Protocol Title:

Efficacy of Novel Agents for Treatment of SARS-CoV-2 Infection Among High-Risk Outpatient Adults: An Adaptive Randomized Platform Trial

NCT Number:

NCT04354428

Document Date:

Version 1.4; 11 April 2020

About this Model Protocol

This model document is a protocol template created to align institutions engaged in evaluating hydroxychloroquine (HCQ), HCQ in combination with azithromycin, and other therapies for SARS-CoV-2 treatment. It contains the following:

- Sections written in black text are mandatory and must be consistent across the participating institutions to maintain scientific integrity of the project
- *Sections written in italics are optional and may be omitted as required (e.g., based on institutional needs and practices)*
- A site-specific protocol addendum template (Appendix 4) is provided to capture information that is specific to a given study site

These materials are provided "AS IS" WITHOUT WARRANTY OF ANY KIND, EITHER EXPRESSED OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. Authors and Sponsors of this document do not accept any responsibility for any loss of any kind including loss of revenue, business, anticipated savings or profits, loss of goodwill or data, or for any indirect consequential loss whatsoever to any person using these materials or acting or refraining from action as a result of the information contained in these materials. Any party using these materials bears sole and complete responsibility for ensuring that the materials, whether modified or not, are suitable for the particular use and are accurate, current, commercially reasonable under the circumstances, and comply with all applicable laws and regulations.

Nothing in this model document should be construed to represent or warrant that persons using this document have complied with all applicable laws and regulations. All individuals and organizations using this document bear responsibility for complying with the applicable laws and regulations for the relevant jurisdiction.

CLINICAL STUDY PROTOCOL

Protocol Title:

Efficacy of Novel Agents for Treatment of SARS-CoV-2 Infection Among High-Risk Outpatient Adults: An Adaptive Randomized Platform Trial

Compound:

A. Hydroxychloroquine
B. Azithromycin
C. Ascorbic acid (control)
D. Folic acid (control)

Short Title:

Treatment for SARS-CoV-2 in High-Risk Adult Outpatients

Protocol Version and Approval Date:

Version 1.4; 11 April 2020

Protocol Registry Number:

To be determined

Regulatory Agency Identifying Number(s):

Not applicable

Sponsor Name and Legal Registered Address:

University of Washington
Roosevelt Way NE, Seattle, WA 98115

Funding Agency and Legal Registered Address:

Bill & Melinda Gates Foundation
1300 I St NW, Washington, DC 20005

Principal Investigator:

Dr. Christine Johnston, University of Washington
Phone: +1 206 520 4318; Email: cjohnsto@uw.edu

Sponsor Signatory Name and Title:

_____ _____

Signature **Date**

Medical Monitor name and contact information is provided separately for each site.

Table of Contents

1. Synopsis....6
2. Schedule of Activities....11
3. Introduction....13
3.1. Background....14
3.2. Study Rationale....15
3.2.1. COVID-19 and Antiviral Approaches....15
3.2.2. Antiviral Effects of Chloroquine Analogues Against COVID-19....15
3.2.3. Rationale for Drug Selection of HCQ....16
3.2.4. Rationale for Dosing Schedule of HCQ....16
3.2.5. Antiviral Effects of Azithromycin Against COVID-19....17
3.2.6. Rationale for Drug Selection of Azithromycin....17
3.2.7. Rationale for Dose Selection of Azithromycin....18
3.2.8. Rationale for Ascorbic Acid Control as a Comparator for HCQ....18
3.2.9. Rationale for Folic Acid Control as a Comparator for Azithromycin....19
3.3. Benefit/Risk Assessment....19
4. Objectives and Endpoints....20
5. Study Design....22
5.1. Overall Design....22
5.2. Participant and Study Completion....23
6. Study Population....24
6.1. Inclusion Criteria....24
6.2. Exclusion Criteria....24
6.3. Screen Failures....25
6.4. Recruitment....25
6.5. Co-enrollment Guidelines....26
7. Treatments....27
7.1. Treatments Administered....27
7.2. Risks to the Participants....27
7.2.1. Risks Associated Administration with HCQ....27
7.2.2. Risks Associated with Azithromycin....28
7.2.3. QT Risks Associated with Coadministration of HCQ and Azithromycin....28
7.2.4. Risks Associated with COVID-19 Diagnosis....28
7.3. Strategies to Minimize Risk....29
7.3.1. Dose Selection....29
7.3.2. Management of Participants to Limit Risks of SARS-CoV-2 Transmission....29
7.4. Dose Modification and Toxicity Management....29
7.5. Method of Treatment Assignment....31

7.6. Blinding 31
7.6.1. Patient Blinding 31
7.7. Preparation/Handling/Storage/Accountability 32
7.8. Treatment Compliance 32
7.9. Concomitant Therapy 32
7.9.1. Prohibited Medications 32
7.9.2. Precautionary Medications 33
7.10. Treatment After the End of the Study 33

8. Discontinuation/Withdrawal Criteria 34
8.1. Discontinuation of Study Treatment 34
8.2. Withdrawal from the Study 34
8.3. Lost to Follow-up 35

9. Study Encounters 36
9.1. Screening/Baseline Evaluation: Day 0/1 36
9.2. Day 2 Through Day 13 37
9.3. Days 2, 4, 9, and 14 (±1 day) 37
9.4. Days 14 and 21 37
9.5. Day 28 38

10. Study Assessments and Procedures 39
10.1. Efficacy Assessments 39
10.1.1. Mid-nasal Swab 39
10.1.2. Participant Survey 39
10.2. Adverse Events 40
10.2.1. Adverse Events 40
10.2.2. Serious Adverse Events 40
10.2.3. Treatment-related AE and SAE 41
10.3. Treatment of Overdose 41
10.4. Pregnancy 41
10.5. Safety Assessments 41
10.6. Dried Blood Spot Sub-study (Optional) 41
10.7. Biohazard Containment 42

11. Statistical Considerations 42
11.1. Sample Size Determination 42
11.2. Populations for Analyses 44
11.3. Statistical Analyses 44
11.3.1. Efficacy Analyses 45
11.3.2. Secondary Endpoints 46
11.3.2.1. Safety Analyses 46
11.3.2.2. Hospitalization 46
11.3.2.3. Disease Severity 46
11.3.2.4. Symptom Resolution 46
11.3.3. Pharmacokinetic Analysis 46
11.3.4. Exploratory Exposure-Response Analyses 47

11.3.5. Combined Study Analysis....47

12. References....48

13. Appendices....51

Appendix 1: Abbreviations and Terms....51
Appendix 2: Protocol Structure....53
Appendix 3: Study Governance Considerations....54
Appendix 4: Site-Specific Protocol Addendum Template....57
Appendix 5: Hydroxychloroquine Label....60
Appendix 6: Azithromycin Label....61
Appendix 7: Physiologically Based Pharmacokinetic Model for Azithromycin Dose Regimen....62
Appendix 8: Pharmacokinetic Sample Collection and Analysis....63
Appendix 9: Physiologically Based Pharmacokinetic Modeling of Hydroxychloroquine Used for Post-exposure Prophylaxis....65
Appendix 10: The inFLUenza Patient-Reported Outcome instrument (Flu-PRO) – Modified for SARS-CoV-2....66
Appendix 11: WHO Ordinal Scale for Clinical Improvement....69

List of Tables

Table 1 Sample Size per Arm Required to Detect a Relative Risk Reduction of 40% to 60% Under Various Control Event and Dropout Rates.... 43

Table 2 Expected Recruitment and Number of Measurable Clinical Outcomes for the First 6 to 8 Weeks of Trial.... 43

1. Synopsis

Narrow Title:

Efficacy of Treatment of SARS-CoV-2 Infection Among High-Risk Outpatient Adults: A Platform Trial

Expanded Title (pending discussions of approach):

Efficacy of Novel Agents for Treatment of SARS-CoV-2 Infection Among High-Risk Outpatient Adults: An Adaptive Randomized Platform Trial

Short Title:

Treatment for SARS-CoV-2 in High-Risk Adult Outpatients

Rationale:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes mild illness confined to the upper respiratory track in 80% of people but can cause severe lower respiratory tract infection (LRTI) in 20%, particularly among those in high-risk groups, defined by advanced age (>60 years) and presence of comorbidities (e.g., cardiopulmonary disease, renal disease, diabetes mellitus). Progression to LRTI appears to frequently result in hospitalization for supplemental oxygen therapy and may lead to need for ventilator respiratory support and ultimately death. Given the rapid spread of the SARS-CoV-2 pandemic, interventions that avert adverse patient outcomes and reduce the strain on the medical system are urgently needed. In addition, prolonged viral shedding has been noted after infection; therapeutic strategies that can effectively reduce viral shedding, and potentially onward transmission, have the potential to shift the trajectory of the pandemic.

There are no licensed therapeutic agents for any human coronavirus disease and many scientists and researchers have proposed potential agents based on *in vitro* and pre-clinical studies. These agents have potential benefits but also have inherent risks and limitations (e.g., scalability of supply). There is urgent need for controlled clinical studies in relevant patient populations to guide both policy makers and other clinical trialists on prioritized drugs, including a path toward future evaluations as data becomes available. This study intends to provide quick and informative data to assist in early decision-making and/or support combined analysis with other concurrently run studies.

Hydroxychloroquine (HCQ) is currently approved for the suppressive treatment (for extrahepatic phase of malaria) and treatment of acute attacks of malaria due to several *Plasmodium* strains. It is also indicated for the treatment of discoid and systemic lupus erythematosus and rheumatoid arthritis. With the first Food and Drug Administration approval in 1955, safety and tolerability of HCQ are well described. *In vitro*, HCQ displays antiviral activity against coronaviruses, including SARS-CoV-2. Pharmacologic modeling based on observed drug concentrations and *in vitro* drug testing suggest that treatment at approved doses (200 to 800 mg daily) could achieve levels in respiratory tract tissues to inhibit virus replication and shedding, potentially reducing adverse outcomes of infection and onward transmission. Preliminary, uncontrolled

studies suggest that HCQ could be clinically beneficial in persons with hospitalized coronavirus disease (COVID-19) and reduce viral shedding. To date, no rigorous treatment studies of outpatient SARS-CoV-2 infection have been reported.

Azithromycin is a broad-spectrum azalide antibiotic that was approved over 30 years for the treatment of a variety of bacterial infections, including pneumonia. Azithromycin has shown antiviral activity in vitro against Ebola, Zika, rhinovirus, and other respiratory viruses [1, 2]. Most recently, the combination of HCQ + azithromycin showed possible evidence of enhanced antiviral activity compared with HCQ alone in patients with COVID19 disease in a small observational series reported by Gautret et al (2020) [1].

This synopsis proposes a randomized platform trial for treatment of high-risk individuals in outpatient settings with SARS-CoV-2 infection, with primary outcomes focused on progression to LRTI, defined by blood oxygen saturation level (SpO2) <93% sustained for 2 readings 2 hours apart AND presence of subjective dyspnea or cough, cumulative incidence of hospitalization or mortality at Day 28, and reduction of upper respiratory viral shedding, defined as time to clearance (2 consecutive negative swabs).

A flexible platform trial design will allow additional candidate agents for SARS-CoV-2 infection that are identified and prioritized for testing to be incorporated into this protocol as additional arms. The design of the trial and the statistical analysis plan will allow seamless integration of new interventions. Some study procedures may be adapted based on a unique safety profile of these new candidates, but the core study elements will be maintained. If an intervention is shown to be effective, this design would also allow replacement of the placebo group with the effective intervention as the comparator.

Design:

Randomized (participant-blinded, treating clinician-blinded, laboratory-blinded, pharmacist-unblinded, and statistician-unblinded), multi-center, placebo-equivalent (ascorbic acid + folic acid)-controlled, and blinded study of HCQ and HCQ + azithromycin for the treatment of SARS-CoV-2 infection in high-risk adults not requiring hospital admission. Additional arms will be added should new potential agents be discovered or combination treatments be proposed.

Population:

Men and women 18 to 80 years of age who test positive for SARS-CoV-2 within the past 72 hours.

High-risk cohort (total of 495 participants, 165 per arm):

Eligible participants will be at increased risk of developing LRTI based on the established risk factors for severe COVID-19 disease (at least one of the following):

i. Age ≥60 years
ii. Presence of pulmonary disease, specifically moderate or severe persistent asthma, chronic obstructive pulmonary disease, pulmonary hypertension, emphysema
iii. Diabetes mellitus (type 1 or type 2), requiring oral medication or insulin for treatment
iv. Hypertension, requiring at least 1 oral medication for treatment

v. Immunocompromised status due to disease (e.g., those living with human immunodeficiency virus with a CD4 T-cell count of <200/mm^3)
vi. Immunocompromised status due to medication (e.g., persons taking 20 mg or more of prednisone equivalents a day, anti-inflammatory monoclonal antibody therapies, or cancer therapies)
vii. Body mass index ≥30 kg/m^2 (self reported)

Low-risk cohort (total of 135 participants, 45 per arm):

Eligible participants will be 18 to 59 years of age, inclusive, without any of the risk factors for developing severe COVID-19 disease (see high-risk cohort above).

Participants will be counseled about the preliminary *in vitro* data on HCQ and HCQ + azithromycin activity against SARS-CoV-2 and equipoise regarding efficacy in humans, given that there are only limited data from an uncontrolled study at this time.

Intervention:

- Intervention A: HCQ 400 mg orally twice on Day 1, followed by 200 mg orally twice daily for an additional 9 days (Days 2 to 10) + placebo (folic acid 800 µg orally once on Day 1, followed by 400 µg orally once daily for an additional 4 days [Days 2 to 5]).
- Intervention B: HCQ 400 mg orally twice on Day 1, followed by 200 mg orally twice daily for an additional 9 days (Days 2 to 10) + azithromycin 500 mg orally once on Day 1, followed by 250 mg orally once daily for an additional 4 days (Days 2 to 5).
- Placebo: Ascorbic acid 500 mg orally twice on Day 1, followed by 250 mg orally twice daily for 9 days + folic acid 800 µg orally once on Day 1, followed by 400 µg orally once daily for an additional 4 days (Days 2 to 5).

