TITLE

Pragmatic Factorial Trial of Hydroxychloroquine, Azithromycin, or both for treatment of SARS-CoV-2 Infection

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Statement of Compliance

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice E6 (ICH-GCP), U.S. Code of Federal Regulations 45 CFR 46 and 21 CFR, and applicable site-specific regulatory requirements.

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1. Protocol Synopsis

Protocol Title: Pragmatic Factorial Trial of Hydroxychloroquine, Azithromycin, or both for treatment of SARS-CoV-2 Infection

Treatment Indication: SARS-CoV-2 Infection requiring hospitalization

Trial Objective: Assess the impact of hydroxychloroquine, azithromycin, or both on clinically relevant outcomes in a real-world population of hospitalized persons with confirmed SARS-CoV-2 infection.

Trial Design: Open-label, factorial randomized clinical trial with two factors and nested randomization. Patients hospitalized with confirmed SARS-CoV-2 infection will be initially randomized to one of two arms in a 1:1 ratio: 1) Standard of care treatment or 2) Standard of care treatment plus 5 days of hydroxychloroquine. Patients with no contraindication to azithromycin who have not received azithromycin in the seven days prior to enrollment will undergo a second randomization in a 1:1 ratio to either receive 1) No azithromycin or 2) 5 days of azithromycin. Randomization will be stratified by hospital and by whether the patient is receiving mechanical ventilation at the time of randomization or not. Participants will be followed for 28 days after randomization or until hospital discharge, whichever is later.

Patient population: A total of 500 hospitalized male and female patients (≥ age 12 years or older), newly diagnosed with SARS-CoV-2 infection by a validated nucleic acid amplification assay and hospitalized due to symptoms of SARS-CoV-2 disease, who do not have documented allergy or intolerance to hydroxychloroquine and who have not received hydroxychloroquine in the 180 days prior to enrollment.

Treatment arms: Study participants will be randomized 1:1 to receive one of the following:

Randomization 1 Treatment

1) Standard of care
2) Standard of care plus hydroxychloroquine 800 mg on day 1, followed by 600 mg daily for a total of 5 days

Participants who have not received azithromycin in the 7 days prior to enrollment and who do not have documented contraindication to azithromycin will undergo a second randomization 1:1 for concurrent treatment:

Randomization 2 Treatment:

1) Treatment dictated by Randomization 1 alone
2) Treatment dictated by Randomization 1 plus azithromycin 500 mg on day 1, followed by 250 mg daily for 4 additional days

Criteria for evaluation:
Primary endpoint:
(1) WHO ordinal scale measured at 14 days after enrollment: The ordinal scale is an assessment of the clinical status at the first assessment of a given study day. The scale is as follows: 1) Death; 2) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO); 3) Hospitalized, on non-invasive ventilation or high flow oxygen devices; 4) Hospitalized, requiring supplemental oxygen; 5) Hospitalized, not

requiring supplemental oxygen - requiring ongoing medical care (COVID-19 [coronavirus disease 2019] related or otherwise); 6) Hospitalized, not requiring supplemental oxygen - no longer requires ongoing medical care; 7) Not hospitalized, limitation on activities and/or requiring home oxygen; 8) Not hospitalized, no limitations on activities.

Secondary endpoints:

(1) Death during the index hospitalization
(2) Number of days on mechanical ventilation
(3) Proportion of patients not receiving mechanical ventilation at baseline who progress to requiring mechanical ventilation during the index hospitalization
(4) WHO ordinal scale measured at 28 days after enrollment
(5) Hospital length of stay in days for the index hospitalization
(6) Days of fever (temperature ≥38.0) after randomization
(7) Days on supplemental oxygen after randomization
(8) All-cause study medication discontinuation
(9) Drug-associated adverse events of special interest

Study sites:

Study participants will be recruited from Duke University Hospital, Duke Regional Hospital, Duke Raleigh Hospital, and the Durham VA Medical Center.

Study duration:

It is anticipated that three months will be required for recruitment and enrollment of study participants. The estimated duration of participation for each study participant is approximately 28 days, including treatment and follow-up.

2. Background Information and Scientific Rationale

2.1 Need for therapeutic agents for SARS-CoV-2

On December 31, 2019, China reported an outbreak of an unknown respiratory illness in Hubei Province, now identified as SARS-CoV-2 (1). The WHO declared a Public Health Emergency of International Concern on January 30, 2020, and a global pandemic on March 12, 2020. As of April 2, 2020, there have been 941,949 cases of COVID-19 and 47,522 deaths globally. In the United States, cases have rapidly risen with the total number of cases currently 216,722, including 5,137 deaths (Johns Hopkins Coronavirus Resource Center accessed at https://coronavirus.jhu.edu/map.html on April 2, 2020).

To date, no randomized, controlled trials (RCTs) exist that demonstrate the efficacy of any therapeutic agent for treatment of SARS-CoV-2. However, several therapeutic agents have been postulated to have some effect against SARS-CoV-2, including remdesivir, lopinivir/ritonavir, tocilizumab, chloroquine/hydroxychloroquine, interferon-1b, and convalescent plasma. Data to support any of these treatments are sparse at best (1). Therefore, in the context of a pandemic, it is critically important that therapeutic agents are investigated in scientifically and ethically sound studies. Large clinical trials are currently underway globally to establish an evidence base therapies used to target COVID-19.

2.2 Scientific rationale

We are proposing to examine the effect of hydroxychloroquine and azithromycin, alone and in combination, on clinically relevant outcomes in hospitalized patients with SARS-CoV-2 infection. Due to its in-vitro activity against SARS-CoV-2 and its wider availability in the United States compared with chloroquine, hydroxychloroquine has frequently been administered to hospitalized COVID-19 patients on an uncontrolled basis. No data from RCTs currently exist to inform clinical guidance on the use, dosing, or duration of hydroxychloroquine for treatment of SARS-CoV-2 infection. Azithromycin has been used in combination with hydroxychloroquine in limited case series and has been postulated to provide additional efficacy.

2.2.1 Mechanism of action of chloroquine and hydroxychloroquine in SARS-CoV-2

Hydroxychloroquine is an anti-malarial 4-aminoquinoline approved by the FDA for treatment in rheumatoid arthritis, systemic lupus erythematosus, and other inflammatory conditions. A derivative of chloroquine, it was first synthesized in 1946 by introducing a hydroxyl group to chloroquine. Hydroxychloroquine has pharmacokinetics similar to that of chloroquine with rapid gastrointestinal absorption and renal elimination, but it has a more favorable risk-benefit ratio than chloroquine due to a wider therapeutic index and better side-effect profile (2, 3).

Investigators have postulated several mechanisms to explain potential activity of chloroquine and hydroxychloroquine against SARS-CoV-2 (4):

1) Inhibition of viral entry. Hydroxychloroquine inhibits biosynthesis of sialic acids and interferes with protein glycosylation, which may disrupt interactions necessary for viral attachment and entry (5, 6).
2) Inhibition of viral release into the host cell. Hydroxychloroquine blocks endosomal acidification, which activates endosomal proteases. These proteases are required to initiate coronavirus/endosome fusion that releases viral particles into the cell (7).
3) Reduction of viral infectivity. Hydroxychloroquine has been shown to inhibit protein glycosylation and proteolytic maturation of viral proteins. Studies on other RNA viruses

have shown a resulting accumulation of non-infective viral particles, or an inability of viral particles to bud out of the host cell (8, 9).

4) Immune modulation. Hydroxychloroquine reduces toll-like receptors and cGAS-STING signaling. It has been shown to reduce release of a number of pro-inflammatory cytokines from several immune cell types (10), such as interleukin-6 thereby blocking the pathway that subsequently leads to acute respiratory distress syndrome (ARDS) (11). By this mechanism, hydroxychloroquine might show benefit in treating cytokine storm that occurs in some critically ill patients with SARS-CoV-2 (12).

2.2.2 Mechanism of action of azithromycin in SARS-CoV-2

In addition to antibacterial effects, azithromycin has anti-inflammatory effect, particularly in the lungs. A mouse model of viral bronchiolitis demonstrated that azithromycin decreased inflammatory mediators in bronchoalveolar lavage fluid and enhanced resolution of chronic airway inflammation (13). Azithromycin has also been associated with improved lung function in patients with cystic fibrosis and diffuse panbronchiolitis, independent of bacterial eradication (14, 15). Macrolides have also been associated with improved survival in patients with community acquired pneumonia when paired with a beta lactam antibiotic, regardless of pneumonia etiology, suggesting an additional effect beyond bacterial killing (16). In a nonrandomized, retrospective analysis of 349 critically ill MERS patients, macrolide therapy (primarily azithromycin) was associated with a nonsignificant reduction in 90-day mortality (odds ratio 0.84, 95% confidence interval 0.47-1.51) (17).

2.3 Evidence for chloroquine and hydroxychloroquine

2.3.1 In vitro studies

Chloroquine has been shown to have *in vitro* activity against a diverse group of RNA viruses (18-20). Furthermore, chloroquine and hydroxychloroquine have been demonstrated to be active against a number of coronaviruses *in vitro*, including SARS-CoV-1 (21, 22).

In one study, chloroquine appeared to block SARS-CoV-2 at low-micromolar concentration in a Vero E6 cells assay (23). This study also demonstrated the proof-of-concept that that chloroquine functioned at both entry and post-entry stages of the SARS-CoV-2 infection in Vero E6 cells. Hydroxychloroquine (EC50=0.72 μM) was shown to be more potent that chloroquine (EC50=5.47 μM) in an *in vitro* anti-SARS-CoV-2 study (12). This study also demonstrated through a PBPK model that lung concentrations of hydroxychloroquine rapidly increased and reached steady state after a loading dose and maintenance doses, and reached levels three times the potency of chloroquine.

2.3.2 In vivo studies of hydroxychloroquine

In February 2020, early results from a number of Chinese clinical trials on chloroquine used for SARS-CoV-2 pneumonia demonstrated that in 100 patients, chloroquine was superior to standard of care in inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting earlier viral conversion, and shortening the disease course (24). Severe adverse reactions to chloroquine were not noted. Based on these data, the National Health Commission of the People's Republic of China recommended inclusion of chloroquine in the Guidelines for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by COVID-19 (25).

On March 25, 2020, a small randomized study from China compared the use of hydroxychloroquine (400 mg per day for 5 days) with standard of care in 30 patients with non-

severe SARS-CoV-2 infection randomized to HCQ or control. There was no difference in respiratory pharyngeal swab viral conversion or clinical outcome at Day 7 between the two groups, but patients received multiple treatments including antivirals (26). Several larger clinical studies are planned or underway, designed to establish the role of chloroquine and hydroxychloroquine for COVID-19 treatment (27) (see section 2.3.5).

A randomized, unblinded study of 62 patients in Wuhan, China (not yet peer-reviewed) with mild SARS-CoV-2 illness examined the impact of hydroxychloroquine 200 mg twice-daily for 5 days, and found a 1-day reduction in fever duration among patients receiving hydroxychloroquine and a higher rate of radiographic resolution at day 6 in the hydroxychloroquine group (25/31, 80.6% improved in the hydroxychloroquine group vs. 17/31, 54.8% in the control group) (28).

