collection Process

i. Process for Identification

Using the system-wide Epic report for COVID-19 testing, the research team will screen for patients. All patients with a pending or positive result will be screened for inclusion. Emergency room physicians, attending physicians, infectious diseases physicians, and clinical pharmacists may also contact the research team to refer a patient for enrollment.

ii. Recruitment

Upon verification of eligibility based on inclusion and exclusion criteria, the research team will contact the patient's attending provider to discuss if the patient is a viable candidate, and if they would like the patient to be approached for consent into the research study. If

in agreement, the attending will mention the study to the patient and notify them that they are eligible for a research study and that a member of the research team will contact them in the near future.

At this time, the other studies actively enrolling patients for the use of investigational agents for the treatment of COVID-19 patients at Methodist and Regions hospital include CARDEA and C3PO (the latter of which is outpatient only). The initiation of this study will be unlikely to influence enrollment of these ongoing studies, as they are distinct in their enrollment criteria. In the event that a patient simultaneously meets criteria for more than one study, the research team will contact the patient's attending to discuss whether one study may be a more appropriate option to present to the patient given their clinical status. If, in the attending's opinion, the patient is an equally good candidate for both studies, study staff will present both options to the patient and permit the study participant to decide which research study (if any) they would choose to participate in.

If additional COVID-19 studies are to be initiated, and could potentially conflict with this application, IRB will be updated with a plan for mitigating these concerns. Additionally, there is a COVID-19 registry being created locally and these patients will be a part of that registry. Study staff involved in the Colchicine/LDN study are also involved in the COVID-19 registry started in April of 2020. As many of the medical history elements captured in the registry are of interest to the investigators of this study, the research team will utilize medical history data documented in the COVID-19 registry for the study. This will avoid burdening the study staff with excessive data entry responsibilities and permit more efficient enrollments. The list of data points that will be extracted from the registry for this study are identified in the data collection tool sample that is included with this submission.

iii. Consent

Considering the highly infectious nature of COVID-19, and the unique strains the pandemic is placing on healthcare and supply chain industries, we are requesting permission to utilize an altered approach to patient consent. This altered approach may require some patients to provide verbal consent under the supervision of a witness prior to obtaining the patient's signature for consent. The processes outlined below present the best scenarios for obtaining patient consent while minimizing unnecessary patient interactions, healthcare worker exposure, and consumption of personal protective equipment. This process has been successfully used by the CCRC since March 2020 to ensure that research staff does not enter the patient's room and meets all requirements for compliance. The CCRC follows the FDA's guidance document, "FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Public Health Emergency", to consent patients during the COVID pandemic.

1) Prior to contacting the patient (or the patient's legally authorized representative/LAR) directly, the study team will reach out to the attending to request permission to approach the patient. If the attending gives permission, he/she may introduce the study prior to research staff outreach.

-The attending may provide important information about patient's capacity to consent for themselves and, if the patient is unable to do so, may inform the study team of a family member or other legal guardian who would be an appropriate LAR who could provide consent for research on the patient's behalf.

-If the patient or LAR primarily speaks a language other than English, study staff will procure a copy of the HealthPartners Short Form Consent Form and ensure the informed consent process is completed with help from a certified medical interpreter. As the consent form will not be translated in full, the study team will ensure that an impartial witness is present (listening in by phone) during the entire consent process and will attest to this by signing the Short Form Consent Form. This will be a fourth individual (other than the study participant/LAR, interpreter, and person obtaining informed consent) who is neither (1) part of the study team, nor (2) serving as the patient's primary provider (MD/PA/NP).

2) Once the team determines what conditions need to be in place to obtain informed consent, a research team member will call the patient or LAR to introduce the study and assess if the patient/LAR can access a link to the REDCap electronic consent form on a cell phone, tablet, or other device.

   *Patients who require time to think about the study before making a decision about participating, or who want an easy-to-reference guide about what is involved, will be given a copy of the participant information sheet. This will have contact information about the study team on it so they may reach out with any questions they may have.

3) If the patient or LAR can receive the link to the consent form, the researcher will send them the consent link and guide the patient through consent over the phone while the patient reviews it on their device. Patients/LARs will sign consent electronically through REDCap if they agree to participate in the research study. Both the full informed consent document and the Short Form will be made available electronically. Study staff obtaining consent will countersign the form in REDCap. If applicable, an impartial witness will also sign the consent form.

If the patient/LAR does not have a device with which they can review and sign the consent form via REDCap in real-time:

a) Research staff will provide a paper copy of the full informed consent form (or Short Form, if consent is provided through an interpreter) to a member of the patient's care team. This person will enter the room to deliver the form for the patient to sign.
b) If possible, research staff will stay on the phone with the patient to review the signature pages and ensure (as much a possible) that the form has been signed and dated appropriately.

c) The provider who entered the room to deliver the consent form will collect the signed document. Research staff will meet the provider outside the patient's room and will obtain the consent form (using gloves) and place the signed document in a paper bag.
d) The date and time of consent form collection will be noted on the bag, and it will be placed in quarantine for a minimum of 3 days to avoid possible COVID-19 infection among the research team.
e) The study staff member completing the consent process will document the method of consent as "paper" and will countersign the form electronically in REDCap.
f) If the consenter is an LAR, depending on hospital visitation restrictions, research staff may meet them in person in or outside of the hospital to obtain a signature on the paper consent form.
g) In rare circumstances, an LAR may be able to provide neither an electronic consent nor an in-person consent. In this case, a neutral witness privy to the telephone consent conversation may sign the witness line of the consent form to attest that the LAR was in agreement.

