Protocol

Protocol for: Chamma-Siqueira NN, Negreiros SC, Ballard S-B, et al. Higher-dose primaquine to prevent relapse of *Plasmodium vivax* malaria. N Engl J Med 2022;386:1244-53. DOI: 10.1056/NEJMoa2104226

This trial protocol has been provided by the authors to give readers additional information about the work.

Protocol and Analysis Plan Summary

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Original Protocol
Approved on December 20, 2017

Efficacy of Three Regimens of Chloroquine and Primaquine for the Treatment of *Plasmodium vivax* Malaria in Cruzeiro do Sul, Acre, Brazil

Investigator(s) and Institutions

- Dr. Suiane Negreiros do Valle, MD, PhD, Health Secretariat of Acre State (Hospital Regional do Jurua). Dr. Negreiros will be responsible for implementation and general oversight of the study.
- Giselle Rachid Viana, PhD, Instituto Evandro Chagas. Dr. Viana will be responsible for quality assurance and control or laboratory procedures used in this study.
- Paola Marchesini, MD, PhD, Instituto Evandro Chagas. Dr. Marchesini will be responsible for overall project, making sure it fits into the needs of the national malaria control program in Brazil.
- Marinete Marins Povoa PhD, Instituto Evandro Chagas. Dr. Povoa will be responsible for supervising all laboratory procedures associated with this project.
- Venkatachalam (Kumar) Udhayakumar, PhD, Malaria Branch, CDC (IRB number 13427). Dr. Kumar will be responsible for supervising and troubleshooting the molecular typing to be done as part of this study. He will not have access to any personal identifier.
- Michael Green, PhD, Entomology, CDC (IRB number 17614). Dr. Green will be responsible for supervising and troubleshooting the antimalarial blood level determination to be done as part of this study. He will not have access to any personal identifier.
- Sarah-Blythe Ballard, MD, PhD, MPH, Malaria Branch, CDC (SEV 7105). Dr. Ballard will be responsible for technical assistance to the protocol and project implementation. Dr. Ballard will likely interact with study subjects at the time of study implementation and supervisory visits. He will not have access to stored personal identifiers.
- Alexandre Macedo de Oliveira, MD, MSc, PhD, Malaria Branch, CDC (IRB number 9885). Dr. Macedo de Oliveira will be responsible for technical assistance to the protocol and coordination between colleagues in Brazil and at CDC. Dr. Macedo de Oliveira will likely interact with study subjects at the time of study implementation and supervisory visits. He will not have access to stored personal identifiers.

Conflict of interest

None of the authors have any conflict of interest to report.

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Summary

Background: The World Health Organization recommends that antimalarial treatment policies be evaluated every few years to check their efficacy. *P. vivax* malaria is the most common species in Brazil and cases are concentrated in the Amazon Region in Brazil.

Objectives: Assess the efficacy of 3 different regimens of chloroquine and primaquine for the treatment of *P. vivax* infections in Cruzeiro do Sul, Acre, Brazil.

Methods: An in vivo drug efficacy study will be conducted in Cruzeiro do Sul, Acre State, Brazil. A total of 257 study participants ≥5 years of age with parasitologically confirmed *P. vivax* monoinfections will be included. Patients will be divided in 3 different groups: treatment with regular dose of primaquine (0.5 mg/kg per day for 7 days) with directly observed therapy; regular dose of primaquine without directly observed therapy; and increased total dose of primaquine (0.5 mg/kg per day for14 days) with directly observed therapy. All patients will receive chloroquine (CQ) for three days at a daily dose of approximately 25 mg/Kg in accordance with the Brazilian National Malaria Control guidelines. Primaquine will be given for 7 or 14 days under supervision or not, depending on the study group. Clinical and parasitologic parameters will be monitored over a 28-day follow-up period to evaluate drug efficacy and for a total period of 168 days (24 weeks) to evaluate chances of recrudescence, relapse, or reinfection. Blood samples will be taken to measure the CQ levels in blood on Day 7 and day of failure, if occurring in the initial 28 days of follow up. In addition, a blood sample will be collected on filter paper on first day and on day of suspected failure to help differentiate parasite genotypes using techniques based on polymerase chain reaction. Results from this drug efficacy study will be used to assist the Brazilian Ministry of Health in assessing their national malaria treatment policy for *P. vivax* malaria.

Background

The impact of malaria on the health and economic development of human populations is greatest in the tropics and subtropics. The World Health Organization (WHO) has estimated 212 million episodes of malaria in 2015, of which 90% of those in in Africa. There were a total of 429,000 malaria deaths worldwide, the majority in in children under 5 years of age (WHO 2015). Although the majority of deaths occur among children in sub-Saharan Africa, malaria accounts for considerable morbidity in the Americas, particularly in the Amazon Basin.

Most countries in the Americas have adopted the WHO Global Strategy for Malaria Control, which relies on prompt and effective antimalarial treatment as the major means of reducing malaria morbidity and mortality (WHO 2008). The ultimate success of this strategy rests on the ability of ministries of health to provide antimalarial drugs with proven efficacy. Although a wide variety of methods have been used to assess resistance to antimalarial drugs in vivo methods, in vitro drug sensitivity testing, and molecular analyses, most national malaria control programs rely on data from in vivo efficacy trials to assess the efficacy of the current first- and second-line drugs and to decide if charges in malaria treatment policy are needed.

The most widely used approach to conduct in vivo drug efficacy trials in the Americas follows the guidelines of the WHO (WHO 2009, WHO 2015) with the modifications recommended by the Pan American Health Organization for studies in the Americas (PAHO 2003). The goal of such studies is to assess antimalarial drugs currently being used for first-line treatment of uncomplicated malaria. Much of the effort to monitor antimalarial efficacy in the Americas has been done as part of the Amazon Network of Antimalarial Resistance Monitoring and the Amazon Malaria Initiative (PAHO 2012). This information is critical for guiding the development of rational antimalarial drug policies in endemic areas.

Chloroquine-resistant *P. vivax* was first reported from Papua New Guinea in 1989 in two Australian soldiers (Rieckmann, Davis et al. 1989). In 1995, a study in Irian Jaya, Indonesia showed resistance in at least 44% of the P. vivax patients treated with chloroquine (CQ) (Baird, Basri et al. 1995). Several investigators have reported cases of CQ-resistant P. vivax in South America. In 1996, in Guyana, Phillips et al. reported three patients in whom 25 mg/kg of CQ failed to eliminate parasitemia despite adequate therapeutic blood levels of CQ (Phillips, Keystone et al. 1996). Three years later, in the Brazilian Amazon region, Alecrim et al. reported a 12-year old girl with P. vivax malaria who continued to have parasitemia after receiving a supervised course of 25mg/kg of CQ (Alecrim, Alecrim et al. 1999). More recently, Soto et al. reported three cases of CQ-resistant P. vivax in Colombia (Soto, Toledo et al. 2001).

In addition, recent studies have shown high reinfection rates by *P. vivax* even after treatment with recommended dose of primaquine (0.5 mg/kg per day for 7 days) (Durand, Cabezas et al. 2014, Negreiros, Farias et al. 2016). Despite the fact that primaquine is the only drug currently available for the terminal treatment of *P. vivax* and *P. ovale* infections, variable reinfection rates have been reported in the literature (Hill, Baird et al. 2006, Durand, Cabezas et al. 2014). WHO recommends daily dose of 0.25 mg/kg/day (infections from temperate regions) and 0.50 mg/kg/day (infections from tropical regions with high rates of relapses) all over 14 days, with a total dose of 3.5 mg/kg or 7.0 mg/kg, respectively, after reports of relapses in Asia and Oceania (Collins and Jeffery 1996, Baird and Hoffman 2004, WHO 2015). This concern about frequent relapses has led CDC to change its recommendation to increase the total dose of primaquine for terminal treatment 7.0 mg/kg to be given over 14 days for terminal treatment for *P. vivax* infections from all regions of the world (Centers for Disease Control and Prevention 2015).

Because of the serious public health implications of CQ-resistant and primaquine-resistant *P. vivax* in the Americas, it is critically important to limit reports to well-confirmed cases. In most cases, this will require measurement of CQ blood levels and genotyping of parasites from the initial infection and any suspected recrudescence. *P. falciparum* in vivo trials take advantage of well-established molecular markers that help differentiate cases of recrudescence and reinfection, by polymerase chain reaction (PCR) techniques. Although no universally accepted technique for this purpose exists for *P. vivax.* Microsatellites, base pair repeats in the parasite genome, described by Imwong et al (Imwong, Sudimack et al. 2006) have been used for this purpose with variable results. We believe that having PCR-corrected analysis is especially important in the context of the long follow-up period, six months, we aim for this study.

Most malaria cases in the Americas are reported in Brazil (Silveira 2001, Oliveira-Ferreira, Lacerda et al. 2010). In 2016, 151,620 malaria cases were reported to the Brazilian National Reportable Disease Information System. Most of these cases (99.7%) occurred in the Amazon region, which encompasses the states of Acre, Amazonas, Amapa, Para, Maranhao, Mato Grosso, Roraima, Rondonia, and Tocantins. In this region, socio-economic and environmental conditions, such as presence of natural breeding sites and abundance of Anopheles mosquitoes, favor malaria transmission. Amazonas, Rondonia, Para, and Acre states were responsible for 85.5% of malaria cases in 2011 according to the Brazilian National Reportable Disease Information System. As in other regions of the world, malaria is seasonal in Brazil, cases increase during or after the rainy season (Costa 2009).