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To test the efficacy of HCQ or HCQ + azithromycin compared to placebo to prevent progression to LRTI, among persons with SARS-CoV-2 infection who are at high risk of progression • To test the efficacy of HCQ or HCQ + azithromycin compared to placebo to reduce SARS-CoV-2 viral shedding	• LRTI, defined by resting SpO2<93% sustained for 2 readings 2 hours apart AND presence of subjective dyspnea or cough *The trial is statistically powered for this endpoint in the high-risk population.* • Cumulative incidence of hospitalization or mortality, measured at Day 28 *The trial is statistically powered for*

	this endpoint in the high-risk population. • Time to clearance of nasal SARS-CoV-2, defined as 2 consecutive negative swabs (swabs collected daily Day 1 through Day 14) *Trial is statistically powered for this endpoint in both the high- and lower-risk populations, analyzed separately.*
Secondary	
• To test the safety of HCQ or HCQ + azithromycin compared to placebo for treatment of high-risk outpatients with SARS-CoV-2 infection	• Serious adverse events (including death and hospitalization) and adverse events resulting in treatment discontinuation
• To test whether HCQ or HCQ + azithromycin has an effect on the duration of hospitalization among persons who become hospitalized with COVID-19 disease	• Days of hospitalization
• To test whether HCQ or HCQ + azithromycin has an effect on disease severity compared to placebo	• Peak score on WHO Ordinal Scale for Clinical Improvement • Peak score on modified Flu-PRO within the first 14 days
• To test whether HCQ or HCQ + azithromycin decreases the resolution rate for symptomatic SARS-CoV-2 infection/COVID-19 disease compared to placebo	• Proportion of days with fever after randomization • Proportion of days with respiratory symptoms after randomization • Proportion of days with SpO2<93% for >1 hour/day without supplemental oxygen after randomization
• To test whether HCQ or HCQ + azithromycin is associated with decreased viral shedding from self-collected nasal swabs over 14 days compared to placebo	• Proportion of days with SARS-CoV-2 detected from mid-nasal swabs by PCR • Median quantity of SARS-CoV-2 detected from mid-nasal swabs by PCR
Exploratory	

• To assess pharmacokinetics and exposure-response relationship of HCQ • To assess pharmacokinetics and exposure-response relationship of HCQ + azithromycin	• HCQ blood concentration in DBS • Azithromycin blood concentration in DBS

COVID-19: coronavirus disease; DBS: dried blood spot; Flu-PRO: inFLUenza patient-reported outcome; HCQ: hydroxychloroquine; LRTI: lower respiratory tract infection; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SpO2: blood oxygen saturation level; WHO: World Health Organization.

Sample Size:

The study will initially enroll 495 high-risk participants (165 per arm) to achieve 90% statistical power at 2-sided type I error rate of 5% for a pairwise comparison against the control to detect a treatment effect of relative risk reduction of 50% for the primary outcome(s) assuming control event rate of 30% for the primary outcome and 5% dropout rate. A smaller cohort of 135 participants (45 per arm) aged 18 to 59 years and without risk factors for progression to LRTI will be enrolled for the co-primary virologic outcome. Eligible participants will be randomized at an equal allocation ratio to study treatment (HCQ + placebo [folic acid], HCQ + azithromycin, or placebo [ascorbic acid + folic acid]). If a second eligible patient is in the same household, both will be assigned to the same regimen. A blinded sample size re-assessment may be done to possibly increase the target sample size, conditional on the observed incidence rate and within household correlation. With an expected recruitment rate of approximately 83 to 124 patients per week, it can be expected that 53 to 79 patients from the control group will be observed for clinical outcome in the high-risk group in the fourth week. Should a new intervention be added, allocation ratio will be adjusted to favor the new arm, ensuring that control arm participants are still enrolled concurrently with active arms; an empirical Bayesian information borrowing method will be used to incorporate past control arm data for the comparison of new intervention that is added later.

Duration:

Twenty-eight days of clinical follow-up per participant.

Enrollment over 4 weeks: Recruitment rate of 83 to 124 patients per week.

Completion in 6 weeks from first patient in (without sample size re-assessment).

Proposed Sites (up to 6): University of Washington, University of Maryland, SUNY, Tulane, NYU, and others to be discussed

2. Schedule of Activities

Procedure	Screening	Self-quarantine									EOS
		Treatment Period									
	Day 0[a]	Day 1[a]	Day 2[a]	Day 3	Day 4[a]	Days 5 to 8	Day 9[a]	Days 10 to 13	Day 14[a]	Day 21	Day 28[b]
Informed consent	X										
Demography	X										
Sign HIPAA form	X										
Past and current medical conditions, including known pregnancy and/or lactation status	X										
Concomitant medications	X										
Laboratory documentation of COVID-19 testing	X										
Inclusion and exclusion criteria	X										
Randomization	X										
Mid-nasal swab		X	X	X	X	X	X	X	X	X	X
Study therapy (HCQ, azithromycin, ascorbic acid, folic acid)		##	#	#	#	#[c]	#	#[d]			
Daily Survey (including dosing and swab adherence, concomitant medications)		X	X	X	X	X	X	X	X	X	
Modified Flu-PRO		X	X	X	X	X	X	X	X	X	X
WHO Ordinal Scale for Clinical Improvement									X	X	X
Participant collected daily vitals (including temperature, pulse, SpO2, QT monitoring, respiratory rate)		X	X	X		X	X	X	X	X	X
Contact with study clinician or staff		X	X	X[e]	X	X[e]	X	X[e]	X	X[e]	X
Exit Contact Survey (including concomitant medications, symptoms, etc.)											X
Adverse event review		X	X	X	X	X	X	X	X	X	X
If in sub-study: dried blood spot sample for HCQ and azithromycin concentration and anti-SARS-CoV-2 antibodies		*X*	*1 to 5 samples (no more than 1 per day) after dosing start*								*X*

Procedure	Screening	Self-quarantine									EOS
		Treatment Period									
	Day 0[a]	**Day 1[a]**	**Day 2[a]**	**Day 3**	**Day 4[a]**	**Days 5 to 8**	**Day 9[a]**	**Days 10 to 13**	**Day 14[a]**	**Day 21**	**Day 28[b]**

COVID-19: coronavirus disease; EOS: end of study; Flu-PRO: inFLUenza patient-reported outcome; HCQ: hydroxychloroquine; HIPAA: Health Insurance Portability and Accountability Act of 1996; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SpO2: oxygen saturation; WHO: World Health Organization.

Note: ## denotes either 400 mg HCQ twice a day + placebo (800 µg folic acid) once a day, 400 mg HCQ twice a day + 500 mg azithromycin once a day, or placebo (500 mg ascorbic acid twice a day + 800 µg folic acid once a day) (depending on treatment arm assignment).

Note: # denotes either 200 mg HCQ twice a day + placebo (400 µg folic acid) once a day, 200 mg HCQ twice a day + 250 mg azithromycin once a day, or placebo (250 mg ascorbic acid twice a day + 400 µg folic acid once a day) (depending on treatment arm assignment).

[a] Screening and Day 1, 2, 4, 9, and 14 evaluations will be conducted through a web-based screening tool, HIPAA-compliant video conference (Telehealth), telephone, or text messaging. Screening (Day 0) and Day 1 evaluations may occur on the same day.

[b] For patients who are hospitalized on Day 28, the last visit will be ±3 days after discharge.

[c] Last day of study medication will be administered on Day 5 for azithromycin and folic acid.

[d] Last day of study medication will be administered on Day 10 for HCQ and ascorbic acid.

[e] These evaluations will be as needed/requested by study participant.

3. Introduction

This is a randomized, multi-center, placebo-equivalent (ascorbic acid + folic acid)-controlled, blinded platform trial for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in high-risk adults not requiring hospital admission. The trial will start with hydroxychloroquine (HCQ) (Intervention A) and HCQ + azithromycin (Intervention B) as experimental interventions. Additional arms will be added as new agents or combinations are prioritized. In addition, if other agents are found to be effective, HCQ or HCQ + azithromycin may become the control group against which new interventions are measured. Evaluations include safety and tolerability, SARS-CoV-2 viral shedding, and development of lower respiratory tract infection (LRTI). Initially, there will be 3 study arms (Arm 1: HCQ + placebo [folic acid], Arm 2: HCQ + azithromycin, Arm 3: placebo [ascorbic acid + folic acid]) enrolled to assess a daily dosing regimen administered for 10 days to prevent LRTI and decrease viral shedding.

Up to 165 eligible high-risk adults (18 to 80 years of age) per arm will be randomized (at the level of household) in a 1:1:1 ratio to receive one of the following therapies:

- HCQ 400 mg orally twice on Day 1, followed by 200 mg orally twice daily for an additional 9 days (Days 2 to 10) + placebo (folic acid 800 µg orally once on Day 1, followed by 400 µg orally once daily for an additional 4 days [Days 2 to 5])
- HCQ 400 mg orally twice on Day 1, followed by 200 mg orally twice daily for an additional 9 days (Days 2 to 10) + azithromycin 500 mg orally once on Day 1, followed by 250 mg orally once daily for an additional 4 days (Days 2 to 5)
- Placebo: Ascorbic acid 500 mg orally twice on Day 1, followed by 250 mg orally twice daily for 9 days + folic acid 800 µg orally once on Day 1, followed by 400 µg orally once daily for an additional 4 days (Days 2 to 5)

A second group of 135 adults (18 to 59 years of age) without risk factors for progression to LRTI per arm will be randomized in a 1:1:1 ratio to the same interventions.

HCQ and ascorbic acid will appear similar, and taste will be partially masked as HCQ can be bitter and ascorbic acid will be sour. Azithromycin will not appear similar to folic acid. If there are more than 1 participant in the same household, all will be assigned to the same randomized group, preserving some degree of masking.

During the study, participants will perform the following:

- Collect mid-nasal swabs for viral detection for the co-primary trial endpoint
- Complete daily physical assessments for symptoms of LRTI and measurement of temperature, respiratory rate, pulse, and oxygen saturation (SpO2), QT monitoring, respiratory rate
- Complete surveys that will include questions about symptoms from both the drug regimen and respiratory and systemic symptoms, review of concomitant medications, and other pertinent topics

During the 28 study days, participants will take the medication, complete surveys, collect mid-nasal swab for viral quantification, and assess symptoms for progression to LRTI. Physical assessments will include daily temperature, SpO2, pulse, QT monitoring, and respiratory rate.

3.1. *Background*

SARS-CoV-2 is a coronavirus novel to the human population discovered in December 2019; it is currently the cause of a global pandemic [3,4,5]. The World Health Organization (WHO) named the novel coronavirus SARS-CoV-2 and the disease caused by the virus as COVID-19.

As of 25 March 2020, nearly 500,000 persons were infected, with over 21,000 deaths reported from around the globe. Person-to-person transmission has occurred in China, across temperate Asia, Europe, and North America, with sporadic cases in Africa and person-to-person transmission in the southern hemisphere. Accurate reporting is limited by availability of diagnostic testing. WHO declared the COVID-19 pandemic a Public Health Emergency of International Concern on 30 January 2020 [6], and the United States declared a national emergency on 13 March 2020 [7].

Most deaths and severe pneumonitis have occurred in the elderly or in persons with underlying pulmonary or cardiac comorbidities or diabetes. In healthy adults, including pregnant women, it can cause a febrile, self-limited pneumonia. Infection appears less symptomatic in children and younger adults [8]. Nevertheless, the burden of this pandemic to the global health and economic systems is expected to be substantial. No acquired immunity to this novel viral infection appears to exist in the human population globally, and no effective treatment or preventative agent is licensed at this time.

As with many infectious epidemics, household contacts, first responders, caregivers, and medical personnel attending persons with COVID-19 are at high risk of infection. The incubation time requires 14 days of quarantine for exposed individuals not wearing personal protective equipment [9], and on 03 March 2020, WHO declared a global shortage of personal protective equipment, leaving doctors, nurses, and other frontline workers dangerously ill-equipped to care for COVID-19 patients [10]. Extensive absences from the care network and health system will degrade the ability to care not only for those with COVID-19 but also for routine healthcare issues as well. At the height of local epidemic, the health care system becomes overburdened with patients with respiratory illness. To date, rigorous self-isolation and lockdown have been required to contain the SARS-CoV-2, leaving entire societies to abruptly stop normal life. Interventions are urgently needed to stop viral spread and to decrease the morbidity and mortality cause by the infection. The ability to stop viral replication to prevent transmission of the virus and to prevent LRTI, which is associated with need for hospitalization and possibly mechanical ventilatory support, will be of benefit to the individual, the hospital system, and the health of the public. In addition, treating those at highest risk of progression to LRTI and hospitalization will have the greatest impact on the pandemic. Including a cohort without risk factors for LRTI for the co-primary virologic outcome will provide additional data to inform whether the intervention is likely to have a public health benefit by reducing transmission in situations where self-isolation is not feasible.

3.2. *Study Rationale*

3.2.1. *COVID-19 and Antiviral Approaches*

SARS-CoV-2 is a novel betacoronavirus of zoonotic origin, similar to the coronaviruses severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). Based on current evidence, case fatality rate for SARS-CoV-2 is about 3%, which is significantly lower than that of SARS-CoV (10%) and MERS-CoV (40%) [11]. However, SARS-CoV-2 has potentially higher transmissibility (R0: 1.4 to 5.5) than both SARS-CoV (R0: 2-5) and MERS-CoV (R0: <1).

Our understanding of the viral pathogenesis of SARS-CoV-2 remains limited. However, it appears that the virus cell entry depends on the binding of the viral spike (S) proteins to cellular receptors and on S protein priming by host cell proteases. SARS-CoV-2, like SARS-CoV, uses the same receptor angiotensin converting enzyme 2 (ACE2) on pulmonary epithelial cells for entry and the transmembrane serine protease 2 for S protein priming [12]. The receptor binding domain of lineage B betacoronaviruses is a single, continuous domain that contains all of the structural information necessary to interact with the host receptor. Fusion is mediated at the cell membrane, delivering the viral nucleocapsid inside the cell for subsequent replication. ACE2 expression is found in the lung epithelial cells, vascular endothelium, renal tubular epithelium, and epithelia of the small intestine. Viral shedding has been localized primarily to respiratory droplets and fecal samples [4].

Medications to treat and/or prevent SARS-CoV-2 need to inhibit aspects of the viral life cycle, ultimately blocking replication. Already-approved and available medications are ideal for immediate evaluation for SARS-CoV-2 infection treatment and prevention. Two potential targets for anti-SARS-CoV-2 medications are viral polymerases and proteases [13]. Pilot clinical studies are already ongoing for SARS-CoV-2 using various repurposed antiviral medicines (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov). Similarities between SARS-CoV-2 with SARS-CoV and MERS suggest that antivirals with *in vitro* efficacy against SARS-CoV and MERS may be promising agents as SARS-CoV-2 post-exposure prophylaxis (PEP) [13,14].

3.2.2. *Antiviral Effects of Chloroquine Analogues Against COVID-19*

Chloroquine (CQ) was discovered in 1934 by Bayer and was used in 1945 as an antimalarial, which became one of the most prescribed drugs globally, prior to the emergence of widespread drug resistance in *Plasmodium falciparum* [15]. CQ was found to be effective against rheumatoid tenosynovitis in 1951 [16]. HCQ was licensed in the United States in 1955 as an antimalarial and as a drug for rheumatoid arthritis, and it was widely marketed for the latter due to a favorable safety profile with chronic use [17]. The mechanisms of action for HCQ for treatment of rheumatoid arthritis and other autoimmune diseases are still not fully understood despite widespread use over the past 60 years [18].

