STUDY PROTOCOL

PROTOCOL TITLE:
The CHloroquine for Influenza Prevention (CHIP) Trial.
A randomised, double-blind, placebo controlled trial of chloroquine for the prevention of influenza:
evaluation of its clinical pharmacology and relationship between drug levels and protective efficacy

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CHIP

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SITE CLINICAL PRINCIPAL INVESTIGATOR:
Dr Lawrence Lee, Assistant Professor, Dept of Medicine, NUHS

OTHER INVESTIGATORS:
A/Prof Annelies Wilder-Smith, Consultant, Department of Medicine, NUHS (Site Clinical Co-Principal Investigator)
Prof Nicholas Paton, Visiting Professor, Dept of Medicine, NUHS (Trial Chief Investigator)
Dr Sophia Archuleta, Consultant, Dept of Medicine, NUHS (Site Clinical Investigator)

STUDY SITE:
Investigational Medicine Unit, NUHS
5 Lower Kent Ridge Road, Level 6, Kent Ridge Wing 2
Singapore 119074

COLLABORATORS:
Prof Yin Bun Cheung, Head of Biostatistics, SCRI
A/Prof Eng Eong Ooi, Emerging Infections Programme, Duke-NUS
Dr Gerard Wong, Deputy Director, Investigational Medicine Unit, NUHS
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Study Protocol Number <CHIP>, Version <2.6>, Dated < 1 May 2010>
1.1. Rationale and justification for the Study

a. Rationale for the Study

Current measures for prevention of influenza infection

There is a very limited range of measures available to prevent influenza infection. Vaccination is the mainstay of prevention efforts, and seasonal influenza vaccine has a protective efficacy of up to 70% against clinical infection with seasonal influenza strains. Oseltamivir has been shown to have a protective efficacy of 89% against laboratory-confirmed clinical influenza when used for 7 days as post-exposure prophylaxis following contact with an infected household contact. (Welliver, Monto et al. 2001) When used as pre-exposure prophylaxis for 6 weeks during the winter season in several US cities, oseltamivir had a protective effect of 74% against laboratory-confirmed clinical influenza. (Hayden, Atmar et al. 1999) The limitations of current prevention methods are illustrated by the ongoing transmission of influenza each year (estimated more than 600,000 cases per year in Singapore). (Ng, Pwee et al. 2002) The need for new measures is particularly acute at the present time, due to the emergence of the H1N1 pandemic.

A vaccine against H1N1 will not be available for many months, and then only in sufficient quantity to provide coverage to a small proportion of the world’s population. It will therefore have to be prioritised to high risk groups and will not be widely available to the general population. As the number of H1N1 cases increases, the limited stockpiles of oseltamivir will need to be restricted to the treatment of infected individuals, and then further prioritised to those who are at the highest risk of hospitalisation. (Gani, Hughes et al. 2005) As a consequence, the availability of oseltamivir for post-exposure prophylaxis will become extremely limited, and there is no prospect of the widespread use of oseltamivir for pre-exposure prophylaxis in any population. Even if sufficient stockpiles were available, widespread use of oseltamivir in the community would likely accelerate the emergence of oseltamivir resistance, which has already been noted in the pandemic H1N1 strain. Therefore there is an urgent need to identify additional pharmacological prevention measures that might be rolled out to the general population, preferably for use as pre-exposure prophylaxis in order to decrease the risk of infection, decrease the severity of infection, or decrease the infectiousness of the infected individual to others.

The ideal candidate drug would be simple to take (single daily dose, or less), be well tolerated with a well-established safety profile that means it can be administered safely without the need for laboratory monitoring, be currently available in sufficient quantities worldwide for an immediate roll-out programme once efficacy is demonstrated, be sufficiently economical for use in resource poor countries (which often have the largest susceptible populations and are least likely to obtain vaccine rapidly), be free from interactions, and have a putative mechanism of action that is unlikely to be compromised by the development of antiviral resistance.

Chloroquine for influenza

Chloroquine (CQ), and its analogue hydroxychloroquine (HCQ), have been in widespread clinical use for more than 50 years in the treatment and prevention of malaria and the treatment of rheumatological diseases. In vitro studies have shown them to have activity against a number of viruses including HIV and SARS associated coronavirus. (Sperber, Kalb et al. 1993; Chiang, Sassaroli et al. 1996; Savarino, Gennero et al. 2001; Vincent, Bergeron et al. 2005) Clinical trials have been conducted in HIV infection that confirmed that this is matched by in vivo activity.(Sperber, Louie et al. 1995; Sperber, Chiang et al. 1997) CQ is concentrated in the acidic organelles such as the endosome, lysosome and Golgi vesicles, where it increases the pH. This change in pH disrupts a number of enzymes and the post-translational modification of newly synthesised proteins and it is thought that this underlies the broad-spectrum antiviral activity of CQ. (Savarino, Boelaert et al. 2003)

CQ also has in vitro activity against influenza, although the pH dependency of the virus appears to vary between strains. (Ooi, Chew et al. 2006; Di Trani, Savarino et al. 2007) However, efficacy of
CQ has been demonstrated against both H1N1 and H3N2 strains in independent laboratories at concentrations that are achievable in vivo at the doses of CQ used for malaria prophylaxis and treatment of connective tissue diseases. (Ooi, Chew et al. 2006; Di Trani, Savarino et al. 2007) There has been one limited animal model study in which CQ did not diminish the weight loss experienced by mice infected with a mouse-adapted H1N1 strain (mortality, viral replication, and other usual outcome parameters were not reported), but did produce a significant reduction in nasal virus titre at day 4 in an adapted H3N2 strain infection in ferrets. (Vigerust and McCullers 2007) Given the numerous differences in viral and host factors between humans and the animal models, (Mizgerd and Skerrett 2008) the absence of clear evidence of benefit of CQ against clinical illness in these animal models does not negate the possibility of activity of CQ against influenza infection in humans, and is of particularly questionable relevance for predicting efficacy of CQ in prevention of influenza (rather than treatment of clinical illness).