All consents, no matter the form, will include time for patients to ask questions, consult with their treating team or family members, and be asked a few comprehension questions from the research team to ensure the patient understands what they are consenting to take part in.

iv. Data Sources/steps for data acquisition

Patient data will be collected by the research team members that are listed in the IRB personnel section of the application. With the exception of informed consent, all data will be collected through manual chart review/abstraction in Epic. Any necessary correspondence with the patient or care team will happen via phone if possible. Below is the planned schedule of events:

Schedule of Events									
Study time point/activity	**Screening**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Up to Discharge**
Baseline Enrollment Information									
Inclusion & Exclusion Criteria	1								
Informed consent, randomization	1								
Demographics	1								
Past medical history	1								
Detailed baseline O2 needs	1								
Pregnancy testing (as needed)	1								
Clinical Course Monitoring (can be documented following day)									
Safety assessment and reporting		1	1	1	1	1	1	1	1
Concomitant medications	1	1	1	1	1	1	1	1	1
New Drug-Drug Interactions	1	1	1	1	1	1	1	1	1
Colchicine dose frequency (Daily/BID, with rationale if changed)	1	1	1	1	1	1	1	1	1
Study Drug Administration	1	1	1	1	1	1	1	1	1
Max O2 needs, SpO2 range, both q12h	1	1	1	1	1	1	1	1	1
Tmax	1	1	1	1	1	1	1	1	1
Chest imaging (yes/no)	1	1	1	1	1	1	1	1	1
EKG or continuous telemetry (yes/no)	1	1	1	1	1	1	1	1	1
LMWH > 0.5 mg/kg/dose (not cumulative daily dose) or heparin drip?	1	1	1	1	1	1	1	1	1
Level of care	1	1	1	1	1	1	1	1	1
Length of hospitalization									1
Mortality									1
Laboratory Monitoring									
Study time point/activity	**Screen**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Up to Discharge**
BNP	1	1	1	1	1	1	1	1	1
CBC with differential	1	3	1	1	1	3	3	3	4
C-reactive protein	1	3	1	1	1	3	3	3	4
D-dimer	1	3	1	1	1	1	1	1	4
Ferritin	1	3	1	1	1	1	1	1	4
Hepatic Function Panel	1	3	1	1	1	1	3	1	4
Procalcitonin (Serum creatinine)	1	1	1	1	1	1	1	1	1
SCr	1	2	2	2	2	2	2	2	2
Troponin	1	1	1	1	1	1	1	1	1
Key	**1 = Record**	**2 = Minimum of every 48 hours**		**3 = unless ordered as standard of care within the past two days, order on this study day**			**4 = unless ordered as part of standard of care, order within 24 hours of meeting the primary endpoint**		

**Screening and Day 1 procedures may occur on the same day

Additional Clarification - Schedule of Events

Baseline Enrollment Information	
Inclusion & Exclusion Criteria	Assessment of study eligibility at time of hospital admission
Informed consent, randomization	Enrollment in study and assignment to study arm
Demographics	Documentation of patient characteristics (age, gender, race, etc.). See data collection plan for more information
Past medical history	Documentation of diagnoses from problems list at baseline; relevant medical history data extracted from COVID-19 registry. See data collection tool sample for more information
Detailed baseline O2 needs	Documentation of need for supplemental home oxygen therapy at baseline (yes/no); if yes, amount in L
Pregnancy testing (as needed)	Rule out pregnancy for female participants of child-bearing age

Clinical Course Monitoring – Performed daily until discharge	
Safety assessment and reporting	Review of chart for AEs by study staff
Concomitant medications	Review of medications taken in hospital in addition to study drugs (reviewed by study staff/ study pharmacists)
New Drug-Drug Interactions	Assessment of new/updated medications to determine risk of new drug interaction based on change in concomitant medications/revised information about any medication the was previously on.
Colchicine dose frequency (Daily/BID, with rationale if changed)	Documentation regarding any changes in colchicine dosage and reason for doing so
Study Drug Administration	For interventional arms: Administration of colchicine twice per day (08:00, or when morning drugs are given, and 20:00, or when afternoon drugs are given), unless change in patient's health status warrants temporarily stopping or reducing medications. Administration of naltrexone will occur once per day at 08:00 (dose on day 1 can be at any time) or when morning drugs are given.
Max O2 needs, SpO2 range, both q12h	Maximum O2 needs for continuous 12-hour periods beginning at hospital admission; highest and lowest SpO2 during each time period.
Tmax	Maximum recorded temperature patient reached during the study day 0:00-23:59.
Chest imaging (yes/no)	Documentation of whether patient received SOC chest imaging on the study day
EKG or continuous telemetry (yes/no)	Documentation of whether patient received an SOC EKG or is on continuous telemetry on study day
LMWH > 0.5 mg/kg/dose (not cumulative daily dose) or heparin drip? (yes/no)	Documentation of need for more than .5mg/kg total dose of Low Molecular Weight Heparin on study day
Level of care	Documentation of clinical scale score; hospital unit through which patient is being treated (Gen Med, ICU) and any transfers that occur

