Pre-exposure Prophylaxis for SARS-Coronavirus-2: A Pragmatic Randomized Clinical Trial

Short Title: PREP COVID-19

UMN IRB Number: STUDY00009414

ClinicalTrials.gov: NCT04328467

Principal Investigator: Radha Rajasingham MD

Draft or Version Number: *1.0*

27 **March 2020**

CONFIDENTIAL

STATEMENT OF COMPLIANCE

This protocol will utilize a single institutional review board (IRB) registered with the Office of Human Research Protections (OHRP) and issued a Federal Wide Assurance (FWA). The research will be reviewed and approved by the IRB and will be subject to continuing review [45 CFR 46.103(b)].

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Signed:

Site Investigator:	Radha Rajasingham, MD Assistant Professor of Medicine	Date:	March 26, 2020

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
CFR	Code of Federal Regulations
COVID19	coronavirus disease 2019
SRS-CoV	SARS coronavirus (i.e. circa 2003)
SARS-CoV-2	SARS coronavirus 2 (i.e. circa 2019)
CRF	Case Report Form
CRO	Contract Research Organization
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDS	Investigational Drug Services
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
MERS-CoV	Middle East respiratory syndrome coronavirus

MOP	Manual of Procedures
N	Number (typically refers to subjects)
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
US	United States
WHO	World Health Organization

Summary

Full Title:	Pre-exposure prophylaxis for coronavirus: A Pragmatic Randomized Clinical Trial
Short Title:	PREP COVID-19
Clinical Phase:	III
Sponsor:	Investigator-Initiated Protocol, University of Minnesota
Principal Investigators:	Radha Rajasingham, MD
Accrual Ceiling	3500
Study Population	Healthcare workers at high risk for exposure to COVID-19 cases
Objective	Test if pre-exposure prophylaxis with hydroxychloroquine can prevent development of COVID-19 disease
Study Design	Double-blind, randomized clinical trial Internet-based trial driven by self-report. Study medicine delivered by courier to consented participants.
Intervention Arms:	Hydroxychloroquine 200mg tablet. 1. 400 mg orally once, followed by 400mg 6 to 8 hours later, thereafter 400mg weekly for the duration of follow up, up to 12 weeks 2. 400mg orally once, followed by 400mg 6 to 8 hours later, thereafter 400mg twice weekly for the duration of follow up, up to 12 weeks
Control Arm:	Placebo 2 tabs once, followed by 2 tabs 6 to 8 hours later, thereafter two tabs weekly or twice weekly for the duration of follow up, up to 12 weeks
Primary Endpoint	• COVID-19-free survival

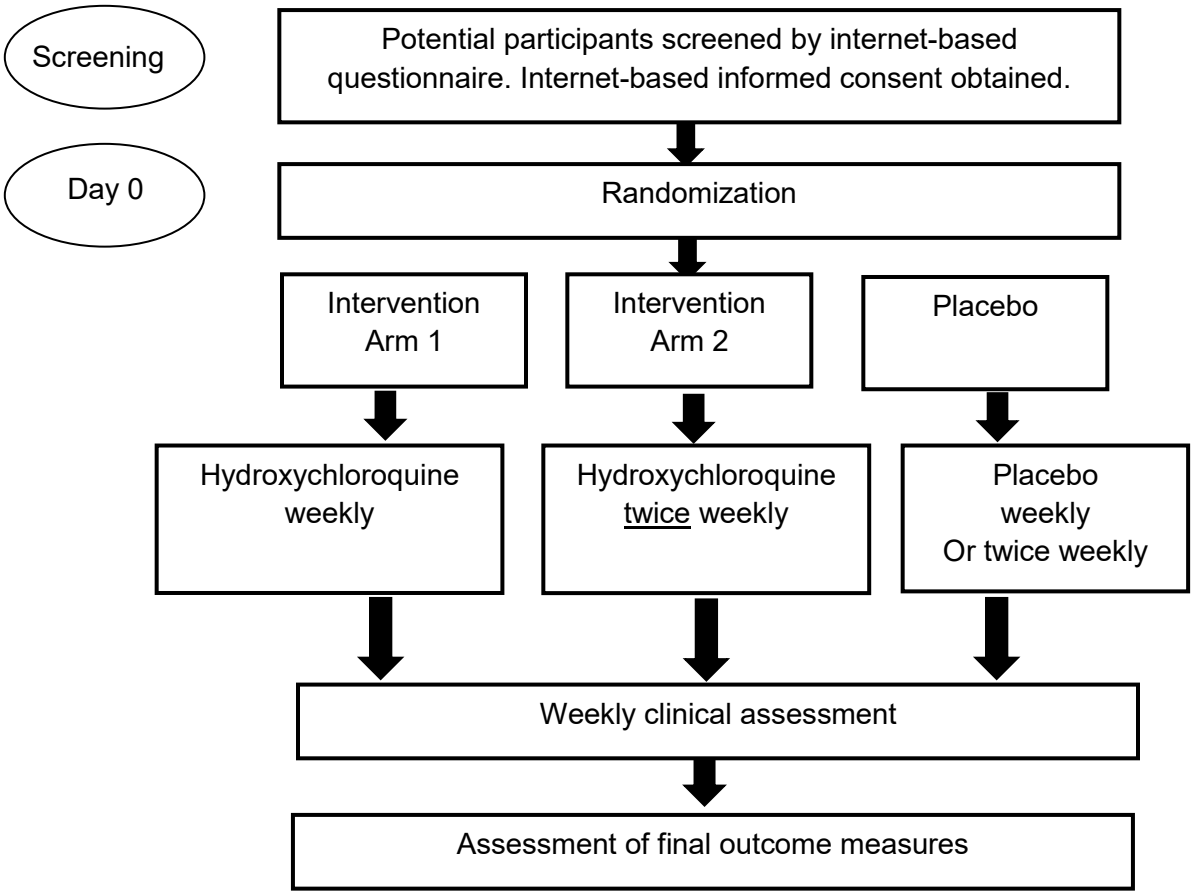
Secondary Endpoints	1. Incidence of confirmed SARS-CoV-2 detection 2. Incidence of possible COVID19 symptoms 3. Incidence of all-cause study medicine discontinuation 4. Ordinal Scale of COVID-19 Disease maximum severity if COVID-19 diagnosed 5. Incidence of Hospitalization for COVID-19 or death 6. Incidence of study medication-related side effects
Duration of Participation	● Recruitment and follow up will be internet-based. All patients will be followed for at least 4 weeks. Recruitment is expected to take 4 weeks, for a total study duration of 8 weeks. But the total study duration will depend on the actual rate of enrollment. Study drug will be given for a maximum of 12 weeks.

Inclusion Criteria	● Healthcare worker, at high risk of COVID-19 exposure:
	  ● Persons primarily working in emergency departments (physicians, nurses, ancillary staff, triage personnel)
	  ● Persons primarily working in intensive care units (physicians, nurses, ancillary staff, respiratory therapists)
	  ● Persons performing aerosol generating procedures: ex. Anesthesiologists, nurse anesthetists (CRNAs)
	  ● First responders: ex. EMTs, paramedics
	● Age >=18 years of age
	● Provision of Informed Consent

Exclusion Criteria	● Active COVID-19 disease ● Confirmed prior COVID-19 disease ● Current fever, cough, shortness of breath ● Contraindication or allergy to chloroquine or hydroxychloroquine ● Prior retinal eye disease ● Known Chronic Kidney disease, Stage 4 or 5 or dialysis. ● Known glucose-6 phosphate dehydrogenase (G-6-PD) deficiency. ● Weight <40 kg ● Current use of hydroxychloroquine or cardiac medicines of flecainide, amiodarone, digoxin, procainamide, or propafenone ● Current use of medications with known significant drug-drug interactions: artemether, lumefantrine, mefloquine, tamoxifen, or methotrexate.

Statistical Assumptions	● 10% transmission rate for high-risk healthcare workers with no treatment ● Alpha = 0.025 (to account for two treatment groups vs. placebo comparisons) ● Assuming 15% lost to follow up in the placebo group and 5% lost to follow up in each treatment group ● N=3500 has 80% power to detect a hazard ratio of 0.65, using a two-sided log-rank test

Schematic of Study Design:



At the close of the study, any participant with ongoing illness will continue to be assessed for up to a total of 90 days after the common closing date.