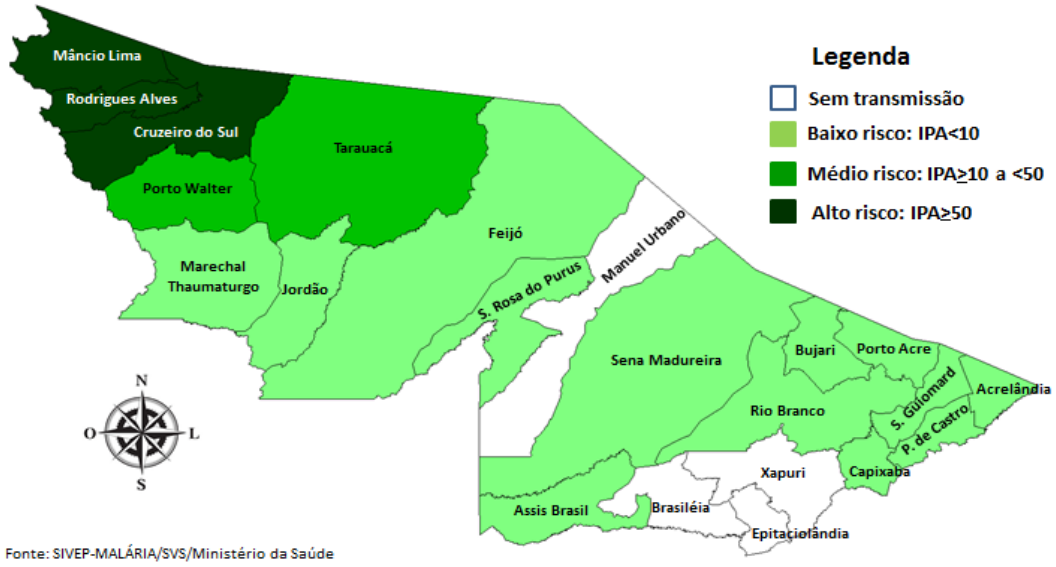
In 2016, 73.9% of malaria cases in Brazil were due to *P. vivax* alone, 9.47% to *P. falciparum* alone, and the rest due to mixed infections with these two species. *P. malariae* is rarely seen in Brazil (regular surveillance data from Brazil, SIVEP, 2017). Table 1 shows malaria cases over the last few years.

Acre state is responsible for a huge proportion of cases in the Brazilian Amazon region, especially in the municipalities of Cruzeiro do Sul, Mâncio Lima and Rodrigues Alves (Figure 1). In 2016, Acre had 35,209 cases in addition to 4,098 positive cases detected during treatment response control slides (TRCS), a total of 39,307. Cruzeiro do Sul had 20,591 cases, with additional 2,195 cases by TRCS, a total of 22,786 cases. (SIVEP, 2017).

Table 1. Malaria cases in Brazil.

Region	2012	2013	2014	2015	2016
	Cases	Cases	Cases	Cases	Cases
Brazil	234,545	169,231	138,936	137,947	123,935
North	234,497	169,187	13,8919	137,925	123,904
Acre	27,005	33,755	30,982	26,632	34,382
Cruzeiro do Sul	16,053	19,701	17,179	13,936	19,027

Figure 1. Acre state and malaria risk by region, 2008.



Source: Acre state Health Secretariat.

Legend: Alto Risco, High risk; Medio Risco, Medium risk, and Baixo Risco, Low risk.

Although currently there is no evidence of CQ-resistant or primaquine-resistant *P. vivax* in Cruzeiro do Sul, Acre State, Brazil, there is concern about the tolerance to primaquine. The Ministry of Health would like to assess the efficacy of three regimens of CQ and primaquine for the treatment of uncomplicated P. vivax malaria as part their effort to study antimalarial drug resistance within the country.

Objectives

The objective of this study is to evaluate the therapeutic efficacy of CQ and primaquine in the treatment of *P. vivax* malaria in Cruzeiro do Sul, State of Acre, Brazil using three primaquine schemes. These are (1) routine standard dose in Brazil, WHO dose for temperate regions with supervised treatment; (2) double dose, WHO dose recommended for tropical regions with supervised treatment, and (3) standard dose treatment from Brazil without supervision.

Methods

Study site

This study will take place at the malaria treatment posts in Cruzeiro do Sul, Acre State, Brazil. Cruzeiro do Sul is a municipality with 85,000 residents in Northwest Brazil. Both *P. vivax* and *P. falciparum* malaria are endemic in the region with an annual parasite incidence (API) of 233.4/1,000 (SIVEP, 2016). In accordance to Brazilian national policies, malaria diagnosis and treatment are offered free of charge in the region. Road access to public health facilities is relatively easy in the urban and peri urban areas of the city. It is common practice in this region for patients to present to health facilities for diagnosis and first treatment dose. Patients could be then visited at home by health agents for directly observed therapy of the following doses.

The study will take place with patients recruited on spontaneous demand who attend the Emergency Room of the Juruá Regional Hospital (HRJ). This malaria post has a malaria diagnostic laboratory running 24 hours continuously, and it also registers the highest number of malaria cases in the city, according to statistics from the Brazilian malaria information system (SIVEP). Three other malaria diagnostic stations will also be selected for this study, health posts located in the neighborhoods of Aeroporto Velho, Miritizal, Cruzeirinho, and others, and will be coordinated by the mobile team of the study. After the period of recruiting patients, the teams will be installed in the Clinical Research Room located at Santa Casa de Misericórdia de Cruzeiro do Sul.

Timing of study

The study will be conducted from February 2018 to December 2018 during peak malaria transmission season. Close communication will be maintained with regional and local health officials to determine the most appropriate time for the study to begin. Due to the 6-month follow-up period (168 days), we expect to conclude patient follow-up by December 2018 and data analysis by May 2019.

Study teams

At least two clinical teams will be required to conduct this study and interact with patients. Each team will consist of a nurse and a nursing assistant. One team will be based in the HRJ outpatient clinic and the other in the malaria research room. The team based in the clinical research room will be a mobile team responsible for admitting and following patients at least three malaria diagnostic post that are not at the HRJ (Cruzeirinho, Aeroporto Velho, Miritizal, and others). In addition to the clinical teams, one supervising physician and three microscopists will be part of the study.

Groups of study

This evaluation will have three distinct patient groups:

1. Group 1. Treatment with supervised CQ and unsupervised primaquine with usual primaquine dose of 3.5 mg/kg (0.50 mg/kg / day for 7 days).
2. Group 2. Treatments with CQ and primaquine supervised with usual primaquine dose of 3.5 mg/kg (0.50 mg/kg/day for 7 days).
3. Group 3. Treatments with supervised CQ and primaquine with increased primaquine total dose of 7.0 mg/kg (0.50 mg/kg/day for 14 days).

Group 3 will be the comparison group for efficacy and reinfection at the 24-week follow-up. Treatment response rates at Day 28 and during complete follow-up for reinfections will be compared between Groups 1 and 3, and between Groups 2 and 3.

Study procedures

The physician will act as study coordinator and will be responsible for overall study supervision, study forms, and administrative issues. The study doctor will supervise the entire clinical staff at all times and will be available to handle unexpected events.

The nurse will be responsible for evaluating the patients at the time of admission to the study, obtaining a clinical history, conducting a physical examination, administering the study medication (CQ and primaquine

after confirmation of normal levels of glucose-6-phosphate dehydrogenase activity [G6PD]), visits on the first four days of follow-up (Days 0, 1, 2, and 3) and preferably Day 7, either in the study centers or at home visits (see below).

The nursing assistant will be responsible for monitoring primaquine doses for 7 or 14 days (after confirmation of normal levels of G6PD activity) depending on the study group, through visits to the patients at their homes for follow-up visits and patients' remaining check-ups. Since primaquine therapy can only be started after confirmation of normal G6PD activity, this drug will not be initiated on Day 0 when CQ will be initiated. We expect to have G6PD results within one week; therefore, primaquine will likely be initiated during the first week of follow-up. The doses of medicament, CQ and primaquine, will be administered with direct supervision, either by the nurse or by the nursing assistant, the two supervised treatment groups. In the unsupervised primaquine treatment group, only the doses of CQ will be supervised. Both the nurse and the nursing assistant can collect the blood samples, via fingerprick and venous puncture, recommended with part of this study.

As a routine practice in Ministry of Health facilities in Brazil, all febrile patients have a thick blood smear or rapid diagnostic test (RDT) for malaria examined before antimalarial treatment is administered. This usually takes place at a health facility where sick patients present. The staff of these malaria diagnostic posts, hospital and other health centers, will be informed of the study. Patients attending the study sites, HRJ or the health posts, who have a positive blood smear or RDT for *P. vivax* will be approached by a member of the study team or told by a health facility staff about this in vivo trial.

The study team nurse will assess inclusion and exclusion criteria and explain details about the study. If eligible patients agree to take part in the study, they, or the patient's parent (in case of patient's age < 18 years), will be asked to sign the informed consent or parental permission form (and also obtain verbal assent from children 7 through 17 years of age). After that, patient will be undergo blood collection for malaria diagnosis confirmation, medical evaluation, and all other study procedures. In case of refusal, patients will be referred back to health facility staff to receive the standard treatment for *P. vivax* malaria without any sort of penalty.

Enrolled patients will be asked to complete the CQ treatment at the health facility they were admitted at if they can. The study team nurse will schedule home visits for those patients who do not wish to come back to the health facility for follow-up during the period the patient is receiving CQ. Once a normal activity level of G6PD is documented, primaquine will be initiated and give also as supervised or not supervised doses,

depending of the study group. On and after Day 3, when CQ treatment ends, arrangements will be made with the patient to be seen at home. The nurse assistant will make arrangements to visit patients at home for the remaining doses of primaquine. Study team will encourage patients to come back to health facilities, but we expect that most patients will prefer to be visited at home as this is common practice in Cruzeiro do Sul for malaria treatment and follow-up.

We believe that after CQ treatment is over and patient's health status has improved, the study team nurse assistant will be able to appropriately manage the follow-up visits. These nurse assistants will be in close contact with the nurse of their respective team and arrangements for re-evaluations will be made if necessary.

Study microscopists will be responsible for reporting blood smears results on the same day they are taken. Details on how the laboratory work will be organized are provided below. The physician will serve as a study coordinator, being responsible for the overall supervision of the study, study forms, and administrative issues. All study team personnel will be trained in their respective tasks before the start of this study. This training will take part in a 5-day period with participation of collaborators from the Centers for Disease Control and Prevention (CDC), Atlanta and Instituto Evandro Chagas, de Belem, Para. We will also invite the Unidade De Boas Praticas Clinicas da Fiocruz (Good Clinical Practices Unit at Fiocruz) to take part in the study.