CQ and HCQ have been proposed as potential agents for treatment and prevention against other infectious agents beyond malaria [19,20]. The mechanism of action differs according to the pathogen: against intracellular bacteria and fungi by alkalinizing vacuoles containing the

microorganisms, restoring the activity of other antibiotics, and against viral replication through alkalization of acidic organelles, namely, endosomes, lysosomes, and Golgi vesicles.

CQ is effective *in vitro* against SARS-CoV coronavirus in Vero E6 cells with a half-maximal effective concentration (EC_{50}) of ~8 μM [21] and had shown evidence of prevention activity *in vivo* [22]. Hence, these re-purposed drugs were obvious hits for testing against SARS-CoV-2. *In vitro* inhibition in Vero E6 cells against the novel coronavirus, SARS-CoV-2, has been published in recent weeks. Wang et al (2020) showed that the EC_{50} and EC_{90} for CQ in Vero E6 cells are 1.13 and 6.90 μM, respectively [23]. Yao et al (2020) showed that the EC_{50} for CQ treatment of infected cells at 48 hours was 5.47 μM, whereas HCQ appeared slightly more potent, with EC_{50} of 0.72 μM at 48 hours [24]. These levels appear to be within the range of exposures that could be achieved with standard HCQ treatment, and likely prophylaxis, due to concentrations of the drug achieved in the lung tissue. No *in vitro* data in the lung epithelial cells or any animal model data are available.

Multiple observational and small Investigator-initiated COVID-19 pneumonia treatment trials using CQ, HCQ, and variety of other medications are ongoing in China (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov). Gao et al reported anecdotal efficacy of CQ as treatment for COVID-19-associated pneumonia [25].

3.2.3. *Rationale for Drug Selection of HCQ*

The trial will test a daily regimen of HCQ for 10 days for treatment of SARS-CoV-2 (package insert, Appendix 5). Daily dosing has the highest likelihood to achieve sustained required drug levels for viral inhibition, as shown in the physiologically based pharmacokinetics (PBPK) modeling (Appendix 9). HCQ is commonly used daily in doses up to 600 mg of HCQ sulfate (465 mg base) per day for rheumatoid arthritis or systemic lupus erythematosus initially, with a usual maintenance dose of 200 mg (155 mg base) for maintenance therapy. HCQ and CQ are both commonly used in a weekly dosing schedule for malaria chemoprophylaxis.

HCQ is associated with a better safety profile for daily and chronic use than CQ, including 5 decades of experience with use in these dose ranges in adults and the elderly. It is on the WHO Essential Medicines List for use in rheumatic disorders and is widely prescribed as an anti-inflammatory for rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune syndromes. Based on the limited *in vitro* data available, HCQ appears to be slightly more potent than CQ against SARS-CoV-2 [24].

As the COVID-19 epidemic remains very fluid and new data are emerging from observational and clinical trials daily, this protocol is written to allow adaptation to incorporate additional medications beyond HCQ and HCQ + azithromycin.

3.2.4. *Rationale for Dosing Schedule of HCQ*

HCQ is a long-acting drug with a terminal half-life of approximately 40 days. It is well absorbed, moderately protein bound, and will accumulate in tissues including the lung, heart, liver, and kidneys. It is typically given with a loading dose of approximately 2-fold the standard dose to accelerate achieving steady-state drug concentrations [26]. Since the drug will be used in those

with SARS-CoV-2 upper respiratory tract infection, it is desirable to quickly achieve adequate drug levels to decrease viral replication.

A PBPK model was built (SIMCYP simulator version 18) using physical and chemical parameters of HCQ obtained from the literature [27]; pharmacokinetic (PK) parameters (liver intrinsic clearance, fa, ka) were determined from clinical data [28]. This PBPK model was used to simulate HCQ concentrations in plasma and lung fluid following 5 proposed dosing regimens in order to select an optimal regimen for the Peking University Third Hospital's ongoing trial of HCQ in China. The combination of *in vitro* antiviral concentration-effect and predicted drug concentrations in this study were used to propose a loading dose of 400 mg HCQ twice on Day 1, and drug levels will remain above the EC_{50} for at least 14 days of treatment and potentially beyond Day 21.

A second study (BYSY-DCTC-CPPO-HCQ-PBPKAR) was undertaken to simulate HCQ concentration-time profiles in plasma, whole blood, and lung. Since elderly patients have reduced glomerular filtration rate (GFR), simulations were conducted using a healthy Caucasian healthy population with renal injury (GFR 30 to 60 mL/min) and compared to a population with normal renal function to support the study design of therapeutic use of HCQ.

This protocol will investigate a single dosage of HCQ. Participants will receive a loading dose of 400 mg twice on Day 1, followed by 200 mg twice a day for 9 additional days. Subsequent investigations will be encouraged to undertake a more rigorous exposure-response assessment to define optimal dosing, including exploration of the lowest possible effective dose, and possible alternate dosing schedules (i.e., weekly instead of daily).

3.2.5. *Antiviral Effects of Azithromycin Against COVID-19*

Azithromycin is a broad-spectrum azalide antibiotic used to treat a number of bacterial infections, including pneumonia. Azithromycin has shown antiviral activity *in vitro* against Zika, Ebola, rhinoviruses, and other respiratory viruses [1, 2]. Although the mechanism of its antiviral activity is not clear, some findings suggest it may be associated with augmentation of interferon response [2]. Alternatively, azithromycin may convey antiviral activity by increasing the pH of cell organelles such as endosomes and the *trans*-Golgi network [29]. Changing the pH of intracellular vesicles may alter the glycosylation of ACE2, a key receptor for cell entry for SARS-CoV-2 [30,31].

3.2.6. *Rationale for Drug Selection of Azithromycin*

The trial will test a daily regimen of azithromycin in combination with HCQ in the treatment of SARS-CoV-2 (Zithromax package insert, Appendix 6). In the Gautret study [1], among 20 participants with confirmed COVID-19 who were treated with HCQ, 6 received azithromycin (500 mg on Day 1 followed by 250 mg/day on Days 2 to 5) to prevent bacterial pneumonia superinfection. On Day 6, all participants who were treated with the HCQ + azithromycin combination showed virologic cure as measured by PCR of nasopharyngeal samples compared with 57.1% of participants treated with HCQ alone.

Azithromycin, which has been approved for use for >30 years, is associated with a favorable safety profile. It is on the WHO's List of Essential Medicines, and WHO classifies it as critically

important for human medicine. Azithromycin is widely available worldwide. Common short-term drug toxicities include nausea, vomiting, diarrhea and upset stomach.

3.2.7. *Rationale for Dose Selection of Azithromycin*

The dose of azithromycin selected for this study aligns with the Dosage and Administration recommendations in the United States prescribing information (USPI) for the treatment of community-acquired pneumonia. This regimen is 500 mg as a single dose on Day 1, followed by 250 mg once daily on Days 2 through 5. Azithromycin does not undergo significant metabolism and does not complex with or induce cytochromes P450, thus minimizing the potential for drug interactions. Because azithromycin has a long terminal half-life (79 hours with 500 mg oral dosing), once daily or single-dose treatments are possible [32].

The dose regimen of 500 mg as a single dose on Day 1, followed by 250 mg once daily on Days 2 through 5 was used in the recently published study of HCQ and azithromycin in patients with COVID-19 [1]. Using a PBPK model, azithromycin lung concentrations were simulated for this dose regimen to ensure that physiologically relevant lung exposure can be reached. Results are presented in Appendix 7. Based on the simulations, it appears that a dosing regimen of 500 mg for 1 day followed by 250 mg once daily for 4 days is able to attain concentrations of azithromycin in the lung that would approach the in vitro EC_{50} value reported for inhibition of SARS-CoV-2 by azithromycin [33].

3.2.8. *Rationale for Ascorbic Acid Control as a Comparator for HCQ*

In healthy adults, COVID-19 disease is likely to present as an upper respiratory viral infection, characterized by a febrile disease with cough and fatigue [3,34]. Symptom reporting may vary based on the participants' perception as to whether they are taking HCQ or ascorbic acid [35], but the primary study endpoints of LRTI and viral shedding are not affected. There is no rigorously proven therapy for individuals with outpatient COVID-19 disease, although multiple therapies are under investigation.

Because there is no established therapy, use of a control is acceptable and ethical for both the participants' health and safety as well as ensuring the most rigorous trial design to evaluate an intervention for COVID-19 disease caused by SARS-CoV-2. Participants will be blinded to their allocation to the extent possible, particularly for the comparison of ascorbic acid and HCQ. Both of those medications have a taste, tablets will not be labeled as either HCQ or ascorbic acid, and an ascorbic acid tablet that is comparable in size and shape to HCQ will be used. If >1 individual from the same household is enrolled, all will be assigned to the same randomized group.

The dose of ascorbic acid chosen for this protocol is safe. All participants, regardless of assigned group, will be able to take additional ascorbic acid (e.g., over the counter vitamins or through food) should they choose, as there is no known maximum daily safe dose of ascorbic acid. Clinical trial evidence has demonstrated that ascorbic acid, alone or in combination with other micronutrients, does not substantially reduce the risk of upper respiratory infections or severe consequences of infectious processes [36,37]; thus, ascorbic acid is not expected to have a prevention effect for SARS-CoV-19 and is considered a placebo-equivalent product for this study.

3.2.9. *Rationale for Folic Acid Control as a Comparator for Azithromycin*

Folic acid, also known as vitamin B9 is one of the B vitamins and commonly used as a dietary supplement. Specifically, folic acid is also used as a supplement during pregnancy to reduce the risk of neural tube defects in the baby. Folic acid is on the WHO's List of Essential Medicines, widely available worldwide, and inexpensive.

Folic acid has no known antiviral activity. The risk of toxicity from folic acid is low, because folic acid is water-soluble and excreted through urine. The 100% daily recommended for folic acid is 400 µg, and the upper intake level is established as 1000 µg/day. No health risks have been associated with high intake of folic acid from food sources

As folic acid is not expected to have a preventative effect for SARS-CoV-19 it is considered an acceptable placebo-equivalent product for this study.

3.3. *Benefit/Risk Assessment*

COVID-19 disease can be unpredictable in its severity, but a 3.4% mortality rate has been observed among clinical pneumonia cases. The elderly (>60 years of age) and those with medical comorbidities (e.g., cardiopulmonary disease, renal disease, diabetes mellitus) are at highest risk of poor outcomes [3,4,5]. Moreover, transmission in younger persons amplifies infection in communities, putting susceptible persons at risk. There is no proven drug for treatment of those with COVID-19 disease. HCQ has an excellent safety record at the proposed doses for many years and both agents have potent viral suppression *in vitro*. The safety profile of azithromycin at the proposed dose is similarly well characterized with safety considerations and monitoring guidance provided in the label. There is equipoise as to whether the *in vitro* efficacy of HCQ and azithromycin, or any other drug or drug combination, will translate into efficacy to prevent LRTI. Thus, the potential benefit-to-risk ratio for testing HCQ and HCQ + azithromycin as treatment is favorable in this population.

4. Objectives and Endpoints

Objectives	Endpoints
Primary	
• To test the efficacy of HCQ or HCQ + azithromycin compared to placebo to prevent progression to LRTI, among persons with SARS-CoV-2 infection who are at high risk of progression • To test the efficacy of HCQ or HCQ + azithromycin compared to placebo to reduce SARS-CoV-2 viral shedding	• LRTI, defined by resting SpO2<93%, sustained for 2 readings 2 hours apart AND presence of subjective dyspnea or cough *The trial is statistically powered for this endpoint in the high-risk population.* • Cumulative incidence of hospitalization or mortality, measured at Day 28 *The trial is statistically powered for this endpoint in the high-risk population.* • Time to clearance of nasal SARS-CoV-2, defined as 2 consecutive negative swabs (swabs collected daily Day 1 through Day 14) *Trial is statistically powered for this endpoint in both the high- and lower-risk populations, analyzed separately.*
Secondary	
• To test the safety of HCQ or HCQ + azithromycin compared to placebo for treatment of high-risk outpatients with SARS-CoV-2 infection	• Serious adverse events (including death and hospitalization) and adverse events resulting in treatment discontinuation
• To test whether HCQ or HCQ + azithromycin has an effect on the duration of hospitalization among persons who become hospitalized with COVID-19 disease	• Days of hospitalization
• To test whether HCQ or HCQ + azithromycin has an effect on disease severity compared to placebo	• Peak score on WHO Ordinal Scale for Clinical Improvement • Peak score on modified Flu-PRO within the first 14 days

• To test whether HCQ or HCQ + azithromycin decreases the resolution rate for symptomatic SARS-CoV-2 infection/COVID-19 disease compared to placebo	• Proportion of days with fever after randomization • Proportion of days with respiratory symptoms after randomization • Proportion of days with SpO2<93% for >1 hour/day without supplemental oxygen after randomization
• To test whether HCQ or HCQ + azithromycin is associated with decreased viral shedding from self-collected nasal swabs over 14 days compared to placebo	• Proportion of days with SARS-CoV-2 detected from mid-nasal swabs by PCR • Median quantity of SARS-CoV-2 detected from mid-nasal swabs by PCR
Exploratory	
• To assess pharmacokinetics and exposure-response relationship of HCQ • To assess pharmacokinetics and exposure-response relationship of HCQ + azithromycin	• HCQ blood concentration in DBS • Azithromycin blood concentration in DBS

COVID-19: coronavirus disease; DBS: dried blood spot; Flu-PRO: inFLUenza patient-reported outcome; HCQ: hydroxychloroquine; LRTI: lower respiratory tract infection; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SpO2: blood oxygen saturation level; WHO: World Health Organization.

5. Study Design

5.1. *Overall Design*

The overarching goal of this study is to assess the effectiveness of interventions on the incidence of LRTI progression among high-risk adult outpatients with SARS-CoV-2 infection to inform public health control strategies.

This is a randomized, multi-center, placebo-equivalent (ascorbic acid + folic acid)-controlled, blinded platform trial. Stratified randomization based on different trial sites will be used. The trial will start with HCQ (Intervention A) and HCQ + azithromycin (Intervention B) to assess the efficacy on the prevention of LRTI progression. The trial may allow for agents to be added and tested against placebo with standardized eligibility criteria, outcomes, and measurements.