2.3.3 In vivo studies of hydroxychloroquine and azithromycin combination therapy

A recent non-randomized study from France of hydroxychloroquine (200 mg every 8 hours x 10 days) (n=26) for patients hospitalized with SARS-CoV-2 who had symptoms of upper and lower tract infections without oxygen requirement found that hydroxychloroquine decreased viral shedding at Day 6 compared to the untreated group (n=16) (70% vs 12.5%, p= 0.001). Six patients in the hydroxychloroquine group were receiving concomitant azithromycin for prevention of secondary bacterial pneumonia. On Day 6, all patients treated with the combination (hydroxychloroquine and azithromycin) demonstrated virologic clearance, compared to 57.1% of patients treated with hydroxychloroquine alone (n= 20) (29).
This study had several limitations, including the following:

1) Six patients in the hydroxychloroquine arm with poor outcomes (including death, ICU transfer, treatment intolerance) were excluded from the analysis.
2) Use of an arbitrary, unvalidated virologic clearance on Day 6 was used as the primary outcome. For example, the authors reported that one patient tested negative for the virus on Day 6 but subsequently tested positive on Day 8, indicating that a single, negative polymerase chain reaction (PCR) test on a nasopharyngeal swab may not indicate persistent virologic clearance.
3) The control group was not systematically selected, introducing allocation bias.

A follow-up manuscript from the same authors (published online on March 28, not yet peer reviewed) reported a non-comparative study of 80 patients hospitalized with SARS-CoV-2 infection without oxygen requirement (only 15% had fevers and 4 patients were asymptomatic) who were treated with the same doses of hydroxychloroquine and azithromycin for 3-10 days. One patient died; 3 transferred to ICU; 15% progressed to oxygen requirement; and 81.3% were improved at discharge. 83% of patients had negative PCR tests of nasopharyngeal swabs by Day 7, and 93% had negative PCR studies by Day 8. 97.5% of patients had negative viral cultures by Day 5. Mean hospital stay was 4.6 days. Toxicity data were not reported. ECG was performed before and 2 days after treatment, but QTc data were not reported (30). A subsequent, smaller study of 11 patients treated with hydroxychloroquine/azithromycin at a different site in France did not suggest any benefit of the combination on viral clearance or clinical outcomes, and one patient had medications discontinued because of QTc prolongation (31).

Given the substantial limitations of existing in vivo studies, further trials are urgently needed to better understand the role of hydroxychloroquine and azithromycin in the treatment of SARS-CoV-2.

2.3.4 How this study complements other planned work

Investigators across the U.S. have recently designed multiple clinical trials to evaluate impact of several experimental pharmacologic agents and other therapies when administered to patients with COVID-19. For example, three multicenter studies are actively enrolling to analyze efficacy and safety of remdesivir for hospitalized patients with moderate or severe disease from COVID-19 (NCT04292730, NCT04292899, and NCT04280705). Each of these studies excludes patients with mild disease, moderate renal insufficiency, or moderate transaminitis. Other U.S. studies actively recruiting patients are assessing efficacy and safety of sarilumab in hospitalized patients with severe COVID-19 (NCT04315298) or nitric oxide inhalational therapy for mild/moderate (NCT04305457) or severe (NCT04306393) disease. Investigators have designed additional studies to analyze the potential impact of numerous other experimental agents, including losartan (NCT04312009), intravenous aviptadil (NCT04311697), PUL-042 inhalation solution (NCT04312997), CD24Fc (NCT04317040), and convalescent plasma (NCT04325672); however, these COVID-19 studies are not yet recruiting patients.

While providers commonly prescribe hydroxychloroquine and/or azithromycin to treat hospitalized patients with COVID-19, no high-quality data exists to demonstrate that either of these treatments are effective. Investigators associated with a single U.S. study plan to evaluate efficacy of hydroxychloroquine versus azithromycin for hospitalized patients with COVID-19 (NCT04329832); however, this study is not yet recruiting patients and does not randomize patients to a control arm where patients receive neither experimental therapy. A randomized, placebo-controlled study in Alberta, Canada will randomize patients to 5 days of hydroxychloroquine versus placebo to examine whether hydroxychloroquine reduces the incidence of severe disease (NCT04329611). The AIDS Clinical Trials Group is planning a multicenter study to examine the combination of hydroxychloroquine/azithromycin in outpatients and plans to collaborate with Novartis to do a similar treatment trial in inpatients; however, it is not clear when those studies will begin, and the Novartis study will reportedly exclude critically ill patients. The NHLBI-funded PETAL network is also planning an inpatient trial of hydroxychloroquine versus placebo (NCT04332991).

Our proposed study is unique and complements other planned studies because our study will allow systemic comparison of clinical outcomes for patients who receive hydroxychloroquine versus azithromycin, as well as for patients who receive **neither** or **both** experimental agents. Other studies evaluating these agents to date have not randomized patients to a control group that receives standard of care (i.e., neither experimental agent); however, **inclusion of a randomized control group is critical to understanding efficacy and safety of hydroxychloroquine and/or azithromycin when used to treat COVID-19**. Finally, many hospitalized patients in the proposed study population would not qualify for treatment via existing clinical trials due to insufficient disease severity, renal dysfunction, or transaminitis. Therefore, this study provides a mechanism for interested patients who would not otherwise qualify for a clinical trial to participate in a study and receive protocolized pharmacologic therapy targeting COVID-19.

3. Descriptions of Study Drugs

3.1 Hydroxychloroquine

3.1.1 Formulation, Appearance, Packaging, and Labeling

Hydroxychloroquine sulfate tablets are provided as a white/off-white, film-coated, peanut-shaped tablets. Each tablet contains 200 mg of the active ingredient (HCQ sulfate). The non-active ingredients in the formulation include black ink, calcium hydrogenophosphate, carnauba wax, cornstarch, magnesium stearate, Opadry White YS-I-7443 and polyethylene glycol 400.

3.1.2 Dosing and administration

Participants will receive an 800 mg loading dose the first day, followed by 600 mg daily for 4 more days. All study drug doses will be administered orally. Study drug will be supplied as 200 mg tablets.

3.1.3 Rationale for selection of dose

Hydroxychloroquine is an aminoquinolone antimalarial agent that is also approved and used for the treatment of autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus.

Both chloroquine and hydroxychloroquine have been reported to have *in vitro* antiviral activity against SARS-CoV-2 (23, 32). *In vitro* testing using Vero cells vary; Yao et al demonstrated an EC50 of 23.90 and 5.47 uL for chloroquine at 24 and 48 hours respectively, compared to 6.14 and 0.72 for HCQ (12). Liu et al reported on four multiplicities of infection (MOIs) (0.01, 0.02, 0.2, and 0.8); the 50% maximal effective concentration (EC50) for chloroquine was (2.71, 3.81, 7.14, and 7.36 μM) and for hydroxychloroquine was (4.51, 4.06, 17.31, and 12.96 μM) (32). Hydroxychloroquine may exhibit higher activity to chloroquine in protecting Vero cells from infection (“prophylaxis”); when Vero cells were pre-treated with drug before SARS-CoV-2 challenge, EC50 for chloroquine was >100 and 18.01 uL at 24 and 48 hours, respectively, compared to 6.25 and 5.85 for HCQ (32). In animals, both drugs share similar tissue distribution patterns, with high concentrations in the liver, spleen, kidney, and lung reaching levels of 200–700 times higher than those in the plasma (33).

The optimal dose of hydroxychloroquine for treatment or prophylaxis is unknown. Absorption after oral dosing is incomplete and variable (~70% [range: 25 to 100%]) (2, 34). Metabolism is primarily hepatic with metabolites including bidesethylchloroquine, desethylhydroxychloroquine, and desethylchloroquine (35). The drug has long half-life elimination of approximately 40 days (2). Standard dosing in other medical conditions include: systemic lupus erythematosus (200-400 mg daily given once or divided BID), malaria treatment (800 x1, followed by 400 mg at 6, 24 & 48 h after initial dose), Q fever (600 mg/day in 1 to 3 divided doses) and rheumatoid arthritis (200-400 mg daily in 1-2 divided doses). The recommended maximum dose of HCQ is 5 mg/kg actual body weight daily.

The dose chosen for this study is 800 mg orally once daily on day 1 as a loading dose followed by 600 mg QD for an additional 4 days. This dose was chosen for several reasons:

- current *in vitro* studies report a wide range of EC50 for SARS-CoV-2,
- variability of absorption and of tissue distribution into the lung,

- due to a lack of phase 1b data for this drug in SARS-CoV-2 infection, the optimal pharmacokinetic/pharmacodynamic target is unknown.

Yao et al made assumptions regarding the absorption and tissue distribution that were optimistic and recommended as an optimal treatment dose 400 mg BID on day 1 as a loading dose followed by 200 mg BID daily for the remainder of the treatment course (12). There is concern that this dose will not meet the necessary pharmacokinetic/pharmacodynamic targets for efficacy, in which case a negative study would not be due to failure of the antiviral efficacy but of suboptimal dosing. Yao et al also noted that the drug exposure was the same for 200 mg BID or 400 mg QD daily dosing. We propose a higher loading dose of 800 mg once daily on day 1 to ensure more rapid steady state tissue distribution and 600 mg daily (versus 200 mg three times daily) dosing for ease of dosing and adherence.

3.2 Azithromycin

Azithromycin is an azalide antibacterial that is FDA-approved for treatment of acute bacterial exacerbations of chronic obstructive pulmonary disease, acute bacterial sinusitis, community-acquired pneumonia, pharyngitis/tonsillitis, uncomplicated skin and skin structure infections, urethritis and cervicitis, and genital ulcer disease. Between 2013-2015, it was one of the most commonly prescribed antibiotics in the United States, with an average of 443 prescriptions per 1,000 Express Scripts beneficiaries (~15 million prescriptions per year) (36).

3.2.1 Formulation, appearance, labeling and packaging

Azithromycin tablets are supplied as pink film-coated tablets containing azithromycin dihydrate equivalent to 250 mg of azithromycin.

3.2.2. Dosing and administration

Azithromycin will be dosed at 500 mg orally on day 1 of treatment, followed by 250 mg once daily on days 2-5 of treatment.

3.2.3 Rationale for dose selection

This dosing is a commonly used dose for community-acquired pneumonia; as the potential mechanism of action and pharmacodynamics of azithromycin when used to treat SARS-CoV-2 are unknown, we chose a dose that has been demonstrated safe in a large number of patients.

Mechanism of Action / Potential activity in COVID19:

- Antibacterial effect: Binds bacterial 50S ribosomal subunit and inhibits RNA-dependent protein synthesis (37). Can potentially serve to prevent bacterial super infection in patients with COVID19.
- Immunomodulatory effect (studied mainly in COPD and Cystic Fibrosis): Inhibits NFκB activation and expression of activator protein-1, reduces number and function of neutrophils leading to lower concentrations of neutrophil elastase, IL-8, and IL-1β. This in turn leads to a reduction in cellular damage/tissue injury (38, 39). Adjunct therapy in moderate to severe ARDS has been shown to improve survival and reduce days on mechanical ventilation, which may be related to this immunomodulatory effect (40).