In order to avoid reinfection due to malaria during the follow-up period, we will instruct patients to use malaria prevention measures. These are long-lasting insecticide-impregnated mosquito nets (ITNs).

Inclusion criteria

1. Age ≥5 years
2. Body weight <120 kg
3. Documented fever (axillary temperature ≥37.5^{o} C) or history of fever during the previous 48 hours in the absence of another obvious cause of fever, such as pneumonia, otitis media, etc
4. Monoinfection with *P. vivax* with parasitemia between 100 and 200,000 asexual parasites/µl as determined by microscopic examination of thick and thin peripheral blood smears
5. Informed consent from the patient or parent/guardian (for those <18 years), assent from child (ages 7 to 17 years inclusive), patients 5 through 6 years old will not need an assent
6. Willingness on the part of the patient to return to the clinic and/or receive home visits for regular check-ups during the 24-week (168 days) follow-up period
7. Place of residence within 30–45 minutes of study site.

Exclusion criteria

1. Presence of malaria danger signs
    a. Unable to drink
    b. Vomiting (more than twice in the previous 24 hours)
    c. Recent history of convulsions (one or more in the previous 24 hours)
    d. Impaired consciousness
    e. Unable to sit or stand
2. Presence of signs of severe malaria (WHO criteria)
    a. Cerebral malaria (unarousable coma)
    b. Severe anemia (hematocrit <15% or clinical signs) hemoglobin <5 mg/ml) (Note: we will use hemoglobin less than 8 mg/ml as exclusion criteria)
    c. Renal failure (serum creatinine >3 mg/dL or clinical signs)
    d. Pulmonary edema
    e. Hypoglycemia (blood glucose <40mg/dL or clinical signs)
    f. Shock (systolic blood pressure <70 mm Hg in adults; 50 mm Hg in children)
    g. Spontaneous bleeding/disseminate intravascular coagulation
    h. Repeated generalized convulsions
    i. Acidemia/acidosis (clinical signs)
    j. Macroscopic hemoglobinuria
    k. Jaundice
3. Self-reported presence of other underlying chronic or severe diseases (e.g., cardiac, renal, hepatic diseases, HIV/AIDS, tuberculosis, malnutrition, psoriasis)
4. History of hypersensitivity reactions to any of the drugs being tested. Mild itching with CQ is not in itself a criterion for exclusion. This occurrence will be evaluated by the study doctor before excluding the patient for this reason alone.
5. Use of drugs with antimalarial activity in the past 30 days. (Annex D)
6. Current pregnancy (either self-reported being pregnant at enrollment or a positive urine or plasma pregnancy test at time of enrollment), previous pregnancy is not an exclusion criteria
7. Hemoglobin <8 mg/mL
8. G6PD deficiency. This will be a late exclusion criteria as soon as the results of G6PD testing becomes available.

Sample size

Sample size was calculated based on the expected proportion of recurrent infection after treatment of *P. vivax* with CQ and primaquine in the study population. A previous study in Cruzeiro do Sul showed a recurrent infection rate of *P. vivax* at a 6-month follow-up of 30% and prevalence of heterologous isolates, reinfection or relapse by different genotype, from 16.6% of cases (Negreiros, 2016 # 124). We estimate that the rate of recurrent infection after a double dose of primaquine (Group 3) is lower than that of 16.6% (most hypnozoites will be treated at the highest dose), and we estimate the recurrent infection rate in the dose group usual and supervised (Group 2) similar to that found by Negreiros and collaborators (30%). For Group 1, based on discussions with Brazilian Ministry of Health, we estimated that, due to the adherence of patients, this will be 40%, higher than that of Group 2.

Considering the above estimates and using Fisher Exact test, we needed 74 patients in each group to compare prevalence of 10% (Group 3) and 30% (Group 2) (power = 90% and 5% level of significance). Likewise, in order to compare difference of 10% (Group 3) and 40% (Group 1), we need 39 patients in each group. Increasing this minimum sample to 50 patients, which is recommended by the WHO as a minimum sample size for in vivo studies (WHO, 2009 # 40), and adding 30% in all groups to accommodate follow-up losses, we have sample size of 96 patients in Groups 2 and 3, and 65 patients in Group 1. The total number of patients in the study was 257 patients.

Informed consent

Informed consent, parental permission forms (TCLE in Portuguese acronym) and assents (TALE in Portuguese consent) are ways in which the participant confirms his or her participation in a clinical study. The TCLE (Appendices A1) is applicable for participants 18 years of age and older. In the case of participants less than age 18, the legal guardian must sign the TCLE for Parents or Legal Entities (Appendix A2), which serves as parental permission. Oral assent participants aged 7 to 17, inclusive, years will be used (Appendices B1 and B2). In the case of participants less than 18 years old at time of enrolment but who turn 18 during follow-up, we will request informed consent (TCLE) at his/her following visit after the 18th birthday. If patient denies written consent, we will withdrawal him/her at that point.

The objectives and procedures of the study and the rights of volunteers will be explained in detail to potential volunteers in Portuguese. The TCLE (Appendix A1) signed with a blue or black in three copies will be requested from all participants older than 18 years. Permission using the same forms (Appendix A2) will be asked from guardians of participants younger than 18 years of age who are willing to participate. A member of the study team should also sign this document. In addition, a TALE (Appendices B1 and B2) will be

requested orally from patients 7–17, inclusive, years of age, according to the participant's age range. The practice of oral assent rather than its signature is due to the fact that the Ethics Committee of Brazil prefers only one document signed as part of the consent and consent process. Assent will not be required from patients 5 to 6, inclusive, years of age. These documents will be applied by the principal investigator or someone trained by him/her.

In the case of illiterate persons, consent forms and parental permission, as appropriate, shall be read to potential participants and obtained verbally. A digital fingerprint, which is a legal form of documentation of consent in Brazil, will be collected from each patient in the consent and parental permission, which will also be signed by two individuals not directly involved in the study as witnesses. All study participants will receive copies of the consent / assent form.

If the patient or his/her guardian refuses to grant authorization for his / her participation through the signing of the TCLE or oral assent in the case of TALE, this will not entail any detriment to patient care. All participants or their guardians may also withdraw their consent at any time. This does not cause any harm to the patient or their follow-up by the routine health service. In case of changes in the TCLE or TALE after the approval of the original protocol by the committees of ethics of law, these should be submitted to afore mentioned committees and only from then on we can be used with new participants.

A specific consent (Appendix C) will be required for patients over the age of 18 and their caregivers in cases of patients aged 5 to 17, inclusive, years for the storage of remaining samples after the study for use in malaria studies.

Patients will receive contact information cards, including phone numbers for after-hours phone calls from the study team. Consents must be filed minimum for 5 years, along with all other documents related to the clinical study. The study coordination will define where these documents will be stored after the study is completed.

Pregnancy testing

In accordance to what is considered childbearing age in Brazil, all women 10 to 49, inclusive, years old being considered for the study will undergo a plasma or urine pregnancy test.

Enrollment procedures

The nurse in the study team will evaluate the inclusion and exclusion criteria and explain the details of the study. When an eligible patient agrees to participate in the study, he or she will be required to sign the Informed Consent Form (TCLE) and give oral assent, if applicable.

In order to expedite the patient registration procedure, one of the microscopists trained for the study will review the blade stained by the health service and estimate the parasitemia on this slide. At this moment the patient is provisionally decided to include or not a patient while waiting for the coloration and reading of the blade taken by the study team.

Study steps are the following:

- Following the randomization table (Annex G), allocate each patient to one of the study groups using a pre populated randomization table obtained with SAS (SAS Institute, NC) or statistical software. Therefore, patient allocation will be determined in advance to the patient encounter itself.
- A Case Report Form, consisting of demographic and clinical information (Appendix E).
- Patient's body weight and axillary temperature will be measured. Results will be recorded on the Case Report Form.
- On Day 0 (day of admission), all blood samples will be collected by one single venous drawing of approximately 7 ml in a dry collection tub. The nurse, nurse assistant, or the laboratory technician will use the drawing to perform two sets of thick and thin smears to confirm malaria diagnosis and estimate parasitemia, he or she will put one drop of blood in each of the four circles of the Whatman® filter paper card, transfer 5.0 ml to a tube with EDTA for G6PD activity determination, and also hemoglobin level determination.
- Women aged 10 to 49, inclusive, years old, will also receive a pregnancy test, using either urine or a 0.5 mL aliquot of the venous blood.
- We will rely on the microscopist hired for this study to provide the first reading, allowing for patient enrollment and treatment initiation. This professional will undergo a refresher training and competency evaluation by laboratorians from Instituto Evandro Chagas before study initiation. Within 24 hours after the first reading, a second microscopist also trained for this study will read slides, blinded to the first reading results (see below).
- Four drops of blood will be collected on a filter paper, FTA classic cards by Whatman®. This sample will be used for differentiation of reinfection vs. recrudescence and relapse during the follow-up period (on or after Day 4). This collection will be repeated in case of parasite reappearance on or after Day 4.

- Five mL of blood will be collected in an EDTA tube on Day 0 to allow for G6PD activity determination.
- One drop of blood will be used to determine the hemoglobin level on Days 0, 14, and 28. Blood for hemoglobin on Days 14 and 28 will be obtained by fingerprick.
- 2 mL of blood will be collected in an EDTA tube on Day 7 to allow for chloroquine level determination. This collection will be repeated in case of parasite reappearance from Day 7 through Day 28.