1 KEY ROLES

Principal Investigator: Radha Rajasingham MD

Co-Investigator: David R. Boulware MD, MPH

Co-Investigator: Akram Khan, MD

Co-Investigator: Bryan Wolf, MD

Co-Investigator: Peter Li, MD

Co-Investigator: Ingrid Mayer, MD

Co-Investigator: Justin Balko, PharmD, PhD

Co-Investigator: Brian Rini, MD

Co-Investigator: Sarah Lofgren, MD

Co-Investigator: Archana Bhaskaran

Co-Investigator: Matthew Pullen, MD

Co-Investigator: Caleb Skipper, MD

Pharmacologist: Melanie Nicol, PharmD, PhD

Biostatistician: Kathy H Hullsiek, PhD

Associate Statistician: Ananta Bangdiwala, MS

Associate Statistician: Nicole Engen, MS

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Significance of Research Question/Purpose:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a rapidly emerging viral infection causing COVID-19. The current strategy uses a public health model of identifying infected cases, isolation, and quarantine to stop transmission. Once exposed, observation is standard-of-care.

No effective therapy currently exists for treatment. The lack of effective therapy diminishes persons presenting for self-quarantine. Having an effective pre-exposure prophylaxis for healthcare workers at high risk of contracting COVID-19, even if only partially effective, may additionally create synergy for the public health strategy of case identification and isolation – if a safe prophylaxis is available.

People who develop COVID-19 disease generally develop signs and symptoms, including mild respiratory symptoms and fever, after an average of 5-6 days after exposure (i.e. mean incubation period). The range of the incubation period is between 1 to 14 days.[1]

Most people infected with the COVID-19 virus have mild disease and recover. Approximately 80% of laboratory-confirmed patients have had mild to moderate disease, which includes non-pneumonia and pneumonia cases, 14% have severe disease, and 6% are critically ill with respiratory failure, shock, and/or multiple organ dysfunction.[2]

Preliminary Data:

Chloroquine has *in vitro* activity in cell lines against SARS-CoV and SARS-CoV2. In a Vero E6 cell line, the half-maximal effective concentration (EC50) activity of chloroquine was 1.13 μM against SARS-CoV2.[3] Hydroxychloroquine is functionally equivalent as chloroquine.

Another compound under treatment trials, remdesivir (Gilead) had an EC50 of 0.77 μM.[3] Remdesivir (Gilead) is not FDA-approved and in limited quantities. Hydroxychloroquine is FDA-approved and globally is inexpensive. Chloroquine is no longer broadly available in the USA.

Existing Literature:

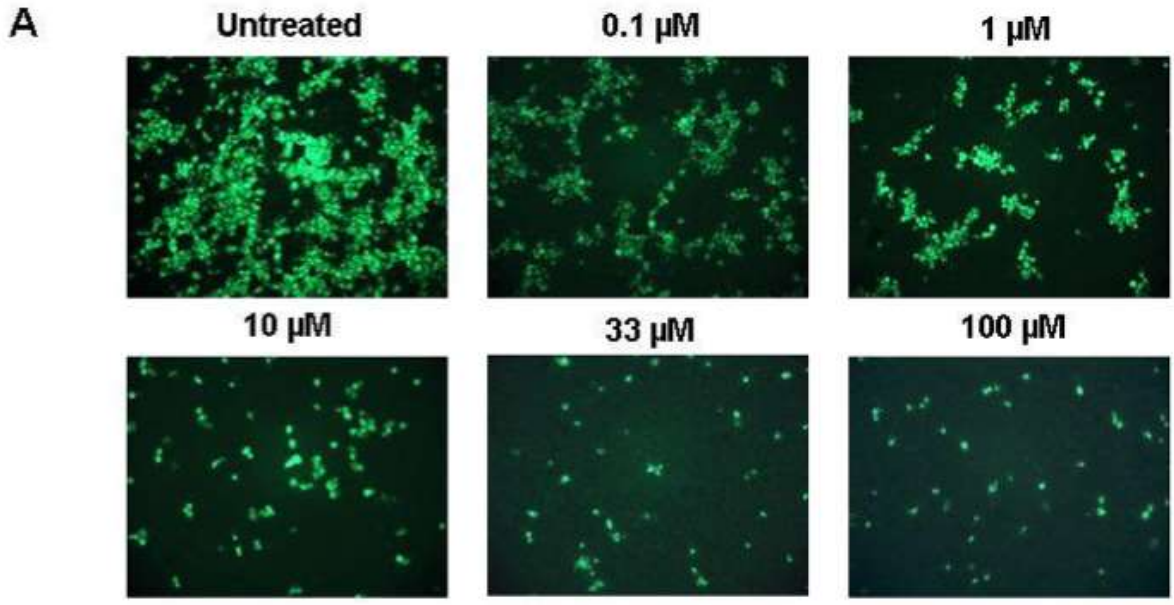
Several drugs, such as ribavirin, interferon, lopinavir-ritonavir, corticosteroids, have been used in patients with SARS or MERS, although the efficacy is poorly understood as a gold standard randomized clinical trial has not been conducted.

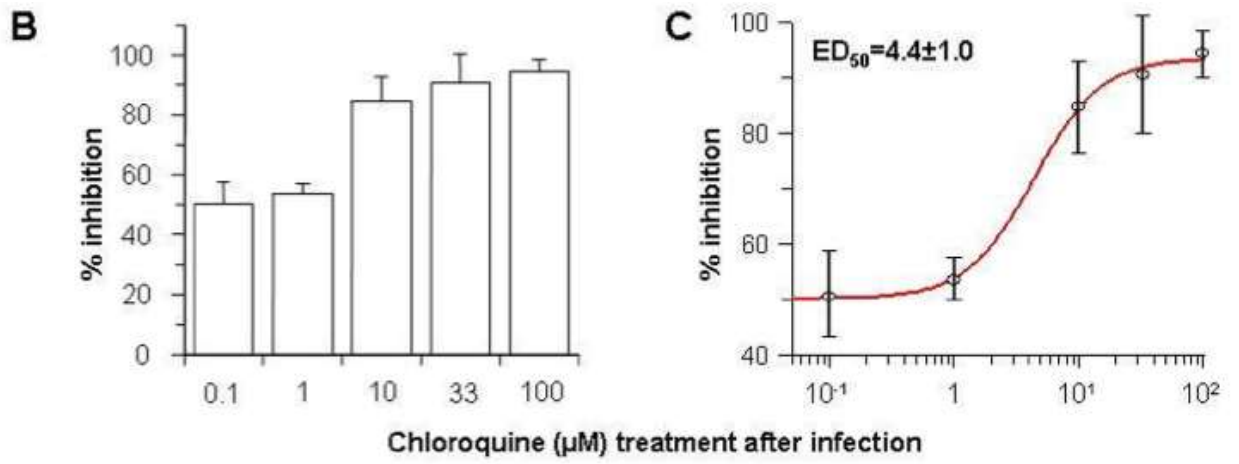
After the original 2003 SARS outbreak, screening of compounds was performed. The Special Pathogens Branch of the Division of Viral and Rickettsial Diseases at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia investigated chloroquine.

CDC investigators reported that post-infection chloroquine treatment was effective in vitro at preventing the spread of SARS-CoV infection in an in vitro cell-based system.[4] Vincent et al reported:

> "In order to investigate the antiviral properties of chloroquine on SARS-CoV after the initiation of infection, Vero E6 cells were infected with the virus and fresh medium supplemented with various concentrations of chloroquine was added immediately after virus adsorption. Infected cells were incubated for an additional 16–18 h, after which the presence of virus antigens was analyzed by indirect immunofluorescence analysis. When chloroquine was added after the initiation of infection, there was a dramatic dose-dependent decrease in the number of virus antigen-positive cells (**Fig. 2A**). As little as 0.1–1 μM chloroquine reduced the infection by 50% and up to 90–94% inhibition was observed with 33–100 μM concentrations (**Fig. 2B**). At concentrations of chloroquine in excess of 1 μM, only a small number of individual cells were initially infected, and the spread of the infection to adjacent cells was all but eliminated. A half-maximal inhibitory effect (EC50) was estimated to occur at 4.4 ± 1.0 μM chloroquine (**Fig. 2C**). These data clearly show that the addition of chloroquine can effectively reduce the establishment of infection and spread of SARS-CoV if the drug is added immediately following virus adsorption."[4]

Figure 2. Post-infection chloroquine treatment reduces SARS-CoV.