Initially, this study will enroll up to 495 eligible adults (18 to 80 years of age) with high risk for LRTI progression at baseline who are PCR-confirmed SARS-CoV-2 infection (165 per arm). An additional cohort of 135 eligible adults (18 to 59 years of age) without risk factors for LRTI progression at baseline who are PCR-confirmed SARS-CoV-2 infection will be enrolled for the co-primary virologic outcome (45 per arm). Eligible participants will be enrolled and randomized in a 1:1:1 ratio to HCQ + placebo (folic acid), HCQ + azithromycin, or placebo (ascorbic acid + folic acid). Recruitment rate will be assessed on a weekly basis starting at the end of second week after the first eligible patient is recruited and randomized. Blinded sample size re-assessment will be done to increase the sample size target, should a lower control event rate (CER) and/or higher dropout rate than expected be observed. A decision for a possible interim analysis will be made during the trial based on the information on the observed recruitment rate, the CER, the dropout rate, and the final sample size target, if applicable. The decision for interim analysis will be made in a blinded manner (e.g., based on pooled number of events). The interim monitoring plan (written by the Study Statistician) will define monitoring bounds to maintain the 2-sided type I error rate at the desired 5% (e.g., 97.5% or higher probability of superiority over the control group). Should new experimental candidates be added during the trial, allocation ratios will be adapted to favor the new arms. For instance, if another experimental intervention (i.e., Intervention C) is added when HCQ, HCQ + azithromycin, and control arm are all actively recruiting, higher allocation ratio in favor of Intervention C will be adopted (e.g. 2:1:1:1).

It is anticipated that additional treatment modalities will be incorporated into this protocol, in an adaptive fashion. Those will be added through later protocol amendments, which will define additional sample size needs and any potential change to randomization (e.g., changing the randomization ratio, continuation/discontinuation of any of the existing arms, etc.).

Participants will be counseled about the preliminary *in vitro* data on HCQ and HCQ + azithromycin activity against SARS-CoV-2 and equipoise regarding efficacy in humans, given that there are only limited data from an uncontrolled study at this time.

Participants may participate in a sub-study where they will be asked to provide a dried blood spot (DBS) sample for therapy concentration and pharmacokinetics of the medications as well as for SARS-CoV-2 antibody testing.

An independent data and safety monitoring board (DSMB) will be convened for this study with expertise in COVID-19 or respiratory viruses and emerging epidemics as well as biostatistics. The purpose of the DSMB is to monitor the study for operational futility, social harms, and efficacy. The DSMB will also review the blinded sample size re-assessment plan and proposed sample size changes and make recommendations on the allocation ratio, in case the new intervention arms are added.

This multi-center study will be conducted in high COVID-19 disease incidence areas in the United States, with the potential to expand enrollment to international sites.

5.2. *Participant and Study Completion*

As an initial target, up to 165 high-risk participants and 45 low-risk participants per arm will be randomly assigned to study treatment or control. This sample size target will likely increase based on the blinded sample size re-assessment.

A participant is considered to have completed the study if he/she has completed all phases of the study including the last scheduled procedure shown in the Schedule of Activities (SoA).

This study will be considered completed when sufficient number of participants complete the study to enable appropriate evaluation of the primary endpoint.

6. Study Population

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

6.1. *Inclusion Criteria*

Participants are eligible to be included in the study only if all of the following criteria apply:

High-risk cohort (total of 495 participants, 165 per arm):

1. Men or women 18 to 80 years of age, inclusive, at the time of signing the informed consent
2. Willing and able to provide informed consent
3. Laboratory confirmed SARS-CoV-2 infection, with test results within past 72 hours
4. Access to device and internet for Telehealth visits
5. At increased risk of developing severe COVID-19 disease (at least one of the following)
    a. Age ≥60 years
    b. Presence of pulmonary disease, specifically moderate or severe persistent asthma, chronic obstructive pulmonary disease, pulmonary hypertension, emphysema
    c. Diabetes mellitus (type 1 or type 2), requiring oral medication or insulin for treatment
    d. Hypertension, requiring at least 1 oral medication for treatment
    e. Immunocompromised status due to disease (e.g., those living with human immunodeficiency virus with a CD4 T-cell count of <200/mm^3)
    f. Immunocompromised status due to medication (e.g., persons taking 20 mg or more of prednisone equivalents a day, anti-inflammatory monoclonal antibody therapies, or cancer therapies)
    g. Body mass index ≥30 (self-reported)

Low-risk cohort (total of 135 participants, 45 per arm):

1. Men or women, 18 to 59 years of age, inclusive without any risk factors for developing severe COVID-19 disease (point 5 above)
2. Willing and able to provide informed consent
3. Laboratory confirmed SARS-CoV-2 infection, with test results within past 72 hours
4. Access to device and internet for Telehealth visits

6.2. *Exclusion Criteria*

Participants are excluded from the study if any of the following criteria apply:

1. Known hypersensitivity to HCQ or other 4-aminoquinoline compounds
2. Known hypersensitivity to azithromycin or other azalide or macrolide antibiotics
3. Currently hospitalized
4. Signs of respiratory distress prior to randomization, including respiratory rate >24
5. Current medications include HCQ

6. Concomitant use of other anti-malarial treatment or chemoprophylaxis
7. History of retinopathy of any etiology
8. Psoriasis
9. Porphyria
10. Chronic kidney disease (Stage IV or receiving dialysis)
11. Known bone marrow disorders with significant neutropenia (polymorphonuclear leukocytes <1500) or thrombocytopenia (<100 K)
12. Concomitant use of digoxin, cyclosporin, cimetidine, amiodarone, or tamoxifen
13. Known cirrhosis
14. Known personal or family history of long QT syndrome
15. History of coronary artery disease with a history of graft or stent
16. History of heart failure, Class 2 or greater using the New York Heart Association functional class
17. Taking medications associated with prolonged QT such as antipsychotic medications or antidepressants (e.g., citalopram, venlafaxine, and bupropion) and unable to stop during the trial
18. Taking warfarin (Coumadin or Jantoven)
19. Known history of glucose-6-phosphate-dehydrogenase deficiency
20. History of myasthenia gravis

Note: Pregnant and lactating persons will be eligible for enrollment into this study. Both HCQ and azithromycin have been shown to be safe in pregnant women.

6.3. *Screen Failures*

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to study treatment. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study (screen failure) can be rescreened if there is a change in their eligibility.

6.4. *Recruitment*

Each site will establish local recruitment and screening methods that operationalize protocol-specified requirements for eligibility determination in a manner that is tailored to and most efficient for the local study setting and target study population. Each site will use a variety of recruitment approaches, including direct recruitment at clinics, referrals from other providers and SARS-CoV-2 testing sites and laboratories, and use of online and social networking websites and apps. Recruitment materials will educate participants about COVID-19, transmission within households, and epidemiology in the community.

The proposed sites have established track records of high-quality clinical research integrated into clinical care settings; annual retention rates in clinical trials conducted in these sites exceed 80% to 90%. The sites have large COVID-19 epidemics with regulation limiting contact to reduce infectious spread.

If additional participants are required to increase sample size or add additional treatment arms, the number will be communicated to approval bodies for authorization.

6.5. *Co-enrollment Guidelines*

Participants may be co-enrolled in other research studies, provided that these are observational studies only. Any other exception requires approval of the Principal Investigators; if a participant clinically worsens, such as requiring hospitalization, it is expected that an exception will be automatically granted and participation in treatment studies permitted. The study team should be consulted for co-enrollment in studies that do not meet this guidance or if there are questions about eligibility for co-enrollment. For any co-enrolled study, combined blood draws should not exceed current Red Cross phlebotomy guidance.

7. Treatments

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

7.1. *Treatments Administered*

Study Treatment Name	Hydroxychloroquine sulfate	Ascorbic acid	Azithromycin	Folic acid
Dosage Formulation	200 mg (155 mg base) tablets	250 mg tablets	250 mg tablets	400 µg tablets
Route of Administration	Oral			
Dosing Instructions	Take 2 tablets twice on Day 1 and 1 tablet twice a day for the subsequent 9 days, for a total of 10 days of treatment. Take at approximately the same time of the day with a meal or a glass of milk. If a dose is missed, it should be taken as soon as possible. If it is less than 4 hours before the next dose, the dose should be skipped.		Take 2 tablets once on Day 1 and 1 tablet once a day for the subsequent 4 days, for a total of 5 days of treatment. Tablets can be taken with or without food. If a dose is missed, it should be taken as soon as possible. If it is less than 4 hours before the next dose, the dose should be skipped.	
Packaging and Labeling	The medication for home delivery will be dispensed in an otherwise-unmarked container with the study label. The container will be labeled with a unique identifier. The container will be packed in a standard box used for mail delivery of medications as needed.			
Manufacturer	Sandoz	Cardinal Health	Greenstone LLC NDC 59762-2198-3 (blister pack) NDC 59762-2198-3 (bottle)	Leader (distributed by Cardinal Health)

7.2. *Risks to the Participants*

7.2.1. *Risks Associated Administration with HCQ*

With tens of millions of doses of HCQ administrated for malaria and autoimmune diseases, the side effect profile of HCQ is well described and the drug is generally well tolerated. With short-term administration (as opposed to chronic/year-long use in rheumatologic disease management), the major AEs are gastrointestinal (nausea, vomiting, dyspepsia, abdominal cramps, and diarrhea) and transient skin rashes. The gastrointestinal symptoms may vary by specific generic manufacturer of HCQ [38] and are best managed by taking the drug with food or a glass of milk (Appendix 5). A transient rash, most commonly morbilliform or psoriasiform, can develop in 10% of participants, often with a sustained loading dose, and is often managed by lowering the dose. To avoid this potential side effect, this protocol is using a short loading dose, not a sustained one. Uncommonly, idiosyncratic leukopenia/thrombocytopenia can occur and the

drug should not be given to those with underlying bone marrow disorders. Lastly, hypoglycemia can occur and those taking insulin or glucose-lowering drugs are at risk; blood glucose should be monitored.

The safety of HCQ for treatment of patients with COVID-19 disease is unknown. COVID-19 may be associated with cardiac effects. HCQ may prolong QT, resulting in arrhythmias. Participants will be monitored for QT prolongation and counseled about this risk.

Long-term manifestations of HCQ, including retinitis, renal and hepatic disease, and cardiomyopathy (Appendix 5), are not likely in short-term exposure.

7.2.2. *Risks Associated with Azithromycin*

The most common adverse reactions to azithromycin are diarrhea, nausea, abdominal pain, or vomiting (Zithromax USPI Appendix 6). Severe side effects include serious allergic reactions, including, but not limited to, anaphylaxis, angioedema, and dermatologic reactions such as Stevens-Johnson syndrome. Abnormal liver function, hepatitis, cholestatic jaundice, hepatic necrosis, and hepatic failure have been reported, some of which have resulted in death. Azithromycin can cause abnormal changes in the cardiac electrophysiology that may lead to a potentially fatal irregular heart rhythm. It may increase the risk of death, especially in those with heart problems, compared with those on other antibiotics such as amoxicillin or no antibiotic. The Food and Drug Administration warning indicated that people with pre-existing conditions are at particular risk, such as those with QT interval prolongation, proarrhythmic conditions, low blood levels of potassium or magnesium, a slower than normal heart rate, or those who use certain drugs to treat abnormal heart rhythms. Elderly patients may be more susceptible to development of torsades de pointes arrhythmias.

7.2.3. *QT Risks Associated with Coadministration of HCQ and Azithromycin*

Combination treatment with HCQ + azithromycin was associated with heart-rate corrected QT interval (QTc) prolongation in a study of 84 patients with SARS-CoV-2 [39]. Maximal QTc prolongation was observed between Days 3 and 4 of treatment. QTc increase from baseline was >40 ms in 30% of the patients and 11% exhibited severely prolonged QTc, defined as a maximum increase to >500 ms. Multivariate analysis showed that the development of acute renal failure rather than baseline QTc was a significant predictor of severe QTc prolongation in these patients.

7.2.4. *Risks Associated with COVID-19 Diagnosis*

Enrollment in this protocol will not impact the public health department's advice for self-quarantine. Enrollment may improve morale during quarantine for COVID-19 infection. COVID-19 infection may be associated with anxiety, and the ability to monitor for LRTI and interact with study clinicians may allay anxiety.

7.3. *Strategies to Minimize Risk*

7.3.1. *Dose Selection*

The recommended dose of HCQ for chronic use in lupus erythematosus is 200 to 400 mg daily, a dose that is safely taken for years including among elderly patients (≥65 years). The selection of the time-limited dosing for this study is likely to be safe with transient adverse events (AEs) (gastrointestinal symptoms and rashes) that are self-remitting.

The recommended dose of azithromycin is 500 mg as a single dose on Day 1, followed by 250 mg once daily on Days 2 through 5. This dose has been safely used for several years for treatment of a wide variety of bacterial infections, including pneumonia.

7.3.2. *Management of Participants to Limit Risks of SARS-CoV-2 Transmission*

To limit the transmission of SARS-CoV-2, participants will receive visits via secure Telehealth in order to limit the movement of persons with potential SARS-CoV-2 and leave clinical space free for ill patients requiring care. Also, to limit exposure in waiting rooms and pharmacies, clinical specimens will be self-collected and medications will be delivered to homes. This will also eliminate exposure of study personnel to SARS-CoV-2.

7.4. *Dose Modification and Toxicity Management*

If a study therapy dose is missed, it should be taken as soon as possible. If it is less than 4 hours before the next dose, the dose should be skipped.

Modification for toxicities is discussed below. Only toxicities related to study medications provided through the study will be considered in the toxicity management section.

Grade 1 or 2

Participants who develop Grade 1 or 2 toxicity (per Division of Acquired Immunodeficiency Syndrome [DAIDS] AE Grading Table; see: https://rsc.tech-res.com/docs/default-source/safety/division-of-aids-(daids)-table-for-grading-the-severity-of-adult-and-pediatric-adverse-events-corrected-v-2-1.pdf) that is considered by the site Investigator to be related to the study medication may continue study treatment at the discretion of the site Investigator with close follow up. If a participant chooses to discontinue study treatment, the site should notify the study protocol team within 7 days. These participants will remain on study, off study treatment, and have all evaluations performed.

Grade 3

- Participants who develop a Grade 3 symptomatic toxicity thought by the site Investigator to be related to study drug should have study product withheld, and the site should consult with the Core Protocol team. The participant should be reevaluated every 2 days until the AE returns to Grade ≤2, at which time study drug may be reintroduced at the discretion of the site Investigator in consultation with the protocol team.
- Participants experiencing Grade 3 toxicity requiring permanent discontinuation of study product should be followed up weekly until resolution of the toxicity. Participants will

have premature study treatment discontinuation evaluations performed. These participants will remain on study, off study treatment, and have all evaluations performed per the SoA.