Aside from its putative antiviral effects, there are additional pathways by which CQ might be of benefit in influenza. Patients with severe influenza have increased levels of pro-inflammatory cytokines, (de Jong, Simmons et al. 2006) and it has been suggested that anti-inflammatory and immunomodulatory agents may therefore be of benefit. (Fedson 2008) CQ and HCQ have been used for decades as immunomodulatory agents in the treatment of T-cell mediated immune diseases such as systemic lupus erythematosus. Although the precise mechanisms responsible for HCQ clinically-relevant actions are not fully understood, it is thought that the cellular pH change interferes with one or more steps in the T-cell activation pathway such as major histocompatibility complex (MHC) class II antigen presentation. (Ziegler and Unanue 1982), and T-cell receptor mediated intracellular calcium signalling. (Goldman, Gilman et al. 2000) HCQ also inhibits T-cell proliferative responses to T-cell mitogens and production of pro-inflammatory cytokines IL1 and IL-6. (Sperber, Quraishi et al. 1993) Thus, even if CQ is not effective in preventing infection with influenza, its immune actions may lead to a reduction in the severity of symptoms, the incidence of severe complications, or the requirement for oseltamivir. This is a further potential advantage to using a pre-exposure prophylaxis approach, as the immune modulating effects of CQ will be present early in the course of infection and are therefore likely to have maximum benefit in modulating the course of disease (as opposed to starting an immunomodulatory agent after symptomatic disease is identified, when the course of disease may be set and it may be too late for an anti-inflammatory agent to abrogate the process).

There are many attributes of CQ that make it an ideal candidate drug for the prevention of influenza. It can be taken once weekly, is well-tolerated, has been in widespread clinical use for 50 years and is known to be very safe (including administration in children and pregnant women) and can therefore be administered without clinical or laboratory monitoring, and it has few significant interactions with other drugs. CQ works on cellular pathways, and hence influenza viral resistance is unlikely to develop. It is widely available, cheap and could be rolled out very rapidly and on a scale that might attenuate the global H1N1 pandemic. The importance of evaluating generic drugs such as CQ that have potential efficacy in a pandemic situation, and the importance of commencing the relevant clinical trials rapidly after the start of a pandemic, has been highlighted in several recent expert reviews. (Fedson 2008; Fedson 2009)

The well-established safety record of CQ together with the urgency of the clinical need, mean that the traditional regulatory strategy of drug development is inappropriate in this situation. What is needed immediately is a randomised controlled trial that will rapidly confirm or refute the hypothesis that CQ can be used to prevent influenza. We propose to conduct such a trial that will provide a definitive answer to this vitally important question.

If the intervention is found to be effective in preventing influenza with a protective efficacy of at least 50% in this trial, it would likely have an immediate influence on clinical practice in Singapore and worldwide. Given the absence of alternatives for pre-exposure prophylaxis measures in the general population, if an effective or partially effective intervention were introduced widely (as would be
possible with CQ) this could have a major impact on reducing the spread of the pandemic and reducing the number of clinical influenza cases.

In addition to evaluating efficacy against the H1N1 pandemic strain, this trial will also evaluate the efficacy of CQ against seasonal influenza strains. Thus the results are likely to be generalisable beyond the current pandemic, and if CQ is found to be effective, this would be of major clinical importance for the long term. Given the considerable annual morbidity and mortality from seasonal influenza, this would potentially have a huge impact on health. Briefly sketch the background to the current proposal, critically evaluate existing knowledge and specifically identify the gaps that the project is intended to fill.

b. **Rationale for Doses Selected**

   The dose of CQ corresponds to the usual dose used in malaria prophylaxis (taken once weekly) in order to maximise convenience and tolerability. This is preceded by the same dose taken daily for the first week in order to achieve levels corresponding to those shown to have efficacy against influenza in vitro (see section 8.1 for further details).

c. **Rationale for Study Population**

   The study population will be drawn from the general community population. This is appropriate as the intervention we are investigating is intended as something that would ultimately be deployed widely in the community, if it is shown to be effective.
   The internet-based screening process for the study is likely to select a relatively young, internet-literate study population which is appropriate given the need for online data collection for the study, and is also appropriate given that the H1N1 virus appears to have a tendency to infect the young disproportionately.

d. **Rationale for Study Design**

   This research will test the efficacy of a novel pre-exposure preventive intervention for influenza in a large, high-quality, definitive randomised double blind placebo controlled trial. A randomised controlled trial study design is the only approach that will generate sufficiently robust evidence to be able to lead directly to a change of clinical practice.
   A placebo control is needed because participants may modify their behaviour if they believe they are taking a potentially preventive medication and this in turn may influence their risk of influenza acquisition. Furthermore, the endpoint is dependent on symptom report which is subjective and may also be influenced by the perception that the participant is on some sort of preventive medication.

2. **HYPOTHESIS AND OBJECTIVES**

2.1. **Hypothesis**

   The main hypothesis is that CQ will decrease the risk of influenza acquisition
2.2. **Primary Objectives**

To determine the efficacy of CQ in the prevention of clinical influenza

2.3. **Secondary Objectives**

(i) To determine the efficacy of CQ in reducing the severity of clinical symptoms of influenza

(ii) To assess the clinical pharmacology of CQ and the relationship between drug levels and protective efficacy

(iii) To establish a large database containing high quality prospective clinical symptom data collected in real-time, influenza virus identification and typing, and epidemiological data collected from a community sample during the midst of an influenza pandemic, together with matching stored plasma and PBMC samples, that can be used for additional translational research studies (either related or unrelated to the effects of CQ).

(iv) To establish the infrastructure for continuing a long-term cohort study of influenza in Singapore

(v) To determine the efficacy of CQ in reducing viral infections other than influenza.

2.4. **Potential Risks and benefits:**

a. **End Points - Efficacy**

If CQ is effective, the participants in this study may benefit from a reduced risk of influenza infection, or reduced severity of symptoms if they contract influenza.

b. **End Points - Safety**

CQ is a very well-established drug with a good safety profile. Participants may experience mild symptoms such as gastrointestinal discomfort, skin itching or blurred vision, but these are often transient and can be managed by symptomatic measures or by dose reduction. Participants will be given instructions on how to manage these symptoms if they occur. There is miniscule risk of any of the serious complications of long term CQ use (such as retinopathy) given the duration of treatment and the dose of CQ used in this trial. This dose has been widely prescribed in the past for malaria prophylaxis without any medical monitoring.