Vincent et al also reported: “Since we observed antiviral effects by chloroquine immediately after virus adsorption, we further extended the analysis by adding chloroquine 3 and 5 h after virus adsorption and examined for the presence of virus antigens after 20 h. We found that chloroquine was still significantly effective even when added 5 h after infection (Fig. 3); however, to obtain equivalent antiviral effect, a higher concentration of chloroquine was required if the drug was added 3 or 5 h after adsorption.”[4]

Further experiments demonstrated that chloroquine impaired the terminal glycosylation of angiotensin-converting enzyme-2 (ACE2) receptor, which is the binding site for the envelope spike glycoprotein of SARS-CoV and SAR-CoV2.

Conversely, chloroquine did not have activity against Middle East respiratory syndrome coronavirus (MERS-CoV), which may be related to MERS binding to CD26 receptor protein.[5]

In a March 9, 2020 publication, hydroxychloroquine was found to have greater activity than chloroquine.[6] The SARS-CoV-2 EC 50 values for hydroxychloroquine were 6.14 μM at 24 hours and 0.72 μM at 48 hours.[6] Conversely, chloroquine EC50 values were >100 μM at 24 hours and 18.01 μM at 48 hours.[6] This inhibition assay was performed with Vero cells using an infectious dose of 100 plaque forming units.

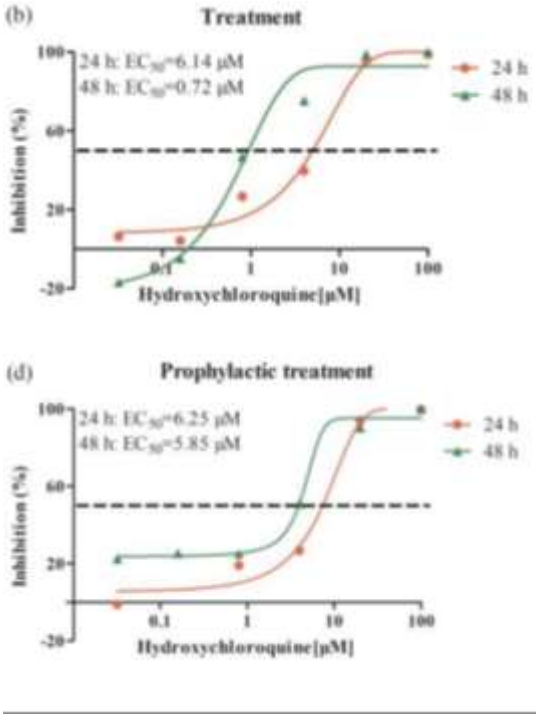


Figure 3 displays the antiviral activities of hydroxychloroquine for treatment or prophylaxis against SARS-CoV-2 *in vitro*.[6]

In a March 18, 2020 publication, hydroxychloroquine was found to be less toxic than chloroquine with slightly lower, but similar potency.[7] At MOI of 0.01, 0.02, 0.2, and 0.8, the EC50 of hydroxychloroquine was 4.51, 4.06, 17.31, and 12.96 μM respectively compared to 2.71, 3.81, 7.14, and 7.36 μM for chloroquine.

On March 20, 2020, a clinical study from France reported improved viral clearance in 20 patients treated with 600 mg per day of hydroxychloroquine for 6 days compared to 16 controls.[8] By 6 days, 70% of those in the hydroxychloroquine group had negative viral PCR on nasopharyngeal swab compared to 13% in the control group. However, the authors noted an additional 6 subjects on hydroxychloroquine that were removed from the analysis because they did not complete therapy- 5 of these were still viral PCR positive at the time of drop-out. Without an adequately powered, randomized clinical trial, the efficacy of hydroxychloroquine against COVID-19 disease remains to be seen but these data are encouraging in regards to a potential antiviral effect.

2.2 Rationale

The current standard of care is observation and quarantine after exposure to COVID-19. There is no approved treatment or prophylaxis for COVID-19.

As of March 6, 2020, the CDC estimates that the transmission of SARS-CoV2 after a U.S. household close contract is 10.5% (95%CI, 2.9 to 31.4%).[9] Among all close contacts, the SARS-

CoV2 transmission rate is estimated at 0.45% (95%CI, 0.12 to 1.6%) by the CDC. These estimates are based on monitoring of travel-associated COVID19 cases. Conversely, in a setting with community transmission, the secondary attack rate in China was 35% (95%CI, 27-44%) based on 48 transmissions among 137 persons in 9 index patients.

Chloroquine or Hydroxychloroquine may have antiviral effects against SARS-COV2 which may prevent COVID-19 disease or reduce disease severity. It is not known at what dosing hydroxychloroquine may be effective for pre-exposure prophylaxis.

We propose to dose for SARS-CoV-2 pre-exposure prophylaxis at:

1. 400mg once weekly (with a loading dose)
2. 400mg twice weekly (with a loading dose)

Study recruitment is expected to take 4 weeks, and all participants will be followed for a minimum of 4 weeks. If study recruitment is slower than anticipated, the study medications will be given for a maximum of 12 weeks.

The projected levels achieved will be approximately 0.72 uM which is at the half-maximal effective concentration (EC50) where 50% viral inhibition would occur. Given chloroquine's and hydroxychloroquine's long half-time, a steady-state concentration may take days to achieve. Thereby, a loading dose may be necessary as seen in emergency prophylactic use of hydroxychloroquine or chloroquine for anti-malarial purposes.[4]

If hydroxychloroquine does not prevent disease for some, it may reduce COVID-19 disease severity. Attenuated disease may, in turn, be associated with reduced rates of transmission.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The short-term use of hydroxychloroquine is well-tolerated with a safe track record since 1955. The most common reported side effects include:

- headache, dizziness, ringing in your ears;
- nausea, vomiting, stomach pain;
- loss of appetite, weight loss;
- mood changes, feeling nervous or irritable;
- skin rash or itching;
- hair loss.

GI side effects are minimized when taken with a meal or with milk. Antacid medications should be spaced apart by at least 4 hours.
Long-term use of hydroxychloroquine at its accepted prophylactic dose is also well-tolerated.

In our recent post-exposure study of hydroxychloroquine, where hydroxychloroquine is given at high dose (800mg) daily for 5 days, 70% of participants reported no side effects. The most common side effects, reported in ~30% of participants were GI-related symptoms such as nausea or vomiting.

In this study, we are using lower dose (400mg) weekly or twice weekly. We expect even fewer side effects, if any.

- Potential adverse effects by system, as listed on the FDA package insert:
  - **Eye**: "Chloroquine retinopathy" is a rare side-effect of chronic use after multiple years of use. This has not occurred with <1 year of continuous use. Furthermore, based on the recommendation by the American Academy of Ophthalmology, screening for retinopathy is warranted with dosing higher than 5 mg/kg/day. The proposed dosing in this trial is order of magnitudes lower.
  - **Dermatologic Reactions**: Bleaching of hair, alopecia, pruritus, skin and mucosal pigmentation, photosensitivity, and skin eruptions (urticarial, morbilliform, lichenoid, maculopapular, purpuric, erythema annulare centrifugum, Stevens-Johnson syndrome, acute generalized exanthematous pustulosis, and exfoliative dermatitis).
  - **Hematologic Reactions**: Various blood dyscrasias such as aplastic anemia, agranulocytosis, leukopenia, anemia, thrombocytopenia (hemolysis in individuals with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency).
  - **Gastrointestinal Reactions:** anorexia, nausea, vomiting, diarrhea, and abdominal cramps. Isolated cases of abnormal liver function and fulminant hepatic failure.
  - **Cardiac Reactions:** Long-term use (>1 year) has been associated with the development of cardiomyopathy and arrhythmias.
  - **Allergic Reactions**: Urticaria, angioedema, and bronchospasm have been reported
  - **CNS Reactions**: Irritability, nervousness, emotional changes, nightmares, psychosis, headache, dizziness, vertigo, tinnitus, nystagmus, nerve deafness, convulsions, ataxia
  - **Miscellaneous Reactions:** Weight loss, lassitude, exacerbation or precipitation of porphyria and non-light-sensitive psoriasis.