Grade 4

- Participants who develop a Grade 4 symptomatic toxicity will have study product permanently discontinued, and the site should notify the Principal Investigator within 72 hours.
- Participants experiencing Grade 4 toxicity requiring permanent discontinuation of study product should be followed up weekly until resolution of the AE or return to baseline. These participants will remain on study, off study treatment, and have all evaluations performed per the SoA.

Specific Management of Toxicities Related to Study-provided Drugs

HCQ

Gastrointestinal disturbance

Gastrointestinal disturbance (nausea, vomiting, dyspepsia, abdominal cramps, and diarrhea) is a common known possible side effect of HCQ. Taking with food or milk may improve tolerability.

Visual disturbances

Suspected visual changes should be evaluated for possible etiologies—if an HCQ-associated visual disturbance is suspected, HCQ should be stopped.

Allergic reactions

HCQ should be discontinued permanently if a serious allergic reaction is suspected. These participants will remain on study, off study treatment, and have all evaluations performed per the standard operating procedure(s).

QT prolongation

HCQ is associated with QT prolongation. QT will be assessed during the study using a portable monitor that can transmit arrhythmias to a central location which will be monitored. Participants will be counseled about this risk.

Hypoglycemia

HCQ can be associated with hypoglycemia, particularly among patients with diabetes mellitus who are also taking insulin or sulfonylureas. Participants will be counseled about this risk.

Azithromycin

Gastrointestinal disturbance

Gastrointestinal adverse reactions (nausea, vomiting, abdominal pain, and diarrhea) are common side effect of azithromycin. Most gastrointestinal side effects do not require treatment discontinuation and participants may receive treatment for symptoms (e.g., antiemetics).

Allergic reaction

Serious allergic reactions, including, but not limited to anaphylaxis, angioedema, and dermatologic reactions such as Stevens-Johnson syndrome have been reported with azithromycin treatment. Azithromycin should be discontinued immediately in the event of a hypersensitivity reaction.

QT prolongation

Azithromycin is associated with QT prolongation. QT will be assessed during the study using a portable monitor that can transmit arrhythmias to a central location which will be monitored. Participants will be counseled about this risk.

7.5. *Method of Treatment Assignment*

Participants will be randomized in a 1:1:1 ratio to HCQ + folic acid, HCQ + azithromycin, or ascorbic acid + folic acid at the level of the household (all eligible participants in 1 household will receive the same intervention). The randomization plan will be overseen by the Study Statistician. The randomization code and resulting allocation list will be generated and overseen by the Study Statistician. The list will be blocked and stratified by site and risk level.

7.6. *Blinding*

7.6.1. *Patient Blinding*

This is a blinded study. However, the medications are not identical-appearing and dosing is different for the 2 active agents so blinding will operate in different ways across those involved in the study, preserving blinding for the participants, those who directly interact with them, and measurement of the study endpoints:

- Participants will be blinded. The bottle of medication they receive will not identify the treatment allocation and only the number and frequency of pills to be taken. If >1 participant per household is randomized, all will receive the same treatment.
- Treating clinicians will be blinded, as the study medication will be dispensed directly to participants.
- Laboratory testing for viral shedding will be blinded, as laboratory staff will not be informed of randomized assignment. It should be noted that the viral shedding endpoint of the trial is an objective assessment, unlikely altered by unmasking, should it occur.
- Study pharmacy staff will be unblinded, as they will prepare the study medication.
- The study statistician will be unblinded for analysis purposes.

Eligible participants will receive one of the following therapies:

- HCQ 400 mg orally twice on Day 1, followed by 200 mg orally twice daily for an additional 9 days (Days 2 to 10) + placebo (folic acid 800 µg orally once on Day 1, followed by 400 µg orally once daily for an additional 4 days [Days 2 to 5])

- HCQ 400 mg orally twice on Day 1, followed by 200 mg orally twice daily for an additional 9 days (Days 2 to 10) + azithromycin 500 mg orally once on Day 1, followed by 250 mg orally once daily for an additional 4 days (Days 2 to 5)
- Placebo: Ascorbic acid 500 mg orally twice on Day 1, followed by 250 mg orally twice daily for 9 days + folic acid 800 µg orally once on Day 1, followed by 400 µg orally once daily for an additional 4 days (Days 2 to 5)

HCQ and ascorbic acid will appear similar, and taste will be partially masked as HCQ can be bitter and ascorbic acid will be sour. Azithromycin and folic acid have the same dosing schedule but are different in shape. Because participants in 1 household will all receive the same assignment and study clinicians will not see the study medication, the blind will be maintained.

The participants will be blinded to their randomization group once assigned. At enrollment, the unblinded Study Pharmacist will use the randomization code revealed at the point of randomization to provide the participant with their group assignment and dispense the allocated study medication in a bottle marked with the study label. The medication and medication information, mid-nasal swabs sufficient to complete the study procedures, *DBS sampling kit, if within the sub-study,* and study instructions will be delivered to the participant.

7.7. *Preparation/Handling/Storage/Accountability*

Drugs should be stored at room temperature, as per package insert. Records must be maintained that document receipt, release for dosing, disposal, or return to the Sponsor.

7.8. *Treatment Compliance*

The participant will be contacted to ensure that they received the box of study supplies, were able to collect the first mid-nasal swab and store it appropriately, and took their first day of medication. Participants will be asked to complete a survey that includes information regarding treatment administration. Medication errors, including under-dosing, will be recorded in the survey. *In a sub-study, HCQ and azithromycin concentrations via a DBS will also be evaluated.*

Consultation via Telehealth, text messaging, or telephone will be available to provide support to the participant to complete study procedures.

7.9. *Concomitant Therapy*

Participants will be asked about concomitant medications at the screening/baseline evaluation visit. During the study, participants will be asked to complete surveys (Daily Survey and Exit Contact Survey) that include information regarding any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant receives during the study. At each contact, the Investigator should question the participant about any medication taken.

7.9.1. *Prohibited Medications*

Prohibited medications for concomitant administration with HCQ include digoxin, cyclosporin, amiodarone, cimetidine, and tamoxifen, anticoagulants (e.g., warfarin), anticonvulsants,

rifampin, antifungals, immunosuppressants, antipsychotic medications, and antidepressants (citalopram, venlafaxine, and bupropion). Refer to Appendix 5 for medications prohibited for coadministration with HCQ.

Prohibited medications for concomitant administration with azithromycin include quinolone antibiotics (e.g., levofloxacin, moxifloxacin), macrolide antibiotics (e.g., erythromycin, clarithromycin), and antiarrhythmic drugs such as Class I (e.g., quinidine, procainamide, disopromide) and Class III (e.g., dofelitide, ibutilide, sotalol). Refer to Appendix 6 for medications prohibited for coadministration with azithromycin.

7.9.2. *Precautionary Medications*

Use of medications classified as precautionary is not study prohibitory but will be discussed with the study clinician and the participant. These medications include androgens, antidiabetic agents, phenothiazines, artemether, beta-blockers, cardiac glycosides, aminoquinolines, dapsone, escitalopram, herbs, lumefantrine, maitake, mefloquine, monoamine oxidase inhibitors, nelfinavir, pegvisomant, prothionamide, quinolones, salicylates, and selective serotonin reuptake inhibitors, and warfarin. Careful monitoring is needed during concomitant use of azithromycin with digoxin, colchicine, or phenytoin.

7.10. *Treatment After the End of the Study*

No additional treatment will be provided at the end of the study.

8. Discontinuation/Withdrawal Criteria

8.1. *Discontinuation of Study Treatment*

Study treatment will be discontinued for the following reasons:

- Hospitalization (at the discretion of the inpatient provider, hospitalized participants may continue to receive study treatment if maintained on the originally randomized treatment regimen)
- Requirement for prohibited concomitant medications or other contraindication to study product
- Occurrence of an AE requiring discontinuation of study product, including prolonged QT
- Request by participant to terminate study treatment
- Clinical reasons believed to be life-threatening by the physician, even if not addressed in Section 7.2

Participants who stop study product should continue study participation off study product with continued evaluations as per the SoA. The reason for study product discontinuation should be recorded.

Hospitalized participants will be followed through hospitalization and the last visit will be within 3 days of discharge.

8.2. *Withdrawal from the Study*

- A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time for the following reasons:
  - At the request of the primary care provider if he/she thinks the study is no longer in the best interest of the participant
  - Participant is judged by the Investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results
  - At the discretion of the Institutional Review Board/Ethics Committee or government agencies as part of their duties, Investigator, or industry supporter
- If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such withdrawal of consent.
- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.
- See SoA for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

8.3. *Lost to Follow-up*

A participant will be considered lost to follow-up if he/she is unable to be contacted by the study site.

The following actions must be taken if a participant fails to comply with required study procedures:

- The site must attempt to contact the participant as soon as possible and counsel the participant on the importance of maintaining the assigned procedure schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. Study Encounters

The current COVID-19 pandemic has placed a significant burden on the healthcare system. For this study, specimen and data collection will be conducted to minimize impact of non-ill participants within the healthcare system. If the participant is assessed as eligible, contact between study participants and study personnel will occur via a Health Insurance Portability and Accountability Act (HIPAA)-compliant video conference (Telehealth). Optionally, contact with the study clinician or staff after Day 1 may be conducted by telephone.

Participants will be instructed to seek clinical care should they manifest any signs or symptoms of LRTI requiring medical intervention and notify their physician about trial participation.

9.1. *Screening/Baseline Evaluation: Day 0/1*

Participants will be assessed for study eligibility through a screening conducted through a web-based screening tool, HIPAA-compliant video conference (Telehealth), telephone, or text messaging.

Day 0 evaluations are as follows:

- Informed consent
- Collection of demographic information
- Sign HIPAA form
- Collection of past and current medical conditions, including known pregnancy and/or lactation status
- Collection of concomitant medication information
- Collection of information regarding exposure to the index case (including laboratory documentation of COVID-19 testing)
- Check of inclusion and exclusion criteria

Eligible participants will be randomized. Participants will receive a monitoring kit via courier, which will include thermometer, SpO2 device, and QT monitor. They will be instructed on how to self-assess respiratory rate. In addition, they will receive a swab kit via courier or mail, which includes a Quick Start Instruction Card, swabs, plastic tubes for swab collection, a return box with affixed Category B UN3373 label, as required by International Air Transport Association (IATA) guidelines [40], and a pre-paid return shipping label.

The participant will do the following on Day 1:

- Collect mid-nasal swab for PCR
- Complete Daily Survey (online, telephone, or text messaging). This survey will include confirmation of adherence to study therapy dosing and daily swab collection, concomitant medication review, AEs, and symptoms review and objective measures including SpO2, respiratory rate, temperature, pulse, and QT. In addition, the modified inFLUenza patient-reported outcome (Flu-PRO) survey will be completed.
- Take study therapy (HCQ + placebo [folic acid], HCQ + azithromycin, or placebo (ascorbic acid + folic acid), as assigned

- *If in DBS sub-study, collect DBS sample for analysis of HCQ and azithromycin concentrations.*

Instructions for skin puncture and DBS sample preparation are provided in Appendix 8. A study team member will be available via Telehealth, telephone, or text messaging to provide support for completion of this study procedure.

Screening (Day 0) and Day 1 procedures can occur on the same day.

The SARS-CoV-2 positive test results will be confirmed through laboratory records.

9.2. *Day 2 Through Day 13*

The participant will do the following every day from Day 2 through Day 13, inclusive:

- Collect mid-nasal swab for PCR
- Take study therapy until Day 5 (inclusive) for azithromycin or folic acid (as assigned) and until Day 10 (inclusive) for HCQ or ascorbic acid (as assigned)
- Complete Daily Survey (online, telephone, or text messaging). This Survey will include confirmation of adherence to study therapy dosing and daily swab collection, concomitant medication review, AEs, and symptoms review and objective measures including SpO2, respiratory rate, temperature, pulse, and QT. In addition, the modified Flu-PRO survey will be completed.
- *If in DBS sub-study, collect DBS samples for analysis of HCQ and azithromycin concentrations at any time during this period (1 to 5 times) after study drug dosing has commenced. No more than 1 sample per day should be collected.*

A study team member will be available via Telehealth, text messaging, or telephone to provide support for completion of study procedures. The courier will collect the swabs at minimum within 1 or 2 days of Day 7 and Day 13 and potentially as frequently as daily.

9.3. *Days 2, 4, 9, and 14 (±1 day)*

Contact with study clinician or staff will be conducted via telemedicine (Telehealth) or telephone. Participants will be clinically assessed for signs and symptoms of respiratory distress and will have in person assessment of AEs. As needed, additional contact with the study clinician or staff will be conducted at the request of the participant (e.g., if developing concerning symptoms or an adverse event) or if needed to clarify study procedures or follow-up symptoms.

9.4. *Days 14 and 21*

The participant will do the following on Days 14 and 21:

- Collect mid-nasal swab for PCR
- Complete Daily Survey (online, telephone, or text messaging). This survey will include confirmation of adherence to study therapy dosing and daily swab collection, concomitant medication review, AEs, and symptoms review and objective measures

including SpO2, temperature, pulse, and QT. In addition, the modified Flu-PRO and WHO Ordinal Scale for Clinical Improvement surveys will be completed.

The courier will collect the swabs at minimum within 1 or 2 days of Day 14 and Day 21.

9.5. *Day 28*

The participant will do the following on Day 28:

- Collect mid-nasal swab for PCR
- Complete the Exit Contact Survey (online, telephone, or text messaging). This survey will include confirmation of adherence to study therapy dosing and daily swab collection, concomitant medication review, AEs, and symptoms review and objective measures including SpO2, temperature, pulse, and QT. In addition, the modified Flu-PRO and WHO Ordinal Scale for Clinical Improvement surveys will be completed.
- *If in DBS sub-study, collect DBS sample for analysis of HCQ and azithromycin concentrations as well as detection of anti-SARS-CoV-2 antibodies.*

For participants who are hospitalized on Day 28, the Day 28 procedures will be performed within 3 days after discharge from the hospital.

A study team member will be available via Telehealth, text messaging, or telephone to provide support for completion of study procedures.

The courier will collect the swabs at minimum within 1 or 2 days of Day 28.

Clinical outcomes will be confirmed through the electronic health record, if possible.

Participant Reimbursement

Participants will be reimbursed on Day 28 or at the post discharge visit. No reimbursement will be provided to participants for referral of their close contacts. No reimbursement will be provided for unscheduled Telehealth visits requested by the participants for support with study procedures.

10. Study Assessments and Procedures

- Study procedures and their timing are summarized in the SoA.
- Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All baseline evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management and obtained before signing of the informed consent form may be utilized for screening or baseline purposes provided that the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.
- *Blood samples will only be collected as a part of a sub-study. The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 10 mL.*

10.1. *Efficacy Assessments*

10.1.1. *Mid-nasal Swab*

Participants will collect daily mid-nasal swabs for on Days 1 to 14, 21, and 28 for viral detection.