3. **STUDY POPULATION**

3.1. **List the number of subjects to be enrolled.**

The trial will enrol 1500 participants from the general population in Singapore. The trial will be widely advertised and we anticipate attracting a group of participants who are mainly young and internet-literate. The minimum age for this study is 18 years, and there are no exclusions based on race (although we require participants to be English-speaking in order to complete the study symptom diary).

3.2. **Procedure for Recruitment**

Rapid recruitment of participants is essential for the success of this trial and to facilitate this we will use a web-based screening tool. The address of the study website will be widely advertised.
through multiple avenues in the local media (television, print, radio, online). This system will allow us to disseminate as widely as possible the information about the trial, and will allow suitable and interested individuals to identify themselves and make an appointment for a screening visit at the IMU. Once the individual has accessed the website, there will be 5 steps required in sequence, and individuals will be able to exit and return at any stage:

1. Read participant information sheet (they will be able to print this out if desired)
2. Register their contact details (email, phone numbers, address) and receive a CHIP User ID and Password. Participants over the age of 18 but under 21 years will also be required to provide contact details for their parent or legal guardian.
3. Enter demographic information and indicate responses to a checklist of basic study entry criteria to test eligibility. Answers that appear to violate the inclusion criteria will be flagged and they will either be informed that they are not eligible (and provided with a reason), or will be given a number of the trial hotline to call for further clarification.
4. Answer a series of multiple choice questions to test their understanding of the key components of the trial: e.g. the fact that half of participants will receive a placebo; the concept of randomisation; the requirements for study visits and blood tests; the requirements for recording symptoms at home; the steps taken to protect confidentiality. At the end of these questions, they will be provided with the correct answers and a paragraph of explanation (based on the information sheet) for any questions where they give an incorrect answer. For these items they will be required to confirm that they now understand these points, or be encouraged to call the trial hotline if further clarification is needed.
5. Book an appointment for screening. They will be able to view and select from available screening slots. Once a screening appointment has been selected the participant will be provided with directions to the IMU and will be given the contact telephone number for the trial hotline. The online system will also allow them to log in again (using their User ID and password) in order to change or cancel a screening appointment. Participants over the age of 18 but under 21 years will be instructed to bring their parent or guardian with them to the screening visit.

This procedure will (a) facilitate the consent process by providing information to participants to read at leisure (b) facilitate the booking arrangements and the rapid screening process for a large number of individuals over a short time period and (c) provide an opportunity for participant online entry of contact information. Initially entry to the study will be exclusively by online registration. This will be reassessed after the first 2 weeks of recruitment, and if this appears to be a barrier to recruiting sufficient numbers to the trial, then the manpower of the study hotline will be increased in order to allow a parallel system of telephone assessment and booking (the same steps as the online system will be followed, and copies of the information sheet will be sent to participants by post).

At the screening visit at the IMU, the eligibility criteria will be checked, and the participant will be asked to provide a written signature on a copy of the consent form.

### 3.3. Inclusion Criteria

The participant must meet the following inclusion criteria to participate in this study:

a. Age 18 -65
b. Have the ability to provide informed consent
c. If a woman of child-bearing potential, she should be willing to use contraception for the
period of the trial.

3.4. Exclusion Criteria

Potential participants meeting any of the following exclusion criteria will be excluded from participation:

a. Acute influenza-like illness at screening (screening can be postponed until 1 week after recovery, if still within the recruitment period of the trial)
b. History of psoriasis, porphyria cutanea tarda, epilepsy, myasthenia gravis, myopathy of any cause, cardiac arrhythmias, or serious hepatic or renal disease.
c. A woman who is pregnant or breast feeding
d. Current use of medication with known serious hepatotoxic effects
e. Current use of medication with known serious interaction with CQ: amiodarone, anticonvulsants, ciclosporin, digoxin, mefloquine, moxifloxacin
f. Current severe depression (as indicated by current use of antidepressant medication)
g. Known serious retinal disease
h. Current or recent (within the past 30 days) participation in any other clinical intervention trial.
i. Known G6PD deficiency
j. Vaccination for influenza (seasonal or H1N1 strain) within the 3 months prior to screening

3.5. Withdrawal Criteria

Participants may discontinue study medication if they wish to do so, but will be asked to give a reason if possible. Participants will be encouraged to continue the trial medication to the end of the study even if they develop an episode of influenza-like illness during the study as it will not be possible to tell with certainty, until the laboratory tests are analysed, whether that episode was influenza or a simple URTI. The Principal Investigator or other investigators may advise the participant to stop taking trial medication if they believe that there is evidence, based on symptom report, that the medication is causing serious toxicity. If a participant stops taking trial medication they will still be encouraged to continue with other study procedures (symptom diary, nasal swabs as required) and return for the 12 week follow up visit.

3.6. Subject Replacement

Participants who drop out will not be replaced.

4. TRIAL SCHEDULE

Participants will be required to attend for only two visits: a screening visit and a week 12 follow-up visit. Details are given below and in the trial schedule table.

5. STUDY DESIGN
5.1. **Summary of Study Design**

This is a randomised, double-blind, placebo-controlled, parallel group trial. It is a phase II trial using a medication (CQ) that is licensed for other medical indications but that has not been previously tested for its efficacy in prevention of influenza. The recruitment phase is expected to last 4 weeks. Each participant will take study medication for 12 weeks and will be assessed at the end of that time.

6. **METHODS AND ASSESSMENTS**

6.1. **Randomisation and Blinding**

Participants will be randomly assigned to CQ or placebo in a 1:1 ratio. A randomisation list will be prepared by the trial statistician and the code will only be supplied to the computer programmer and the personnel responsible for packaging study drug. Randomisation will be performed on the computer by the study coordinator in the IMU once all the screening procedures have been completed and while the participant remains in the clinic. The participant will be allocated a unique prescription number and this will be matched with the corresponding bottle of study medication.

Un-blinding will generally be discouraged during the study, and the appropriate management for all patients will be to assume that they are receiving active study medication (i.e. CQ). If a health-care worker attending to the patient during the follow up period considers it essential for clinical management to know whether or not a participant is taking active product, this will first be discussed with the principal investigator or co-PI. If it is agreed that un-blinding is required then the principal investigator or deputy will contact the trial statistician or other individual with access to the randomisation list, and that individual will then contact the external healthcare worker directly to inform them of the treatment allocation of the participant.