2.3.2 Known Potential Benefits

- There are no known benefits in humans for preemptive treatment.
- *In vitro* antiviral activity against SARS-CoV and SARS-CoV-2 viruses.
- Chloroquine and Hydroxychloroquine have a long history of safe, effective use as an antimalarial, both acutely and long-term use.
- Chloroquine is being used therapeutically for severe COVID-19 disease in China and Korea.
- Commonly used as a chronic medication for autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus).

- Recent studies have shown potential broad antiviral effects *in vitro* at dosages below currently recommended clinical dosing, reducing risk of potential adverse effects listed above.

3 OBJECTIVES

3.1 Study Objectives

To determine if pre-exposure prophylaxis with hydroxychloroquine is effective at prevention of COVID-19 disease.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measure

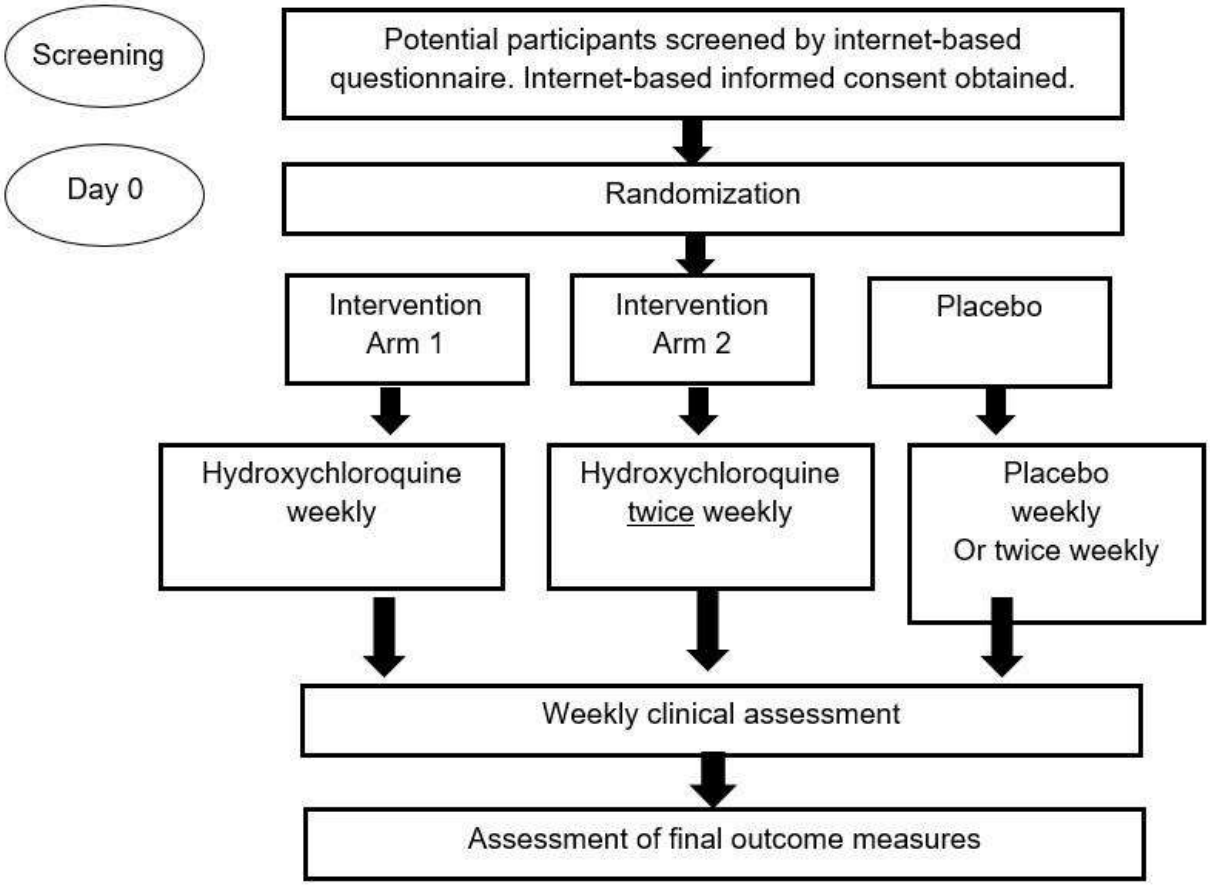
- COVID-19-free survival

3.2.2 Secondary Outcome Measures

- Incidence of confirmed SARS-CoV-2 detection
- Incidence of symptoms compatible with COVID19 (possible disease)
- Incidence of all-cause study medicine discontinuation or withdrawal
- Ordinal Scale of COVID-19 Disease Severity (for those who develop COVID-19)
    - No illness
    - Illness with outpatient observation
    - Hospitalization (or post-hospital discharge)
    - Hospitalization with ICU stay or death
- Incidence of hospitalization or death
- Self-reported adherence
- Self-reported study medication side effects

Primary outcome of COVID-19-free survival will be confirmed by positive test. Assessment of other outcome measures will be primarily by self-report. As necessary, COVID-19 disease will be verified from public health records, medical records, or death certificates.

4 STUDY DESIGN



At the close of the study, any participant with ongoing illness will continue to be assessed for up to a total of 90 days after the common closing date.

4.1 DESIGN

Randomized, double-blind clinical trial, parallel design. This is an event-driven trial. Subjects will be followed until study termination. Study drug will be prescribed for up to 12 weeks.

4.2 Study participant duration

- Weekly internet-based virtual visits for the duration of follow up
- Study drug is prescribed to be taken for up to 12 weeks
- For participants ill with COVID-19 disease at the common closing date for the study, observational follow up will extend to up to 90 days after the common closing date to assess final outcome status.

- Pregnant women have their fetal outcome assessed post-partum.

4.3 Study procedures

4.3.1 Screening

- Baseline screening for eligibility via self-administered questionnaire
- Informed consent

4.3.2 Randomization

- Participants will be randomized at the investigation pharmacy
- The investigational pharmacy will dispense masked study medicine (hydroxychloroquine or placebo)
- The study medicine will be shipped to participants to arrive ~1 day after enrollment

4.3.3 Day 1 Virtual visit

- Verify receipt of study medicine
- Clinical status check-in
- Begins taking study medicine (2 tabs), then 2 tabs in 6-8 hours
- Query for symptom status
- Query for hospitalization or SAEs

4.3.4 Weekly Virtual visit

- Clinical status check-in for COVID-19 symptoms
- Assessment of adherence and side effects by self-report
- Continue study medicine up to 12 weeks
- Query for study medicine side effects since enrollment
- Query for SARS-CoV-2 testing
- Query for hospitalization or SAEs

4.3.5 Study termination virtual visit (to be completed on or shortly after the common closing date for the study)

- Clinical status check-in for COVID-19 symptoms
- Query for study medicine adherence and side effects since enrollment
- Query for SARS-CoV-2 testing
- Query for hospitalization or SAEs
- Query for pregnancy status
- Final outcome assessment

4.4 Individually identifiable health information: Name, date of birth, and phone number will be collected so as to prescribe study medication. Email addresses will be collected for communication. If participants are hospitalized, the hospitalization date will be collected.

4.5 Sub studies

Participants in Minneapolis, MN, Portland, OR, and Nashville TN, will have the opportunity to participate in an optional sub-study evaluating peripheral blood correlatives. Please see full details in Appendix A.

5 STUDY ENROLLMENT AND WITHDRAWAL

Participants will undergo screening via internet-based google forms. The screening and inclusion criteria will be based on self-report.