Length of hospitalization	Documented time from first study drug dose (or when it be if in the SOC arm) to discharge; check daily and document where patient discharged to (home, TCU, etc.)
Mortality	Documentation of patient death at any time during the hospital stay

Laboratory Monitoring	
BNP	Documented during patient's hospital stay as performed per SOC up to the point of discharge
CBC with differential	Documented during patient's hospital stay as performed per standard of care with supplemental research-only labs added as need according to the schedule above
C-reactive protein	Documented during patient's hospital stay as performed per standard of care with supplemental research-only labs added as need according to the schedule above
D-dimer	Documented during patient's hospital stay as performed per standard of care with supplemental research-only labs added as need according to the schedule above
Ferritin	Documented during patient's hospital stay as performed per standard of care with supplemental research-only labs added as need according to the schedule above
Hepatic Function Panel	Documented during patient's hospital stay as performed per standard of care with supplemental research-only labs added as need according to the schedule above
Procalcitonin	Documented during patient's hospital stay as performed per SOC up to the point of discharge
Serum Creatinine (SCr)	Documented during patient's hospital stay as preformed per SOC up to the point of discharge. Research-only labs will be ordered as needed to ensure no more than 48 hours passes between draws up to the point of discharge.
Troponin	Documented during patient's hospital stay as performed per SOC up to the point of discharge

d. Interventions/treatment

Dosing strategy

Colchicine

Patients randomized to a colchicine-containing treatment arm will receive colchicine 0.6 mg twice daily for up to 28 days. On the day of enrollment, provided the first dose can be given prior to 16:00 that day, patients are eligible to receive two doses; the second dose will be scheduled for 22:00. Patients experiencing gastrointestinal side effects (nausea, vomiting, and diarrhea) on twice daily dosing may have the dose decreased to 0.6 mg daily. Dosing will continue twice daily unless there is a change that requires a dose adjustment or an exclusion criterion is met. Dosing deviations above the study protocol will be allowed if medically necessary for the treatment of an additional indication (e.g. colchicine for viral pericarditis).

Renal dosing:

- CrCl* ≥50 mL/min: colchicine 0.6 mg twice daily (standard dose timing to cluster cares of 08:00 and 20:00 will be used.
- CrCl* 30-49 mL/min: colchicine 0.6 mg every 24 hours
- CrCl* 11-29 mL/min**: colchicine 0.6 mg every 48 hours
- CrCl* ≤10 mL/min: doses will be held
-

*Calculated using the Cockcroft-Gault formula
**CrCl < 30 mL/min at the time of screening excludes enrollment at that time point

Hepatic dosing: If severe hepatic function impairment is present, use is not recommended for the purpose of this study.

Drug interactions (54-55): All participants, in all arms of the study will have an evaluation of all current medications for drug interactions, as is the standard of care in hospitalized patients at Methodist or Regions Hospital.

The following two lists contain agents with drug-drug interactions with colchicine, and may exclude patients from enrollment based on a combination of interactions and renal function. Patients requiring continued treatment with medications in the following two lists will require study clinical pharmacist review for eligibility and colchicine dosing (if applicable).

1) **Concomitant medications that may be contraindicated due to CYP3A4 and/or P-glycoprotein metabolic interactions:**

Patients requiring continuation of atazanavir, bocepravir, clarithromycin, cobicistat, cyclosporine, darunavir (with ritonavir), dasabuvir, dronedarone, elvitegravir (with ritonavir), erythromycin, idelalisib, indinavir (with ritonavir), itraconazole, ketoconazole, lopinavir (with ritonavir), nefazodone, nelfinavir, ombitasvir, paritaprevir (with ritonavir), posaconazole, ranolazine, ritonavir, saquinavir (with ritonavir), tacrolimus, telaprevir, tipranavir (with ritonavir), telithromycin, or voriconazole will require study clinical pharmacist review for eligibility and colchicine dosing (if applicable).

2) **Concomitant medications that may pose drug interactions due to CYP3A4 and/or P-glycoprotein metabolic interactions:**

Patients requiring continuation of abametapir, amiodarone, amprenavir, aprepitant, crizotinib, delavirdine, fluconazole, fluvoxamine, fosnetupitant, imatinib, interferon alfa-2A, lasmiditan, letermovir, mibefradil, mifepristone, netupitant, reserpine, tacrolimus, or venetoclax will require study clinical pharmacist review for eligibility and dosing (if applicable).