Participants will receive a swab kit either via courier or mail, which includes a Quick Start Instruction Card, swabs, plastic tubes for swab collection, a return box with affixed Category B UN3373 label, as required by IATA guidelines [40], and a pre-paid return shipping label. Participants are instructed to place their self-collected nasal swabs directly into the plastic tube that is pre-labeled with a unique barcode. Next, participants are instructed to place the plastic tube containing the self-collected nasal swab into a specimen bag, pre-packaged with an absorbent sheet, and then place the specimen bag into the provided return shipping box. Swab kits may contain either viral transport media, 0.9% saline, or phosphate-buffered saline, which have been shown to be equivalent for storage of SARS-CoV-2 samples based on validated assays [41]. Previous testing has demonstrated that respiratory viral ribonucleic acid (RNA) is stable in room temperature for up to 1 week.

The used swabs will be collected by the courier or returned via the postal service.

Swabs will be subjected to RNA amplification and tested for SARS-CoV-2.

10.1.2. *Participant Survey*

Participants will be asked to complete surveys (Daily Survey and Exit Contact Survey) that will include questions about symptoms from both the drug regimen and respiratory and systemic

symptom, review of concomitant medications, and other pertinent topics. A modified self-reported Flu-Pro instrument will be completed each day through Day 14 and on Day 21 and Day 28 to assess degree of illness [42] (Appendix 10).

10.2. *Adverse Events*

Participants will be asked to complete surveys (Daily Survey and Exit Contact Survey) that include information on any symptoms that they are experiencing. In addition, AE review by a staff member (via telephone, Telehealth, or text messaging) will be performed.

All AEs must be recorded on the electronic case report forms (eCRFs) if any of the following criteria have been met:

- All AEs meeting SAE definition
- All AEs leading to discontinuation of study medication
- All AEs judged by the site Investigator to be associated with study medication

10.2.1. *Adverse Events*

An AE is any untoward medical occurrence that occurs in a patient or clinical investigation subject administered a pharmaceutical product, which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the product.

AEs may include the onset of new illness and the exacerbation of pre-existing medical conditions. An AE can include an undesirable medical condition occurring at any time, including baseline or washout periods, even if no study treatment has been administered.

10.2.2. *Serious Adverse Events*

An SAE is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is an important medical event that may not be immediately life-threatening or result 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

All AEs that are recorded must have their severity graded. To grade AEs, sites should refer to the DAIDS AE Grading Table, corrected Version 2.1, July 2017, which can be found on the DAIDS Regulatory Support Center website at https://rsc.tech-res.com/docs/default-source/safety/division-of-aids-(daids)-table-for-grading-the-severity-of-adult-and-pediatric-adverse-events-corrected-v-2-1.pdf.

10.2.3. *Treatment-related AE and SAE*

A treatment-related adverse event is defined as any new AE that begins, or any preexisting condition that worsens in severity, after at least 1 dose of study treatment has been administered until 30 days after the end of study visit (i.e,. Day 28) and is considered by the Investigator to be related to the study medication.

All AEs and SAEs should have attribution recorded as treatment- or not treatment-related, in the judgment of the site Investigator.

10.3. *Treatment of Overdose*

Overdose of HCQ or azithromycin should be managed according to the labeling information (see Appendix 5 and Appendix 6). Study drug overdose, including misuse or abuse of the product and medication errors, should be reported in the eCRF in the clinician notes.

Ascorbic acid and folic acid exhibit low toxicity; risks from overdose are expected to be minimal.

10.4. *Pregnancy*

Pregnancies occurring in participants enrolled in this study must be reported and followed to outcome. Pregnancy alone is not regarded as an AE unless there is a possibility that the study drug may have interfered with the effectiveness of a contraceptive medication. Elective abortions without complications should not be considered AEs unless they were therapeutic abortions. Hospitalization for normal delivery of a healthy newborn should not be considered an SAE. Pregnancy is not considered an SAE unless there is an associated serious outcome. Spontaneous abortions should always be reported as SAEs.

10.5. *Safety Assessments*

Safety will be assessed via participant Surveys, as shown in the SoA.

Participants will be asked to complete surveys (Daily Survey and Exit Contact Survey) that include questions about their health, healthcare seeking, symptoms, illness within their household, contact, and mobility. Qualifying events will be recorded on the eCRF and reported as AEs, as described in Section 10.2.

10.6. *Dried Blood Spot Sub-study (Optional)*

Up to 200 participants will be enrolled in the DBS sub-study. DBS will be requested, but not required, of all participants. For those study sites not wishing to participate in the DBS sub-study, this will not be considered a protocol deviation.

Participants will receive instructions for DBS self-collection in writing, with telephone, Telehealth, and text messaging options as support. Once cards have been dried, they will be collected by the study courier and returned to the laboratory.

The aim of the DBS sub-study is to evaluate HCQ and azithromycin drug concentrations as an adherence measure and the PK of HCQ or azithromycin. If serological assays for SARS-CoV-2 are available, DBS may be tested for SARS-CoV-2 antibodies.

Pharmacokinetics

The exposure-response relationship of HCQ and azithromycin of treated for SARS-CoV-2 has not been established. Population PK analyses can be used to further inform dose selection in other populations and support concentration-response investigations with efficacy and safety outcomes.

To accomplish this, sparse PK sampling techniques can be employed. This would involve collection of whole blood at 1 to 5 times after dosing has commenced. The time of collection post-dose can be random; however, no more than 1 sample per day should be collected.

Given the long half-lives of HCQ and azithromycin, samples for several weeks after the last dose are also informative to the population PK model development.

The basic requirements for PK sampling are as follows:

1. Accurate record of time of the dose prior to the blood sampling (dd:mm:yy; hh:mm)
2. Accurate recording of time of blood sampling (dd:mm:yy; hh:mm) for each blood sampling
3. Whole blood can be obtained by venipuncture or capillary blood by skin puncture using a lancet
4. Approximately 100 µL of blood is then applied to filter paper as outlined in Appendix 8

Anti-SARS-CoV-2 Antibody Testing

DBS sample for serology will be collected at Day 28 and tested for SARS-CoV-2 antibodies provided that an appropriate test is available.

10.7. *Biohazard Containment*

As the transmission of SARS-CoV-2 and other respiratory droplet pathogens can occur through contact with respiratory droplets and contaminated surfaces, precautions will be employed by all personnel in the handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All dangerous goods and materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by Code of Federal Regulations 42 Part 72. Please refer to instructions detailed in the IATA Dangerous Goods Regulations.

11. Statistical Considerations

11.1. *Sample Size Determination*

The sample size required to show a 50% treatment efficacy (i.e., relative risk reduction of 50%) is entirely dependent on the CER. Sample size calculations used an estimated 30% CER.

The table below shows the sample size required at various treatment effects, CER, and dropout rates for operating characteristics of 90% statistical power and 2-sided type I error rate of 5%. Power may be lower depending on CER and dropout rate (Table 1). As the initial sample size target, 165 per arm was chosen for each experimental group (HCQ and HCQ + azithromycin) to achieve 90% power with 0.05 two-sided type I error for a pairwise comparison against the control (ascorbic acid and folic acid) to detect at least 50% treatment efficacy in reducing the progression to LRTI (primary endpoint) assuming a CER of 30% and 5% dropout rate.

Table 1 Sample Size per Arm Required to Detect a Relative Risk Reduction of 40% to 60% Under Various Control Event and Dropout Rates

RRR	Dropout Rate=0%			Dropout Rate=5%			Dropout Rate=20%		
	CER=10%	CER=20%	CER=30%	CER=10%	CER=20%	CER=30%	CER=10%	CER=20%	CER=30%
40%	962	437	261	1013	460	275	1202	546	327
50%	578	263	158	609	277	**165**	723	329	198
60%	375	171	103	395	180	108	469	214	128

CER: Control event rate; RRR: Relative risk reduction.
Sample size calculation was done with the desired operating characteristics of 90% statistical power and 5% type I error rate (2-sided).

As illustrated in the table above, the statistical power will depend heavily on the observed CER. Given the uncertainty of the target population, the final target sample size will be re-estimated using the observed CER and dropout rate in the control group as a blinded sample size re-assessment. As it is difficult to predict the enrollment rate, the recruitment rate will be assessed on a weekly basis starting at the end of second week. It is expected that the enrollment rate will be fast, and to enroll the initial target size of 495 participants (165 per three groups), it will take 4 to 6 weeks, resulting in an approximate recruitment rate of approximately 83 to 124 participants per week. At the end of fourth week, it can be expected that 53 to 79 participants from the control group have been followed-up with their clinical follow-up providing reasonable estimates on CER and dropout rate (Table 2).

Table 2 Expected Recruitment and Number of Measurable Clinical Outcomes for the First 6 to 8 Weeks of Trial

Time	Recruitment Rate of 83 Participants/Week		Recruitment Rate of 124 Participants/Week	
	Number of Enrolled Total Participants	Number of Total Measurable Outcomes	Number of Enrolled Total Participants	Number of Total Measurable Outcomes
End of Week 1	83	0	124	0

End of Week 2	166	0	248	0
End of Week 3	249	79	372	118
End of Week 4	332	158	495	236
End of Week 5	415	237	495	354
End of Week 6	495	316	495	471
End of Week 7	495	395	--	--
End of Week 8	495	471	--	--

The number of enrolled participants at the end of each week was calculated with the recruitment rate assumptions of 83 and 124 participants per week. The number of total measurable outcome was calculated based on 14 days of clinical follow-up required for each participant and under the assumption of 5% dropout rate.

The procedure for the blinded sample size re-assessment will be described in an Interim Monitoring Plan approved by the DSMB. In brief, the study statistician will assess the CER and dropout rate and re-calculate the sample size required to achieve 90% statistical power at 5% 2-sided type I error rate to detect a treatment effect size of 50% relative risk reduction. This will be done with proper firewall to maintain blinding of the information on the re-calculated sample size. If study findings indicate that a study arm should be dropped and additional arms added, the sample size required will be calculated based on accumulated data.

11.2. *Populations for Analyses*

For analysis purposes, the following populations are defined and will be described in greater detail in the trial Statistical Analysis Plan:

Population	**Description**
Intention to Treat (ITT)	All enrolled participants: high-risk group for the primary clinical endpoint; high- and low-risk groups (separately) for the primary virologic endpoint.
PK evaluable	Participants from the DBS sub-study with at least 1 interpretable PK sample

DBS: dried blood spot; PK: pharmacokinetic; PCR: polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

11.3. *Statistical Analyses*

The Statistical Analysis Plan will be developed and finalized before database lock and will describe the participant populations to be included in the analyses, the detailed analytical plans with endpoints and procedures for accounting for missing, unused, and spurious data. An Interim Monitoring Plan will also be developed to describe approaches for re-estimation of sample size

and any planned interim analyses. This section presents a brief summary of the planned statistical analyses of the primary and secondary endpoints (these are listed in Section 4).

11.3.1. *Efficacy Analyses*

Demographic characteristics (age, sex, race) of each study group will be tabulated.

The mean age (plus range and standard deviation) by sex of the enrolled participants, as a whole and per group, will be calculated.

Primary analyses: The primary analyses will be conducted on the intend-to-treat population overall and by cohort (high-risk and low-risk). Participants randomized to each active treatment will be compared to participants randomized to placebo (ascorbic acid + folic acid). The primary analysis will make use of all participants randomized to placebo, whether contemporaneously enrolled or not, using an Empirical Bayesian information borrowing method (described below). Due to anticipated heterogeneity in risk of disease progression, pre-specified baseline variables, including age, BMI, and days of symptoms at time of enrollment will be included in the model to increase precision.

LRTI will be analyzed using logistic regression stratified by site.

Sensitivity analysis: The primary analyses will be repeated, replacing randomization arm by actual treatment to account for possible off-label use of and noncompliance to the investigational products.

Subgroup analyses: All subgroup analyses will be pre-specified in the Statistical Analysis Plan. Any further subgroup analyses will be considered ad hoc.

Empirical Bayesian information borrowing method

In the case that a new experimental treatment is added into this platform trial, there will already be data already collected from patients who are enrolled prior (past data). To incorporate the past control data with the concurrent control data from patients who are concurrently enrolled, empirical Bayesian information borrowing method will be used [43]. Instead of using concurrent data only, combining past data with the concurrent data can potentially be advantageous in terms of statistical power and lower number of participants that are needed to be randomized to the control arm. This is particularly important for possible improved ethics and feasibility for the context of conducting a platform trial for SARS-CoV-2. Additionally, in the case that relevant external trial data become available, this empirical information borrowing method may be used. The DSMB, which will contain clinical experts, will make on whether to include or ignore the external trial data. The Study Statistician will assess the similarities of the external trial(s) to this clinical trial in terms of eligibility criteria, trial location, data collection procedures, and the reliability and comprehensiveness of the available dataset. If deemed appropriate, the empirical Bayesian information borrowing method will be used to determine the degree of the information that can be “borrowed” from the external dataset and be used for the statistical comparison.

As there are multiple randomized clinical trials that are either ongoing or being planned right now for SARS-CoV-2 with potentially relevant interventions, it is important to plan for an approach, such as this empirical Bayesian borrowing method, that can potentially incorporate these external data with the internal data. An important feature of this method is the avoidance of

use of any subjective or informative prior distributions that may become a point of dispute in certain schools of thought. To our knowledge, there are currently 2 published clinical trials with potentially relevant interventions as the ones that are proposed for this adaptive platform trial [1, 44]. In the Chinese trial registry, there are more than 300 interventional trials that have been registered for SARS-CoV-2. It is very likely that external data source will become available during the trial.

The full technical details on the empirical Bayesian information borrowing method will be described in the Statistical Analysis Plan.

Missing Data

Due to the design of the study and retention activities, measurable outcomes are expected for all participants. However, in the unlikely event of a missing test result, the missing data will be imputed.

11.3.2. *Secondary Endpoints*

All secondary endpoints will be assessed in the Intention-to-Treat population overall and by cohort.

11.3.2.1. Safety Analyses

All safety analyses will be performed on the Intention-to-Treat population. AEs will be compared by study group.

11.3.2.2. Hospitalization

Hospitalization rates between the groups will be compared using logistic regression stratified by site. Number of days hospitalized will be described graphically and by median and interquartile rage.

11.3.2.3. Disease Severity

Disease severity, as measured by the WHO Ordinal Scale for Clinical Improvement (Appendix 11), will be compared between the groups using a proportional odds model.

Disease severity over the first 14 days and at Days 21 and 28 will also be measured by the modified Flu-PRO survey (Appendix 10). Results will be analyzed as for the WHO Ordinal Scale for Clinical Improvement.