5.1 Subject Inclusion Criteria

- A healthcare worker at high risk for COVID-19 exposure (defined below)
- >= 18 years of age
- Provision of informed consent

5.2 Subject Exclusion Criteria

- Active COVID-19 disease
- Confirmed prior COVID-19 disease
- Current fever, cough, shortness of breath
- Contraindication or allergy to chloroquine or hydroxychloroquine
- Prior retinal eye disease
- Known Chronic Kidney disease, Stage 4 or 5 or dialysis.
- Known glucose-6 phosphate dehydrogenase (G-6-PD) deficiency.
- Weight <40 kg
- Current use of hydroxychloroquine or cardiac medicines of flecainide, amiodarone, digoxin, procainamide, or propafenone
    - Current use of medications with known significant drug-drug interactions: artemether, lumefantrine, mefloquine, tamoxifen or methotrexate.

Rationale for inclusion / exclusion criteria:

Healthcare workers at high risk for COVID-19 exposures include:

- *Persons primarily working in emergency departments (physicians, nurses, ancillary staff, triage personnel)*
- *Persons primarily working in intensive care units (physicians, nurses, ancillary staff, respiratory therapists)*
- *Persons performing aerosol generating procedures: ex. Anesthesiologists, nurse anesthetists (CRNAs)*
- *First responders: ex. EMTs, paramedics*

In clinical practice of tropical medicine, chloroquine or hydroxychloroquine are prescribed without any baseline laboratory testing or monitoring.

Age criteria are meant to increase the propensity to follow up and minimize dropouts.

Chronic use of hydroxychloroquine for >1 year can cause retinopathy or cardiomyopathy, thus persons with baseline conditions will be excluded. Study medicine is excreted via the kidney with dose reduction recommended in CrCl <30 cc/min (Stage 4 Kidney Disease). G-6-PD deficiency is listed as a caution on the FDA label. G-6-PD testing is not routinely performed in clinical care prior to giving hydroxychloroquine prescriptions (unlike with primaquine). Medication exclusions are for possible drug-drug interactions, particularly with cardiac arrhythmia medicines with a caution on the FDA-package insert.

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

Participants will be randomized via permuted block randomization. Randomization will be recorded on an electronic log by the pharmacy. Study investigators and subjects will be blinded.

5.3.2 Masking Procedures

Participants will be provided masked study medicine, shipped by courier (e.g. FedEx). The intervention vs. placebo will not be identical; however, participants and outcome assessors will be masked to their assignment.

5.3.3 Reasons for Withdrawal

Participants may withdraw at any time point at their discretion.

5.3.4 Handling of Withdrawals

Withdraws will be counted as failures for the secondary endpoint of completion of study medication.

5.3.5 Termination of Study

This study may be prematurely terminated if there is sufficient reasonable cause, including but not limited to:

- Unexpected, significant, or unacceptable risk to subjects
- Interim analyses by the DSMB.
- Insufficient compliance with protocol requirements
- Data are not sufficiently complete and/or not evaluable

- Regulatory authorities decide that study should be terminated

If the study is prematurely terminated for harm, current subjects will complete follow up, and no further subjects will be enrolled. If the study is terminated due to benefit, then the study will immediately convert into an open-label prospective cohort to collect further observational data on the safety and efficacy of the intervention, up to the IRB approved recruitment limit.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

6.1.1 Acquisition

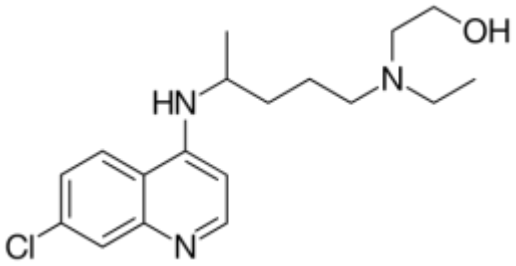
FDA-approved formulation of hydroxychloroquine will be purchased.

6.1.2 Formulation, Packaging, and Labeling

The study medicines will be packaged by the Investigational Drug Services. Dispensed medications will be delivered by courier (e.g. FedEx) to study participants.

6.1.3 Drug Description:

Hydroxychloroquine sulfate

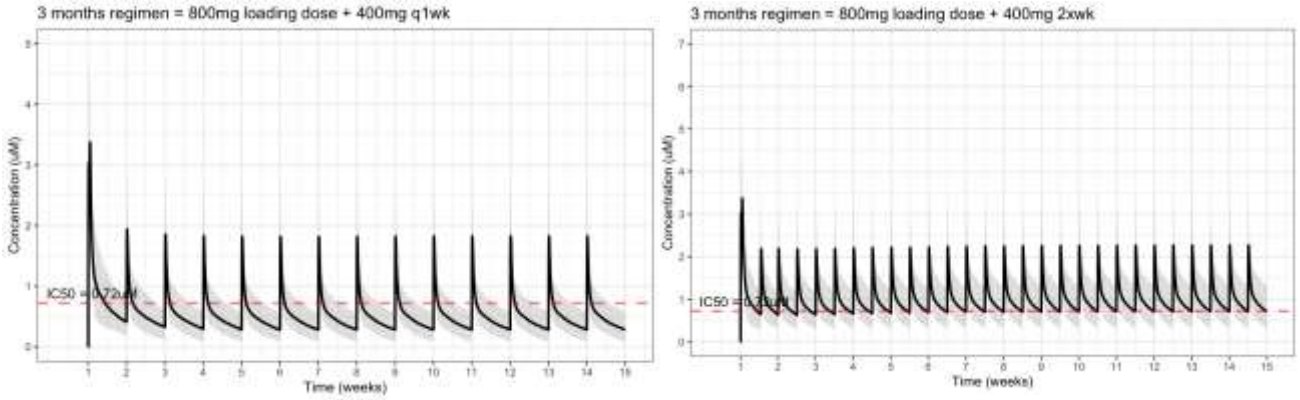


6.1.4 Formulation:

200mg tablet (= 155 mg base of chloroquine)

6.1.5 Pharmacokinetics:

- Absorption: Rapid and almost completely
- Distribution: Widely distributed into body tissues
- Metabolism: Partially hepatic to main metabolyte of desethylchloroquine
- Excretion: Urine (>=50% as unchanged drug); acidification of urine increases elimination
- C_{max} = 1.2 nmol/mL = 1.2 μmol/L = 1.2 μM at 400mg single dose.[5,7]
- T_{max} = 2.4 hours
- $T_{1/2}$ = 172 $\pm$ 39 hours = 7.1 +1.6 days
- AUC_{last} = 75.4 $\pm$ 47 nmol/h/mL
- This C_{max} is in the therapeutic window for SARS-COV2 activity.
- Steady-state trough concentrations for 400mg twice-weekly dosing is expected to be above 0.72 μM, which is above the EC50 of viral inhibition.



6.1.6 Product Storage and Stability

Store at room up to 30° C (86° F). Dispense in a tight, light-resistant container.

6.2 Dosage, Preparation, and Administration of Investigational Product

6.2.1 Drug/Device Handling: Hydroxychloroquine or placebo will be dispensed by the investigational Pharmacy. To do so, study investigators will send a prescription to the Pharmacy, the pharmacy will randomize the subject, and dispense the appropriate study medicine. The medicine will then be provided to research volunteers via courier delivery in the United States.

6.3 Modification of Investigational Product for a Participant

With mild side effects, participants may be instructed to split the 2 tablet weekly dosing into multiple times on the same day (for example, one tablet in the morning, followed by one tablet at night).

In the event of substantial side effects, participants may discontinue the study medication.

In the event of the development of acute kidney injury with creatinine clearance <30 mL/min, the dose should be reduced by half.

6.4 Accountability Procedures for the Investigational Product:

Accountability will be via self-report at weekly virtual visits.