- Patients receiving fibrates or gemfibrozil during their hospitalization will need to be discontinued prior to the first dose of colchicine (if assigned to either of the two colchicine-containing treatment arms) due to increased risk of myopathy and rhabdomyolysis with concomitant use. Study team members will consult with the attending physician and/or study pharmacists to see if this is an appropriate and safe option for a potential study participant prior to consent and randomization.

- The combination of colchicine and statin therapy (atorvastatin, fluvastatin, lovastatin, pitavistatin, pravastatin, rosuvastatin, or simvastatin) may have an increased risk of myopathy and rhabdomyolysis. Our approach for patients receiving statin therapy will follow the standard of care for this interaction as follows:

    - Patients receiving a statin for **primary** prevention of cardiovascular disease must be, in the opinion of the attending physician, an appropriate candidate for discontinuation of their statin for the duration of the study to be eligible for enrollment. If they are randomized into a colchicine-containing arm of the study, their statin therapy will be held.

    - Patients receiving a statin for **secondary** prevention of cardiovascular disease must be, in the opinion of the attending physician, able to tolerate substitution of their current statin therapy for rosuvastatin 20 mg once daily. If they are randomized into a colchicine-containing arm of the study, statin therapy will be resumed with rosuvastatin 20 mg daily. This is being done in an effort to minimize interruptions in statin therapy, and drug-drug interaction potential.

Low-Dose Naltrexone

Patients randomized to an LDN-containing treatment arm will receive naltrexone 4.5 mg once daily. The first dose can be given at any time during the day of enrollment/randomization, and will be timed at 08:00 daily thereafter (with AM colchicine dose, if in combined colchicine/LDN arm) for up to 28 days (unless new contraindication or exclusion criteria met).

Naltrexone is most commonly used in the treatment of alcohol and opioid dependence at standard doses of 25 to 100 mg daily. At these doses, naltrexone acts as a non-selective, pure opioid receptor antagonist and blocks the effects of opioids by competitively binding to the receptors. However, naltrexone exhibits different effects in relation to its concentration. LDN is considered a daily dose ranging from 1 – 4.5 mg. At these lower doses, naltrexone antagonizes Toll-like receptor 4 on glial cells, reducing the inflammatory immune response (47, 56).

Additional Procedures: Drug Interaction Assessment at Screening/Enrollment

As previously mentioned, to ensure patients are properly screened and identified as eligible/ineligible with respect to prescribed medications, this research team will identify a group of study pharmacists and physicians (including the PI, Dr. Daniel Delaney, and a pain specialist, Dr. Joseph Johnson), who will be available as-needed to field questions regarding any medications a patient is taking during or prior to their hospital stay that may or may not preclude study participation.

Interaction of LDN and Colchicine

As previously described, the use of LDN and colchicine in combination for study purposes is presumed safe, as there are no known drug interactions with these treatments, nor any known interactions affecting metabolism. As this study was designed and developed by a team of pharmacists, many of whom will be involved in safety monitoring and dosing regimen adjustments, all appropriate measures will be taken in response to AEs identified in study participants regardless of their assigned arm, including those receiving both colchicine and LDN.

Randomization

Randomization will be stratified by baseline severity score (2 or 3) to ensure balanced baseline severities between the four arms. Block randomization will be used to ensure equal group sizes. As the final sample size is unknown, we will use small blocks of eight. This is being done in an effort to ensure evenly distributed treatment arms. A research team member will create an Excel file to determine randomization assignments. The Excel file will be numbered from one to 180 (the largest possible sample size). Each number will represent a subject ID, for each ordered block of 8 rows; they will generate 8 random numbers between 0 and 1 with 3 decimal places and no replacement. The subjects with the smallest two values (tier 4) will receive colchicine + standard supportive care (group A), the subjects with the second lowest set of digits (tier 3) will receive colchicine + LDN + standard supportive care (group B), the two subjects with next highest set of digits (tier 2) will receive LDN alone + standard supportive care (group C) and the subjects with the largest two values (tier 1) will receive standard supportive care and no drug (group D). Separate spreadsheets will be used for patients with a clinical scale score of 2 vs 3 at the time of randomization to ensure a balance in baseline in severity among all the arms. When a research team member enters a consented subject into this database, the subject will be considered randomized. Any subject who consents but is not randomized will not receive a sequential study ID, as this would interrupt the Excel file's randomization scheme. Instead, subjects who are not randomized may be referred to as subjects a, b, c, etc. and will be tracked in a separate file.

Patients will be randomized in a 1:1:1:1 fashion to the following treatment:

Randomized Intervention Group			
A	B	C	D
*Colchicine 0.6 mg BID + low dose naltrexone 4.5mg daily + standard supportive care	*Colchicine 0.6 mg BID + standard supportive care	Low-dose naltrexone 4.5 mg daily + standard supportive care	Standard of care

*can receive two doses of colchicine on day 1 if enrollment completed/first dose given before 16:00.