The randomization allocation will be revealed to participants in a “thank you” letter/email which will be sent to them with a summary of the results, when all relevant analyses of the study have been completed.

6.2. **Contraception and Pregnancy Testing**

A urine pregnancy test will be performed at the screening visit for females of childbearing age. Although CQ is regarded as safe in pregnancy, women will be required to use adequate methods of contraception (preferably barrier methods) during the trial.

6.3. **Study Visits and Procedures**

   a. **Screening Visits and Procedures**

The following procedures will be performed at the screening/ baseline visit:
(i) Review of medical, drug and dietary supplement history, vaccination history, current symptoms.
(ii) Collection of demographic data and household data.
(iii) Physical examination: body weight, height, temperature and blood pressure will be measured. Further directed physical examination will be performed only if indicted by the participant’s symptom report (e.g. chest auscultation)
(iv) A urine pregnancy test will be performed for women of childbearing potential
(v) 15ml of blood will be collected for storage (for batched virology testing, see below)
(vi) An optional additional 20ml blood sample will be collected for peripheral blood mononuclear cell (PBMC) storage in the first 300 participants who give consent (consent will be obtained separately using a tick-box on the informed consent document).

b. Study Visits and Procedures

There are no scheduled study visits other than the screening / baseline visit and a week 12 (window of -1 to + 7 days) follow up visit. The majority of follow-up data collection for this trial will be done by participant self-assessment and recording, supplemented by data obtained (by participant consent) from routine healthcare consultations during this time period.

Medical follow up during the study period
Given the nature of the influenza pandemic, it is essential that medical follow up of trial participants for all respiratory illnesses, as well as all other medical management, be provided through the usual community channels. Participants will be able to call the study hotline to ask for advice on any side effects of study medication, but any medical consultations during the study period will need to be arranged through the normal community channels (i.e. GPs).

Participants will be given a card with the trial hotline number and the site website address along with a brief summary of the trial and will be asked to present this to any healthcare practitioner that they see during the trial. The healthcare practitioner will be able to contact the study hotline and speak to the trial coordinator or doctor, or access the trial website for the general information about the trial and to view the participant information sheet. If the patient is hospitalised or develops any serious medical condition, the healthcare provider will be instructed to report this to the study team via the study hotline without delay.

Clinical symptom diary
The approach to symptom collection is based on that used successfully in the MRC FluWatch study, a large influenza cohort study currently conducted in the UK. It is anticipated that this will be particularly successful in Singapore, given the high rate of internet connectivity and mobile phone usage.

Participants will be asked to keep a diary record of any symptoms they have during the 12-week follow-up period. At the screening visit, participants will be instructed on the use of the online symptom diary, and will also be given a paper backup diary and a thermometer to take home. Participants with access to the internet (anticipated to be the majority) will be asked to complete the diary online, in preference to the paper diary. The symptom diary consist of a checklist of the following symptoms: sneezing, sore throat, runny nose, blocked nose, dry cough, coughed up phlegm, wheeze, headache, diarrhoea, muscle aches, fatigue, feeling of fever. Participants will be asked to grade each of these symptoms as none, mild, or moderate/severe. Participants will also be asked to report any other symptoms they have experienced and be asked to grade these in the same way. On the diary day they will be asked to take their temperature using the thermometer provided and record this information in the diary. A combination of a regular weekly diary record, and a daily diary record completed during periods of
influenza like-illness will be used.

Weekly record:
At a minimum, participants will be expected to make a diary record once weekly, covering any symptoms they have had in the previous week. Participants will receive a once-weekly email / SMS text message on the day the diary is due to be completed. Participants who fail to complete the diary online will receive a call from the study coordinator the following working day as a reminder. This weekly record will start with a question about whether they have had any symptoms in the previous week. If they answer yes, they will be asked to indicate the start date and stop date of each of these symptoms (following the usual checklist format) and grade each of the symptoms. They will also be asked whether they have had any days off school/college/work due to illness, and whether they have had contact with any healthcare professionals due to illness. They will also be asked whether they have taken their study medication that week and asked whether they have taken any other medication: common ‘flu medications will be listed as options.

Daily record:
Participants will be asked, in addition to completing the weekly diary record, to start a daily diary record if they develop any ‘flu-like symptoms, and to continue this daily record until the symptoms resolve.

Record of healthcare consultations
Participants will be instructed that if they become unwell during the trial, they should seek medical care from their normal healthcare provider. Participants will ask their healthcare provider to complete a simple, one-page form to record the name, address and phone number of the healthcare provider/clinic, the date of the consultation, the initial diagnosis that was made, the treatment prescribed, and whether any nose or throat swabs or other samples were taken for diagnosis of influenza. The healthcare provider will be asked to provide the participant with a hard copy of any results obtained on influenza diagnostic tests. This information will be passed to the IMU coordinator at the week 12 trial follow-up visit. If the participant is seriously unwell or requires hospitalisation, the healthcare provider will be asked to telephone the study hotline to provide further detailed information. This form will also give the healthcare provider some background information about the trial, and about interactions with the study medication.

Additional blood sample (optional) for participants getting influenza vaccination during the study period
If the participant decides to undergo vaccination for influenza during the study, they will be encouraged to come back to the IMU for an additional blood draw (15 mL, to be used for batched virology testing as at other study visits) before they have the vaccination.

Nasal swabs
Participants will be given a nasal swab pack to take home and will be trained at the baseline visit on how to use this. They will be provided with a simple step by step instruction sheet, and with the contact number for the trial hotline if they have any questions. The pack will contain two nasal swabs and PrimeStore (Longhorn), a RNA stabilizing agent that has previously been shown to be effective in preventing the viral RNA from deterioration, and will be stored in the participant’s home at room temperature until needed. If the participant develops any fever (feeling feverish or documented fever), runny nose or cough for more than 24 hours during the follow up period they will be asked to take a swab from each nostril and place this in the transport media and store the swab in the refrigerator. This method has been used successfully in the UK FluWatch study. The PrimeStore will lyse the viruses thereby inactivate the virus while preserving its genomic material, and this will permit PCR to be
performed later. When the participant has taken a nasal swab they will be asked to call the hotline and a replacement pack will be sent to them by post approximately 1 week later to use in the event of any subsequent symptomatic episode.

c. **Final Study Visit:**

There will be a single scheduled follow-up visit for this study at 12 weeks following the baseline visit. The appointment for the single follow-up visit will be arranged at the time of the baseline visit, and participants will receive an automated email and text reminder of this visit at one week prior to the scheduled visit, and again one day prior to the scheduled visit. They will be able to reschedule the visit using the online booking system or by calling the study hotline.