- Direct antiviral effect: *invitro studies;* Suppresses viral replication of Rhinovirus in bronchial epithelial cells (exact mechanism unclear) (41-43), inactivates endocytic activity of A(H1N1)pdm09 blocking internalization in early infection (exact mechanism unclear) (44). Azithromycin has no studied direct antiviral effect with the Coronaviridae family.
- Combination of Hydroxychloroquine (HCQ) and Azithromycin studied in a small prospective non-randomized trial could suggest that Azithromycin might augment the effects of HCQ, but this is still unclear in true effect, clinical significance as well as underlying mechanism (29)

Oral Pharmacokinetics:

- Immediate release, bioavailability 34 – 52% with a half-life of 68 – 72 hours.
- Extended release bioavailability, 28 – 43% with a half-life of 59 hours.
- Metabolized hepatically to inactive metabolites and excreted in both bile and urine with 50% and 6 – 14% being unchanged, respectively.

Drug-drug interactions:

- CYP3A4 substrate
- P-glycoprotein/ABCB1 inhibitor
- Potentially drug-drug interactions relevant to our study include:
    - Chloroquine; QT prolongation and ventricular arrythmias
    - Other QT prolonging drugs such as antiemetics, antidepressants, antipsychotics, and other antimicrobials

Safety and tolerability (Oral, Adults):

- Single dose regimens tend to be associated with higher frequency of adverse events, however this is not relevant to our study given that the dosing of azithromycin will be a daily oral dose for 5 days.
- The majority of adverse events are gastrointestinal with an incidence of approximately >10%: Loose stools (≤14%), diarrhea (2% - 9%), nausea (≤7%), and vomiting (≤2%).
- Other (systems based) adverse events occur at lower rates of 1 – 10%: Cardiovascular: Chest pain (≤1%), palpitations (≤1%); Central nervous system: Dizziness (≤1%), drowsiness (≤1%), fatigue (≤1%), headache (≤1%), vertigo (≤1%); Dermatologic: Skin rash (≤5%), pruritus (≤2%), skin photosensitivity (≤1%); Endocrine & metabolic: Increased lactate dehydrogenase (1% – 3%), increased gamma-glutamyl transferase (1% –2%), increased serum potassium (1% – 2%), decreased serum bicarbonate (≥1%), decreased serum glucose (>1%); Gastrointestinal: Abdominal pain (1% – 7%), anorexia (≤2%), dysgeusia (≤1%), dyspepsia (≤1%), flatulence (≤1%), gastritis (≤1%), melena (≤1%), mucositis (≤1%), oral candidiasis (≤1%); Genitourinary: Vaginitis (≤3%), genital candidiasis (≤1%); Hematologic & oncologic: decreased hematocrit (>1%), decreased hemoglobin (>1%), increased neutrophils (>1%), thrombocythemia (adults: >1%), eosinophilia (≥1%), lymphocytopenia (≥1%); Hepatic: Increased serum ALT (≤6%),

increased serum AST (≤6%), increased serum bilirubin (≤3%), cholestatic jaundice (≤1%); Neuromuscular & skeletal: Increased creatine phosphokinase (1% – 2%); Renal: Increased serum creatinine (≤6%), increased blood urea nitrogen (≤1%), nephritis (≤1%); Respiratory: Bronchospasm (≤1%).

- Other (rare) adverse events potentially relevant to our study include:
    - QT prolongation; Azithromycin can inhibit the rapid component of the delayed rectifier K^+ current channels and induce QT prolongation, however several studies and analyses have shown that Azithromycin-induced QT prolongation does not lead to Torsade de Pointes (45, 46).
    - Increased risk of death from cardiovascular events; Some studies have suggested that Azithromycin has been associated with death from cardiovascular events in high risk individuals, however several subsequent studies and analyses have not shown an association (47, 48).
- Pregnancy: Azithromycin crosses the placenta but is generally considered safe in pregnancy (Category B) (37, 49, 50); a recent study did associate macrolides with an increased incidence of genital malformations, but azithromycin was not specifically studied (51).
- Breast Feeding: Azithromycin is present in breast milk and has been shown to have a half-life of ~15 hours, with some studies detecting Azithromycin in breast milk up to 30 days after single dose administration (52, 53). There is conflicting data of the incidence/nature of macrolide-induced infantile hypertrophic pyloric stenosis in infants exposed in the first 2 weeks of life (54, 55). Some infants may experience adverse gastrointestinal events such as diarrhea and should be monitored while the mother is receiving therapy (55). It is generally considered safe in breast feeding mothers (Category B).
- Contraindications: Hypersensitivity to macrolides, history of macrolide induced hepatitis/cholestatic jaundice

4. Objectives

4.1 Definitions

The World Health Organization ordinal scale is defined as an assessment of the clinical status of a study participant at the first assessment of a given study day. For purposes of this study, this assessment will be considered to have occurred at 9 a.m. on the study day.

The ordinal scale is an assessment of the clinical status at the first assessment of a given study day. The scale is as follows: 1) Death; 2) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO); 3) Hospitalized, on non-invasive ventilation or high flow oxygen devices; 4) Hospitalized, requiring supplemental oxygen; 5) Hospitalized, not requiring supplemental oxygen - requiring ongoing medical care (COVID-19 related or otherwise); 6) Hospitalized, not requiring supplemental oxygen - no longer requires ongoing medical care; 7) Not hospitalized, limitation on activities and/or requiring home oxygen; 8) Not hospitalized, no limitations on activities.

4.2 Primary

(1) To compare the efficacy of each experimental treatment (azithromycin and hydroxychloroquine) to the efficacy of supportive care, using the World Health Organization ordinal scale measured on day 14 after enrollment

4.3 Secondary

(1) To compare the rates of death during the index hospitalization among participants receiving each experimental treatment and participants receiving supportive care alone
(2) To compare the number of days on mechanical ventilation among participants receiving each experimental treatment and participants receiving supportive care alone
(3) To compare the proportion of participants not receiving mechanical ventilation at baseline who progress to requiring mechanical ventilation during the index hospitalization among participants receiving each experimental treatment and participants receiving supportive care alone
(4) To compare the World Health Organization ordinal scale measured on day 28 after enrollment between participants receiving each experimental treatment and participants receiving supportive care alone
(5) To compare the total length of hospitalization during the index hospitalization among participants receiving each experimental treatment and participants receiving supportive care alone
(6) To compare the duration of fever (defined as temperature ≥38.0 C measured at any site) after randomization among participants receiving each experimental treatment and participants receiving supportive care alone
(7) To compare the duration of supplemental oxygen use after randomization among participants receiving each experimental treatment and participants receiving supportive care alone
(8) To describe the rates of all-cause discontinuation of study medication among patients receiving experimental treatment
(9) To compare the rates of drug-associated adverse events of special interest among patients receiving each experimental treatment and participants receiving supportive care alone during the first fourteen days after enrollment

5. Study Design

This will be a pragmatic, randomized, open-label, incomplete factorial with nested randomization clinical trial evaluating the efficacy and safety of two potential treatments for hospitalized patients with confirmed SARS-CoV-2 infection. Participants who are hospitalized and have a positive nucleic acid amplification test for SARS-CoV-2 will undergo an initial randomization in a 1:1 ratio to one of the following regimens:

Arm 1: Supportive care alone

Arm 2: Supportive care plus hydroxychloroquine

Participants who meet eligibility criteria to receive azithromycin will undergo a second randomization in a 1:1 ratio to receive additional concurrent therapy. This will effectively result in four treatment groups:

1) Supportive care alone
2) Supportive care plus hydroxychloroquine
3) Supportive care plus azithromycin
4) Supportive care plus hydroxychloroquine plus azithromycin

6. Study Population

500 hospitalized male and female patients (≥ age 12 years or older) diagnosed with SARS-CoV-2 related infection, and who meet all study eligibility criteria will be enrolled in the study. Participants will be enrolled at all three DUHS hospitals: Duke University Hospital, Duke Regional Hospital and Duke Raleigh Hospital, as well as the Durham VA Medical Center. We anticipate that most participants enrolled in the study will be residents of the 12-county Raleigh/Durham Combined Statistical Area. Estimated time of screening to end of study for an individual subject will be 28 days, including treatment and follow up.

Participant eligibility will be confirmed by one of the study co-investigators prior to enrollment.

Inclusion Criteria

To participate in the study, participants must meet ALL of the following criteria:

1) Admitted to participating hospital with symptoms suggestive of SARS-CoV-2 infection OR develop symptoms of SARS-CoV-2 during hospitalization
2) Subject (or legally authorized representative) can provide written informed consent (in English or Spanish) affirming intention to comply with planned study procedures prior to enrollment
3) Male or female aged 12 years or older at the time of enrollment
4) Has laboratory-confirmed SARS-CoV-2 infection determined by a validated nucleic acid amplification assay (public health or commercial) in any respiratory specimen collected within 14 days of randomization
5) Illness of any duration that includes
    - Radiographic evidence of pulmonary infiltrates (chest X-ray or CT scan) OR
    - Clinical documentation of lower respiratory symptoms (cough, shortness of breath, or wheezing) OR
    - Any documented SpO2 ≤ 94% on room air OR
    - Any inpatient initiation of supplemental oxygen regardless of documented cause

Exclusion Criteria

Participants with ANY of the following criteria will be excluded from the study:

1) Participating in any other clinical trial of an experimental agent for SARS-CoV-2
2) On hydroxychloroquine at any time during hospitalization, or within 180 days of hospitalization for COVID-19 regardless of indication
3) History of cirrhosis, long QT syndrome or porphyria of any classification
4) Most recent ECG prior to time of screening with QTc of ≥500 msec
5) Known hypersensitivity to hydroxychloroquine or 4-aminoquinoline derivatives
6) Weight less than 40 kg
7) Death anticipated within 48 hours of enrollment
8) Inability to obtain informed consent from the patient or designated medical decision maker

Inclusion of Vulnerable Subjects

Hydroxychloroquine has been shown to permeate across the placenta, with cord blood concentrations equivalent to those found in maternal blood. Despite theoretical concerns of retinal toxicity and ototoxicity, numerous cohort studies have demonstrated its safety in

pregnant women exposed to the medication. Hydroxychloroquine has been used extensively as an immunomodulatory agent in pregnant women and the American College of Rheumatology recommends continuation of the drug among pregnant women with SLE who were initiated prior to pregnancy. After careful assessment of available data, consultation with local experts, and the lack of alternative therapeutic options for persons with SARS-CoV-2, the study team deemed that inclusion of pregnant women in the trial was warranted.

Persons under the age of 18 are significantly less likely present with symptoms warranting hospitalization than the rest of the population. As a result, little clinical trial data exist on therapeutics for COVID-19 in this subset of the population. This trial presents an opportunity to systematically collect data on the impact of two widely available, low-cost agents on a potentially life-threatening infection, with no known therapeutic options. Both azithromycin and hydroxychloroquine have been shown to be safe in adolescents. Based on the risk/benefit analysis for prospective hospitalized patients in this age group, inclusion of adolescents (age 12-18) in the trial is warranted.

Prisoners will not be enrolled in this study because it does not involve one of the Health and Human Services permissible categories of research involving prisoners.

Lifestyle Considerations
Study participants will be asked to avoid participation in other trials of pharmacologic agents with possible activity against SARS CoV-2.

Screen Failures
At completion of prospective participant screening, the principal investigator (or designee) will review eligibility criteria to determine the patient's suitability for study inclusion. Data on reason for ineligibility will be collected for all screen failures. Persons who fail study screening will be notified about the reason for their ineligibility. Patients who are deemed ineligible due to eligibility criteria based on clinical findings/data can be re-screened again at a subsequent time based on investigator's discretion.