Schedule of study procedures

The following chart outlines the procedures and treatment for study subjects:

Tasks	Day 0 (Enrollment)	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28 to Day 168 (monthly, 6 times)	Any other day (after Day 3, if fever)	Day of any recurrent parasitemia (on or after Day 4)
Blood smear	X		X	X	X	X	X	X	X	X
Axillary temperature	X	X	X	X	X	X	X	X	X	X
Focused history and physical exam	X									
Review of symptoms and physical exam	X	X	X	X	X	X	X	X	X	X
Blood for PCR in filter paper	X									X
Blood for G6PD (EDTA-coated tube)	X									
Blood for CQ levels (EDTA-coated tube)					X					X[1]

Hemoglobin level	X					X		X, on Day 28 only, not monthly afterwards		
Check G6PD result		X	X	X	X					
Treatment										
CQ[2] dose	X	X	X							
Primaquine[3] dose										

1. Blood for chloroquine level will only be taken during the first 28 days of follow up.
2. See chloroquine doses on Tables 2 and 3.
3. Primaquine should be initiated as soon as result of G6PD activity is available. Primaquine course should be of at least seven or 14 consecutive days, depending on the study group of the patient with doses adjusted for body weight as recommended (Table 4a and 4b).

Blood smear

A Giemsa-stained thick blood film will be examined at 1,000x to identify parasite species and determine the parasite density. Laboratory refresher training to be provided by colleagues from Instituto Evandro Chagas will cover these procedures. Thick smears should be stained with diluted Giemsa stain (1:50, vol/vol) (or another) for 20 minutes. Thin smears should be stained with diluted Giemsa stain (1:50, vol/vol) (or another) for 30 minutes. Parasite density will be calculated by counting the number of asexual parasites using a hand tally counter against 200 white blood cells (WBCs) in the thick and thin smear, based on a WBC count of 6,000 WBC/μl. The parasite density per microliter will be calculated using the following formula:

$$\text{Parasite density}/\mu l = \frac{\text{number of parasites counted} \times 6{,}000}{\text{number of WBC counted}}$$

If the parasite count is <100 parasites/200 WBC, counting will be continued until 500 WBCs have been counted. If no asexual parasites are found after counting 500 WBCs, the count will continue until 1,000 WBCs are counted. In other words, a total of 1,000 WBCs will be examined before a blood smear is considered negative. Gametocytes will also be counted and number of gametocytes per microliter will be estimated on the same manner.

All blood smears will be examined by two independent microscopists. The first microscopist will be based at the clinic at the Hospital Regional do Jurua and the second at the malaria office at the Santa Casa de Cruzeiro do Sul. This way the two initial readings will be done by professionals in Cruzeiro do Sul. Blood smears with differences in species diagnosis or asexual parasite density of >50% between the two microscopists will be re-examined by a third, independent microscopist at Instituto Evandro Chagas or at the reference laboratory in Cruzeiro do Sul. Asexual parasite density will be calculated by averaging the counts of the two concordant microscopists using geometric mean.

Asexual parasite densities will be calculated by averaging the results of the two concordant microscopists using geometric mean. Gametocitemia will be the arithmetic means of the same two readings. The use of geometric and arithmetic means is decided based on the expected distribution of these values in previous similar studies.

Antimalarial therapy

All treatments will be provided free of charge to patients. CQ (150 mg base) and primaquine (15 mg base and 5 mg of base) tablets will be obtained from reputable sources to guarantee quality. We will use the medication procured by the Brazilian Ministry of Health, which undergo regular quality control testing. All

doses of CQ and primaquine will be administered under supervision of a study team member for Groups 2 and 3. Only CQ doses will be overserved for Group 1. Primaquine treatment will only be started for patients with documented normal activity of G6PD and will be given for seven or 14 consecutive days. Patients with abnormal G6PD activity level will not undergo primaquine treatment, will be excluded from the study and referred for follow up at health facility. This patients will need to be replaced to reach sample size.

Patients will be observed for 30 minutes after treatment for adverse reactions or vomiting. Any subject who vomits during this period will be retreated with the same dose of CQ or primaquine and observed for an additional 30 minutes. If a subject vomits a second time, he/she will be referred for alternative treatment respecting referral policies in Cruzeiro do Sul. Patient will be withdrawn from the study.

Following national recommendations of Brazil, dosing of both CQ and primaquine will be adjusted by weight according to Tables 2 (a and b) and 3 (a and b) for the treatment of 7 or 14 days.

Table 2a. Dosing of CQ and primaquine for *P vivax* malaria treatment (Groups for 7 days) for patients ≤24 Kg.

Weight (kg)	Number of pills per day						
	Day 0		Day 1		Day 2		Additional 4 days
	CQ	Primaquine (5 mg tablet)*	CQ	Primaquine (5 mg tablet)*	CQ	Primaquine (5 mg tablet)*	Primaquine (5 mg tablet)*
5–9 Kg	1/2	1	1/4	1	1/4	1	1/2
10–14Kg	1	2	1/2	1	1/2	1	1
15–24 Kg	1	2	1	2	1	2	2

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.

Table 2b. Dosing of CQ and primaquine for *P vivax* malaria treatment (Groups for 14 days) for patients ≤24 Kg.

Weight (kg)	Peso (kg)	Número de comprimidos por dia							
		Day 0		Day 1		Day 2		Additional 4 days	7 additional days
		CQ	Primaquine (5 mg tablet)*	CQ	Primaquine (5 mg tablet)*	CQ	Primaquine (5 mg tablet)*	Primaquine (5 mg tablet)*	Primaquine (5 mg tablet)*
5–9 Kg	5 a 9 kg	1/2	1/4	1/4	1/4	1/4	1/4	1/4	1
10–14Kg	10 a 14 kg	1	1	1/2	1	1/2	1	1	1 and 1/2
15–24 Kg	15 a 24 kg	1	2	1	2	1	2	2	2

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.

Table 3a. Dosing of CQ and primaquine for *P vivax* malaria treatment for patients >25 kg (Groups for 7 days).

Weight (kg)	Number of pills per day						
	Day 0		Day 1		Day 2		Additional 4 days
	CQ	Primaquine (15 mg tablet)*	CQ	Primaquine (15 mg tablet)*	CQ	Primaquine (15 mg tablet)*	Primaquine (15 mg tablet)*
25–34 Kg	2	1	2	1	2	1	1
35–49 Kg	3	2	2	2	2	2	1
≥50 Kg**	4	2**	3	2**	3	2**	2**

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.
** Patients ≥70 Kgs will have to have the primaquine dose corrected by weight as shown in Tables 4a and 4b.

Table 3b. Dosing of CQ and primaquine for *P vivax* malaria treatment for patients >25 kg (Groups for 14 days).

Weight (kg)	Peso (kg)	Número de comprimidos por dia						
		Day 0		Day 1		Day 2		11 additional days
		CQ	Primaquine (15 mg tablet)*	CQ	CQ	Primaquine (15 mg tablet)*	CQ	Primaquine (15 mg tablet)*
25–34 Kg	25 a 34 kg	2	1	2	1	1	1	1
35–49 Kg	35 a 49 kg	3	1 1/2	2	1 1/2	1 1/2	1 1/2	1 1/2
≥50 Kg**	≥ 50 kg**	4.	2**	3	2**	2**	2**	2**

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.
** Patients ≥70 Kgs will have to have the primaquine dose corrected by weight as shown in Tables 4a and 4b.

In case of patients 70–120 Kgs, primaquine should be taken as a daily dose of 30 mg (2 adult pills) for a number of days according to patient weight (Tables 4a and 4b).

Table 4a. Dosing of primaquine for *P vivax* malaria treatment for patients >70 kg (Groups 1 and 2).

Weight	Number of treatment days with primaquine (two 15 mg tablets per day)*	Total dose (mg)**
70–79 Kg	8	240
80–89 Kg	9	272
90–99 Kg	10	304
100–109 Kg	11	336
110–119 Kg	12	368

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.

** Total dose estimated.

Table 4b. Dosing of primaquine for *P vivax* malaria treatment for patients >70 kg for groups taking 14 days of primaquine (Group 3).

Weight	Number of treatment days with primaquine (two 15 mg tablets per day)*	Total dose (mg)**
70–79 Kg	17	510
80–89 Kg	18	540
90–99 Kg	21	630
100–109 Kg	23	690
110–119 Kg	26	780

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.

** Total dose estimated.

Concomitant treatment

Using standard healthcare practices in Brazil, study team members or clinic personnel will administer supportive treatment to subjects as necessary. This include paracetamol, which will be given for axillary temperatures >38.0^{o} C and parents/guardians will be instructed in the use of tepid sponging for children. Other conditions that may require additional treatment or evaluation will be referred to the reference center for specific follow up.

Follow-up procedures

Subjects will be asked to return to each one of the study sites on Days 1, 2, 3, 7, 14, 21, 28, and every four weeks until Day 168 (24 weeks mark). On those visits a clinical assessment and blood collection via finger prick (except for Day 7, when venous puncture will be made) will take place. We will not collect blood on Day 1. In case patients cannot or do not wish to return to the health facility, arrangements will be made for patients to be visited at home by a study team member: the nurse during the period he/she has to take CQ, or the nurse assistant afterwards. All CQ doses will be given as directly observed therapy. Doses of primaquine will be observed only in patients in Groups 2 and 3. Tolerance of +/- 1 day will be allowed for visits on Day 14 or after.

At all times, patients and any member of the study staff can count on the physician part of this study to evaluate patients and manage medical situations that might arise. If unexpected situations arise, patients will be referred for treatment at routine services in Cruzeiro do Sul, including emergency services and hospitalizations if needed. A schedule with dates of follow-up visits and contact information of study staff will be provided to participants.

Since only ambulatory patients with uncomplicated malaria and a drug with well-known safety profile is being assessed as part of this protocol, there is no need for daily follow-up after treatment is finished. Subjects and parents/ guardians (in the case of participants less than 18 years old) will be encouraged to reach study team for further assessment and/or treatment at any point during follow-up at which the subject is perceived to be ill. Telephone numbers and address of key study personnel will be provided to all participants at the time of enrollment. Compensation for transport to and from the clinic can also be provided, for those patients that incur on transportation costs to come for the visits, or we can arrange a study staff to visit you at your home or pick up you up for the visit.