They will bring back any unused medication, and any paper diaries or swabs that were used during the 12 week follow-up period. Participants will also be asked to bring any information from healthcare consultations obtained during the study period, and especially the results of any investigations performed for influenza.

The symptom diary (electronic or paper) will be reviewed at the week 12 follow up visit and patients will be asked further questions about any symptoms reported as moderate or severe during the study period. These will be reviewed and classified at that time by the study team using a standard set of criteria for grading adverse events (the 2004 Division of AIDS toxicity grading scale). Details of any influenza vaccinations, influenza medications taken and influenza risk factors/exposure during the follow up period will be obtained.

If these are not provided, the results of any laboratory samples taken for the diagnosis of influenza-like illness will be sought by the research team from the appropriate laboratory (with the participant’s consent).

A follow up blood (20ml) sample will be obtained for serological testing and for CQ drug levels. An optional additional 20ml blood sample will be collected for peripheral blood mononuclear cell (PBMC) storage in the participants who had this performed at baseline, and in an additional 50 participants who report having had influenza-like symptoms during the follow-up period (to increase the sample size for an additional cross-sectional analysis of the effects of CQ on immune response to influenza). A saliva sample (2.5 ml) will also be obtained for CQ drug levels.

**Laboratory investigations**

**Serology**

The paired serum samples obtained at baseline and follow up will be tested for a rise in influenza titre. We will use haemagglutination inhibition assay (HI) to diagnose infection with the pandemic influenza strain. We will use a number of different strains of human strains of influenza virus (H1N1 and H3N2) to determine specificity of our approach and by using geometric mean titre comparison. (WHO 2002) We will determine if the resulting seroconversion, if any, were due to infection with the pandemic strain of H1N1 or other seasonal influenza viruses. For confirmation of the HI assay data, we will also perform microneutralization assay on randomly selected HI positive and negative samples. (WHO 2002). The blood samples may also be tested later for other viruses which may account for acute febrile episodes (eg RSV and adenovirus).

**PCR on nasal swabs**
All nasal swabs returned at this visit will be tested as follows. RT-PCR for influenza A virus will be carried out using a previously published protocol which targets the M gene of the virus. (Fouchier, Bestebroer et al. 2000) This protocol is currently in use in Eng Eong Ooi's laboratory to investigate the aetiology of patients enrolled into the early dengue infection and outcome (EDEN) study but are negative for the dengue virus. (Low, Ooi et al. 2006; Tanner, Schreiber et al. 2008) It has the advantage of being able to detect a wide range of influenza A viruses as it targets a highly conserved region of the M gene, which is shared by all influenza A viruses. Confirmation that the infection among the RT-PCR positive samples are due to the pandemic strain of H1N1 will be carried out using primers specific to the pandemic strain when the trial is underway, or by sequencing the H gene, whichever is appropriate given the antigenic drift that might take place between this proposal submission and the implementation of this study, if approved. The nasal swabs may also be tested later for other viruses which may account for acute febrile episodes (eg RSV and adenovirus).

CQ drug levels
CQ levels will be measured in whole blood and saliva using a standard liquid chromatography mass spectrometry (LCMS) assay performed in NUHS.

PBMC storage (subset of patients)
In the subset of patients who agree to have additional blood taken at baseline and week 12, PBMCs will be separated using standard approaches and will be stored at -70°C until analysed. The immunological assays performed on these samples will be related to defining the immunological profile of patients at risk of developing influenza, and to defining the immunological consequences of influenza. The immunological tests will be submitted as a separate sub-study protocol for ethics review at a later stage.

d. Post Study Follow up and Procedures

The study will end with the week 12 visit (which will occur 1 week after the last dose of CQ). Participants will be given a letter to their GP to follow up with any remaining adverse events.

e. Discontinuation Visit and Procedures

If the participant discontinues study medication they will be encouraged to continue all other study procedures (symptom diary and swabs if needed) and to come for the week 12 visit.

If the participant wishes to withdraw immediately from all study procedures, they will be encouraged to come to IMU for a final visit at which all study materials will be collected, and a follow up blood sample obtained. After this they will no longer participate in the study. Participants will be given a letter to their GP to follow up with any remaining adverse events.
f. Optional additional visit

In view of the long half-life of CQ, there may be ongoing protective efficacy against influenza after the drug is discontinued at week 12. In order to maximise the chances of detecting an effect of CQ on influenza infection, we will request that participants continue with an optional 12 weeks of additional follow-up to week 24. During this period, participants would continue to complete routine weekly diaries and to complete daily diaries and take a nasal swab if they develop influenza-like illness symptoms. They would come back for a final study visit at week 24 which would involve the same procedures as the week 12 visit. The follow-up beyond week 12 is entirely optional and additional consent will be taken.

In anticipation of converting the trial subjects into a long-term cohort, we will request subjects who attend the week 24 visit to continue to complete routine weekly diaries and to complete daily diaries and take a nasal swab if they develop influenza-like illness symptoms, till December 2010. If funding becomes available for the long-term cohort, we will ask them to come back at week 48 for another visit. The follow-up beyond week 24 is also entirely optional and additional consent will be taken.

7. TRIAL MATERIALS

7.1. Trial Product (s)

Chloroquine
Chloroquine will be provided as capsules containing CQ phosphate 250mg (equivalent to 150mg base). The study medication will be prepared by a commercial company in Singapore and meets all the necessary regulatory standards. Participants will be instructed to take two capsules once a day, preferably with food for the first 7 days of the trial, commencing on the day after randomisation. Thereafter they will take 2 capsules on the same day each week (the day of study entry) for a total of 10 doses – the last dose of study drug will be taken at week 11, one week prior to the final study visit.