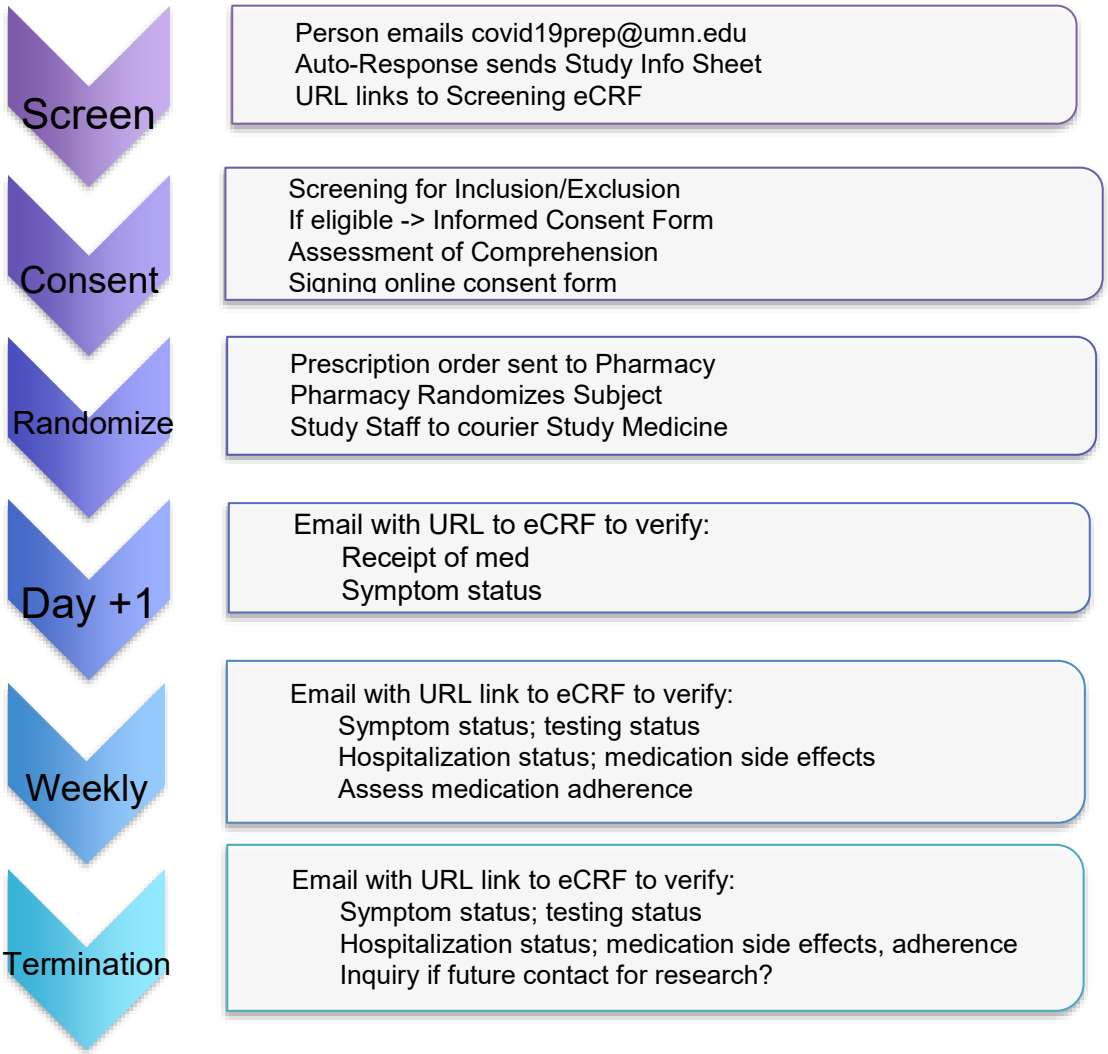
6.5 Assessment of Subject Compliance

Adherence will be via self-report at weekly virtual visits.

6.6 Concomitant Medications/Treatments

Participants may receive other concomitant medications or therapies and will be asked to report these.

Study Schedule



6.7 Screening

- Baseline screening for eligibility
- Informed consent by self-administered
- This will be performed via a web-based form. Eligibility criteria will be by self-report.

6.8 Enrollment/Baseline

6.8.1 Randomization (Day 0)

- Participants will be randomized by a computer-generated algorithm using a permuted block randomization sequence
- Investigational pharmacy will dispense the masked study medicine (12 week supply)
- Study personnel will then courier study medicine to the participant
- Participant will be sent an email to expect the medication to arrive the following day

6.9 Follow-up

- Day 1 Visit
  - Participant will confirm receipt of study medication

- Weekly visits
  - Participant clinical status check-in
  - Assess adherence to study medication and side effects

6.10 Final Study Visit

- Participant clinical status check-in
- Assess adherence to study medicine

6.11 Early Termination Visit

If participants develop symptoms of coronavirus, they will be directed to their healthcare provider and/or local public health authority. We request to follow up hospitalized healthcare workers with suspected COVID-19 up to 90 days beyond the closing date to assess final outcomes.

6.12 Unscheduled Visit

Subjects will be provided a central email contact: faq.covid19prep@gmail.com as a contact point for questions or concerns. This email will forward to an on-call study physician who will call the participant to resolve their concerns.

Additionally, email communications will contain a URL for a sick visit web-form that participants can note their current development of symptoms of medication side effects. This will be the same follow-up eCRF sent on weekly visits. This can be completed multiple times.

7 STUDY PROCEDURES/EVALUATIONS

7.1 Clinical Evaluations

Clinical evaluations will be by self-report.

7.2 Laboratory Evaluations

There are no laboratory evaluations in the protocol. There is an opportunity for participants to participate in an optional lab-based sub-study. Details in Appendix A.

Clinical outcomes are by self-report.

SARS-COV2 positivity is by self-report.

Informed consent will request permission to contact local public health authorities or their medical provider in the event of loss to follow up or COVID-19 disease. Public health authorities or medical providers may verify confirmatory laboratory results.

There is no incentive to be dishonest, and we believe healthcare workers in particular will take their responsibilities seriously.

8 ASSESSMENT OF SAFETY

8.1 Specification of Safety Parameters

Hydroxychloroquine has a track record of safety since its FDA-approval in 1955. As an already, FDA-approved medicine, this trial is designed as a pragmatic trial in the setting of a public health emergency.

8.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

8.2.1 Adverse Events

Hydroxychloroquine has an excellent safety track record, being first FDA-approved in 1955. Adverse events will not be captured unless they result in hospitalization. See Serious Adverse Events below.

Expected adverse events would include normal events within the general population as well as COVID19-related disease events which may include the need for hospitalization, pneumonia, respiratory failure, sepsis, and death.

8.2.2 Reactogenicity (for Vaccine Studies and Some Therapeutic Trials)

Not applicable

8.2.3 Serious Adverse Events

Hospitalization or death are protocol-defined endpoints.

8.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Not applicable

8.3 Reporting Procedures

8.3.1 Serious Adverse Events

An AE or suspected adverse reaction is considered a serious adverse event (SAE) if it results in any of the following outcomes:

- Death,
- a life-threatening adverse event (as below),
- hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening or require hospitalizations may be considered serious when based upon appropriate medical judgment they may jeopardize the participant or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Life-threatening adverse event. An AE is considered “life-threatening” if, in the view of either the site principal investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death. For life-threatening AEs, subjects would be recommended /expected to be hospitalized.

Based on the known safety track record of hydroxychloroquine, this pragmatic protocol will focus on death, life-threatening AEs, and hospitalizations. Incapacity / permanent disability is a possibility with COVID19, but this is not associated with hydroxychloroquine. In the event of incapacity, the subject would be expected to be hospitalized.

Hydroxychloroquine and chloroquine are not known to cause teratogenic events and are viewed as safe in pregnancy, especially with short term use. With this trial’s sample size, this will not further delineate this risk. COVID-19 disease may indeed be teratogenic. For women who are pregnant, we will ask to follow them through the end of their pregnancy.

Thus the hospitalization or death secondary endpoint will capture relevant SAEs.

8.3.2 Regulatory Reporting

As hydroxychloroquine is an FDA-approved medicine being used at standard dosing for malaria prophylaxis, reporting to regulatory authorities will occur in summary format after each DSMB reports and at a frequency of at least annually.

Serious unexpected suspected adverse reactions (SUSARs) which are not expected with COVID19 nor listed in the FDA package insert will be reported to the IRB.

Those SUSARS which are deemed by an independent medical monitor to be related to the study medication will be reported to the FDA and IRB.