11.3.2.4. Symptom Resolution

Days with fever after randomization, respiratory symptoms after randomization, and Sp02<93% after randomization will be modeled using Poisson regression stratified by site with an offset for number of days of observation.

11.3.3. *Pharmacokinetic Analysis*

Sparse PK from DBS will be analyzed using standard population PK analysis methodologies using standard software such as NONMEM® version 7.4 or Phoenix NLME version 8.2.

11.3.4. *Exploratory Exposure-Response Analyses*

PK-evaluable participants will have post-hoc individual concentration profiles and exposure estimates determined for exploratory exposure-response analyses against primary and secondary efficacy and safety endpoints. Exploratory PK/pharmacodynamic analyses will be performed as the data allow.

11.3.5. *Combined Study Analysis*

This protocol is being published as a model protocol for other institutions to consider as they undertake studying treatments to prevent development of LRTI among outpatients with SARS-CoV-2 infection. It is hoped that individual participant data from similar studies can be pooled into a combined study analysis. De-identified data from the present study will be made available for these purposes in accordance with the funder's open access policy (https://www.gatesfoundation.org/how-we-work/general-information/open-access-policy).

12. References

1. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrobial Agents 2020; Epub 2020 Mar.
2. Menzel M, Akbarshahi H, Bjermer L, et al. Azithromycin induces anti-viral effects in cultured bronchial epithelial cells from COPD patients. Sci Rep. 2016 Jun;6:28698.
3. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb;395(10223):497-506.
4. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Feb;382(8):727-733.
5. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Feb;395(10223):507-513.
6. WHO News Release. Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). 2020 Jan 30.
7. White House Press Release. https://www.whitehouse.gov/presidential-actions/proclamation-declaring-national-emergency-concerning-novel-coronavirus-disease-covid-19-outbreak. 2020 Mar 13.
8. Cai J, Xu J, Lin D, et al. A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features. Clin Infect Dis. 2020 Feb. pii: ciaa198.
9. Linton NM, Kobayashi T, Yang Y, et al. Incubation period and other epidemiological characteristics of 2019 novel coronavirus infections with right truncation: a statistical analysis of publicly available case data. J Clin Med. 2020 Feb;9(2). pii: E538.
10. WHO News Release. Shortage of personal protective equipment endangering health workers worldwide. 2020 Mar 03.
11. Chen J. Pathogenicity and transmissibility of 2019-nCoV-a quick overview and comparison with other emerging viruses. Microbes Infect. 2020 Mar;22(2);69-71.
12. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. 2020 Mar; Cell. pii: S0092-8674(20)30229-4 (pre-publication).
13. Li CC, Wang XJ, Wang HR. Repurposing host-based therapeutics to control coronavirus and influenza virus. Drug Discov Today. 2019 Mar;24(3):726-736.
14. Lee JS, Adhikari NKJ, Kwon HY, et al. Anti-Ebola therapy for patients with Ebola virus disease: a systematic review. BMC Infect Dis. 2019 May;19(1):376.
15. Slater AF. Chloroquine: mechanism of drug action and resistance in Plasmodium falciparum. Pharmacol Ther. 1993 Feb-Mar;57(2-3):203-235.
16. Mackenzie AH. An appraisal of chloroquine. Arthritis Rheum. 1970 May-Jun;13(3):280-291.
17. Shippey EA, Wagler VD, Collamer AN. Hydroxychloroquine: an old drug with new relevance. Cleve Clin J Med. 2018 Jun;85(6):459-467.

18. Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol. 2020 Mar;16(3):155-166.
19. Rolain JM, Colson P, Raoult D. Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infections in the 21st century. Int J Antimicrob Agents. 2007 Oct;30(4):297-308.
20. Savarino A, Shytaj IL. Chloroquine and beyond: exploring anti-rheumatic drugs to reduce immune hyperactivation in HIV/AIDS. Retrovirology. 2015 Jun;12:51.
21. Keyaerts E, Vijgen L, Maes P, et al. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun. 2004 Oct;323(1):264-268.
22. Vincent MJ, Bergeron E, Benjannet S, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J. 2005 Aug;2:69-78.
23. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020 Mar;30(3):269-271.
24. Yao X, Ye F, Zhang M, 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 Inf Dis. 2020 Mar. pii: ciaa237.
25. 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 Feb. doi: 10.5582/bst.2020.01047.
26. Furst DE, Lindsley H, Baethge B, et al. Dose-loading with hydroxychloroquine improves the rate of response in early, active rheumatoid arthritis: a randomized, double-blind six-week trial with eighteen-week extension. Arthritis Rheum. 1999 Feb;42(2):357-365.
27. Collins K, Jackson K, Gustafson D. Hydroxychloroquine: a physiologically based pharmacokinetic model in the context of cancer-related autophagy modulation. J Pharmacol Exp Ther. 2018;365(3):447-459.
28. Tett SE, Cutler DJ, Day RO, Brown KF. Bioavailability of hydroxychloroquine tablets in healthy volunteers. Br J Clin Pharmacol. 1989;27(6): 771-779.
29. Poschet JF, Perkett EA, Timmins GS, et al. Azithromycin and ciprofloxacin have a chloroquine-like effect on respiratory epithelial cells. 2020. bioRxiv preprint. doi: https://doi.org/10.1101/2020.03.29.008631.
30. Belouzard S, Millet JK, Licitra BN, et al. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses. 2012 Jun;4(6):1011-33.
31. Wrapp D, Wand N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020 Mar 13;367(6483):1260-1263.
32. Ballow CH, Amsden GW. Azithromycin: the first azalide antibiotic. Ann Pharmacother. 1992;26(10):1253-61.
33. Touret F, Gilles M, Barral K, NougairédeA, Decroly E, de Lamballerie X, Coutard B. *In vitro* screening of a FDA approved chemical library reveals potential inhibitors of SARS-CoV-2 replication. 2020 bioRxiv preprint doi:https://doi.org/10.1101/2020.04.03.023846.

34. WHO. Report of the WHO-China joint mission on coronavirus disease 2019 (COVID-19). 2020 Feb 16-24.
35. Barrett B, Brown R, Rakel D, et al. Placebo effects and the common cold: a randomized controlled trial. Ann Fam Med. 2011 Jul-Aug;9(4):312-322.
36. Fujii T, Luethi N, Young PJ, et al. Effect of vitamin C, hydrocortisone, and thiamine vs hydrocortisone alone on time alive and free of vasopressor support among patients with septic shock: the VITAMINS randomized clinical trial. JAMA. 2020 Jan 17. doi: 10.1001/jama.2019.22176. [Epub ahead of print].
37. Graat J, Schouten E, Kok F. Effect of daily vitamin E and multivitamin-mineral supplementation on acute respiratory tract infections in elderly persons: a randomized controlled trial. JAMA. 2002 Aug;288(6):715-721.
38. Srinivasa A, Tosounidou S, Gordon C. Increased incidence of gastrointestinal side effects in patients taking hydroxychloroquine: a brand-related issue? J Rheumatol. 2017 Mar;44(3):398.
39. Chorin E, Matthew D, Shulman E et al. The QT interval in patients with SARS-CoV-2 infection treated with hydroxychloroquine/azithromycin. 2020. preprint doi: https://doi.org/10.1101/2020.04.02.20047050.
40. UN3373 Medical Packaging. Biological Substance Category B. Available at: https://www.un3373.com/category-biological-substances/category-b. Updated 2018 Jun. Accessed 2020 Mar 01.
41. Rodino KG, Espy MJ, Buckwalter SP, et al. Evaluation of saline, phosphate buffered saline and minimum essential medium as potential 2 alternatives to viral transport media for SARS-CoV-2 testing. J Clin Microbiol. 2020. 2020 Mar 30.
42. Yu J, Powers JH, Vallo D, et al. Evaluation of efficacy endpoints for a Phase IIb study of a respiratory syncytial virus vaccine in older adults using patient-reported outcomes With laboratory confirmation. Value Health. 2020 Feb;23(2):227-235.
43. Schoenfeld D, Zheng H, Finkelstein D. Bayesian design using adult data to augment pediatric trials. Clinical Trials. 2009;6:297-304.
44. Cao B, Wang Y, Wen D, et al. A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. N Engl J Med. 2020 Mar 18. doi: 10.1056/NEJMoa2001282.

13. Appendices

Appendix 1: Abbreviations and Terms

Term	Definition
ACE2	Angiotensin converting enzyme 2
AE	Adverse event
CER	Control event rate
COVID-19	Coronavirus disease
CQ	Chloroquine
DAIDS	Division of Acquired Immunodeficiency Syndrome
DBS	Dried blood spot
DSMB	Data and safety monitoring board
EC_{50}	Half-maximal effective concentration
eCRFs	Electronic case report forms
Eligible	Qualified for enrollment into the study based upon adherence to inclusion/exclusion criteria
Flu-PRO	InFLUenza patient-reported outcome
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
HCQ	Hydroxychloroquine
HIPAA	Health Insurance Portability and Accountability Act
IATA	International Air Transport Association
ICF	Informed consent form
Index case	Term used throughout the protocol to denote the person with confirmed or suspected SARS-CoV-2 infection to whom the study participant was exposed
IRB	Institutional Review Board
LRTI	Lower respiratory tract infection
MERS-CoV	Middle East respiratory syndrome coronavirus
Participant(s)	Term used throughout the protocol to denote the enrolled individual(s)
PBPK	Physiologically based pharmacokinetics
PCR	Polymerase chain reaction
PEP	Post-exposure prophylaxis
PK	Pharmacokinetic(s)
QTc	Heart-rate corrected QT interval
RNA	Ribonucleic acid
SAE	Serious adverse event

SARS-CoV	Severe acute respiratory syndrome coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SoA	Schedule of Activities
SpO2	Oxygen saturation
USPI	United States prescribing information
WHO	World Health Organization
WIRB	Western Institutional Review Board

Appendix 2: Protocol Structure

The protocol for this trial will be structured to be modular. Given the perpetual nature of this trial, there may be modifications and/or additions to the main protocol. Each modification and/or addition to the main protocol will be subject to the data and safety monitoring board who will receive recommendation from other study committees (see Appendix 3).

The main protocol will contain all the background and rationale for this trial and all generic information to the trial, the research approach, the trial design and conduct, and the overall trial governance, and ethical considerations. Other appendices for study governance, site-specific protocol addendum template, hydroxychloroquine (HCQ) label, pharmacokinetic modelling, and pharmacokinetic sample collection and analysis are provided below. The Intervention-Specific Appendices (e.g., HCQ label appendix) will contain the information about the interventions. There will be a specific appendix for each of the intervention arm with features of the given intervention strategy and how it will be delivered, and any additional endpoints and data collection that are not covered in the Main Protocol.

Appendix 3: Study Governance Considerations

Investigators and Institutional Affiliations

The following Investigators and Institutional Affiliations were established at the time of protocol authoring. Designees may be provided, as appropriate. Other institutions may utilize this model protocol with permission from the Principal Investigator.

Name	**Role**	**Institution**	**Contact Information**
Dr. Christine Johnston, MD, MPH	Principal Investigator	University of Washington	Phone: +1 206 520 4318 Email: cjohnsto@uw.edu
University of Washington Coordinating Center			
Dr. Elizabeth R. Brown, ScD	Study Statistician	Fred Hutchinson Cancer Research Center	Phone: +1 206 667-1731 Email: erbrown@fredhutch.org
Dr. Jared Baeten, MD, PhD	Co-Investigator	University of Washington	Phone: +1 206 520-3808 Email: jbaeten@uw.edu
Dr. Connie Celum, MD, MPH	Co-Investigator	University of Washington	Phone: +1 206 520-3800 Email: ccelum@uw.edu
Dr. Ruanne Barnabas, MD, PhD	Co-investigator	University of Washington	Phone: +1 206 520 3813 Email: rbarnaba@uw.edu
Dr. Joshua Schiffer, MD	Co-investigator	Fred Hutchinson Cancer Research Center	Phone: +1 206 667 7359 Email: jschiffe@fredhutch.org

Committees Structure

The Trial Steering Committee will take the overall responsibility for the trial design and conduct. All committees will act in accordance to the International Clinical Harmonization Guidelines for Good Clinical Practice (GCP) Principles.

Study Team Monitoring

The study team will monitor the conduct of the study through monthly summary reports of arms of accrual and baseline characteristics and quarterly reports of data pooled over treatment arms of data completeness, specimen collection, and adverse events (AEs). The study team will review individual participant-level safety data frequently to assess the relation of all reported AEs to study treatment. On a weekly basis, the study team will review by-arm summaries of premature study discontinuations and premature study treatment discontinuations (and reasons) and AEs.

Independent Monitor

Study conduct will be monitored by an independent monitor. Monitors will visit participating clinical research sites to review the individual participant records, including consent forms, electronic case report forms, supporting data, laboratory specimen records, and endpoints through laboratory and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect the sites' regulatory files to ensure that regulatory requirements are being followed and the sites' pharmacies to review product storage and management.

Data and Safety Monitoring Board

An independent data and safety monitoring board (DSMB) will be convened for this study with expertise in coronavirus disease (COVID-19) or respiratory viruses, antiviral therapies and shedding, and emerging epidemics and a biostatistician. The purpose of the DSMB is to monitor the study for operational futility, social harms, and efficacy. The DSMB will evaluate the progress of the project, including periodic assessments of accrual, retention, safety, performance and variation of the project sites, and other factors that can affect project implementation.

The DSMB will review and approve modifications to the overall enrollment target based on the event rate. Due to the anticipated rapid speed of enrollment and the short duration of the study, it is unlikely that pre-specified stopping rules for efficacy and futility in terms of the efficacy of HCQ or HCQ + azithromycin will be reached before all participants are enrolled. The DSMB will review severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) endpoints.

The DSMB will conduct interim reviews (if specified) when adequate data have been accrued and convene by teleconference. Open reports containing accrual and retention rates, participant characteristics, and serious adverse events will be sent to the protocol team and DSMB members the week prior to the DSMB meeting. Only the DSMB members and the unblinded biostatistician will receive password-protected closed reports of SARS-CoV-2 endpoints by randomization arm.

Regulatory and Ethical Considerations

The study will be conducted according to GCP, the Belmont Report, and the Declaration of Helsinki. The study protocol, site-specific informed consent forms (ICFs), participant education and recruitment materials, and other requested documents—including any subsequent modifications—will be reviewed and approved by Western Institutional Review Board (WIRB), as the single IRB of record, responsible for oversight of research conducted at the study sites. Subsequent to initial review and approval, the WIRB will review the study at least annually.

Informed Consent Process

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented in each clinical study before any protocol-specified procedures or interventions are carried out. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant, and this fact will be documented in the participant's record.

A participant who is rescreened is not required to sign another ICF; eligibility for the study must be re-checked prior to enrollment.