Once randomized, the research team will place an order for the correct study medication panel through Epic to be prepared by pharmacy staff. The research team will use the subject ID generated during randomization to order the correct order panel.

Nursing staff will receive education on how to administer the medication via the research team. This education will include that the study medications should be dosed per the Epic orders. The education will also include a list of side effects to watch for in the study patients and contact information to notify the research team 24/7 if any of these adverse events are observed.

To assess for outcomes and adverse events, patients will continue to be monitored per the schedule of events above until they are discharged. Upon discharge from the hospital, patients

will not be prescribed study medications unless for an alternate indication (e.g. continuation of colchicine for viral pericarditis).

Due to feasibility issues, and concerns with unnecessary healthcare worker exposure and consumption of personal protective equipment, we will use a standard treatment arm in lieu of a placebo-controlled arm. Daniel Delaney, PharmD will be the pharmacy liaison at Methodist Hospital and assist in identifying where study medications will be kept, prepped, and distributed. At Regions, study medications will be stored and distributed with the assistance of Jason Sloan, PharmD.

e. Outcome/endpoint and other variable definitions, instruments used

Primary Outcome

Relative to standard supportive care, the primary aim of this study is to determine the impact of colchicine and LDN, alone or in combination, on the progression of moderate COVID-19 to more severe disease. Moderate illness is defined as patients requiring hospitalization due to laboratory confirmed COVID-19, meeting one or more of the following:

- Dyspnea limiting usual activities on baseline O2 needs
- Respiratory rate >/= 30/min at or above baseline O2 needs
- Blood oxygen saturations <94% on room air (or on baseline O2 needs if on home oxygen therapy prior to presentation at the hospital).
- Requiring supplemental 02 above baseline needs (i.e. new receipt of supplemental O2 or an increase over typical oxygen requirements in patients on existing O2 regimen)

Disease progression measured by severity increase from moderate disease to severe disease/critical illness, defined as a minimum 2-point increase within 14 days of enrollment or by hospital discharge, whichever occurs first based on the following 6-point scale:

Clinical Score Scale

Severity	Score	Definition
N/A	1	Discharged or ready for discharge (i.e. not requiring further medical care or monitoring - afebrile, O2 sats >94% on room air or baseline O2 needs, respiratory rate < 24 per minute [all x 48 hours])
Hospitalized, moderate disease	2	Hospitalized, not requiring supplemental O2 (but requiring ongoing medical care or monitoring). If decreasing from a 3, must be at baseline O2 requirements for 24 hours.
	3	Hospitalized requiring supplemental oxygen via nasal cannula (for a minimum of 24 hours if score increasing from 2 or decreasing from a 4
Hospitalized, severe disease	4	Hospitalized on high-flow nasal cannula (HFNC) or noninvasive positive-pressure ventilation (NIPPV) support, (for a minimum of 24 hours if score is increasing from 3 or decreasing from a 5). Patients with baseline use

		of NIPPV, e.g. nocturnal CPAP/BiPAP for obstructive sleep apnea must also require qualifying O2 support during the day to obtain a score of 4.
Hospitalized, critical disease	5	Mechanically ventilated, or a required transfer for ECMO
N/A	6	Death

This scale is a modified version of the World Health Organization R&D Blueprint Ordinal Clinical Scale (57). Modifications were made to fit the study population, as this study focuses on patients requiring hospitalization for laboratory confirmed COVID-19, and does not delineate severity among those requiring mechanical ventilation (patients will have already met primary outcome).

Based on inclusion/exclusion criteria, at minimum patients will have a score of 2 at the time of enrollment, and a maximum score of 3.The primary endpoint is met with progression in severity that is correlated to a 2-point increase, i.e. progression to at least requiring HFNC/NIPPV for patients with an admission score of 2 (2 to 4) or intubation for a patient with an admissions score of 3 (3 to 5) would designate them as “progressed to severe/critical disease”. As described, randomization will be stratified by baseline severity score (2 or 3) to ensure balanced baseline severities between the four arms

Secondary Outcomes

To investigate the effects of colchicine with or without LDN relative to standard supportive care on the following:

- Obtained via Epic
    - Composite in-hospital mortality
    - Total duration of hospitalization (hours) from first dose of any study medication (or when first dose would be for standard of care arm) to hospital discharge (or when ready for discharge but remains hospitalized for non-medical reasons [e.g. transitional care unit placement delays])
    - Total duration of ICU admission (hours)
    - Total duration of intubation (hours)
    - Total duration of HFNC or NIPPV (hours), (excluding baseline NIPPV use, as above) from first dose of study drug (or anticipated first dose) to discharge.
    - Total time above baseline oxygen requirements (hours) from first dose of study drug (or anticipated study drug, if in SOC arm) to discharge (i.e. patients not on supplemental oxygen prior to presentation at the hospital = time on any supplemental oxygen after first dose/anticipated dose of study drug to discharge); for patients on home oxygen therapy of two liters per minute via nasal cannula = time on oxygen >2 liters per minute via nasal cannula or any other oxygen delivery device from first drug dose/anticipated dose to discharge).
    - In-hospital days with a fever ≥38 degrees Celsius (00:00 to 23:59:59) from first dose of study drug (or anticipated first dose) to discharge.