Strategies for Recruitment and Retention
Recruitment
All patients admitted to study site hospitals for SARS-CoV-2 will be screened for participation in the study. No external recruitment efforts will occur beyond this subset of patients. Study coordinators will track the clinical course of every hospitalized SARS-CoV-2 patient in DUHS, with particular attention to whether or not they were offered enrollment into a trial involving another investigational agent. Through review of the electronic health record, the study coordinator will determine the reason patient was not enrolled. If failure to enroll was not due to one of this protocol's exclusion criteria, or a broad declination of participation in SARS-CoV-2 - associated clinical trials (available in OnCore), the patient will be screened and approached for inclusion in the study. Targeted groups of clinicians within the health system will be notified about the trial prior to the study's commencement and on the day the study is open to enrollment via e-mail. Clinician groups to be targeted include: emergency department faculty, trainees and staff; critical care faculty, trainees and staff; hospitalist physicians; infectious diseases faculty trainees and staff; transplant faculty, trainees, and staff; Internal Medicine, General Surgery and Anesthesia house staff. Study details will also be available to all DUHS faculty, trainees and staff via the COVID-19 clinical trials section of CustomID.

The IRB will approve the recruitment process and all materials prior to any recruitment to prospective subjects directly.

To minimize unnecessary exposure to SARS-CoV-2, study staff will be instructed to conduct as much screening for eligibility by electronic health record review as possible, and informed consent will be preferentially obtained by telephone.

Retention
Significant participant attrition is not expected given that this study primarily follows patients while hospitalized. Study investigators will be available to the participant's clinical team to discuss alterations in clinical course that may influence their decision to keep the patient in the trial.

Compensation
Participants will not be compensated for their participation in the study.

Costs
Participants will be charged for study drugs as part of their hospital care. Participants will be informed of this by the study coordinator prior to obtaining informed consent.

7. Enrollment and Randomization

7.1 Enrollment Procedures

Individuals who are hospitalized with a positive nucleic acid amplification test for SARS-CoV-2 and who are not enrolled in another therapeutic trial for SARS-CoV-2 will be invited to participate in this study. Interested individuals will be provided with information about the study including risks and potential benefits of all study procedures. If site staff are satisfied that the potential participant understands the information and the potential participant is willing, the potential participant will be asked to participate in the study. Study-specific procedures will be initiated only after the participant has provided written, informed consent. The consent process will be performed by providing the patient or designated healthcare provider a copy of the consent form (this will be given to an individual who is going into the room; the study coordinator will not enter the room). The patient will be provided an opportunity to review the consent, and when they are ready, the consent process will occur by e-consent on study iPads. If the patient is unable to provide written, informed consent and they have a designated medical decision maker, that person will be approached either in person or by telephone with information about the study. The consent process with the medical decision maker will occur using e-consent also.

7.2 Randomization

This will be a randomized trial. Randomization and treatment arm assignment will be generated with randomly permuted blocks of size 2, 4, 6, and 8. Participants will undergo two randomizations. The first randomization (to standard of care or hydroxychloroquine) will be stratified by whether or not the patient is receiving mechanical ventilation at the time of enrollment and by hospital site. Eligible patients who have provided informed consent will be randomized with equal probability to each arm. Participants who meet inclusion criteria for the second randomization (to add or not add azithromycin) will be randomized with equal probability to each of these treatments. Random assignment sequences will be generated in a way that

limits the imbalance between arms within strata while ensuring that the sequence is not predictable based on previous assignments.

8. Study Procedures

8.1 Ascertainment of potential participants

Potential participants will be ascertained in one of two ways: 1) Daily review of all positive SARS-CoV-2 tests from the relevant hospital laboratories and 2) Referral from treating providers.

8.2 Baseline screening and eligibility determination

By chart review prior to patient encounter:

- Confirm positive SARS-Cov2 test
- Confirm patient meets demographic criteria
- Confirm patient meets clinical criteria
- Ascertain in OnCore research portal that patient is not enrolled in any other COVID-19 related clinical trial
- Review chart for azithromycin within 7 days and hydroxychloroquine use within 180 days of planned baseline encounter (including outpatient records)
- Review medical history for exclusionary conditions
- Review allergy record for evidence of hypersensitivity to azithromycin or hydroxychloroquine or related drugs
- Locate and have investigator review EKG in electronic health record obtained within 7 days of planned encounter for evidence of prolonged QTc

At enrollment encounter (Day 1):

- Locate hospital and unit where patient is located
- Verify with treating clinical team that patient is appropriate for the trial
- Determine whether the patient can provide informed consent. If not, identify patient's legally authorized representative.
- If the patient cannot provide informed consent, patient's legally authorized representative should be contacted and the informed consent process should be executed through them.
- The patient or legally authorized representative should be provided a copy of the written informed consent document to review.
- Informed consent should be preferentially obtained by e-consent as outlined above rather than in person.

8.3 Initial assessment and treatment assignment

Study Intervention Procedures

Immediately post enrollment (Day 1):

- Conduct randomization procedure
- Inform treating team of patient's enrollment. Study coordinator (in conjunction with on-call investigator) will advise the team on their patient's treatment assignment, and will advise the team to write treatment orders accordingly.

- Inform team to perform ECG (if none available in the preceding 7 days). For women of childbearing potential (and no clear evidence of pregnancy), inform team to obtain urine qualitative HCG.

Other activities on Day 1:

- Complete case report form (CRF) in a timely manner. Obtain the following data from EHR to complete CRF including the following:
    - Demographic data
    - Most recent height and weight
    - Chronic medical conditions related to study eligibility
    - Date of onset of COVID-19 symptoms
    - Data on hospitalization including date of admission, date of ICU transfer (if applicable), presenting complaint, vitals on presentation, selected laboratory data on presentation
    - Medications administered (with dates) for index illness prior to presentation and during hospitalization to date
    - Medication allergies on record
    - Findings of last documented lung examination
    - Results of chest X-ray or CT scan on presentation
    - Most recent vital signs prior to time of enrollment
    - Most recent complete blood count, basic metabolic panel and hepatic function panel obtained prior to the time or enrollment
    - QTc of most recent ECG
    - Urine pregnancy test (if applicable)
    - Respiratory support status (ECMO, invasive mechanical ventilation, non-invasive ventilation, high-flow nasal cannula (with oxygen flow rate), standard nasal cannula (with oxygen flow rate)
    - Level of care (ICU, stepdown, standard)
    - WHO ordinal scale value (in conjunction with study investigator)

For patients enrolled at DUHS, all of this data will be automatically captured in Redcap as part of the overall COVID research plan, so the coordinator will not need to extract any data. For patients at other sites, data will need to be entered into the Redcap database.

8.4 Assessment during study

Daily Assessment (Days 2-13):

- Study coordinator will complete CRF based on clinical data abstracted from chart with the following information:
    - Vital signs conducted most proximal to CRF entry
    - Maximum temperature in prior 24 hours (9am-8:59 am)
    - New medications administered (with dates) for index illness during interval since last assessment
    - Interim complete blood count, basic metabolic panel and hepatic function panel
    - QTc of most recent EKG conducted in interim
    - Respiratory support status (ECMO, invasive mechanical ventilation, non-invasive ventilation, high-flow nasal cannula (with oxygen flow rate), standard nasal cannula (with oxygen flow rate)

  - Level of care (ICU, stepdown, standard)
  - Adverse event documentation
- For patients enrolled at DUHS, all of this data will be automatically captured in Redcap as part of the overall COVID research plan, so the coordinator will not need to extract any data. For patients at other sites, data will need to be entered into the Redcap database.

Day 14 Assessment:

- Study coordinator will complete CRF based on clinical data abstracted from chart with the following information:
  - Vital signs conducted most proximal to CRF entry
  - Medications related to index illness still being administered, or written for discharge
  - Most proximal chest X-ray or CT scans
  - Most proximal complete blood count, basic metabolic panel and hepatic function panel
  - QTc of most recent ECG conducted in interim
  - Review for possible drug-related adverse events of special interest
  - Need for supplemental oxygen on discharge (mode of delivery, and supplemental oxygen requirement)
  - Discharge disposition
  - If hospitalized, level of care (ICU, stepdown, standard)
  - WHO ordinal scale value (in conjunction with investigator)
- For patients enrolled at DUHS, all of this data will be automatically captured in Redcap as part of the overall COVID research plan, so the coordinator will not need to extract any data. For patients at other sites, data will need to be entered into the Redcap database.

Day 28 Assessment

- Study coordinator will complete CRF based on clinical data abstracted from chart as follows:

  If hospitalized:
  - Number of days since enrollment on which the participant had a measured temperature ≥38.0 C
  - Number of days since enrollment on which the participant was on mechanical ventilation
  - Number of days since enrollment on which the participant was on noninvasive ventilation
  - Number of days since enrollment on which the participant was on supplemental oxygen
  - Level of care (ICU, stepdown, standard)
  - WHO ordinal scale value (in conjunction with investigator)

If discharged:

- Interim visit to emergency department or urgent care
- Interim admission to hospital
- Interim admission into ICU
- Supplemental oxygen requirement
- Level of long-term care required (skilled nursing facility, assisted living, home)
- Functional status reported by outpatient provider (when available)
- Recurrence of fever or respiratory symptoms
- Lung examination reported by provider (when available)
- Follow up lung imaging (when available)
- WHO ordinal scale value (in conjunction with investigator)

Day 45 Assessment:

- Review of medical record to determine vital status and hospitalization status
- Count of total hospital length of stay

WHO Ordinal Scale

- The WHO Ordinal Scale for Clinical Improvement is an assessment developed by the World Health Organization as part of their COVID-19 therapeutic trial (World Health Organization. "WHO R&D Blueprint: Novel Coronavirus COVID-19 Therapeutic Trial Synopsis" 2020, Geneva, Switzerland URL: https://www.who.int/blueprint/priority-diseases/key-action/COVID-19_Treatment_Trial_Design_Master_Protocol_synopsis_Final_18022020.pdf. Accessed 3/31/2020). The assessment tool is intended to report on the clinical status of COVID-19 patients in therapeutic trials on any given day. Details of the scale are below:

Patient State	Descriptor	Score
Uninfected	No clinical or virologic evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of activities	2
Hospitalized, Mild Disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal cannula	4
Hospitalized, Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Mechanical ventilation + additional organ support (pressors, renal replacement therapy, ECMO)	7
Death	Death	8

-
- All designations of WHO ordinal scale will be made by the study PI or co-investigators.
- A study physician licensed to make medical diagnoses and listed on the delegation log will be responsible for all trial-related decisions.

8.5 Concomitant Medications

The use of all non-study drugs from enrollment until 14 days after enrollment will be monitored and recorded. Per standard of care, all potential drug interactions with hydroxychloroquine and azithromycin will be reviewed prior to administration and discussed with the treating team. If a

potential interaction between hydroxychloroquine and a concurrent medication is deemed potentially harmful by the investigator or treating team, a formal discussion of risks and benefits of hydroxychloroquine will take place between the investigator and the treating team. If either the investigator or the treating team deem that the risk of the concurrent medication outweighs potential benefit, hydroxychloroquine will not be administered and the patient will be withdrawn from the study.

8.6 Laboratory Evaluations

Laboratory evaluations will be performed per standard of care under the direction of the treating team.