8.3.3 Reporting of Pregnancy

Chloroquine and hydroxychloroquine are not known to be teratogenic. Chloroquine and hydroxychloroquine can accumulate in neonatal eyes. Conversely, the risk of severe COVID-19 infection is unknown, but could represent a heightened risk in pregnant women. The CDC states, “We do not have information on adverse pregnancy outcomes in pregnant women with COVID-19. Pregnancy loss, including miscarriage and stillbirth, has been observed in cases of infection with other related coronaviruses (SARS-CoV and MERS-CoV) during pregnancy. High fevers during the first trimester of pregnancy can increase the risk of certain birth defects.”

Thus, the risk/benefit would favor the enrollment of women who may be or are pregnant, so as to not discriminate against pregnant women.

For women who are pregnant, we will ask to have follow through until the end of their pregnancy to assess the outcome of the pregnancy via a brief survey.

8.4 Type and Duration of Follow-up of Subjects after Adverse Events

Participants who are hospitalized for COVID-19 or SAEs will have up to 90 day follow up after the common closing date, to assess their final outcome. Management will be as per the participant’s local healthcare provider.

8.5 Safety Oversight (DSMB)

A data and safety monitoring board (DSMB) will oversee the trial. The quorum will include three members and a biostatistician. The PI will be a non-voting observer, providing input as requested.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial subjects are protected and that the reported trial data are accurate, complete, and verifiable. Clinical monitoring also ensures that the conduct of the trial is in compliance with the currently approved protocol/ amendment(s), ICH, GCP, and with applicable regulatory requirement(s) and Sponsor requirements. Clinical monitoring will also verify

that any critical study procedures are completed following specific instructions. Monitoring will be the responsibility of the University of Minnesota.

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypotheses

We hypothesize that Hydroxychloroquine is superior to placebo for preventing COVID-19 disease. We hypothesize, that if COVID-19 develops while a person is taking hydroxychloroquine prophylaxis, then the COVID-19 disease would be less severe.

10.2 Sample Size Considerations

The planned sample size is up to 3500 participants. There will be two comparisons: treatment once weekly compared to placebo, and treatment twice weekly compared to placebo. With 1165 participants in each of the two treatment groups and in one placebo group, a two-sided log-rank test achieves 80% power at alpha = 0.025 significance level to detect a hazard ratio of 0.65 when COVID-19 free survival is 90% in the placebo group.

The study is powered at alpha = 0.025 to account for the two treatment versus placebo comparisons. The two treatment groups will be compared to the same placebo group. Assumed loss to follow-up is 5% in the treatment groups and 15% in the placebo group. With 3500 participants approximately 310 COVID-19 or death events are anticipated.

Sample Size Re-estimation:

At the time of the second DSMB review, a sample size re-estimation will occur based on the disease transmission rate in the control group. The conservative *a priori* assumption (based on limited data) is 10% transmission risk. The table below shows that if the transmission rate is 8% (lower than assumed) then the sample size would need to be increased to a total of 4353 participants. On the other hand, if the transmission rate is greater than the assumed 10% then the sample size can be decreased.

Sample size requirements to detect a hazard ratio of 0.65 based on COVID19 transmission rates without treatment (assuming 80% power, alpha = 0.025, 15% loss to follow-up in the placebo group and 5% loss to follow-up in the treatment groups)

COVID 19 Transmission Rate Without Treatment	Number Per Group	Total Sample Size (3 Groups)
8%	1451	4353
10%	1163	3489
12%	971	2913
15%	778	2334
18%	650	1950
20%	580	1740

10.3 Planned Interim Analyses

Interim analyses will focus on the statistical testing of the primary endpoint with descriptive reporting of the secondary endpoints as well. Interim analyses will be conducted after enrollment of approximately: 25%, 50%, 75%, and 100% of participants are enrolled. This interval may be modified by the DSMB.

A Lan-DeMets spending function analog of the O'Brien-Fleming boundaries will be used for the outcome of COVID-19-free survival. The provided table assumes four interim analyses with the final analysis with an overall alpha=0.025. The boundaries are truncated at |Z| = 3.01.

Interim Analysis	Sample Size	| Z |	P-value
1	~875 (25%)	> 3.1	.0019
2	~1750 (50%)	> 3.1	.0036
3	~2625 (75%)	> 2.78	.0179
Final	3500 (100%)	> 2.30	.025

Should a stopping boundary be crossed, we would recommend an analysis to determine whether the findings are consistent across secondary endpoints, such that a clear answer is achieved. **In the event of early halting due to efficacy, the study will immediately convert to an open-label observational cohort of hydroxychloroquine prescribed to all consented participants.**

Based on the public health situation, the DSMB has the prerogative to alter the stopping rules.

Futility Analysis:

If the conditional power is <20% at the time of the second interim analysis with approximately 50% of participants enrolled, discontinuation should be considered as a possible recommendation by the DSMB. The sample size re-estimation will be considered here as well as the pace of enrollment.

10.4 Final Analysis Plan

We will assessCOVID-19-free survival in high-risk healthcare workers who receive hydroxychloroquine compared to those who receive placebo.

We will compare survival in a time-to-event analysis for COVID-19-free survival using Log-rank test with survival displayed as a Kaplan-Meier curve. Analysis will be intention-to-treat. Persons who develop confirmed COVID-19 or die will be considered failures. Persons lost to follow up will be censored.

Secondary endpoints for incidence will be assessed via Fisher's Exact test for the proportions by study arm. The secondary endpoint on the ordinal scale will be assessed with a proportional odds model.

Among those with symptoms, severity of symptoms (recorded on a 0-10 visual analog scale) will be compared via Mann-Whitney U by study arm.

A priori subgroup analyses will include assessment by:

- Days from Exposure
- Decile of age
- Sex as a biological variable
- Censored subjects, who became symptomatic before receipt of the first dose of study medicine, will be separately analyzed and reported.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Source documents will include internet forms self-completed by participants directly entered into a RedCAP database.

This protocol is based on self-report.

This internet-based protocol is meant to enable a large number of participants to be recruited quickly as well as maintain the safety of the research staff. In-person visits may cause delays, compliance issues, and public health concerns. The internet-based design allows us to rapidly enroll participants across the country, in the setting of a public health emergency.

Participants will be asked to provide consent to obtain medical records from their healthcare provider or public health official, if there is the need to verify outcomes, for SARS-CoV-2 test results or hospitalizations.

12 QUALITY CONTROL AND QUALITY ASSURANCE

Study medications will be FDA-approved following Good Manufacturing Practice.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Ethical Standard

This study is to be conducted according to US and international standards of Good Clinical Practice (International Conference on Harmonization guidelines), Declaration of Helsinki, and International Ethical Guidelines for Biomedical Research Involving Human Subjects, applicable U.S. government regulations and institutional research policies and procedures. All investigators must have received human subject protection and GCP training prior to human subject involvement.

13.2 Institutional Review Board

Prior to the initiation of the study, the protocol, all informed consent forms and the participant Information materials will be submitted to and approved by the single IRB of record. Likewise, any future amendments to the study protocol will be submitted and approved by the IRB before implementation. This protocol and any amendments will undergo review and approval by the Human Subjects Board at the University of Minnesota under DHHS Assurance FWA00000312.

13.3 Informed Consent Process

- Written informed consent will be obtained via an English-language, internet-based web form. If potential participants have questions, they may contact faq.covid19prep@gmail.com to reach a study staff member to answer their questions about research, either via email or a phone call.
- After completion of reading the form, participants will be assessed for comprehension, querying:
    - Concept of Randomization to hydroxychloroquine (2 different doses) or vitamin placebo
    - Whether hydroxychloroquine is known to be effective in preventing disease
    - Duration of the study? (90 days)
    - Duration of taking the study medicine (12 weeks)
    - When follow up surveys will be sent (Days 1, weekly)
    - If hydroxychloroquine can be shared? (No)

13.3.1 Informed Consent/Assent Process (in Case of a Minor)

- Persons under 18 years of age are not eligible to participate. COVID19 has 0% mortality in children, and rate of progression to symptomatic disease may likely be different. Furthermore, pediatric dosing is weight-based, making remote administration more complicated.

13.4 Exclusion of Women, Minorities, and Children

- Persons under 18 years of age are not eligible to participate. COVID19 has 0% mortality in children and young adults.
- Non-English speaking adults are not eligible as the webpage and consents will only be available in English.