CQ is generally well-tolerated, and adverse effects are unusual at the dose used in the induction period in this trial, and very rare at the once weekly dose used in the maintenance phase (which is the same as that used for malaria prophylaxis). At the dose used in the induction phase, gastrointestinal side effects occur at moderate to severe level in less than 10%, and skin reactions, headache and visual disturbances occur at moderate to severe level in less than 5% of patients. These are likely to be transient and resolve with the reduction in dose during the maintenance phase. Such side effects rarely lead to discontinuation of CQ used in malaria prophylaxis.

Participants will be given an information sheet about the possible side effects and how they can be managed. For example, gastrointestinal intolerance can be managed by symptomatic treatment initially (antacids can be used 4 hours apart from study drug) and itch can be managed by antihistamines. CQ is very toxic in overdose and although the quantities of CQ dispensed to participants in this trial will be relatively small, an emergency procedure will be put in place to deal with un-blinding in the event of an overdose or suspected overdose.

Placebo
This will be provided as identical capsules to the active drug, but instead containing lactose placebo. The medication schedule will be the same as for the active drug.
7.2. Storage and Drug Accountability

The study drug will be stored at room temperature, in a secure environment with limited access to authorised personnel. The site personnel will ensure accountability of the study drug during study period.

8. TREATMENT

8.1. Rationale for Selection of Dose

This dose was selected to represent the best compromise between the high degree of convenience and tolerability that would be required for CQ (if shown to be effective in this trial) to gain widespread acceptance as an influenza pre-exposure prophylaxis regimen taken for many months by healthy members of the community, and the minimum levels likely to be effective in prevention of influenza virus infection. Given the long half-life of CQ (months) the weekly dose should maintain the levels achieved in the once-daily induction phase. (Wetsteyn, De Vries et al. 1995) CQ pharmacokinetics shows considerable inter-individual variation, but the levels of CQ obtained with this regimen should approximate the IC50s of the H1N1 and H3N2 strains used in the in vitro studies (of around 1uM), and the efficacy may be further enhanced as with sustained use the drug is highly concentrated in many tissues including the lung, as well as in white cells and macrophages. (Wetsteyn, De Vries et al. 1995; Ducharme and Farinotti 1996; Ooi, Chew et al. 2006; Di Trani, Savarino et al. 2007)

8.2. Study Drug Formulations

The study drug will be dispensed to the subjects as capsules containing 250mg CQ phosphate or lactose.

8.3. Study Drug Administration

Participants will be instructed to take two capsules once a day, preferably with food for the first 7 days of the trial, commencing on the day after randomisation. Thereafter they will take 2 capsules on the same day each week (the day of study entry) for the remaining 10 weeks of the study (a total of 10 further doses).

Participants will be warned that the study medication can be dangerous in overdose and that they should not exceed the stated dose. They will be instructed that if they cannot remember whether they have taken their medication on a particular day they should wait until the next dose is due, rather than take a further dose “just in case”.

Participants will be contacted after 5-7 days to check on adherence to the study medication and be provided with counselling / advice regarding any reported side effects. Adherence will be reinforced by an automated email and text reminder sent by the web-based system once weekly on the designated day when they are due to take their medication (in conjunction with the reminder to complete the diary).
8.4. Specific Restrictions / Requirements

CQ has relatively few significant interactions with other medications.

The study drug will be discontinued in participants who need to start a contra-indicated medication (amiodarone, ciclosporin, digoxin, mefloquine, moxifloxacin) or in participants who need to start any drug that is known to commonly cause hepatotoxicity (e.g. isoniazid or rifampcin).

Alternatives to CQ or mefloquine will be used if the participant needs malaria prophylaxis or treatment (e.g. atovaquone/proguanil or doxycycline).

Patients will be asked not to take any antacids within the 4 hours before or after taking study medication. If patients require an H2 antagonist, they will asked to use ranitidine in preference to cimetidine (the former has no interaction).

Participants who are prescribed oseltamivir will continue the study medication. There have been no interaction studies performed to date, but based on the known pathways of metabolism of CQ (metabolised by hepatic cytochromes 2C8/9, 2D6 and 3A4) and oseltamivir (extensively converted to oseltamivir carboxylate by esterases located predominantly in the liver, and oseltamivir carboxylate is then eliminated in the kidney; neither oseltamivir nor oseltamivir carboxylate is a substrate for, or inhibitor of, cytochrome P450 isoforms) there is no reason to expect an interaction.

8.5. Blinding

The study medication will be blinded. This will be by means of using an identical placebo. The code will only be known to the trial statistician. See section 6.1 above for further details.

8.6. Concomitant therapy

All medications taken by the participant will be documented.

9. Safety Measurements

9.1. Definitions

UPIRTSO events and serious adverse events are defined below. Events will be reviewed and classified by the PI, or co-PI or other investigator. Severity will be classified using a standard set of criteria for grading adverse events (the 2004 Division of AIDS toxicity grading scale). The relationship of the event to the study drug and whether the event is an expected event or not will be assessed using the listing of adverse effects contained in the summary of product characteristics for CQ.
9.2. Collecting, Recording and Reporting of “Unanticipated Problems Involving Risk to Subjects or Others” – UPIRTSO events to the NHG Domain Specific Review Boards (DSRB)

UPIRTSO events refers to problems, in general, to include any incident, experience, or outcome (including adverse events) that meets ALL of the following criteria:

1. Unexpected
   In terms of nature, severity or frequency of the problem as described in the study documentation (Summary of product characteristics).

2. Related or possibly related to participation in the research
   Possibly related means there is a reasonable possibility that the problem may have been caused by the procedures involved in the research; and

3. Risk of harm
   Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Reporting Timeline for UPIRTSO Events to the NHG DSRB.
1. Urgent Reporting: All problems involving local deaths, whether related or not, should be reported immediately – within 24 hours after first knowledge by the NHG investigator.

2. Expedited Reporting: All other problems must be reported as soon as possible but not later than 7 calendar days after first knowledge by the NHG investigator.

9.3. Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to the Health Science Authority (HSA)

All SAEs that are unexpected and related to the study drug will be reported to HSA.

“A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that:

- Results in death.
- Is life-threatening (immediate risk of death).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in congenital anomaly/birth defect.
- Is a Medically important event.

Medical and scientific judgment will be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes, the important medical event will be reported as serious.”