ECG testing will be recommended as a standard of care intervention prior to and during study drug administration for participants with baseline cardiac conduction abnormalities or electrolyte abnormalities that might predispose to cardiac arrhythmias (see section 11.6).

8.7 Loss to Follow Up

Patients who are discharged from the hospital prior to 14 days after study enrollment will be contacted to ascertain their status on day 14 after enrollment. Patients who cannot be contacted after at least three separate attempts will be assigned a World Health Organization ordinal scale based on health status at the time of hospital discharge.

8.8 Management of Participants Who Discontinue Study Treatment

Participants who prematurely discontinue study treatment will be followed as for other participants and will not receive any additional study treatment during the hospitalization. Participants assigned to receive two study drugs who only discontinue one drug should complete the full course of the other study drug unless there is a contraindication.

If the study is terminated early, study treatment may be continued at the discretion of the investigator and treating team if it is deemed in the participant's best interest.

8.9 Collection of specimens for pharmacokinetic analysis

While no specific specimens will be collected from study participants, we will request permission to use blood left over from routine laboratory blood draws for potential future pharmacokinetic/pharmacodynamic analyses. Blood will either be stored at -80C or will be stored at room temperature as dried blood spots. The future pharmacokinetic/phamacodynamic analyses would consist of measuring the concentrations of study drug (hydroxychloroquine and azithromycin) and correlating these concentrations with clinical outcomes.

9 Study Schedule

The schedule of study activities is summarized in Table 1 below.

Table 1. Schedule of study activities.

	Screen	Baseline	Treatment Period	End of Treatment	Inpatient Follow-up	Day 28 follow-up
Day +/- Window	**-1 or 1**	**1**	**1 to 5**	**5**	**6-14**	**28 ± 2**
ELIGIBILITY						
Demographics & Medical History Review	X					
Review SARS CoV-2 results	X					
Hospital Course Review	X					
Clinical Data Review (including EKG for QTc screen)	X					
Informed consent	X					
STUDY INTERVENTION						
Randomization		X				
Study medication administration			X			
STUDY PROCEDURES						
Vital signs including SpO2		X	Daily		Daily	
Clinical data collection		X	Daily	X	Daily	
Review of laboratory/ECG data		X	Daily		Daily	
Targeted medication review		X	Daily		Daily	
Adverse event evaluation		X	Daily		Daily	X
WHO ordinal scale adjudication		X	Daily	X	Daily	X
EHR review for re-admission or death						X
SAFETY INVESTIGATIONS						
Pregnancy test for females of childbearing potential (if no clear clinical evidence/history of pregnancy)		X				

In addition to the activities outlined above, there will be a single evaluation at the time of hospital discharge to assess length of hospital stay and WHO ordinal scale, if discharge occurs later than 28 days after enrollment.

10 Study Intervention/Investigational Drugs

10.1 Study Drugs

The study drugs are hydroxychloroquine and azithromycin. Each of the study drugs is described in section 3.

The study drugs will be combined into the following study treatment regimens:

Arm 1 (control) Standard of care

Arm 2: Hydroxychloroquine orally for 5 days

Arm 3: Azithromycin orally for 5 days

Arm 4: Hydroxychloroquine orally for 5 days plus azithromycin orally for 5 days

10.2Study Drug Acquisition

As this is a pragmatic trial using readily available FDA-approved medications, study drug will be supplied by the pharmacy of the hospital where the patient is receiving treatment.

10.3Administration and Dosage of Study Drugs

Hydroxychloroquine will be administered orally or via feeding tube at a dosage of 800 mg on day 1, followed by 600 mg daily on days 2-5.

- Patients who do not tolerate the 600 mg daily dose may be dose-reduced to a 400 mg daily dose at the discretion of the study investigator

Azithromycin will be administered orally or via feeding tube at a dosage of 500 mg on day 1, followed by 250 mg daily on days 2-5. If oral azithromycin is not available, intravenous azithromycin at the same dosages may be substituted.

10.4Criteria for Discontinuation of Study Drugs

Participants who meet any one or more of the following criteria will be discontinued from study treatment:

- Participant request for premature discontinuation of study treatment
- Any clinical adverse event, laboratory abnormality, or other medical condition occurs such that continued administration of study treatment is deemed not in the best interest of the patient

11 Assessment of Safety

11.1 Definition of Adverse Events

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease that occurs to a study participant during the course of the study.

Adverse Event Grading

- Mild (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's normal functioning level.

- Moderate (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of functioning, but it is not hazardous to health.

- Severe (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.

- Potentially life-threatening (Grade 4): Extreme limitation in activity, significant assistance required, significant medical intervention or therapy required, or hospitalization required.

[Note: 'Life-threatening' as a Grade 4 severity refers to a 'potential' threat to life whereas 'life

threatening' as a 'serious adverse event' refer to an 'immediate' threat to life.]

11.2 Definition of Serious Adverse Events

- An AE is considered to be 'serious' if it results in one of the following outcomes. An AE needs to meet only one of the above criteria to be considered serious.
  - Death,
  - Life-threatening event (the participant is at immediate risk of death),
  - Requires inpatient hospitalization or prolongation of existing hospitalization
  - Results in persistent or significant disability/incapacity (a substantial disruption of a person's ability to conduct normal life functions),
  - Results in congenital anomaly/birth defect
  - Important medical event that may not be immediately life-threatening or results in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above.

11.3 Assessment of Adverse Events

Both hydroxychloroquine and azithromycin have a track record of safety since their FDA-approval in 1955 and 2002, respectively. There is extensive experience with these drugs in clinical practice. However, because the medicines have not been used in COVID-19 patients, and the use of hydroxychloroquine and azithromycin in combination can increase the risk for major cardiac toxicity including QT prolongation, only drug-specific AEs that are listed in the FDA Warning List for the study drugs and only AEs Grade 3 or higher will be collected.

Hydroxychloroquine has been used for decades for the treatment and prevention of malaria and for autoimmune conditions including rheumatoid arthritis and lupus. The following system abnormalities have been listed under the FDA Warning List for Hydroxychloroquine (mostly with long-term use for autoimmune conditions)
Hypoglycemia: may be severe necessitates close serum glucose monitoring.

Dermatologic: Erythema multiforme, may precipitate a severe attack of psoriasis and porphyria

Nervous system: Dizziness, vertigo, tinnitus, proximal myopathy and muscle weakness, extrapyramidal symptoms, seizure, and suicidal behavior. Close observation in patients with neurological or seizure disorders.

Ocular: irreversible retinal damage has occurred with only with prolonged use (> 5 years) and in individuals with subnormal glomerular filtration, and those with concurrent macular disease (up to 7.5%)

Cardiac: risk of QT prolongation and torsades de pointes, AV block, and ventricular arrythemia. Risk is increased in in patients with underlying QT-prolongation and/or on concurrent QT-prolonging agents.

G6PD deficiency**:** Although warning exists regarding the potential hemolysis in G6PD-deficient patients, studies indicate the risk is very low for hydroxychloroquine and treatment can be initiated while awaiting G6PD test results.

Risk of fetal toxicity**:** no increase in rate of birth defects has been reported; however as the data are considered limited, birth outcomes will be collected at the end of pregnancy.

Drug-drug interactions: See University of Liverpool drug interaction checkers: http://www.covid19-druginteractions.org

The following drugs should not be co-administered: amiodarone, bepridil, mexiletine, rifampicin, and rifapentine

The following drugs may require dose monitoring, alteration of dosage or timing of coadministration: digoxin, propofol, sevoflurane, tizanidine, hydrocodone, methadone, disopyramide, dofelitide, propafenone, quinidine, azithromycin, clarithromycin, erythromycin, levofloxacin, moxifloxacin, ofloxacin, clofazimine, delamanid, bedaquiline, pyrazinamide, telithromycin, mefloquine

Use in children: safety has not been established in children. Fatalities have been reported following accidental ingestion of chloroquine but not of hydroxychloroquine.

Azithromycin has been used extensively for treatment of acute bacterial sinusitis, traveler diarrhea, and community-acquired pneumonia. The following system abnormalities have been listed under the FDA Warning List for arithromycin:

Hypersensitivity: angioedema, anaphylaxis, and dermatologic reactions including Stevens Johnson Syndrome and toxic epidermal necrolysis have been reported rarely

Cardiac: potential risk of QT prolongation and torsades de pointes have been seen, usually in settings of proarrhythmic conditions such as hypokalemia, hypomagnesemia, bradycardia, or those receiving hydroxychloroquine, or antiarrhythmic drugs (quinidine, procainamide, defetilide, amiodarone, sotalol), Risk is also higher in elderly patients. Monitoring for QT prolongation, particularly in setting of concomitant drugs with risk of QT prolongation.

Hepatotoxicity: abnormal liver function, hepatitis, cholestatic jaundice, hepatic necrosis, and hepatic failure have been reported

Clostridium difficile-associated diarrhea: this risk is associated with all antibiotic use

Risk of adverse pregnancy outcome: A recent study suggested that macrolides may confer a higher risk of birth defects compared to penicillins according to a recent study (51). This study compared 100,000 women who had either a single course of a macrolide antibiotic or a penicillin during gestation. The prevalence of major congenital malformations (primarily cardiovascular) associated with first trimester exposure was 28 per 1000 live births compared to 18 in the penicillin group. Second and third trimester prescriptions were not associated with malformations. Macrolide prescriptions in any trimester were associated with genital malformations (mainly hypospadias) in 5 vs 3 per 1000 live births. The absolute numbers of azithromycin compared to other macrolides (clarithromycin and erythromycin) were too small to analyze separately and so it is not clear that the conclusions apply to azithromycin specifically.

11.4 Adverse Event Reporting

Non-serious adverse events will not be collected with the exception of adverse events of special interest possibly related to hydroxychloroquine, which are as follows:

- Arrhythmias (ventricular)
- Hepatic Failure
- Bone marrow failure
- Aplastic anemia
- Prolonged QT interval

- Angioedema
- Exfoliative dermatitis
- Acute generalized exanthematous pustulosis (AGEP)
- Psychosis
- Suicidal Ideation
- Seizure

Serious adverse events which occur between initial dose of study medication and day 14 will be recorded in the electronic case report form. These events, along with the adverse events of special interest listed above, will be entered into the study database and provided to the Data and Safety Monitoring Board upon safety review as required.

Hydroxychloroquine and azithromycin are FDA-approved medicines being used at standard dosing. Reporting to the IRB will occur in a summary format after each Data and Safety Monitoring Board review,

11.5 Reporting of pregnancy outcome

For participants who are pregnant women, we will obtain permission to review the medical record of both the participant and the infant to assess for any complications of the pregnancy and the outcome of the pregnancy. Specific pregnancy-associated outcomes that will be collected are as follows:

- miscarriage in the first trimester
- any fetal deaths and gestational age
- spontaneous preterm birth
- premature rupture of membranes
- fetal growth restriction
- gestational diabetes
- indicated preterm birth
- preeclampsia and severity
- gestational age at delivery
- mode of delivery
- neonatal birth weight
- fetal malformation
- APGAR scores
- neonatal length of stay
- Neonatal intensive care unit admission
- Neonatal death

11.6 Management of adverse events

For adverse events that in the investigator's judgment may be due to study drugs, one of the protocol investigators will discuss the adverse event with the treating physician, and a joint decision will be made whether to discontinue study therapy. If study therapy has already been completed at the time of the adverse event, the patient will be followed per protocol by the study team to assess for resolution of the adverse event. If resolution of the adverse event has not occurred by the day 14 assessment, the adverse event will be reassessed at the day 28 assessment to determine whether resolution has occurred.