13.5 Subject Confidentiality

- Interaction will be via internet-based RedCAP ECRFs conforming to required U.S. privacy and server security standards.
- Clinical data will be entered into a study-specific database by designated staff on a regular basis from completed electronic Case Record Forms (eCRF). Access to the database will be given to authorized personnel only (members of the immediate study team). eCRF and trial documents will be kept in a secure database.
- Documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without the prior written approval of the participant except as necessary for monitoring by the IRB or public health authorities
- No participant identifying information will be disclosed in any publication or at any conference activities arising from the study.

13.6 Future Use of Stored Specimens

- No specimens are to be collected.

14 DATA HANDLING AND RECORD-KEEPING

14.1 Data Management Responsibilities

Investigators will maintain a REDCAP database of study records.

Survey forms will be self-completed by study participants.

14.2 Data Capture Methods

- Data will be obtained via internet-based REDCAP forms.

14.3 Types of Data

- Participants will be asked to provide data regarding COVID19 exposure timing and location. They will also be asked to provide ongoing symptom reports during the follow-up period.

14.4 Timing/Reports

- An enrollment progress report will be generated monthly
    - Participants Enrolled
    - Participants on study
    - Participants completed the study
    - Lost to Follow Up
    - Cumulative COVID19 (pooled, both arms)
    - Cumulative Hospitalizations (pooled, both arms)
- A data safety monitoring board (DSMB) will review data after every 450 participants complete 90 days of follow-up.
- De-identified data will be shared with the research team members for analysis.

14.5 Study Records Retention

- No paper documents will be retained or stored.
- Digital records will be kept in a secure server setting.

14.6 Protocol Deviations

Protocol violations will be reported to the IRB of record.

15 PUBLICATION POLICY

Publication will be expeditiously made with a full, de-identified data made available.

16 LITERATURE REFERENCES

1. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. *Annals of internal medicine* 2020.
2. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *The New England journal of medicine* 2020.
3. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell research* 2020; **30**(3): 269-71.
4. Vincent MJ, Bergeron E, Benjannet S, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virology journal* 2005; **2**: 69.
5. Cong Y, Hart BJ, Gross R, et al. MERS-CoV pathogenesis and antiviral efficacy of licensed drugs in human monocyte-derived antigen-presenting cells. *PloS one* 2018; **13**(3): e0194868.
6. 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). *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2020.
7. Liu J, Cao R, Xu M, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell discovery* 2020; **6**: 16.
8. 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. *International journal of antimicrobial agents* 2020: 105949.
9. Burke RM, Midgley CM, Dratch A, et al. Active Monitoring of Persons Exposed to Patients with Confirmed COVID-19 - United States, January-February 2020. *MMWR Morbidity and mortality weekly report* 2020; **69**(9): 245-6.

Appendix A: Sub study

Sub study Title	Pre-exposure Prophylaxis for SARS-Coronavirus-2: A Pragmatic Randomized Clinical Trial: A sub-study of peripheral blood correlatives
Objectives	This is a sub study for correlative sample collection of blood to evaluate: 1) Circulating antibody (IgG or IgM) presence specific for COVID-19 virus protein at baseline a. This is determined by ELISA from serum or plasma using baseline samples 2) Seroconversion from IgG/IgM negative-to-positive during the treatment period a. This is determined by ELISA from serum or plasma using baseline samples and end-of-study samples 3) Evaluation of HLA-types and germline factors associated with outcomes. a. This is determined by Illumina MEGA^{EX} 6 million SNP array or targeted next generation sequencing of HLA alleles, or other germline DNA testing.
Blood Collection Schedule	Baseline (within 3 days of starting hydroxychloroquine) Study termination or at positive test for SARS-CoV2
Number of Participants	300
Duration of Study	Up to ~ 100 days

Background:

Due to the nature of COVID-19 infection, which is often asymptomatic in many individuals, it is conceivable that some population of enrollees in the parent trial “Pre-exposure Prophylaxis for SARS-Coronavirus-2: A Pragmatic Randomized Clinical Trial”, may have already been exposed and recovered. Due to the pragmatic and emergent

nature of the trial, participants for primary prophylaxis, the absolute nature of prior exposure cannot be determined in real time, or across all participants in the trial.

The purpose of this sub study is to allow for optional enrollment for collection of peripheral blood in health care workers at academic institutions with capabilities to collect and process peripheral blood. The peripheral blood will be used to accomplish several objectives. First, it will allow for post-hoc analyses that can determine which participants enrolled in the sub arm have had prior exposure through assessment of IgG and IgM antibodies specific to COVID-19 viral proteins. Emerging literature suggests that exposed and recovered patients or individuals develop persistent antibodies that can be detected in serum or plasma. Thus, participants with prior exposure (i.e. had presence of antibodies at baseline) can be excluded in secondary efficacy analyses. Secondly, end-of-study collection will allow for definitive assessment of the percentage of healthcare workers who seroconvert (i.e. negative at baseline, and positive at end-of-study) during the trial period in both dosing arms and the comparable placebo arms. Finally, preservation of buffy coat (white blood cells) will serve as a source of DNA and RNA that can be used for subsequent host-derived factors that contribute to drug efficacy or exposure/seroconversion rates.

OBJECTIVES

In participants from the PREP COVID-19 parent protocol, who choose to participate in this sub study, we will evaluate:

Objective 1: Circulating antibody (IgG or IgM) presence specific for COVID-19 virus protein at baseline

Endpoint 1: ELISA from serum or plasma using baseline samples

Objective 2: Seroconversion from IgG/IgM negative-to-positive during the treatment period

Endpoint 2: ELISA from serum or plasma using baseline samples and end-of-study samples

Objective 3: Evaluation of HLA-types and germline factors associated with outcomes

Endpoint 3: Illumina MEGA^{EX} 6 million SNP array or targeted next generation sequencing of HLA alleles, or other germline DNA testing

STUDY CALENDAR

Parameter	Pre-Treatment	End of Treatment
Demographics	**X**	
Blood collection		**X**

Note: The sample collection schedules outlined above are based on an ideal subject. The sample schedule should be followed as closely as is realistically possible; however, the schedule may be modified (± 4 business days) due to problems such as scheduling delays or conflicts (e.g., clinic closure, poor weather conditions, vacations, etc.).

1. Within 4 days prior to starting study drug, unless otherwise noted.

2. At the end of 90 days of study drug as per parent protocol or at positive test for SARS-CoV2

3. One 10 mL EDTA tube (lavender top)

STORAGE AND SUPPLY

EDTA tubes (lavender top): Institutional own supplies will be utilized, kits will not be shipped to participating sites

Collected/ Processed/ Aliquoted Sample Storage

For ALL peripheral blood specimens collected, processed, and aliquoted:

Samples should be stored at -80C and shipped or transferred below on dry ice. Samples should be removed from -80C storage and placed directly in a dry ice-containing shipper with suitable volume of dry ice for overnight transport, taking special care in summer/warm months.

If shipment is not required (e.g. collected and processed at VUMC), samples can be batch couriered or transferred weekly to the Balko lab.

Blood Samples

Tubes should be packed according to the Lab manual and sent immediately (within 24 h).

Peripheral blood will be collected in participants prior to initiating hydroxychloroquine under the parent protocol guidelines, and at the end of treatment (90 days or at positive test for SARS-CoV2. The molecular correlates for each biological sample/time point are listed below.

Tube	Amount	Baseline (within 3 days of enrollment)	End of study (end of 90 days or at positive test for SARS-CoV2)
EDTA tube (lavender top)	10mL (1x10mL)	X	X

Sample Source	Test	Needs immediate specialized processing	Needs immediate shipping
Buffycoat	RNA and DNA extraction	YES	NO
Plasma	ELISA for COVID-19 IgG and IgM		

STATISTICAL CONSIDERATIONS

Correlative analyses will be compared to clinical endpoints on the parent protocol, particularly the primary endpoint of seroconversion during the treatment period. These exploratory analyses will be performed using the baseline and end of treatment blood collections, and are primarily descriptive in nature.

Correlative analyses endpoints will be categorized as dichotomous variables. In exploratory analyses, patients will be stratified by clinical response (i.e. lack of seroconversion) to determine if the change in endpoint is associated with patient-specific clinical benefit from treatment.