When the research subject is female and pregnancy occurs before the end of the treatment with chloroquine and primaquine, there will be interruption of their participation in the research and it will be referred to the

assistance of the municipality regarding the care of pregnant women with malaria. If a patient becomes pregnant after treatment with chloroquine and primaquine, the participant will remain in the study.

Chloroquine blood levels

To aid in the interpretation of treatment failures during the first 28 days of follow-up (reappearance of parasitemia between Days 4 and 28), blood levels of CQ and its major metabolite desethylchloroquine (DCQ) will be measured on Day 7 and at the time of any reappearance of parasitemia from Day 7 to Day 28. A blood level of CQ plus DCQ >100 mg/ml, in the presence of *P. vivax* parasites in the blood is highly suggestive of CQ resistance.

To permit this assessment, 2 ml of blood will be taken by venipuncture from all patients on Day 7 and again at the time of any recurrent parasitemia on or before Day 28, in an EDTA-coated tube and stored in a cold chamber at 4° C. Chloroquine and DCQ levels will be measured by high-performance liquid chromatography (HPLC) by reference laboratories at laboratories in Brazil (Patchen, Mount et al. 1983).

Molecular analysis

We plan to use the microsatellite markers described by Imwong et al. (Imwong, Sudimack et al. 2006) for *P. vivax* in this study. In brief, in case of parasitemia appearing on or after Day 4, blood collected on the day of the parasite reappearance will be processed in pair with sample collected on Day 0. Polymerase chain reaction (PCR) aiming to amplify the microsatellites previously described will be conducted. Fluorescent-labeled PCR products will be analyzed and when the size of an allele was at least different for more than two base pairs from other alleles, it will be considered a different allele. This work will be conducted by collaborators at Instituto Evandro Chagas in Brazil with support from CDC/Atlanta, but we can extract the DNA from the parasite contained in the collected material and send this aliquot to the CDC laboratories in Atlanta if necessary for processing and quality control.

During the consent procedure, we will ask patients and their guardians to keep samples for future malaria studies (Annexes C1, C2, C3). The sample collected represents the minimum necessary for the purposes described in this study and the remaining aliquots will be minimal. We will not increase volumes of samples in order to have leftovers for stocking. In the case of non-concurrence with leftover storage, any leftover filter paper will be destroyed 2 years after the study and even during this period will only be used for assays related to this study and already described in this protocol, such as in need of repeating assays to confirm results.

Outcome measures

The efficacy and lack of efficacy of the drug being evaluated will be based on an assessment of the parasitologic and clinical outcomes recommended by WHO (WHO 2009). These definitions are valid only for the initial 28 days of follow-up. These are:

- Early Treatment Failure (ETF), if patient develops one of the following conditions during the first three days of follow-up:
    - Development of danger signs or severe malaria on Days 1, 2, or 3, in the presence of asexual parasitemia;
    - Asexual parasitemia on Day 2 >Day 0 count in disregard to presence of fever;
    - Asexual parasitemia on Day 3 in the presence of axillary temperature ≥37.5°C;
    - Asexual parasitemia on Day 3 >25% of Day 0 count.
- Late Treatment Failure (LTF), if patient develops one of the following conditions during the follow-up period from Day 4 to 28:
    - Late clinical failure (LCF)
        - Development of danger signs or severe malaria in the presence of asexual parasitemia on any day from Day 4 to Day 28, without previously meeting any of the criteria of ETF, or
        - Axillary temperature ≥37.5°C (or history of fever), in the presence of asexual parasitemia on any day from Day 4 to 28, without previously meeting any of the criteria of ETF.
    - Late parasitological failure (LPF)
        - Presence of asexual parasitemia on any day between Days 7 and 28 and axillary temperature <37.5ºC without previously meeting any of the criteria of ETF or late clinical failure.
- Adequate Clinical and Parasitological Response (ACPR), if patient shows the following conditions during the 28-day follow-up period:
    - Absence of asexual parasitemia between Days 14 and 28 without previously meeting any of the criteria of ETF or LTF.

The resurgence of malaria symptoms with proven parasitemia between Day 28 and Day 168 will be classified as Failure in Extended Follow-up. PCR results will be used to elucidate whether these cases are due to homologous isolates (same genotypic profile between Day 0 isolate and day of reinfection) or heterologous (different genotypic profiles between Day 0 isolate and day of reinfection).

We will perform analysis per protocol and intention to treat, and plan to report both on study reports. In all cases of therapeutic failure, patients will be withdrawn from the study and referred to the routine treatment service according to Brazilian Health Ministry regulations.

Loss to follow-up

Loss to follow-up occurs when, despite all reasonable efforts, an enrolled patient cannot be found. Include failure to find a patient in the community after he or she misses a scheduled follow-up visit. Except for the period patient is taking CQ, it is acceptable for patients to be seen on the day before or day after the schedule visit before he/she is considered loss of follow-up. In addition, any patient who leaves the study area is also considered loss of follow-up. Patients who are lost to follow-up should be distinguished from those who voluntarily leave the study. If a patient is lost to follow up during the period she/he is receiving medications, all efforts will be made by the study team to locate patient and guarantee he/she takes all doses.

Withdrawal from study

A study patient (or a study patient's parent or guardian) who decides not to participate any further in the study is referred to as a voluntary withdrawal. An example of a cause for involuntary withdrawal would development of a concomitant illness that would interfere with the clear interpretation of study outcomes.

Participation interruption

In some instances, a participant may be removed from the study because of an event that does not allow for continued accurate interpretation of response to treatment. Examples include missed treatment dose, detection of a mixed infection during follow-up, or a credible report of additional antimalarial drug use outside the study protocol (such as self-medication).

Subjects meeting any of the following criteria will be withdrawn from follow-up (include reasons for voluntary and involuntary withdrawal):

1. Withdrawal of consent
2. Failure to complete the CQ treatment as prescribed. We will tolerate one day for the primaquine doses
3. Low levels of G6PD activity, these patients will require another patient to be admitted as a replacement
4. Persistent vomiting during the treatment with either CQ or primaquine
5. Erroneous inclusion of a patient outside of the inclusion/exclusion criteria

6. Severe side-effects (see below)
7. Occurrence during the follow-up of concomitant disease that would interfere with a clear classification of the treatment outcome
8. Need for or receipt of blood transfusion
9. Detection of another malaria species infection during the follow-up. These patients will be referred for appropriate treatment.
10. Antimalarial (or antibiotics with antimalarial activity) treatment administered by a third party or self-medication with antimalarial (or antibiotics with antimalarial activity) (doxycycline, clindamycin, etc)
11. Failure to attend scheduled visits within the +/- 1 day of tolerance on or after Day 14 Severe malaria occurring after enrollment
12. Misclassification of a patient due to a laboratory error (parasitemia) leading to the administration of the rescue treatment

The cause of withdrawal from the study or loss to follow-up will be recorded in the Case Report Form. In case of items from 1 to 6 listed above, patients will be excluded from all analysis in the other instances, we will censor patient data until the day of the event.

Adverse events

Occurrence of side-effects associated with the study drugs or procedures will be closely monitored and recorded on appropriate form (Annex F). Patients with side-effects necessitating medical treatment or hospitalization will be referred for appropriate treatment. Study participation may be discontinued if it is established that study procedures cannot be safely continued, such as study drug allergic events during treatment or G6PD deficiency, for that study participant. Of note, patients with skin rash or any severe allergic reaction will be withdrawn from the study, while patients experiencing only itching will not be withdrawn from the study. In addition, adverse events that are unexpected, likely related to the research study, and that place the participants at greater risk of harm will be reported to the local and CDC IRBs in accordance with required reporting schedules after their occurrence, i. e., in no later than 2 working days of CDC awareness.

Alternative treatment

Patients with intolerance to the therapy, therapeutic failure, or reinfection with *P. viva*x infection will be referred to reference health facility with alternative treatment. Alternative treatment will be decided by attending physician.

Limitations

The findings of this study are limited to the catchment area of the study health facilities in Northwest Acre state. We acknowledge that results may not be representative of the whole country. In addition, only consenting participants will be included, which may introduce selection bias in the study. These points will be taken into consideration for interpretation of results.

Ethical considerations

Consent procedure: Informed consent or permission will be asked from adult participants and child's parents or guardian (in case of <18 years old) for those who meet the study inclusion criteria. The age of majority in Brazil is 18 years (Appendices A1 and A2). For participants 7 to 17, inclusive, years old, we will also ask for his/her assent (Appendices B1 and B2); assent will not be asked for children 5 through 6 years old. In case of illiterate participants, we will read the consent/ assent and two witnesses not associated with the study will be asked to sign the forms; and the participant's or guardian's fingerprint, which is a legal form of documentation of consent in Brazil, will be added to the respective forms. Signed consent forms will be kept separate from case report forms. The study details, participant benefits, and possible risks will be explained in Portuguese, the national language in Brazil. All patient information will be kept confidential. Unique numerical identifiers, and not personal identifiers, will be used for data entry and analysis.

Any patients who decide not to enter the protocol will be evaluated as usual by the healthcare facility personnel as customary with no prejudice to the patient or his/her family. In case of confirmed malaria, the patient will be treated in accordance to the guidelines from the National Malaria program in Brazil.

Procedure and risks: There is an inherent minimal risk associated with the finger sticks and venipunctures, which are to be performed as part of this study. The patient may experience a brief moment of discomfort and/or fear during the finger stick or venipuncture and the puncture area may get infected or bruised. We estimate that a total of 10 finger pricks will be needed for each study participant, in addition two blood drawing, one of 7 ml on Day 0 and the other of 2 ml on Day 7, will be taken from the patients. It may be required to collect additional blood in case of suspicion of recurrent infection. Blood collections were reduced to the minimal necessary and we will make sure trained phlebotomists and/or laboratory technicians perform those to minimize risks and discomfort. In addition, to reduce risks, we tried to combine blood collections to be done on the same day in a way to collect blood with just one of the procedures. Separate consent/assent (Annexes C1, C2, C3) will be required for storage of remaining material from the samples. If

no authorization is given, all material from that patient will be destroyed upon completion of the analysis of the study, estimated at two years after its closure. This material will only be used for malaria assessments.