Participants who experience adverse events requiring temporary or permanent discontinuation of study drug will still considered to be part of the study and will be followed as outlined above.

11.6.1 Monitoring and management of QTc interval prolongation

The management will be according to the recommendations of the Duke COVID-19 Therapeutic Group as follows:

All patients who are randomized to hydroxychloroquine and/or azithromycin will be placed on telemetry

After 2nd dose of study drug (approx. >24h), repeat ECG

a. If QTc change is <50ms, continue telemetry, no further ECGs
b. If QTc change >50ms or QTc>500, the patient is considered a 'QT reactor': continue telemetry, mitigate QT prolonging factors: including correcting any K^+ or Mg^+ abnormality and stop azithromycin (if patient is in the azithromycin group), repeat ECG in 24h
c. If further prolonged, or remains >500ms, stop hydroxychloroquine

11.7 Data and Safety Monitoring Board

A data and safety monitoring board (DSMB) will be assembled consisting of three individuals with complementary expertise. At least one member will have expertise in infectious diseases or critical care, and at least one member will have expertise in clinical trials. DSMB members will not otherwise be involved in the study. The DSMB will be apprised of ongoing study results and will meet approximately monthly, or more often as needed.

11.8 Interim Monitoring and Analyses by the Data and Safety Monitoring Board

Evaluation of potential adverse events and tolerability will be performed as close to "real time" as possible by the study team and will be regularly reviewed by the DSMB. As the planned Bayesian analysis permits "real-time" looks at the efficacy results without adjustment, an interim efficacy analysis will be available at each DSMB meeting. The DSMB may recommend early closure of either experimental arm or the trial if, in their judgment, interim evidence is sufficiently strong that one of the trial interventions is clearly indicated/contraindicated because of differences in safety and/or efficacy. The interim results will be presented as described in section 12.3.

12 Statistical Considerations

12.1 Study Hypotheses

1) The World Health Organization ordinal scale measured at day 14 after enrollment will be higher (better) in participants receiving hydroxychloroquine than in participants who do not receive hydroxychloroquine.
2) The World Health Organization ordinal scale measured at day 14 after enrollment will be higher (better) in participants receiving azithromycin than in participants who do not receive azithromycin.
3) The proportion of participants not on mechanical ventilation at baseline who progress to requiring mechanical ventilation during the index hospitalization will be lower in participants receiving hydroxychloroquine than in participants who do not receive hydroxychloroquine.

4) The proportion of participants not on mechanical ventilation at baseline who progress to requiring mechanical ventilation during the index hospitalization will be lower in participants receiving azithromycin than in participants who do not receive azithromycin
5) The World Health Organization ordinal scale measured at day 28 after enrollment will be higher (better) in participants receiving hydroxychloroquine than in participants who do not receive hydroxychloroquine.
6) The World Health Organization ordinal scale measured at day 28 after enrollment will be higher (better) in participants receiving azithromycin than in participants who do not receive azithromycin.
7) The median length of hospitalization during the index hospitalization will be lower among participants receiving hydroxychloroquine than in participants who do not receive hydroxychloroquine.
8) The median length of hospitalization during the index hospitalization will be lower among participants receiving azithromycin than in participants who do not receive azithromycin.

12.2 Analysis Groups

Intention to treat (ITT): Includes all participants who receive a treatment assignment

Modified intention to treat (mITT): Includes all participants who received at least one dose of study medication

Per protocol (PP): Includes all participants who received all assigned study medication

12.3 Analysis Plan

All major treatment group comparisons will be performed according to the principle of "intent-to-treat;" that is, participants will be analyzed (and outcomes attributed) according to the randomized treatment, regardless of actual treatments received. The primary analysis of all primary and secondary endpoints will be performed in the modified intent-to-treat population. Treatment group comparisons will involve the following randomized treatment groups:

A. Standard of care plus hydroxychloroquine

A1. Standard of care + hydroxychloroquine + sub-randomization to azithromycin

A2. Standard of care + hydroxychloroquine + sub-randomization to no azithromycin

A3. Standard of care + hydroxychloroquine, not eligible for azithromycin sub-randomization

B. Standard of care without hydroxychloroquine

B1. Standard of care + azithromycin

B2. Standard of care + no azithromycin

B3. Standard of care, not eligible for azithromycin sub-randomization

The two major treatment group comparisons to be performed are:

- Comparison #1: Hydroxychloroquine versus no hydroxychloroquine.
  - Aggregate of (A1 + A2 + A3) versus (B1 + B2 + B3)
- Comparison #2: Azithromycin versus no azithromycin.
  - Aggregate of (A1 + B1) versus (A2 + B2)

12.3.1. Primary efficacy analysis

The primary analysis of the primary efficacy endpoint, the World Health Organization scale measured on day 14 after enrollment, will be performed using the proportional odds model for ordered categorical data. The proportional odds methodology is an extension of binary logistic regression to accommodate $k > 2$ ordered outcome categories. The model considers all possible ways of dichotomizing k categories into a binary outcome and assumes that the set of all possible binary outcomes is related to covariates through a set of ordinary binary logistic regression models. Treatment group differences in the proportional odds model are expressed as odds ratios with an odds ratio less than 1 suggesting that treatment is better than the control and an odds ratio greater than 1 suggesting that treatment is worse than the control. The magnitude of the odds ratio describes the extent to which treated and untreated patients tend to have outcomes shifted toward better or worse ranking categories. To increase power and enhance interpretability, the proportional odds model may be adjusted for a set of relevant covariates, such as enrolling site and ventilator status at baseline. Covariates will be carefully selected and defined in the trial's Statistical Analysis Plan document. The comparison of hydroxychloroquine versus no hydroxychloroquine will be further adjusted for azithromycin assignment and the comparison of azithromycin versus no azithromycin will be adjusted for hydroxychloroquine assignment. Interaction (effect modification) between hydroxychloroquine and azithromycin will be specifically examined, and estimates of the potential magnitude and credible intervals around that interaction term will be generated.

Parameters of the proportional odds model will be estimated in a Bayesian statistical framework by specifying a prior probability distribution for unknown parameters. The output of a Bayesian analysis is a posterior probability distribution describing the relative likelihood of different numerical estimates for the unknown parameter values. This posterior distribution can be used to determine the likelihood of a clinically important treatment benefit or harm in light of the study data. The Bayesian approach provides an especially useful perspective for interpreting results of small studies that are likely to be accompanied by substantial statistical uncertainty. The Bayesian approach allows decisions to be made based on the estimated probability of a clinically important treatment benefit, as opposed to an arbitrary dichotomization of results as "significant" or "non-significant."

12.3.2 Secondary efficacy analyses

Mechanical ventilation.

The proportion of participants not on mechanical ventilation at baseline who progress to requiring mechanical ventilation will be compared for patients belonging to the following treatment groups as defined above at the beginning of section 12.3:

1) Hydroxychloroquine versus no hydroxychloroquine.
2) Azithromycin versus no azithromycin.

The comparison will be performed using logistic regression and will be adjusted for the same covariates as the primary endpoint proportional odds model.

World Health Organization ordinal scale measured at day 28

The World Health Organization ordinal scale at day 28 will be compared for the following treatment groups as defined above at the beginning of section 12.3:

1) Hydroxychloroquine versus no hydroxychloroquine.
2) Azithromycin versus no azithromycin.

Analysis will be based on the proportional odds model and will be similar to the analysis of the primary endpoint as described above.

Length of hospitalization

The distribution of days from randomization to hospital discharge will be compared for the following treatment groups as defined above at the beginning of section 12.3:

1) Hydroxychloroquine versus no hydroxychloroquine.
2) Azithromycin versus no azithromycin.

The comparison will be based on a semi-parametric or rank based analysis. For participants who die during the enrollment hospitalization, the time from randomization to discharge will be regarded as infinite.

12.3.3 Safety analyses

There will be two comparisons for the primary safety analysis; both will consider the modified intention to treat population during the first fourteen days after enrollment. Comparison 1 will compare the rate of severe adverse events in participants who received hydroxychloroquine versus the rate in participants who did not receive hydroxychloroquine. Comparison 2 will compare the rate of severe adverse events in participants who received azithromycin versus the rate in participants who did not receive azithromycin. An experimental arm will be deemed less safe than the standard of care arm if the observed rate of severe adverse events is greater than the control arm and the 95% credible interval (using a neutral prior distributions) around the difference in rates excludes zero.

The secondary safety analyses will examine the rates of all-cause study drug (hydroxychloroquine and azithromycin) discontinuation in the modified intention to treat population, and will describe them as point estimates and 95% credible intervals (using a neutral prior distribution).

12.4 Sample Size Considerations

Table 2 summarizes frequentist considerations of sample size, based on comparison of the primary outcome between the group of participants who received hydroxychloroquine and those who did not receive hydroxychloroquine. This assumes that the aggregate probabilities of all trial participants to have a day 14 World Health Organization ordinal scale score of 1-8 are as follows: Death 5%, mechanical ventilation/ECMO 10%, noninvasive ventilation/high flow oxygen 15%, hospitalized on supplemental oxygen 35%, hospitalized not requiring supplemental oxygen 5%, hospitalized not requiring ongoing medical care 5%, not hospitalized, limitation on activities and/or requiring home oxygen 5%, not hospitalized, no limitations on activities 20%.

Table 2. Frequentist estimates of power with given odds/win ratios and sample sizes.

Two-Sided	Odds	Win	Power (Two-Group Trial With Total Sample Size N)			
Alpha	Ratio	Ratio	N = 200	N = 300	N = 400	N = 500

0.05	0.75	1.26	20%	29%	36%	44%
	0.70	1.33	29%	41%	51%	61%
	0.65	1.41	40%	55%	67%	77%
	0.60	1.50	52%	70%	82%	89%
	0.55	1.61	66%	83%	92%	96%
	0.50	1.73	78%	92%	97%	99%
	0.45	1.89	88%	97%	99%	>99%
	0.40	2.07	95%	99%	>99%	>99%
0.10	0.75	1.26	31%	40%	49%	56%
	0.70	1.33	41%	53%	64%	72%
	0.65	1.41	52%	67%	78%	85%
	0.60	1.50	64%	80%	89%	94%
	0.55	1.61	76%	89%	96%	98%
	0.50	1.73	86%	96%	99%	>99%
	0.45	1.89	93%	99%	>99%	>99%
	0.40	2.07	98%	>99%	>99%	>99%

The table above is based on the Wilcoxon rank sum test. Group-specific probabilities are assumed to be described by a proportional odds model i.e. randomization to hydroxychloroquine decreases the odds of being in one of the k worst outcome categories and the same odds ratio applies to all possible choices of k. When OR=0.5 the group-specific probabilities are HCQ: 3.41%, 7.28%, 12.14%, 34.39%, 5.61%, 5.79%, 5.95%, 25.43% and standard care: 6.59%, 12.72%, 17.86%, 35.61%, 4.39%, 4.21%, 4.05%, 14.57%. The Win Ratio implied by these two sets of probabilities is 1.73.