Study Records

Each study site will establish a standard operating procedure for confidentiality protection. Each site will ensure that study records including administrative documentation and regulatory documentation as well as documentation related to each participant enrolled in the study, including ICFs, locator forms, case report forms, notations of all contacts with the participant, and all other source documents are stored in a secure manner.

Confidentiality

Participants' study information will not be released without their written permission, except as necessary for oversight by:

- The protocol Principal Investigators or designees
- Study funders
- WIRB
- University of Washington IRB

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. The exceptions are SARS-CoV-2 testing results, which are subject to local and state reporting which is name-based. Local public health may contact participants diagnosed with SARS-CoV-2 for the purpose of surveillance and contact notification. Participants will be informed prior to SARS-CoV-2 testing that results are reportable and may lead to contact by local public health if results are positive for infection.

All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB, Office for Human Research Protections, other local, United States, and international regulatory entities as part of their duties or the industry supporters or designees.

Appendix 4: Site-Specific Protocol Addendum Template

PURPOSE: The use of this site-specific protocol addendum is recommended with a multi-site study.

The purpose of this site-specific protocol addendum is to obtain information describing the local site-specific elements for conduct of the Core Protocol. The descriptions should focus on who, what, and where of the local study activities.

INSTRUCTIONS:

1. Add the Name of the study site to the Title of the Protocol in the header of the document.
2. Please complete each section below. If general description language already appears in the Core Protocol, which adequately describes the local activity at the study site, a notation can be inserted in the given section that reads, "As described in the Core Protocol."
3. Please identify the completed addendum document using a version number and date.
4. Please note: the site-specific protocol addendum will require review and approval by the local Institutional Review Board (IRB)/Ethics Committee (EC) of record for the study site.

Study Site Information:

Name of Institution/Company:

Address:

Study Site Information for the Site Investigator:

Name of Site Investigator:

Title:

Institution/Affiliation:

Address:

Telephone Number:

Cell/Other Number:

Fax Number:

Study Site Number:

Assurance Information:

United States Department of Health and Human Services Office for Human Research Protections Assurance Federal Wide Assurance number:

Expiration Date:

IRB/Ethics Committee Information:

Name of Human Subjects Protection oversight office for study site:

Name and number of reviewing IRB/EC:

Telephone Number at Office:

Fax Number at Office:

If Available, Point of Contact at IRB/EC:

Name:

Telephone Number:

Local Site-specific Information:

1. Identify key study personnel (include name, title, address, point of contact information).
2. Describe the key study personnel roles and responsibilities.
3. Describe the local recruiting procedures and strategies. Provide copy of any site-specific recruitment material. Identification of personnel responsible for completing tasks.
4. Describe the local consenting process. Provide a copy of the site-specific consent form(s). If an Ombudsman is named for the study site, provide name, title, and point of contact information.
5. Identify local study collaborations at the site such as pharmacy, laboratories, and other institutional departments.
6. Describe the local specimen/sampling procedures in place. Include acquisition, disposition, storage, and unique coding. If samples will be kept for future use, describe procedures and the security measures for short-term and long-term management. Provide name of repository.
7. Describe the plan for on-site management of study records and data, and participant study records. Explain procedures and security measures in place for short- and long-term management. Declare who will have access to data.
8. Describe the local measures in place to promote privacy and confidentiality.
9. Describe the local procedures in place for provision of care for the participant regarding research-related injuries.
10. Identify who the participant can contact locally should the participant have any questions regarding the research. Identify who the participant can contact should the participant

have questions regarding their rights as a study participant. Include the points of contact information (name, title, and telephone number).

11. Describe the procedures in place to address Health Insurance Portability and Accountability Act requirements. If a separate authorization form will be used at the study site, provide a copy or ensure appropriate language has been included in the consent document(s).
12. Describe any unique site-specific study procedures or supplemental activities.
13. Declare any unique study population/cultural influences, socioeconomic conditions, etc.
14. Declare any other site-specific reporting obligations and procedures. Name any additional oversight boards or committees.
15. Define abbreviations that may apply to the specific study site.

Appendix 5: Hydroxychloroquine Label

Generic hydroxychloroquine label (current as of June 2018) is available online at https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=b82bbda6-64f2-4426-b4ec-254eeea895ae and provided below.

Appendix 6: Azithromycin Label

The Azithromycin label is available online at
http://labeling.pfizer.com/ShowLabeling.aspx?format=PDF&id=511 and provided below.

Appendix 7: Physiologically Based Pharmacokinetic Model for Azithromycin Dose Regimen

A physiologically based pharmacokinetic (PBPK) model for azithromycin was developed previously and verified against clinical data (Johnson et al., 2016). With the exception of the B:P ratio which was set at 2.28, the input parameters for the azithromycin model remained the same. This updated model was then used to predict exposures in plasma and lung after administration of 500 mg for 1 day and 250 mg QD for 4 days, a dosage regimen that was used by Gautret et al. (2020) to prevent bacterial super-infection in patients receiving hydroxyl-chloroquine as treatment for SARS-CoV-2. Based on physicochemical data, the predicted lung to tissue ratio (Kp) was 16.33, which was lower than the observed ratio of 50.5 (Lucchi et al., 2008). Both predicted and observed ratios were used in simulations (100 subjects aged 20 – 50 years; 50% female) of plasma and lung concentration-time profiles which were then compared against *in vitro* values of 2.12 (EC_{50}) and 8.65 µM (EC_{90}) reported for inhibition of SARS-CoV-2 by azithromycin (Touret et al., 2020) (Figure 1). It should be noted that the unbound fraction in plasma (fu) was set at 0.69 and the predicted fu in lung tissue was 0.82. Thus, unbound and total concentrations were similar in both plasma and lung.

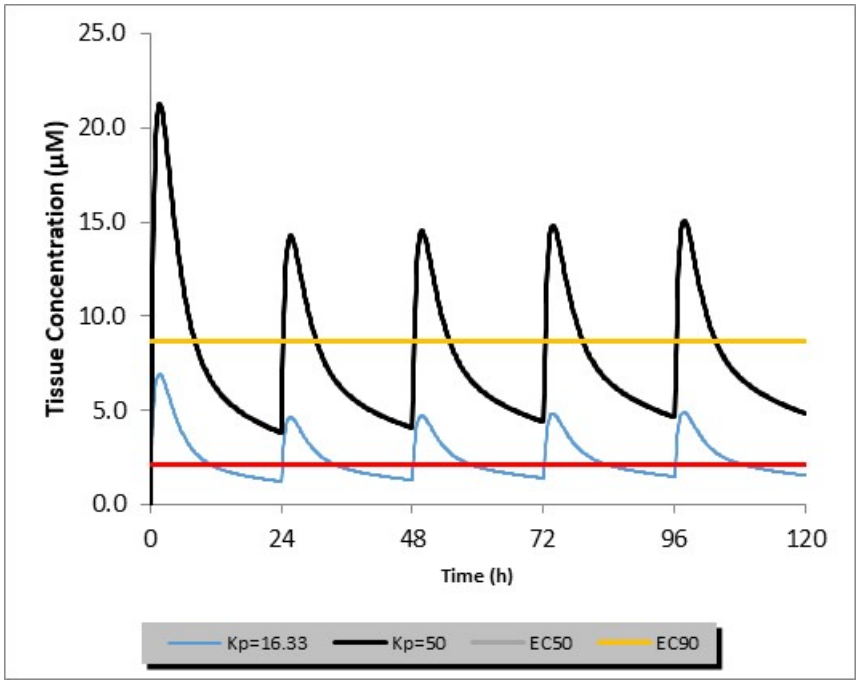

Figure 1. Predicted concentration-time profiles for azithromycin in the lung based on Kp values of 16.33 and 50 in the lung.

Based on the simulations shown, it appears that the dosing regimen of 500 mg for 1 day followed by 250 mg QD for 4 days, is able to attain concentrations of azithromycin in lung that would approach the *in vitro* EC_{50} value determined for azithromycin.

Gautret et al. (2020) Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomised clinical trial. International Journal of Antimicrobial Agents – In Press 17 March 2020: – DOI : 10.1016/j.ijantimicag.2020.10594.

Johnson TN, Jamei M and Rowland-Yeo K (2016). How does in vivo biliary elimination of drugs change with age? Evidence from in vitro and clinical data using a systems pharmacology approach. *Drug Metab Dispos*; 44:1090-1098.

Lucchi M, Damle B, Fang A et al., (2008). Pharmacokinetics of azithromycin in serum, bronchial washings, alveolar macrophages and lung tissue following a single oral dose of extended or immediate release formulations of azithromycin. Journal of Antimicrobial Chemotherapy; 61: 884–891.

Touret F, Gilles M, Barral K, NougairédeA, Decroly E, de Lamballerie X, Coutard B. *In vitro* screening of a FDA approved chemical library reveals potential inhibitors of SARS-CoV-2 replication. bioRxiv preprint doi: https://doi.org/10.1101/2020.04.03.023846.

Appendix 8: Pharmacokinetic Sample Collection and Analysis

Collection of Blood

Skin puncture

1. Put on a pair of disposable gloves.
2. Before skin puncture, the participant should warm his/her hands. The finger is massaged anterogradely to enrich the blood flow toward the puncture site.
3. Clean the skin of the palmar side of the tip of the distal phalanx of the third or fourth finger of the nonwriting hand with a suitable disinfectant, for example, 70% isopropyl alcohol. Puncture the skin by a single-use safety lancet. The finger should be held in such a position that the gravity facilitates the collection of blood from the fingertip.
4. When collection of capillary blood by skin puncture is complete, place a bandage on the fingertip.

Venipuncture

To be conducted by trained personnel per standard procedures.

Preparation of Blood Spots

Preparation from blood collected by skin puncture

1. Wipe off the first drop of blood with a gauze pad because it may contain excess tissue fluids. Massage the finger again to increase blood flow at the puncture site. Transfer the following drop to one of the circles of a filter card without touching the surface directly with the fingertip. Allow the blood to be soaked into the texture of the filter by capillary forces only.
2. Let the next large drop of capillary blood form on the fingertip and collect it in the next circle. Continue this procedure until all necessary circles are filled or blood flow stops.

3. Do not squeeze or "milk" the finger excessively if the blood flow is not sufficient to fill all the required circles of the filter card. If blood flow stops, place a bandage on the fingertip. Perform a second skin puncture on another finger if more blood is needed for the examination.
4. For blood obtained by venipuncture, use syringe to apply approximately 100 µL of blood on the filter paper.

Drying of Blood Spots

To dry the blood spots, put the filter cards on a clean paper towel and let them dry, preferably overnight (but for at least 4 hours), at room temperature in the absence of any external source of heat. When the drying process is complete, the blood spots have a uniformly dark brownish color and no red areas are visible anymore.

Storage and Transportation of Dried Blood Spots

NOTE: Processing of the blood spots can be interrupted after drying. The filter cards can now be stored.

1. For storage, put the filter paper card in a single, gas-impermeable zipper bag, containing 1 to 2 desiccant sachets to protect the specimens from moisture. Optionally, add a humidity indicator card.
2. Transfer this bag to a freezer as soon as possible. If freezers are not available under field conditions, storage at -4°C or even at ambient temperature is feasible for up to 14 days.
3. Transport frozen dried blood spots specimens on dry ice. For filter cards initially kept at ambient temperature, use a triple packaging system, which consists of the zipper bag(s) as the inner container(s) as well as an inner and an outer envelope. No content markings are required on the outer envelope for shipment by regular mail, but the international biohazard symbol must be affixed to the primary inner container.
4. Exclude the filter cards from further processing if the desiccant packs and/or the additional humidity indicator card changes to a pink color.

On the filter paper, the participant should record the following:

1. His/her name and date of birth
2. Date and time of sample
3. Date and time of last dose

Samples that appear to be collected according to the schedule of activities, which have the required amount of blood for a 5- to 6-mm punch and have the minimal required information (1 through 3 above), will be processed for hydroxychloroquine (and metabolite) and azithromycin concentration.

Appendix 9: Physiologically Based Pharmacokinetic Modeling of Hydroxychloroquine Used for Post-exposure Prophylaxis

Appendix 10: The inFLUenza Patient-Reported Outcome instrument (Flu-PRO) – Modified for SARS-CoV-2

DAILY SURVEY (D1-14, D21, D28)

inFLUenza patient-reported outcome (Flu-PRO): 32 items validated, can score across domains.

***FYI: This takes about 5 minutes to complete

How are you feeling today?

FLU-PRO	Not at all	A little bit	Somewhat	Quite a bit	Very much
Nose					
Runny or dripping					
Congestion or stuffy					
Sneezing					
Sinus pressure					
Lack of smell					
Throat					
Sore throat					
Scratchy or itchy throat					
Difficulty swallowing					
Lack of taste					
Eyes					
Teary or watery eyes					
Sore or painful eyes					
Eyes sensitive to light					
Chest/Respiratory					
Trouble breathing					
Chest congestion					
Chest tightness					
Dry or hacking cough					

Wet or loose cough

Sputum (coughing up sputum or phlegm)

Wheezing

Gastrointestinal

Felt nauseous

Stomach ache

Vomit

Diarrhea

Body/Systemic

Felt Dizzy

Head congestion

Headache

Lack of appetite

Sleeping more than usual

Body aches or pains

Weak or tired

Chills of shivering

Felt cold

Felt hot

Sweating

How are you feeling today?

0 no symptoms 1 Mild 2 Moderate 3 Severe 4 Very severe

Please rate interference in daily activities due to illness:

1 Not at all 2 A little bit 3 Somewhat 4 Quite a bit 5 Very much

How is your general health?

1 Poor 2 Fair 3 Good 4 Very good 5 Excellent

Have you returned to your usual health today? Yes/no

Have you returned to your usual activities today? Yes/no

TAKE MEDS, TAKE SWAB (Copy post-exposure prophylaxis)

Take your vitals:

- Oxygen level
- Pulse
- Temperature
- Respiratory rate
- Electrocardiogram monitor

PM Time:

- Take meds
- Take vitals
- Oxygen level
- Pulse
- Temperature
- Respiratory rate

Anxious mood	Not at all	mild	moderate	severe	very severe

Yu J, Powers JH, Vallo D, et al. Evaluation of efficacy endpoints for a Phase IIb study of a respiratory syncytial virus vaccine in older adults using patient-reported outcomes with laboratory confirmation. Value Health. 2020 Feb;23(2):227-235.

Appendix 11: WHO Ordinal Scale for Clinical Improvement

WHO COVID-19 Core Protocol
SOLIDARITY Trial
Version 10.0, March 22, 2020

Patient State	Descriptor	Score
Uninfected	No clinical or virological 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 prongs	4
Hospitalized Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Ventilation + additional organ support – pressors, RRT, ECMO	7
Dead	Death	8