A minority of patients may experience drug side effects. The side effects most commonly associated with CQ are nausea, vomiting, rash, headache, dizziness, urticaria and abdominal pain (Petersen, 2000 # 42). On rare occasions, higher doses of CQ have been associated with aplastic anemia (Nagaratnam, 1978 # 59). Commonly reported side effects of primaquine include hemolysis in patients with G6PD deficiency and gastrointestinal symptoms (Fernando, 2011 # 60). We note that the 14-day primaquine group is receiving a higher dose of the drug than recommended by the Brazilian malaria co-ordination, but it is within the WHO recommendations (WHO, 2015).

Benefits: The participants in this study have some direct and indirect benefits. Patients will receive supervised treatment for malaria and will be closely monitored to check the efficacy of it. In addition, participants will be examined and referred for treatment of other concurrent illnesses. At each visit, the patient or his/her guardian will be informed of his/her physical status and the medical procedures that will take place. The patient's health condition will be closely monitored during the duration of the study. Patients will be encouraged to return for the follow-up visit and in case they do not return, a member of the investigation team will perform a home visit. Indirectly, this study will also allow a better understanding of the efficacy of *P. vivax* malaria treatment in the Amazon region of Brazil. This will ensure policies in Brazil are in place to reduce the morbidity and mortality of malaria.

Patient selection: Malaria affects both children and adults in Brazil. The methods to monitor malaria and evaluate national policies published by the WHO recommends enrollment of patients that are representative of the population (WHO 2009). For this reason, adults and children ≥5 years old will be enrolled in the present study.

Confidentiality: All study forms and materials will be kept in locked cabinets and locked rooms. Patients' identifiers will be available to Cruzeiro do Sul study personnel only to allow for patient follow-up. Electronic databases will be password protected and will not contain personal identifiers. In spite of all of these precautions to maintain confidentiality of the data, it is possible that the confidentiality could be compromised. We will make our best efforts to avoid dissemination of any personal information.

Experimental products or procedures: No new therapies, products, or procedures will be used in this study. The use of CQ and primaquine is a practice in Brazil and routinely recommended for treatment of *P.*

vivax infections. We will, however, evaluate a higher dose of primaquine that than the one recommended in Brazil, but this dose is already recommended by WHO (Negreiros, 2016 #124)(WHO, 2015 #125).

Data management

All forms and files will be kept at a safe place in Cruzeiro do Sul, Acre state, Brazil. File cabinets and rooms where data are stored will be protected by keys and locks and only study investigators will have access to them. Personal identifiers are collected mainly for follow-up reasons, i.e. to allow for patient to be located in case of no show to follow up appointments. This information will not be entered on electronic databases, nor will it be used during the analysis phase.

Case report forms and other study information will be entered in an electronic database in Microsoft Access or equivalent, which will be under responsibility and ownership of the field investigators in Cruzeiro do Sul. No personal identifier will be entered in the electronic databases. A copy of the database, which by design will not contain any personal identifier, will be shared with co-investigators at CDC and IEC for assistance with analysis. Statistical programs, such as Epi-Info (Centers for Disease Control and Prevention, Atlanta, GA) and SAS (SAS Institute, NC), will be used for data management and analysis.

Statistical analysis

Data will be analyzed using two methods: first, by survival analysis where all enrolled patients (including those who were withdrawn from the study or who were lost to follow-up) will be included; second, by the traditional method where patients excluded from the study or lost-to-follow-up during the course of the project are not included.

The number and proportion of subjects who have treatment failures and ACPRs, as defined above, by Day 28 will be calculated. This is the main and primary outcome measure for treatment efficacy. Potential risk factors (e.g., age, initial parasite density, etc.) will be assessed for an association with the probability of a therapeutic failure and the time to therapeutic failure. Percentages of *P vivax* infections from Day 29 to Day 168 will be evaluated.

Dissemination of results

All results will be shared with the Ministry of Health of Brazil and its partners. The Ministry of Health will make the final determination as to how the results should be presented to the regional and local health officers. Results will also be submitted to peer-reviewed scientific journals and presented at national and international meetings.

At the time participants complete the study (Day 168), or earlier if they have had a therapeutic failure, they will be informed verbally by study staff of the outcome/results of their treatment. At enrollment and at the end the study, the investigation team will inform patients about points of contact at the healthcare facility and Ministry of Health levels to obtain the study findings. The Ministry of Health will determine such points of contact.

Budget

The funds for this study will come from CDC as part of AMI/RAVREDA and will be used to hire project coordinator, nurses, nurse assistants, and microscopists. The budget also accounts for laboratory testing to take place in Cruzeiro do Sul. Other study-related costs, such as study drugs, sample collection supplies, and office supplies, are not included because those refer mainly to materials and consumables that will be purchased directly by partner institutions and shipped to study site.

Table 5. Budget for field costs

Expense	Cost (R$)	Number of months	Total Cost (R$)	Total Cost (US$)	Justification
Principal investigator	1,000.00 per month	12	12,000.00	4,000.00	Study coordination
Nurse	3,200.00 per month	12	38,400.00	12,800.00	This professional will have direct oversight of teams
Nurse	2,600.00 per month	12	31,200.00	10,400.00	This professional will have direct oversight of teams
Nurse assistant	1,000.00 per month	12	12,000.00	4,000.00	Follow up visits
Nurse assistant	1,000.00 per month	12	12,000.00	4,000.00	Follow up visits during enrolment period (8 months only)
Microscopist	1,000.00	12	12,000.00	4,000.00	
Microscopist	1,000.00	12	12,000.00	4,000.00	Part-time
G6PD testing	100.00 per sample	250	25,000.00	8,333.33	
Laboratory supplies (needles, syringes, tubes, etc)	12,000.00	NA	12,000.00	4,000.00	This will be provided at no cost to study team by partner organization.
Total			164,250.00	56,393.67	

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Appendix A1: Consent Form. Informed Consent Form for Patients Aged 18 years and older. (Flesh-Kincaid 7.6)

Project: Efficacy of Three Regimens of Chloroquine and Primaquine for the Treatment of Plasmodium vivax Malaria in Cruzeiro do Sul, Acre, Brazil

Purpose

The Brazilian Ministry of Health is working with the Municipal Health Secretariat, State Department of Health of Acre, the Evandro Chagas Institute and the Centers for Disease Control and Prevention (CDC) in the United States of America. They are working on an evaluation of the malaria treatment in Cruzeiro do Sul, Acre. Malaria is a disease caused by a parasite that infects cells in your blood.

The goal of this research is to see if the drugs used to treat malaria are working. We will evaluate treatment with chloroquine and primaquine for vivax malaria. This is the kind of malaria most common in Brazil.

This evaluation is being coordinated by Dr. Suiane Negreiros do Valle. She is a physician at the State Department of Health of Acre, and she works at the Hospital Regional do Jurua.

We intend to include a total of 257 people aged 5 years and over in this assessment.

If you agree, we intend to follow you during the malaria treatment and for six months after the malaria treatment. We will collect blood samples during this time. This is to know if you are free of malaria. If you choose not to take part, you will be treated in the same place with the same medications without any loss or penalty.

Getting Started

After the positive result for malaria vivax, we will place you in one of three groups in this study. The decision to which group you will enter will be random, which means it is by chance like flipping a coin. One group will receive the three-day treatment with chloroquine under supervision and will take primaquine treatment at home for seven days. This is the recommended practice by the Ministry of Health. Another group will receive chloroquine treatment for three days and primaquine for seven days under supervision. The third group will also receive complete treatment under supervision but primaquine will be given for a total of 14 days under supervision. In this last group, the total dose of primaquine is higher than the one currently recommended in Brazil, but it is within the recommendations by the World Health Organization.

We will ask you some questions to know your general health and condition of your current illness. We will ask you some questions to know your age and for how long you have been sick. You do not have to answer any questions you do not want to answer. In addition, you can decide at any time that you no longer want to take part in this survey without penalty. Your care at the health clinic will not change in quality if you choose not to respond or do not want to take part in this study. We also want to examine you and collect blood samples to confirm that you have malaria. We will collect a small amount of blood (about 7 ml, a tea spoon and a half) of blood from the vein in your arm. This blood will be used to confirm the diagnosis of malaria. It will also confirm you have an active substance in your blood to be able to tolerate the treatment with primaquine. We will do these tests right here in Cruzeiro do Sul. After these initial tests, the blood will be stored for other laboratory tests to compare the response to the treatment in case you have another malaria episode during the follow-up. These new tests will be conducted at the Evandro Chagas Institute in Belém do Pará. A small part of the parasite material may be sent to the Centers for Disease Control and Prevention in the USA.

After blood collection and confirmation of the diagnosis, we will immediately start treatment with chloroquine. The dose of medication and the number of days of treatment depend on your weight. For the chloroquine dose, we follow the guidelines and recommendations of the Ministry of Health of Brazil. This means this medication is the same one that you would be receiving even if you did not take part in this study. Chloroquine should be taken for a total of three days. We will ask you to come to this place to receive treatment from our hands. This is true for all three study groups. We can go to your home if you prefer. Chloroquine is well tolerated. It can cause nausea and some pain in the belly within the first few hours after taking in some cases. There is also the risk of allergy to the medicines. At each of these visits, three more visits after today, we need to collect four drops of blood from the tip of your finger and also give you chloroquine for two more days. We will not need to collect blood from you tomorrow.

When we get the results of your laboratory tests, we will start primaquine treatment. This drug is complementary to chloroquine treatment. It is also routinely indicated by the Brazilian Ministry of Health. It is usually taken for a total of seven days. As part of this study, you may be asked to take it for 14 days. This , which is different than the recommendation in Brazil, but within the limits recommended by the World Health Organization. Depending on your weight, you may need to take primaquine for a few more days. You will need to take the medicine either in front of the health professional or at home, depending on the study group. Since it will not be necessary to collect blood all these days, you can decide if you prefer to come here. We can also come to your house to give the drug.