- Adverse events related to colchicine or LDN
- Incidence of significant adverse outcomes associated with/attributable to COVID-19; including ARDS, thromboembolic disease, myocardial injury, and acute kidney injury. Must be documented in patient's problem list during their hospitalization, but not present or clinically suspected prior to receiving the first dose of any study medication (or when a dose would be due if randomized to standard of care).
- Lab results (captured and recorded per the Schedule of Events below) obtained during hospital admission and coded as low, normal, high per hospital reference range
- Cumulative dose of corticosteroids received in ED/hospital (excluding prior to admission/pre-existing use of steroids for an alternative indication); converted to single equivalent units
- Cumulative dose of remdesivir received
- Discharge anticoagulation needs (i.e. number of patients discharged on a direct oral anticoagulant specifically prescribed for prophylaxis of increased venous thromboembolism risk due to COVID-19)

f. Statistical analysis plan

Primary Aim:

For the primary aim, to determine if Colchicine and LDN effect the odds of progression towards severe/critical COVID-19, we will use logistic regression with intent to treat (ITT) analysis for all randomized subjects. We will also determine whether the concurrent administration of both colchicine and LDN changes the odds of progression towards severe/critical COVID-19 versus each alone. A 2X2 factorial design, rather than an analysis of 4 parallel arms, will be employed. The table below indicates the treatment provided to each group: **A** will receive both colchicine and LDN, **B** will receive only colchicine, **C** will receive only LDN, and **D** will receive neither (standard of care group).

		Colchicine	
LDN		Yes	No
	Yes	A	C
	No	B	D

The study will examine the main effect for both colchicine alone (Group B compared to Group D) and naltrexone alone (Group C compared to group D). We will also look for a potential interaction effect of using both medications together. All analysis for the primary aim will be performed using logistic regression. Our outcome variable will be whether a patient experienced clinical deterioration during hospitalization as defined by an increase of at least 2 points on the clinical score/levels of care scale (see above) relative to their baseline score at enrollment.

The effect on disease progression from Colchicine and LDN as compared to SOC, along with the interaction of the two interventions will be estimated with the following model:

$$logit\big(\mathrm{E}\,(Y|X)\big) = \alpha + \beta_1(Colchicine) + \beta_2(LDN) + \beta_3[(Colchicine) * (LDN)] + \beta_4\ (Baseline\ Severity) + \beta_5(age) + \beta_6\ (sex) + \beta_7(race) + \varepsilon$$

This model will result in log odds and p-value estimates for the effect of colchicine ($\widehat{\beta_1}$) and LDN ($\widehat{\beta_2}$) on experiencing clinical deterioration during hospitalization. The interaction term ($\widehat{\beta_3}$) will allow us to determine if administering LDN and colchicine together significantly changes the odds of severe/critical progression compared to the additive effect of each intervention alone. The model will include adjustments for patient age, biological sex, race, and baseline severity.

The results from the model above will be used to create contrast statements that allow for a pairwise comparison of the four study arms and their estimated odds of experiencing clinical deterioration. Odds ratios, confidence intervals, ratio of ratios, as well as the directionality of the effect will be used to describe any differences in effect between each intervention arm and the SOC group.

Effect	Group Comparison	Odds Ratios
Colchicine alone vs. SOC	B vs D	$e^{\widehat{\beta_1}}$
LDN alone vs SOC	C vs D	$e^{\widehat{\beta_2}}$
Colchicine and LDN vs SOC	A vs D	$e^{\widehat{\beta_1}+\widehat{\beta_2}+\widehat{\beta_3}}$

Secondary Aims

For our secondary aim, we will use the modeling steps described in our primary aim to assess the effect of Colchicine and LDN relative to SOC, along with the interaction effect on each of the many secondary outcome measures. One model will be run for each outcome to estimate the differences between the single-drug intervention groups and SOC with the final step of comparing the interaction effects. These comparisons are considered exploratory and are unpowered:

Outcome	Model
Mortality, adverse events, and categorical lab results	Logistic regression
Duration of hospitalization, ICU admission, intubation, and HFNC or NIPPV; Total time above baseline oxygen requirements; days with fever; cumulative dose of corticosteroids received; cumulative does of remdesivir	OLS or x- and/or y- transformed regression, as appropriate depending on the spread of the data

received; discharge anticoagulation needs; continuous lab results	

All analysis will be performed in SAS 9.4 with two-sided alphas of 0.05

g. Power analysis or statement of precision

The use of colchicine +/- LDN to prevent COVID-19 disease progression requiring HFNC, NIPPV, or intubation is an unstudied area, particularly in the context of concomitant administration of our current SOC (if not otherwise contraindicated, remdesivir and dexamethasone in patients requiring supplemental oxygen). It can be estimated that roughly 20% of hospitalized patients will require ICU-level care, approximately half of which (~10% overall) will require mechanical ventilation. Noting that not all patients needing HFNC or NIPPV require ICU-level care, the proportion of patients reaching a clinical score of ≥4 during their admission will be estimated at 40%. We expect that the majority of these patients will initially present with moderate symptoms, therefore our estimated rate of progression to severe/critical care for moderate COVID-19 patients receiving SOC is 30% to 40%.