All SAEs that are unexpected and related to the study drug will be reported. The PI or co-PI will be
responsible for informing HSA no later than 15 calendar days after first knowledge that the case qualifies for expedited reporting. Follow-information will be actively sought and submitted as it becomes available. For fatal or life-threatening cases, HSA will be notified as soon as possible but no later than 7 calendar days after first knowledge that a case qualifies, followed by a complete report within 8 additional calendar days.

9.4. **Safety Monitoring Plan**

A DSMB will be established, comprising two independent physicians. In view of the well-established safety and toxicity profile of CQ this trial is regarded as low risk to participants. A meeting of the DSMB will be convened if and when 10 SAEs have occurred in the trial. The unblinded trial statistician will provide details of those events, split by treatment group, to the DSMB and if there is a significant safety concern raised by the distribution and nature of those events, the DSMB may recommend to the Principal Investigator that the trial should be stopped. Further meetings of the DSMB may be convened at the discretion of the DSMB and trial statistician as further SAE reports accumulate.

9.5. **Complaint Handling**

Complaints will be handled according to the normal procedures in operation in NUHS.

10. **DATA ANALYSIS**

10.1. **Data Quality Assurance**

All investigators and study personnel will be trained in the use of the online data entry system and will be required to maintain adequate and accurate recording of all data throughout the study.

10.2. **Data Entry and Storage**

For the purpose of this study, the electronic case record forms will serve as the source documentation. The data collected during the study will be entered into the online database, either by the subjects themselves or by the study coordinator. The system will allow for audit tracking.

During the course of the study, auditors from relevant authorities may visit the study site to review protocol compliance, check eCRFs, assess drug accountability and ensure that the study drug is being conducted according to GCP guidelines.

Essential documents will be retained for a minimum of fifteen years after the completion or discontinuation of the study.
11. SAMPLE SIZE AND STATISTICAL METHODS

11.1. Determination of Sample Size

We estimate that the rate of laboratory confirmed clinical influenza will be at least 10% over the 12-week study period. This comprises an estimated rate of 5% for clinical infection with seasonal influenza over this time period that is based on national estimates for Singapore, (Ng, Pwee et al. 2002) and the rate seen in the control arm of a previous 6-week influenza prevention trial conducted during the influenza season in the US. (Hayden, Atmar et al. 1999) plus an additional 5% risk of H1N1 pandemic strain infection, that is likely to be a conservative estimate, given the much higher transmissibility of this strain compared to seasonal influenza. (Fraser, Donnelly et al. 2009) We believe that a 50% reduction in clinical cases would justify widespread clinical use of CQ, and is a realistic effect size given the 74% reduction that has been seen with pre-exposure prophylaxis with oseltamivir. (Hayden, Atmar et al. 1999) To detect a 50% reduction in clinical cases in the CQ treated participants (alpha 0.05; power = 0.9; adjusted for 10% loss to follow up) would require 1370 participants. To allow for correlated data arising from multiple participants from the same household, assuming that on average there are 2 participants per household and the intra-class correlation co-efficient (ICC) is 0.1, the sample size needs to increase by 10%, i.e. total 1500. This level of ICC for a binary outcome is larger than that which is often observed. (Hayes and Bennett 1999; Thomson, Hayes et al. 2009) This sample size would have greater than 95% power to detect a similar degree of efficacy for reducing all laboratory confirmed infections (symptomatic and asymptomatic), assuming the rate of laboratory confirmed infection would be 20% over this time period.

11.2. Statistical and Analytical Plans

a. General Consideration

Primary endpoint
Laboratory-confirmed influenza-like illness, defined as the combination of clinical influenza-like illness and laboratory confirmed infection.
Clinical influenza-like illness: temperature of at least 37.2°C, with at least one respiratory symptom (cough, sore throat or nasal congestion) and at least one constitutional symptom (muscle aches, headache, fatigue, feeling of chills or sweats) occurring in the same 24-hour period.
Laboratory confirmed infection:
A. PCR confirmation on nasal swabs
OR
B. Any reliable report (obtained direct from laboratory or healthcare provider, or by documentation provided by the participant) of a laboratory diagnosis of influenza by culture or PCR obtained from a specimen taken during the trial.
OR
C. Serological confirmation by 4 fold rise in antibody titre to seasonal influenza strain (in participants receiving no vaccine or in participants receiving just H1N1 vaccine)
OR
D. Serological confirmation by 4 fold rise in antibody titre to H1N1 strain (in participants receiving no vaccine or in participants receiving just seasonal vaccine)
OR
E. Serological confirmation by 4 fold rise in antibody titre to either seasonal or H1N1 strain based on pre-vaccine serology.

This definition is based on that used in previous influenza prevention trials, adapted to allow the inclusion of endpoints in participants who undergo influenza vaccination during the course of the trial. (Hayden, Atmar et al. 1999; Welliver, Monto et al. 2001)
Main secondary endpoint
Serologically-confirmed infection (four-fold rise in antibody titre at week 12 compared to the baseline sample), whether symptomatic or asymptomatic.

Other secondary endpoints
Laboratory confirmed infection (as defined above), whether symptomatic or asymptomatic
Laboratory-confirmed influenza-like illness with a temperature of at least 37.8°C
Laboratory-confirmed severe influenza-like illness (temperature of at least 37.8°C with at least 5 symptoms of moderate / severe grading on any 1 day)
Other laboratory-confirmed viral infection
Laboratory-confirmed influenza-like illness (using the primary endpoint definition) at week 24
Days off school/ college or work with influenza-like illness
Total grade 3 / 4 adverse events

Statistical analysis plan
a. Efficacy Analyses
The primary analyses will be by intention to treat, with all participants analysed according to the study arm to which they were randomised. For the main analysis, the proportion of participants who have a laboratory-confirmed influenza like illness will be compared between CQ and placebo arms in terms of protective efficacy=1-risk ratio and intervention attributable reduction=risk difference. (Greenwood 2005) A similar approach will be used for the other secondary endpoints expressed as proportions. Separate subgroup analyses will also be performed for H1N1 infection and non-H1N1 infection. The median number of days off school/college/work, median moderate/severe symptom score during influenza-like illness (an indicator of whether CQ ameliorates clinical disease), and frequency of reported clinical symptoms of moderate / severe grade (by body systems) will be compared between the two groups by quantile regression. To examine the relationship between CQ levels and protective efficacy, a subgroup analysis will be conducted to compare the proportion experiencing the primary endpoint in those who have CQ levels above the median of the intervention group, compared to those with CQ levels below that level (or who are on placebo). Robust standard errors will be used for statistical inference and P<0.05 is considered statistical significance. 95% confidence intervals for parameters will be presented.