Treatment and evaluation will be free of charge. The interview, examination, and blood collection will take about 30 minutes on the first visit. It will take about 15 minutes on the next visits. We recommend you sleep under mosquito net with insecticide. This will protect you from malaria.

Follow-Up

After treatment of malaria, we would like to visit you on a weekly basis for the first 28 days after malaria diagnosis. Afterwards, we would like to visit you once a month until six months are complete. It is important that you can take part in those visits. Please let us know if you think that would not be possible. During those visits, we will check your health and collect two drops of blood from your finger. This is to see whether the malaria has been treated. If the malaria comes back, we will refer you for treatment. The results of those tests are available the same day. We will let you know the results. On the seventh day of follow-up, we need to collect a blood sample of more or less 2 mL (less than a teaspoon) from the vein in your arm. This is to measure the levels of chloroquine. In total, there will be 10 samples from the fingertips and two blood samples from the vein in your arm. We will give you a card with the visit scheduling.

For women, we will perform a urine or blood pregnancy test at the beginning of the study for women less than 50 years old. If you are a woman of any age and become pregnant while taking the medication, we will need to stop your participation. You will be referred for treatment of malaria by a reference service here in Cruzeiro do Sul. If you become pregnant after you have finished the drugs, we will follow you as planned.

If you have any health problems or have any questions, please contact Dr. Suiane at the Hospital do Jurua in Cruzeiro do Sul. You can also contact the person who is seen you today here or at the Sala de Estudos em Malaria na Santa Casa. A member of the team will be able to assess you even if it is not the day of your visit at the clinic. The address of the hospital and sala de Estudos are:

Hospital Regional do Juruá	Santa Casa de Misericórdia
Rua 25 de agosto, 5121	Rua Lauro Muller, 473
BairroAeroporto Velho	Bairro Manoel Terças
	Sala ao lado da CERIMAGEM
Phone: (XX) XXXX XXXX	(XX) XXXX XXXX

Risks and Discomfort

There is some discomfort and a small risk if you decide to take part in this study. You may have minimal pain when the blood is collected. Our team is well trained to avoid this. In rare cases, there occurs light

bleeding or bruising. If any injury occurs, we will take steps for you to receive the proper care. We think it is very unlikely you will have any harm.

Benefits

There will be direct and indirect benefit for you upon taking part in this study. As part of this study, we will closely monitor you with respect to your disease. So, we will be able to offer you any additional treatment very quickly if you need it. Your participation will also assist the Brazilian malaria program to know if the malaria drugs are still working.

Compensation

You will not receive any compensation for participating in this study. However, we can reimburse you for food and transportation costs to attend the clinic visits. If you need to be hospitalized, this will be done at a facility of the public health service in Brazil free of charge for you. Under the care of the study physician.

Confidentiality

We will keep your information confidential to the full extent permitted by the law. Your name will be used only during the time you come for your appointment or we visit your house. We will not share your name with people outside of the study group or outside Cruzeiro do Sul. None of the reports from this study will contain your name. Patient's forms will be kept in locked cabinets. Your personal information will not be used for any purpose during this study.

Contact Information

If you have any questions or feel you have been harmed by being in the study, contact:

- Dr. Suiane Negreiros do Valle, general coordinator of the project in Cruzeiro do Sul, phone (XX) XXXX XXXX.
- Dr. Paola Marchesini, researcher, responsible for the follow-up of this study with the coordination of the Brazilian Malaria Program in Brasilia, phone (XX) XXXX XXXX.

You can contact Dr. Arnaldo (information below) if you have any questions. She is available to answer questions about your rights as a participant. She is able to explain your rights as a patient in this study. She is not directly involved in this study, but her role is to make sure your, rights are respected.

Arnaldo Jorge Martins Filho

Coordenador do Comitê de Ética em Pesquisa do Instituto Evandro Chagas - CEP/IEC
Rodovia Br - 316, km 7 s/n
67.030-000 Levilandia
Ananindeua, Pará, Brasil

Phone: (XX) XXXX XXXX

Voluntary participation, refusal and withdrawal

Several aspects of this study are very important. Remember that:

- You are free to decide if you want to take part in this study or not. If you decide not to take part, you will receive the necessary free routine care and treatment, including for malaria. You will not incur any penalty or loss for not taking part.
- You are free to withdraw from this study at any time. That fact will not affect your treatment or the care received. You will still receive the routine free treatment for malaria.
- In some situations, if you develop side effects to the treatment or you get malaria, we may need to withdraw you from the study.
- There is no cost to you for taking part in this study.
- You may let me know now if you have any questions with respect to the study. You can also ask questions about your participation.

Declaration of Consent

Upon signing or providing a fingerprint indicating legal consent for the appropriate declaration, I agree that:

- I have read this form or the form was read to me by someone else
- I was able to ask questions about the study. My questions have been answered
- I agree in a voluntary manner to participate in this study
- I was informed that I have the right to withdraw from the study at any time. That will not affect the care given to me.

Study participant's name: _____ Date _____

_____

Participant's signature

Right thumb fingerprinting

I bore witness to the informed consent and that it was voluntary. I can also verify that the participant or responsible party was informed of the details, risks and benefits of the assessment, and had the opportunity for their questions to be answered.

_____ _____

Signature of Witness 1 Date

_____ _____

Signature of Witness 2 Date

Declaration by investigator

I have adequately read, or born witness to the exact reading of the consent form, to the participant and the individual had the opportunity to ask questions. I confirm that the individual freely gave their consent.

Printed name of the member of the assessment team _____

Signature of the member of the assessment team _____

Note: Three copies will be made of this consent. One will remain with the patient or individual responsible for him or her, another will remain on file at the institution where the research takes place, and the third will remain with the coordinator of this assessment.

Appendix A2: Parental Permission Form for Parents of Patients Aged 5 Through 17 years. (Flesh-Kincaid 7.6)

Project: Efficacy of Three Regimens of Chloroquine and Primaquine for the Treatment of Plasmodium vivax Malaria in Cruzeiro do Sul, Acre, Brazil

Purpose

The Brazilian Ministry of Health is working with the Municipal Health Secretariat, State Department of Health of Acre, the Evandro Chagas Institute and the Centers for Disease Control and Prevention (CDC) in the United States of America. They are working on an evaluation on malaria treatment in Cruzeiro do Sul, Acre. Malaria is a disease caused by a parasite that infects cells in your blood.

The goal of this research is to see if the drugs used to treat malaria are working. We will evaluate treatment with chloroquine and primaquine for vivax malaria. This is the kind of malaria most common in Brazil.

This evaluation is being coordinated by Dr. Suiane Negreiros do Valle. She is a physician at the State Department of Health of Acre, works at the Hospital Regional do Jurua.

We intend to include a total of 257 people aged 5 years and over in this assessment.

If you agree, we intend to follow your child during the malaria treatment and for six months after the malaria treatment. We will collect blood samples during this time. This is to know if your child is free of malaria. If you choose not to take part, your child will be treated in the same place with the same medications without any loss or penalty.

Getting Started

After the positive result for malaria vivax, we will place your child in one of three groups in this study. The decision to which group your child will enter will be random, which means it is by chance like flipping a coin. One group will receive the three-day treatment with chloroquine under supervision and will take primaquine treatment at home for seven days. This is the recommended practice by the Ministry of Health. Another group will receive chloroquine treatment for three days and primaquine for seven days under supervision. The third group will also receive complete treatment under supervision but primaquine will be given for a total of 14 days under supervision. . In this last group, the total dose of primaquine is higher than the one currently recommended in Brazil, but it is within the recommendations by the World Health Organization.

We will ask you some questions to know his/ her general health and condition of his/her current illness. We will ask you some questions to know his/her age and for how long your child has been sick. You do not have to answer any questions you do not want. In addition, you can decide at any time that you no longer want to take part in this survey.

Your child's care at the health clinic will not change if you choose not to respond or do not want to take part in this survey. We also want to examine your child and collect blood samples to confirm that he/ she has malaria. We will collect a small amount of blood (about 7 ml, a tea spoon and a half) of blood from the vein in your child's arm. This blood will be used to confirm the diagnosis of malaria. It will also confirm your child has an active substance in his/ her blood to be able to tolerate the treatment with primaquine. We will do these tests right here in Cruzeiro do Sul. After these initial tests, the blood will be stored for other laboratory tests to compare the response to the treatment in case your child has another malaria episode during the follow-up. These new tests will be conducted at the Evandro Chagas Institute in Belém do Pará. A small part of the parasite material may be sent to the Centers for Disease Control and Prevention in the USA.

After blood collection and confirmation of the diagnosis, we will immediately start treatment with chloroquine. The dose of medication and the number of days of treatment depend on your child's weight. For the chloroquine dose, we follow the guidelines and recommendations of the Ministry of Health of Brazil. This means this medication is the same one that your child would be receiving even if she/ he did not take part in this evaluation. Chloroquine should be taken for a total of three days. We will ask you to come to this place to receive treatment from our hands. This is valid for all three study groups. We can go to his/ her home if you prefer. Chloroquine is well tolerated. It can cause nausea and some pain in the belly within the first few hours after taking it in some cases. There is also the risk of allergy to the medicines. At each of these visits, three more visits after today, we need to collect four drops of blood from the tip of your child's finger and also give him/ her chloroquine for two more days. We will not need to collect blood from him/ her you tomorrow.

When we get the results of your child's laboratory tests, we will start primaquine treatment. This drug is complementary to chloroquine treatment. It is also routinely indicated by the Brazilian Ministry of Health. It is usually taken for a total of seven days. As part of this study, your child may be asked to take it for 14 days. This, which is different than the recommendation in Brazil, is within the limits recommended by the World Health Organization. Depending on his/ her weight, she/ he may need to take primaquine for a few

more days. He/ she will need to take the medicine either in front of the health professional or at home, depending on the study group. Since it will not be necessary to collect blood all these days, you can decide if you prefer to come here. We can also come to your child's house to give the drug.

Treatment and evaluation will be free of charge. The interview, examination, and blood collection will take about 30 minutes on the first visit. It will take about 15 minutes on the next visits. We recommend your child sleep under mosquito net with insecticide. This will protect him/ her from malaria.