To examine sample size from a Bayesian perspective, we used Monte Carlo simulations to study trial operating characteristics under a range of hypothetical true treatment effects. The operating characteristics of interest were frequentist power, type-I error, and expected study duration. Simulations are based on a two-arm trial that enrolls patients until declaring the treatment of interest to be efficacious, stopping for evidence of harm or inefficacy, or reaching a pre-specified maximum sample size, which we assumed to be N = 200, 300, 400, or 500. The primary endpoint is the WHO 8-level ordinal scale and the primary measure of treatment effect is the win ratio. Treatment group differences were assessed by fitting a proportional odds model with the WHO scale as an ordinal outcome and treatment group as a covariate. Properties of a fully Bayesian analysis were approximated by assuming that the maximum likelihood estimate of the treatment effect coefficient was normally distributed. The prior distribution assigned 2.5% probability to the hypothesis of a true odds ratio below 0.5 (highly efficacious) and 2.5% probability to the hypothesis of a true odds ratio exceeding 2.0 (highly harmful). In each simulated trial we assumed that interim analyses were performed sequentially after each patient beginning after collecting data from the first 20 patients. Signals for harm and efficacy were defined as follows:

- Moderate efficacy: The win ratio exceeds 1.25 with at least 80% Bayesian probability

- Any efficacy: The win ratio exceeds 1.00 with at least 95% Bayesian probability
- Moderate harm: The win ratio is less than 0.90 with at least 75% Bayesian probability
- Inefficacy/any Harm: The win ratio is less than 1.00 with at least 80% Bayesian probability

For simplicity, the simulation assumes that each patient's outcome is known before the next patient is randomized. In each simulated trial, the true probabilities for the 8 WHO categories in aggregate across two treatment groups were assumed to be 5%, 10%, 15%, 35%, 5%, 5%, 5%, 20%. Data were generated under scenarios with true odds ratios ranging from 0.4 (benefit) to 2.0 (harm). We simulated 500 trials per odds ratio scenario and estimated operating characteristics by averaging across simulated trial results.

Table 3. Probability of reaching threshold for moderate or any efficacy as function of true odds ratio if enrollment is capped at 200, 300, 400, or 500 patients.

True	Max N = 200		Max N = 300		Max N = 400		Max N = 500	
Odds Ratio	Moderate	Any	Moderate	Any	Moderate	Any	Moderate	Any
Power								
OR = 0.75	19%	27%	27%	43%	35%	55%	40%	66%
OR = 0.70	24%	36%	39%	55%	46%	71%	55%	80%
OR = 0.65	41%	51%	56%	70%	65%	81%	71%	88%
OR = 0.60	52%	64%	70%	84%	80%	93%	87%	96%
OR = 0.55	62%	71%	81%	90%	89%	96%	93%	99%
OR = 0.50	76%	84%	90%	96%	96%	99%	99%	>99%
OR = 0.45	86%	93%	95%	98%	98%	99%	>99%	>99%
OR = 0.40	93%	96%	99%	99%	99%	>99%	>99%	>99%
Type-I Error								
OR = 1.00	4%	8%	5%	10%	5%	13%	6%	14%
OR = 1.05	4%	5%	5%	7%	5%	8%	5%	9%
OR = 1.10	1%	2%	2%	4%	2%	4%	2%	4%
OR = 1.15	1%	1%	1%	1%	1%	2%	1%	2%
OR = 1.25	1%	1%	1%	1%	1%	1%	1%	1%
OR = 1.50	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
OR = 2.00	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%

Table 4. Probability of reaching threshold for inefficacy or harm as function of true odds ratio if enrollment is capped at 200, 300, 400, or 500 patients.

True	Max N = 200		Max N = 300		Max N = 400		Max N = 500	
Odds Ratio	Harm	Inefficacy	Harm	Inefficacy	Harm	Inefficacy	Harm	Inefficacy
Power								
OR = 1.00	22%	37%	28%	43%	31%	47%	33%	49%
OR = 1.05	32%	45%	37%	52%	40%	55%	42%	59%
OR = 1.10	32%	45%	39%	55%	43%	61%	47%	65%
OR = 1.15	43%	56%	49%	64%	54%	71%	58%	77%
OR = 1.25	55%	67%	65%	76%	70%	81%	74%	87%
OR = 1.50	73%	84%	85%	93%	91%	96%	94%	98%
OR = 2.00	97%	98%	>99%	>99%	>99%	>99%	>99%	>99%
Type-II Error								
OR = 0.75	6%	10%	6%	11%	6%	11%	6%	11%
OR = 0.70	4%	8%	4%	8%	4%	8%	4%	8%
OR = 0.65	2%	5%	2%	5%	2%	5%	2%	5%
OR = 0.60	1%	2%	1%	2%	1%	2%	1%	2%
OR = 0.55	1%	3%	1%	3%	1%	3%	1%	3%
OR = 0.50	<1%	1%	<1%	1%	<1%	1%	<1%	1%
OR = 0.45	<1%	1%	<1%	1%	<1%	1%	<1%	1%
OR = 0.40	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%

Table 5. Expected looks until reaching threshold for moderate efficacy or reaching enrollment cap as function of true odds ratio

Odds Ratio	Max N = 200	Max N = 300	Max N = 400	Max N = 500
OR = 0.75	182	259	327	390
OR = 0.70	181	249	306	355
OR = 0.65	167	218	257	289
OR = 0.60	155	193	218	234
OR = 0.55	145	173	187	197
OR = 0.50	133	150	156	158
OR = 0.45	115	124	127	128
OR = 0.40	101	104	105	105

Table 6. Expected looks until stopping for futility or harm or reaching enrollment cap as function of true odds ratio

Odds Ratio	Max N = 200	Max N = 300	Max N = 400	Max N = 500
OR = 1.00	159	219	274	326
OR = 1.05	149	200	246	289
OR = 1.10	146	196	238	275
OR = 1.15	131	170	203	228
OR = 1.25	117	146	168	184
OR = 1.50	96	107	113	116
OR = 2.00	59	60	60	60

12.5 Interim Analysis

For ethical reasons, interim examination of clinical endpoints and key safety events will be performed at regular intervals (approximately monthly) during the course of the trial. An independent Data and Safety Monitoring Board (DSMB) will be appointed to monitor participant safety and to review overall trial performance. The primary objective of these interim analyses will be to ensure the safety of trial participants and evaluate the accumulating evidence for safety and efficacy. In addition, interim monitoring will involve a review of participant recruitment, compliance with the study protocol, status of data collection, and other factors which reflect the overall progress and integrity of the study.

Interim efficacy analyses will focus on the primary endpoint (World Health Organization ordinal scale) and will be performed both for the comparison of hydroxychloroquine versus no hydroxychloroquine and for the comparison of azithromycin versus no azithromycin. At each interim analysis, we will use the Bayesian proportional odds model to evaluate the probabilities for each of the following hypotheses in light of accruing study data:

<u>Hydroxychloroquine</u>

- Moderate efficacy.
    - Defined as an odds ratio of 0.80 or less favoring treatment hydroxychloroquine over no hydroxychloroquine
- Any efficacy
    - Defined as an odds ratio of less than 1.0, favoring treatment hydroxychloroquine over no hydroxychloroquine
- Inefficacy
    - Defined as an odds ratio greater than 1.0, favoring treatment no hydroxychloroquine over hydroxychloroquine
- Moderate harm
    - Defined as an odds ratio greater than 1.1, favoring treatment no hydroxychloroquine over hydroxychloroquine

<u>Azithromycin</u>

- Moderate efficacy.

    - Defined as an odds ratio of 0.80 or less favoring treatment azithromycin over no azithromycin
- Any efficacy
    - Defined as an odds ratio of less than 1.0, favoring treatment azithromycin over no azithromycin
- Inefficacy
    - Defined as an odds ratio greater than 1.0, favoring treatment no azithromycin over azithromycin
- Moderate harm
    - Defined as an odds ratio greater than 1.1, favoring treatment no azithromycin over azithromycin

The following events will be regarded as signals for review and consideration of early stopping.

- Probability of at least moderate efficacy is >80%.
- Probability of any efficacy is >95%
- Probability of inefficacy is >80%
- Probability of moderate harm is >75%

If any of the above signals are triggered, the DSMB will be notified and enrollment will be temporarily suspended while the DSMB considers whether to recommend continuing or terminating the study.

It is important to emphasize the triggers listed above are intended as a guide for interpreting the interim analyses and not as a strict rule for early termination. The DSMB will consider a variety of results, including efficacy analyses of secondary endpoints, results from other ongoing and recently completed studies, and all aspects of safety.

12.6 Multiplicity Considerations

With the various primary and secondary endpoints, and two major treatment group comparisons, we recognize that there is a multiplicity of analyses to be performed, which leads to an increased probability that at least one of the comparisons could be statistically "significant" by chance. The Bayesian statistical framework addresses multiplicity by focusing on the probability that a treatment is beneficial or harmful as opposed to the probability of observing a significant difference if the treatment effect is literally zero. If the prior distribution is chosen to be a faithful reflection of the prior evidence in favor or against a clinically important treatment benefit, then posterior probabilities are an appropriate reflection of the posterior evidence and can be used for decision making without the need for an ad hoc multiplicity adjustment. Importantly, the results of the trial will not be explicitly tied to a labeling decision or recommendation for practice but will be reported in an objective manner in order to provide evidence to be used by multiple stakeholders. Although the analysis will not be explicitly adjusted to account for multiple comparisons, we will be appropriately conservative in the interpretation of these multiple comparisons, taking into account the magnitude of observed differences and looking for consistency across endpoints and subgroups. Publications presenting trial results will present a balanced interpretation and will call attention to the potential for results to be impacted by random sampling variation.

13 Protection of Human Subjects

This study will be conducted in conformity with the ethical standards set out in the latest version of the Declaration of Helsinki.

13.1 Institutional Review Board

Each participating institution will provide for the review and approval of this protocol and the associated informed consent documents by an appropriate institutional ethics committee (IEC) or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are placed into use.

13.2 Informed consent process

Only individuals who provide written informed consent, or in the absence of ability to provide consent have consent provided by their appointed medical decision-maker, will be enrolled in this study. Written informed consent is required before any study-specific procedures or receipt of study medications. Potential participants and/or their medical decision makers will have the conditions of the study explained to them, including potential harms and benefits, the nature and timing of study procedures, alternatives to study participation, that study participation is voluntary, that a decision to not participate in the study will not affect the quality of their future medical care, and that they may withdraw from participation at any time. Literate individuals will be provided with a language-appropriate document to read; illiterate individuals (i.e. individuals who speak and understand, but do not read and write, the language in which the consent discussion is conducted) will have the contents of the document explained to them by a trained study staff member; such individuals can be enrolled by 'making their mark' on the consent document. Potential participants will have the opportunity to ask questions of the site investigator or delegate, and to discuss participation with their family and/or friends or think about the study prior to deciding whether or not to participate. A copy of the signed informed consent document will be given to the participant for his/her records.

13.3 Participant confidentiality

All records identifying the participant will be kept confidential and, to the extent permitted by the applicable laws and regulations, will not be made publicly available without sufficient de-identification procedures.

All paper study records will be stored in a locked office and electronic study records will be stored on password-protected computers; only designated trained study staff will have access to study records.

Clinical data will be maintained in a secure, HIPAA compliant, password-protected RedCap database managed by the Duke Office of Clinical Research (DOCR) Research Management Team. The database resides on Duke Health Technology Services (DHTS) managed servers behind a Duke firewall and is accessible only by designated study staff with a password protected account.