Using the assumption that colchicine would reduce the proportion of patients who progress in clinical score by 20% (raw difference), with an alpha 0.05 and 80% power, we will require a sample of at least 136 (34 per arm, 68 patients receiving colchicine, groups A and B). Similarly, we expect a 20% difference in proportion of patients progressing to critical status when receiving LDN as compared to SOC, requiring 68 patients total receiving LDN (34 per arm, groups A and C).

Although our study contains four arms, the analysis plan is powered off of a comparison of two unadjusted main effects rather than a formal comparison of each of the three intervention arms to the SOC [A, B, and C to the SOC arm (D)]. Because this is not a parallel-arms study, we will not be able to compare the LDN-only arm with the combined arm (A and C) or the colchicine-only arm with the combined arm (A and B). However, the decision to use a 2X2 factorial design permits us to compare larger pools of study participants and still be appropriately powered to detect a significant difference associated with each intervention and determine if the use of both interventional drugs together changes the odds of progressing to severe/critical disease compared to the use of each alone. The study team acknowledges that any statements about the interaction effect of both medications will necessarily be less precise than those of the main effects.

It is important to emphasize that with the unknowns (disease trajectory, actual effect of study drugs, and population of consentable patients); this is a very rough estimate. If there is a true effect, we may observe it at a much smaller n, or not observe it at all even if we reach our goal recruitment. Thus, our interim assessments will be very important in conducting this study when enrollment hits at least 10 in each arm. At this point, the sample will likely be underpowered to observe an effect if there is one. Absolute rate of progression to severe/critical care will be calculated for the SOC and pooled intervention groups. If there is <

10% difference in absolute progression rates between the SOC arm and the pooled intervention patients, we will take into account resources, rate of recruitment, futility, and ethical considerations when deciding whether to move forward with recruitment at this point. Next steps will be evaluated/determined at that time including a possible second interim assessment when enrollment exceeds 20 per arm. As we are in desperate need of inexpensive, safe, orally administrable, and commercially available treatment options for COVID-19, we are hopeful the IRB will understand this.

Sample size per arm needed to detect effect sizes shown

Rate of Progression to Severe/Critical No Effect (H0)	Effect (Reject H0)	N per arm/Total N
0.3	0.1	34/136
0.4	0.2	42/168

h. Strengths and limitations

The study team is composed of multiple clinical pharmacists, physicians from infectious diseases, hospital medicine, anesthesiology, cardiology, and rheumatology, many of whom have been caring for hospitalized COVID-19 patients for almost 10 months. As a result, the team is very well acquainted with the patient population, the current practices for managing COVID-19 in the hospital, and is motivated to find an oral treatment option that has a favorable safety profile, is inexpensive, and commercially available. The Critical Care Research Center (CCRC) is also familiar with working in the acute care setting and carrying out research protocols like the one proposed. As such, we have a well-rounded group to carry out this robust study. We will have access to patients from two large hospitals allowing a large patient pool to enroll from (see setting/environment/organizational feasibility).

Ideally, our study would be double blind and placebo-controlled, but that was not feasible given limited resources and time during the current COVID-19 surge. As such, we opted to use a SOC control group. While the study is not blinded, we will attempt to have a second blinded physician assess AE's. We will be limited in our sample size due to locations, funds, and availability of patients and study medications. As previously noted, there are many unknowns and calculating a sample size estimate is very challenging and may need to be adjusted as the study progresses. We could improve the study by assessing for viral clearance, but due to the lack of widely available testing, and testing shortages in general, we are unable to include this.

5. Setting/environment/organizational feasibility

This study will be conducted at Methodist Hospital and Regions Hospital in collaboration with clinical pharmacy, infectious diseases, and hospital medicine groups at each location. Dr. Daniel Delaney will serve as the overall PI of the trial, as well as the PI of the Methodist site, supported by Drs. Sadia Ali and Michael Schnaus. Dr**.** Gertner will serve as the PI at Regions with support from Dr. Schnaus

who also sees patients at Regions Hospital, as well as Drs. Rebecca Peglow and Paula Skarda. Enrollment at both of these large hospitals will give us access to a larger and potentially more diverse patient population, and as of December 2020 have had multiple weeks of a combined COVID-19 census of 100-150+ patients. The CCRC will assist with research efforts at both sites and will collaborate with other research staff in the Park Nicollet and Methodist system to carry out this protocol.

Regarding coordination of inpatient care, Dr. Schnaus has been tasked with leading research efforts from the Hospital Medicine perspective, Drs. Ali and Peglow for infectious diseases, and Drs. Daniel Delaney and Jason Sloan for pharmacy. Many of these individuals, in addition to the rest of the research team, are members of the COVID-19 Clinical Research Expert Panel for HealthPartners. As COVID-19 research leaders within the Care Group, they are up to date on current practices, and have access to necessary groups for input and collaboration to carry out these efforts successfully.