b. Safety Analyses
The frequency of reported clinical symptoms of moderate / severe grade (by body systems) will be compared between the two groups by quantile regression. The number of serious adverse events (if any) will be compared between groups by appropriate non-parametric tests.

c. Interim Analyses
No interim analysis is planned for this trial as no data on the primary outcome parameter (which includes laboratory confirmation) will be available until the trial has ended.

d.

e.
12. ETHICAL CONSIDERATIONS

12.1. Informed Consent

A two-stage consent process will be required for this study (see also section 3.2, above). In the first stage, participants will be able to access the patient information sheet on the study website, and will need to provide electronic consent for participation in the study. There will be an interactive process that ensures that participants fully understand the key elements of the study prior to giving electronic consent. They will also be given a telephone number to call if they have further questions about any aspects of the study.

Parental consent will be obtained (in person) for participants between the age of 18 to 21 years.

At the screening visit, the research co-ordinator will answer any remaining questions about the study and will confirm with the participant that they remain willing to enter the trial. The participant will be required to sign a consent form to indicate this.

As participants will need to complete on-line symptom questionnaires in English as part of the trial, we will recruit only English-speaking patients. Therefore, we will not make provision for translating the study materials and internet based questionnaires into other languages.

In addition to the online participant consent, we will use a written consent form for all participants.

An addendum to the consent form will be used to obtain written informed consent for the additional 12 weeks of follow-up to week 24. Additional consent will also be obtained to take 20 mL of blood for PBMCs, in 50 participants who did not consent to this at baseline.

12.2. IRB review

This protocol and the associated informed consent documents will be reviewed and approved by the IRB / NHG DSRB and Health Science Authority prior to initiation of study procedures.

12.3. Confidentiality of Data and Patient Records

All study findings and documents will be regarded as confidential. The investigators and other study personnel must not disclose such information without prior written approval from the Principal Investigator.

Subject confidentiality will be strictly maintained to the extent possible under the law. Subject names must not be disclosed. They will be identified on the eCRFs and other study documents by their initials, birth date, and/or assigned subject number. A record of the contact details will be kept in the online system, as this is necessary for contacting participants by email and SMS text, which is an essential part of the study design. However, this information will be stored securely, and will be removed from the electronic system 6 months after the completion of the trial (or after the completion of the registration process, for people who register but do not take part in the trial). Participants will be informed that their contact information will be held for 6
months at the time of registering contact details on the website.

The blood samples will be destroyed once all analyses have been completed. Information that relates to the diagnostic testing of influenza (based on PCR or serology tests) may be returned to the participant at the end of the trial upon receipt of a written request. No other data from laboratory analyses will be returned to the participant, or their family, or the investigators or any other physician who is treating the subject or who may treat the subject in future. Neither the subject’s insurance company nor employer will have any access to these test results.

13. PUBLICATIONS

The findings from the main study will be submitted for publication to an international peer-reviewed journal that supports open access publication within 12 months. If the nature of the findings warrant rapid publication, then a journal with a mechanism for fast-track publication will be preferred.

The final report or the publication of the study will be furnished to the HSA within 3 months of trial completion or when available.

14. RETENTION OF TRIAL DOCUMENTS

Essential documents will be retained for a minimum of fifteen years after the completion or discontinuation of the study in a secure storage facility. The records will be accessible for inspection by authorized authorities.
APPENDIX A – GENETIC SUBSTUDY

Chloroquine is equally cleared by renal excretion and metabolism in the liver. Cytochromes 2C8, 3A4 and 2D6 have been shown to metabolize chloroquine in vitro. The quantitative contributions of these enzymes in vivo are not known. In addition, the role of drug transporter such as P-glycoprotein is not known.

Genetic factors are also implicated in susceptibility and response to infections including influenza. Genetic factors may also affect the effects of chloroquine.

The primary objective of this optional substudy is to determine the effect of polymorphisms in drug metabolism and transporter genes on the pharmacokinetics of chloroquine and its efficacy (if any).

The secondary objectives are:

1. To discover genetic polymorphisms involved in susceptibility and response to influenza and other infections
2. To discover genetic polymorphisms involved in effects of chloroquine.
3. To establish a pharmacogenetics database which may be used in future studies.

For subjects who consent to having their DNA tested for polymorphisms related to infections and chloroquine, DNA will be extracted from the whole blood sample used to measure chloroquine pharmacokinetics. No additional blood sample to be taken. A coded identification number will be given to subject’s DNA and the link stored in a secure database.

The DNA will then be sent for sequencing or allele-specific PCR to study polymorphisms in CYP 2C8/9, 2D6 and 3A4. Other polymorphisms in drug metabolism and transporter enzymes related to chloroquine may be tested at a later stage. Other genetic tests may be performed to study susceptibility and response to infections, and chloroquine effects. No other genetic tests will be done. DNA samples will be destroyed after these tests are done. Subjects will not get their test results and information will not be entered into the medical records or made publicly available.

The primary analysis will be to compare whole blood chloroquine concentrations in subjects with CYP2C8 polymorphisms (http://www.cypalleles.ki.se/cyp2c8.htm), as this is likely to be the predominant cytochrome involved in chloroquine metabolism. This will be performed with standard statistical tests such as ANOVA and t tests. CYP2D6 and 3A4 will be done next, followed by drug transporters and other drug metabolism enzymes as necessary. Secondary analyses will be performed with other genetic markers.

For subjects who consent to be included in the pharmacogenetics database, their DNA samples will be stored. These samples may be tested for polymorphisms in pharmacokinetic and pharmacodynamic genes at a later stage. Subjects may be contacted to come back for future studies related to these enzymes. After 15 years, the DNA samples will be destroyed.

A separate informed consent document has been provided for this substudy. Informed consent will be taken by study coordinators or the investigators. Ethics approval will be sought for this substudy before implementation.