Study monitors and/or regulatory authorities may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records, primary laboratory data, and pharmacy records for study participants; this information will be provided to

participants during the Informed Consent process. The clinical study site will permit access to such records.

13.4 Study discontinuation

The Data and Safety Monitoring Board may recommend study termination, termination of a study arm, or termination of a study site. If the study is closed or enrollment is terminated at a site, the DUHS Institutional Review Board will be notified. Participants experiencing an adverse event at the time of early termination should be followed until resolution or stabilization of the event. Participants who were receiving study treatment at the time of early termination will continue care per the preferences of their treating medical providers.

14 Data Handling and Record Keeping

Information from the medical record will be directly downloaded into a secure RedCap database. The directly downloaded data will be supplemented with electronic medical record review by one of the investigators, the results of which will also be entered into the RedCap database. After study completion, the secure data will be retained for six years per DUHS policy.

15 Publications and Dissemination of Study Results

Study results will be analyzed and published promptly after approval by all investigators. No identifying information regarding individual participants will be included in any publication.

REFERENCES

- 1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
- 2. Rainsford KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. Inflammopharmacology. 2015;23(5):231-69.
- 3. Sahraei Z, Shabani M, Shokouhi S, Saffaei A. Aminoquinolines Against Coronavirus Disease 2019 (COVID-19): Chloroquine or Hydroxychloroquine. Int J Antimicrob Agents. 2020:105945.
- 4. Devaux CA, Rolain JM, Colson P, Raoult D. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? Int J Antimicrob Agents. 2020:105938.
- 5. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J. 2005;2:69.
- 6. Olofsson S, Kumlin U, Dimock K, Arnberg N. Avian influenza and sialic acid receptors: more than meets the eye? Lancet Infect Dis. 2005;5(3):184-8.
- 7. Yang ZY, Huang Y, Ganesh L, Leung K, Kong WP, Schwartz O, et al. pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. J Virol. 2004;78(11):5642-50.
- 8. Savarino A, Lucia MB, Rastrelli E, Rutella S, Golotta C, Morra E, et al. Anti-HIV effects of chloroquine: inhibition of viral particle glycosylation and synergism with protease inhibitors. J Acquir Immune Defic Syndr. 2004;35(3):223-32.
- 9. Klumperman J, Locker JK, Meijer A, Horzinek MC, Geuze HJ, Rottier PJ. Coronavirus M proteins accumulate in the Golgi complex beyond the site of virion budding. J Virol. 1994;68(10):6523-34.
- 10. Schrezenmeier E, Dorner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol. 2020;16(3):155-66.
- 11. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? Lancet Infect Dis. 2003;3(11):722-7.

- 12. Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Clin Infect Dis. 2020.
- 13. Beigelman A, Mikols CL, Gunsten SP, Cannon CL, Brody SL, Walter MJ. Azithromycin attenuates airway inflammation in a mouse model of viral bronchiolitis. Respir Res. 2010;11:90.
- 14. Schultz MJ. Macrolide activities beyond their antimicrobial effects: macrolides in diffuse panbronchiolitis and cystic fibrosis. J Antimicrob Chemother. 2004;54(1):21-8.
- 15. Equi A, Balfour-Lynn IM, Bush A, Rosenthal M. Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. Lancet. 2002;360(9338):978-84.
- 16. Garcia Vazquez E, Mensa J, Martinez JA, Marcos MA, Puig J, Ortega M, et al. Lower mortality among patients with community-acquired pneumonia treated with a macrolide plus a beta-lactam agent versus a beta-lactam agent alone. Eur J Clin Microbiol Infect Dis. 2005;24(3):190-5.
- 17. Arabi YM, Deeb AM, Al-Hameed F, Mandourah Y, Almekhlafi GA, Sindi AA, et al. Macrolides in critically ill patients with Middle East Respiratory Syndrome. Int J Infect Dis. 2019;81:184-90.
- 18. Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun. 2004;323(1):264-8.
- 19. Keyaerts E, Li S, Vijgen L, Rysman E, Verbeeck J, Van Ranst M, et al. Antiviral activity of chloroquine against human coronavirus OC43 infection in newborn mice. Antimicrob Agents Chemother. 2009;53(8):3416-21.
- 20. Tan YW, Yam WK, Sun J, Chu JJH. An evaluation of Chloroquine as a broad-acting antiviral against Hand, Foot and Mouth Disease. Antiviral Res. 2018;149:143-9.
- 21. Colson P, Rolain JM, Lagier JC, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. Int J Antimicrob Agents. 2020:105932.
- 22. Biot C, Daher W, Chavain N, Fandeur T, Khalife J, Dive D, et al. Design and synthesis of hydroxyferroquine derivatives with antimalarial and antiviral activities. J Med Chem. 2006;49(9):2845-9.

- 23. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020;30(3):269-71.
- 24. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends. 2020;14(1):72-3.
- 25. Gao ZC. [Efficient management of novel coronavirus pneumonia by efficient prevention and control in scientific manner]. Zhonghua Jie He He Hu Xi Za Zhi. 2020;43(0):E001.
- 26. Chen X. Hydroxychloroquine and chloroquine. Clin Infect Dis. 2020.
- 27. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care. 2020.
- 28. Chen Z, Hu J, Zhang Z, Jiang S, Han S, Yan D, et al. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. medRxiv. 2020.
- 29. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents. 2020:105949.
- 30. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and
- azithromycin in 80 COVID-19 patients with at least a six-day follow up: an
- observational study Int J Antimicrob Agents. 2020;Online only(Pre-print).
- 31. Molina JM, Delaugerre C, Le Goff J, Mela-Lima B, Ponscarme D, Goldwirt L, et al. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. Medecine et Maladies Infectieuses. 2020.
- 32. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov. 2020;6:16.
- 33. Popert AJ. Chloroquine: a review. Rheumatol Rehabil. 1976;15(3):235-8.
- 34. Lim HS, Im JS, Cho JY, Bae KS, Klein TA, Yeom JS, et al. Pharmacokinetics of hydroxychloroquine and its clinical implications in chemoprophylaxis against malaria caused by Plasmodium vivax. Antimicrob Agents Chemother. 2009;53(4):1468-75.

- 35. McChesney EW, Conway WD, Banks WF, Jr., Rogers JE, Shekosky JM. Studies of the metabolism of some compounds of the 4-amino-7-chloroquinoline series. J Pharmacol Exp Ther. 1966;151(3):482-93.
- 36. Durkin MJ, Jafarzadeh SR, Hsueh K, Sallah YH, Munshi KD, Henderson RR, et al. Outpatient Antibiotic Prescription Trends in the United States: A National Cohort Study. Infect Control Hosp Epidemiol. 2018;39(5):584-9.
- 37. Lexi-Drugs: Azithromycin (Systemic) Drug Information [Internet]. Wolters Kluwer Clinical Drug Information, Inc. 2020 [cited March 27, 2020].
- 38. Zimmermann P, Ziesenitz VC, Curtis N, Ritz N. The Immunomodulatory Effects of Macrolides-A Systematic Review of the Underlying Mechanisms. Front Immunol. 2018;9:302.
- 39. Kanoh S, Rubin BK. Mechanisms of Action and Clinical Application of Macrolides as Immunomodulatory Medications. Clinical Microbiology Reviews. 2010;23(3):590-615.
- 40. Kawamura K, Ichikado K, Takaki M, Eguchi Y, Anan K, Suga M. Adjunctive therapy with azithromycin for moderate and severe acute respiratory distress syndrome: a retrospective, propensity score-matching analysis of prospectively collected data at a single center. Int J Antimicrob Agents. 2018;51(6):918-24.
- 41. Gielen V, Johnston SL, Edwards MR. Azithromycin induces anti-viral responses in bronchial epithelial cells. Eur Respir J. 2010;36(3):646-54.
- 42. Menzel M, Akbarshahi H, Bjermer L, Uller L. Azithromycin induces anti-viral effects in cultured bronchial epithelial cells from COPD patients. Sci Rep. 2016;6:28698.
- 43. Schogler A, Kopf BS, Edwards MR, Johnston SL, Casaulta C, Kieninger E, et al. Novel antiviral properties of azithromycin in cystic fibrosis airway epithelial cells. Eur Respir J. 2015;45(2):428-39.
- 44. Tran DH, Sugamata R, Hirose T, Suzuki S, Noguchi Y, Sugawara A, et al. Azithromycin, a 15-membered macrolide antibiotic, inhibits influenza A(H1N1)pdm09 virus infection by interfering with virus internalization process. J Antibiot (Tokyo). 2019;72(10):759-68.
- 45. Ohara H, Nakamura Y, Watanabe Y, Cao X, Yamazaki Y, Izumi-Nakaseko H, et al. Azithromycin Can Prolong QT Interval and Suppress Ventricular Contraction, but Will Not Induce Torsade de Pointes. Cardiovasc Toxicol. 2015;15(3):232-40.
- 46. Milberg P, Eckardt L, Bruns HJ, Biertz J, Ramtin S, Reinsch N, et al. Divergent proarrhythmic potential of macrolide antibiotics despite similar QT prolongation: fast

phase 3 repolarization prevents early afterdepolarizations and torsade de pointes. J Pharmacol Exp Ther. 2002;303(1):218-25.

- 47. Svanstrom H, Pasternak B, Hviid A. Use of azithromycin and death from cardiovascular causes. N Engl J Med. 2013;368(18):1704-12.
- 48. Almalki ZS, Guo JJ. Cardiovascular events and safety outcomes associated with azithromycin therapy: a meta-analysis of randomized controlled trials. Am Health Drug Benefits. 2014;7(6):318-28.
- 49. Sarkar M, Woodland C, Koren G, Einarson AR. Pregnancy outcome following gestational exposure to azithromycin. BMC Pregnancy Childbirth. 2006;6:18.
- 50. Ramsey PS, Vaules MB, Vasdev GM, Andrews WW, Ramin KD. Maternal and transplacental pharmacokinetics of azithromycin. Am J Obstet Gynecol. 2003;188(3):714-8.
- 51. Fan H, Gilbert R, O'Callaghan F, Li L. Associations between macrolide antibiotics prescribing during pregnancy and adverse child outcomes in the UK: population based cohort study. BMJ. 2020;368:m331.
- 52. Salman S, Davis TM, Page-Sharp M, Camara B, Oluwalana C, Bojang A, et al. Pharmacokinetics of Transfer of Azithromycin into the Breast Milk of African Mothers. Antimicrob Agents Chemother. 2015;60(3):1592-9.
- 53. Sutton AL, Acosta EP, Larson KB, Kerstner-Wood CD, Tita AT, Biggio JR. Perinatal pharmacokinetics of azithromycin for cesarean prophylaxis. Am J Obstet Gynecol. 2015;212(6):812 e1-6.
- 54. Lund M, Pasternak B, Davidsen RB, Feenstra B, Krogh C, Diaz LJ, et al. Use of macrolides in mother and child and risk of infantile hypertrophic pyloric stenosis: nationwide cohort study. BMJ. 2014;348:g1908.
- 55. Goldstein LH, Berlin M, Tsur L, Bortnik O, Binyamini L, Berkovitch M. The safety of macrolides during lactation. Breastfeed Med. 2009;4(4):197-200.