As this study seeks to answer questions being asked around the world for patients suffering from COVID-19, we hope the results not only influences our local care teams and patients, but also the medical community at large.

6. Risks and benefits

Risks

Each medication has a well-known safety profile and is generally well tolerated. With the appropriate monitoring, avoidance of important drug-drug interactions, and dosing adjustments provided by this protocol, we do not anticipate a notable difference, in adverse events between the treatment and SOC arms (including the combined LDN and colchicine arm).

Colchicine

As gastrointestinal side effects (most commonly diarrhea, potentially nausea, and vomiting) are occasionally seen with colchicine, this may be a specific exception to observed differences in adverse effects, and should be noted even though it is uncommon at low doses of 0.6-1.2 mg/day. Less common (<1%) adverse drug events include hepatotoxicity, bone marrow suppression, and myotoxicity. Renal insufficiency and drug interactions leading to increased colchicine levels are the primary risk factor for side effects; the enrollment process is designed to preclude or minimize this risk, and will be monitored for in enrolled patients regardless (58).

Low-Dose Naltrexone

The medication is well tolerated at much higher doses than outlined in this protocol (25-100 mg vs 4.5 mg daily). At the higher doses, hepatotoxicity can occur; this will be monitored for as part of the study protocol. Trials using LDN for its anti-inflammatory properties have found it to be uniformly well tolerated by participants, and if side effects do occur they're generally mild and constitutional (headache, dizziness, nausea, insomnia or sedation, anxiety, abnormal dreams) (43). In terms of drug interactions, the primary concern would be avoiding opioid withdrawal in patients receiving chronic opioid therapy, and the enrollment process will exclude patients in which this is a concern. For patients requiring management of acute pain with opioids following enrollment, pain control may still be achieved with concomitant LDN and opioid administration, though it may require a higher total

dose of opioids, intentionally separating administration times of the opioid and study medication, or the use of an alternative agent (e.g. ketamine). If there are challenges with pain control during treatment, continued study participation will of course remain voluntary, and a pain specialist, Dr. Joseph Johnson, will be available to provide pain management recommendations as needed.

Benefits

As this is novel research, it cannot guarantee benefit to the patients enrolled. It is our hope that the information gathered will have broad implications in a variety of care settings - which could include, uniquely, rural and underserved communities (not just to those hospitalized in resource rich regions, or those with access to larger, academic/research hospital systems). If proven beneficial, colchicine +/- LDN would be the first treatment option that: has an existing supply chain with broad commercial availability, is generally well tolerated with a very well-known and favorable safety profile, and can be administered orally.

Additionally, the medications considered “standard of care” at this point are in stark contrast to those used in published literature. For example, per our institution’s current treatment algorithm, all patients requiring supplemental oxygen (assuming no contraindications) would be candidates for both remdesivir 200 mg x 1 IV dose followed the next day by 100 mg q24h IV x up to 4 doses, as well as dexamethasone 6 mg q24h x 10 up to 10 doses. None of the patients received remdesivir in the aforementioned studies (42-46). Additionally, the 614 patients received azithromycin and hydroxychloroquine two and three times more often, respectively, than steroids (which was only given to 30.3% of patients).

Confirmation of the potential benefits of colchicine alongside the current SOC within our Care Group is needed. In addition to having the potential to provide a new treatment option to a more diverse patient population, it may mitigate initiation of higher risk (dexamethasone, anticoagulation), more expensive therapies (remdesivir). Taken together with the fact that there are no registered clinical trials investigating the combination of LDN with colchicine, each of the study’s treatment arms will provide novel, actionable information that may prove useful in optimizing patient outcomes and resource utilization (beds, ventilators, and capital).

7. Data Confidentiality and Privacy

All data collected in this study will be stored on servers that only the research team from HealthPartners has access to such as REDCap and the CCRC drive. Data will not be shared with anyone outside of HealthPartners without prior authorization. Only those directly involved with the study will have access to screening and participant information. All data will be de-identified once the team is finished with data collection and identifiers will be destroyed.

8. Timeline

November 2020 -Early January 2021: Study development, IRB application creation, IRB submission, IRB review and approval

January 2021: First patient in

January 2021 - mid 2021: Patient enrollment and data collection

Q3 2021: Statistical analysis

Q4 2021: Manuscript Preparation and Submission

9. Dissemination/Sharing Results/Integration and Impact

This information will be shared with the Infectious Disease and Hospital Medicine groups, and the COVID-19 Command Center. We hope the results can guide the continued use of these medications for more patients with COVID-19 or eliminate them as an option.

After completion of the study, we will attempt to submit our results for publication and presentation in the relevant public health, infectious disease, and hospital medicine journals and conferences.

10. References:

1. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China
2. COVID-19: consider cytokine storm syndromes and immunosuppression.
3. Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients
4. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China
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