Follow-Up

After treatment of the malaria, we would like to visit your child on a weekly basis for the first 28 days after malaria diagnosis. Afterwards, we would like to visit your child once a month until six months are complete. It is important that you can take part in those visits. Please let us know if you think that would not be possible. During those visits, we will check his/ her health and collect two drops of blood from his/ her finger. This is to see whether the malaria has been treated. If the malaria comes back, we will refer your child for treatment. The results of those tests are available the same day. We will let you know the results. On the seventh day of follow-up, we need to collect a blood sample of more or less 2 mL (less than a teaspoon) from the vein in his/her arm. This is to measure the levels of chloroquine. In total, there will be 10 samples from the fingertips and two blood samples from the vein in your arm. We will give you a card with the visit scheduling.

If your child is a girl aged more than 10 years old, inclusive, we will perform a urine or blood pregnancy test at the beginning of the study. If your child of any age becomes pregnant while taking the medication, we will need to interrupt your participation. Your child will be referred for treatment of malaria by a reference service here in Cruzeiro do Sul. If she becomes pregnant after you have finished the drugs, we will follow your child as planned.

If you or your child have any health problems or have any questions, please contact Dr. Suiane at the Hospital do Jurua in Cruzeiro do Sul. You can also contact the person who is seen you today here or at the Sala de Estudos em Malaria na Santa Casa. A member of the team will be able to assess you and your child even if it is not the day of your child's visit at the clinic. The address of the hospital and sala de pesquisa are:

Hospital Regional do Juruá
Rua 25 de agosto, 5121
BairroAeroporto Velho

Santa Casa de Misericórdia
Rua Lauro Muller, 473
Bairro Manoel Terças

		Sala ao lado da CERIMAGEM
Phone:	(XX) XXXX XXXX	(XX) XXXX XXXX

Risks and Discomfort

There is some discomfort and a small risk if you decide to take part in this study. Your child may have minimal pain when the blood is collected. Our team is well trained to avoid this. In rare cases, there occurs light bleeding or bruising. If any injury occurs, we will take steps for your child to receive the proper care. We think it is very unlikely your child will have any harm.

Benefits

There will be direct and indirect benefit for your child upon taking part in this study. As part of this study, we will closely monitor your child with respect to his/ her disease. So, we will be able to offer your child any additional treatment very soon. His/ her participation will also assist the Brazilian malaria program to know if the malaria drugs are still working.

Compensation

You will not receive any compensation for participating in this study. However, we can reimburse you for food and transportation costs to attend the clinic visits. If your child needs to be hospitalized, this will be done at a facility of the public health service in Brazil free of charge for you and your child. Under the care of the study physician.

Confidentiality

We will keep your child's information confidential to the full extent permitted by the law. His/ her name will be used only during the time she/he comes for her/his appointment or we visit your child's house. We will not share your child's name with people outside of the study group or outside Cruzeiro do Sul. None of the reports from this study will contain your child's name. Patient's forms will be kept in locked cabinets. Her/ his personal information will not be used for any purpose during this study.

Contact Information

If you have any questions or feel your child has been harmed by being in this study, contact:

- Dr. Suiane Negreiros do Valle, general coordinator of the project in Cruzeiro do Sul, phone (XX) XXXX XXXX.

- Dr. Paola Marchesini, researcher, responsible for the follow-up of this study with the coordination of the Brazilian Malaria Program in Brasilia, (XX) XXXX XXXX.

You can contact Dr. Arnaldo (information below) if you have any questions. She is available to answer questions about your child's rights as a participant. She is able to explain your rights as a patient in this study. She is not directly involved in this study, but her role is to make sure your and your child's rights are respected.

Arnaldo Jorge Martins Filho
Coordenador do Comitê de Ética em Pesquisa do Instituto Evandro Chagas - CEP/IEC
Rodovia Br - 316, km 7 s/n
67.030-000 Levilandia
Ananindeua, Pará, Brasil
Phone: (XX) XXXX XXXX

Voluntary participation, refusal and withdrawal

Several aspects of this study are very important. Remember that:

- You are free to decide if you want your child to take part in this study or not. If you decide not to take part, he/ she will receive the necessary free routine care and treatment, including for malaria. You or your child will not incur any penalty or loss for not taking part.
- You are free to withdraw from this study at any time. That fact will not affect your child's treatment or the care received. He/ she will still receive the routine free treatment for malaria.
- In some situations, if your child develops side effects to the treatment or she/he gets malaria, we may need to withdraw your child from the study.
- There is no cost to you or your child for taking part in this study.
- You may let me know now if you have any questions with respect to the study. You can also ask questions about your child's participation.

Declaration of Consent

Upon signing or providing a fingerprint indicating legal consent for the appropriate declaration, I agree that:

- I have read this form or the form was read to me by someone else
- I was able to ask questions about the study. My questions have been answered
- I agree in a voluntary manner to participate in this study

- I was informed that I have the right to withdraw from the study at any time. That will not affect the care given to me.

Study participant's name: _____ Date _____

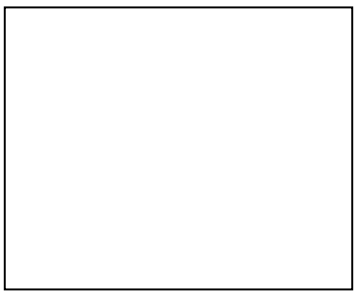
_____

Guardian's name

_____

Guardian's signature

Right thumb fingerprinting



I bore witness to the informed parental permission and that it was voluntary. I can also verify that the participant or responsible party was informed of the details, risks and benefits of the assessment, and had the opportunity for their questions to be answered.

_____ _____

Signature of Witness 1 Date

_____ _____

Signature of Witness 2 Date

Declaration by investigator

I have adequately read, or born witness to the exact reading of the parental permission to the relative (mother or father) of the participant, or respective guardian, and the individual had the opportunity to ask questions. I confirm that the individual freely gave their consent.

Printed name of the member of the assessment team _____

Signature of the member of the assessment team _____

Note: Three copies will be made of this consent. One will remain with the patient or individual responsible for him or her, another will remain on file at the institution where the research takes place, and the third will remain with the coordinator of this assessment.

Appendix B1: Assent Form (for participants aged 7–12, inclusive) (Flesh-Kincaid 3.8)

Project: Efficacy of Three Regimens of Chloroquine and Primaquine for the Treatment of *Plasmodium vivax* Malaria in Cruzeiro do Sul, Acre, Brazil

The Secretariat that takes care of the health of the people in Cruzeiro do Sul will conduct a study. This is to know if the drugs that are used to treat malaria are being good.

Your parents / guardian let you take part. I am asking you now if you want to take part. You are not required to take part. Only if you want.

As part of this study, you will be visited more often by a member of our team than if you were not part of it. You will receive the same routine malaria drugs.

If you agree, you will receive two medications, one white and one orange. They are not in liquid but in pill. Today we will also take a small amount of blood from your arm. This is to do some tests. When we visit you to find out how you are. We will also conduct further tests to see if you are still sick with malaria. We will also want to know if the medicine is still in your blood, and how your body is reacting to the medicine. If you are a woman 10 years of age or older, we will do a pregnancy test. This is done in blood or urine. We will inform your guardian of these results.

You do not have to be afraid to take the sample. The people who will take your blood are already used to doing this. But it may hurt a little. We will also take some blood from your fingers when you come to see us. In one of these days, we will need to take blood from your arm again. If you see or feel anything different after you take medicine tell mom or dad. You can also tell the person who comes to see you. If you already know how to call, you can even call us. We are here to help you.

If you no longer want to receive our visits, you can tell us. This will not cause you any trouble.

Do you understand what I said to you? You can ask your parents or me any questions.

Want to be part of the project?

Appendix B2: Assent Form (for participants aged 13–17, inclusive) (Flesh-Kincaid 4.6)

Project: Efficacy of Three Regimens of Chloroquine and Primaquine for the Treatment of Plasmodium vivax Malaria in Cruzeiro do Sul, Acre, Brazil

The Acre Health Department is working with the Ministry of Health of Brazil and with international partners. We are conducting a study to evaluate the treatment of malaria here in Cruzeiro do Sul.

Your parents / guardian agreed for to you take part. I am asking you now if you want to take part. You are not required to take part if you do not want to.

This study will offer you the same malaria medications you would receive if you were not in the study. However, you will be evaluated by our team more often than if you were not part of it.

If you agree, you will receive the two drugs. They are called chloroquine and primaquine. You will need to come back here or we will come to you 13 more times in the next 6 months. This is to see how you are feeling. If you are not feeling well or have a fever, you may need to return more often. At each visit, we will collect a small amount of blood from your finger. This is to check if you are still sick and what type of malaria you have.

Today and on the seventh day, we will also collect a small amount of blood from your arm. This is to check the medicine in your blood. It will also help us know how your body is reacting to the medicine. If you are a woman, we will do a pregnancy test. This is done in blood or urine. We will inform your parents of the result. We will also ask your parents to save any left-over blood for future studies in malaria.

It can hurt when we stick your finger or arm with the needle. An infection may occur. To prevent this, trained people will do the blood collection. Chloroquine can cause nausea and itchiness. Primaquine can cause heartburn, vomiting and diarrhea. These symptoms are mild. You may have severe symptoms, which is rare.

There will be a person here every day to help you.

You may decide not to continue the study at any time and this will not cause you any problem.

Do you have any questions regarding the study? You can ask your parents or me any questions.

Do you want to be part of the project?

Annex C1. Authorization for storage of remaining samples (Participants aged 18 years and older and Parents of Participants aged five through 17 years) (Flesh-Kinkaid level 7.6)

Invitation

We would like to store what is left of the samples we collect during this study. We will keep these samples at the Institutional biobank of the Evandro Chagas Institute. This is part of the Brazilian Ministry of Health. A biobank is a place where we keep samples from people. We respect people and the samples they give us to keep. We need your free and informed consent to keep your/ your child's samples. We say the following things to make sure that you are taking part because you want to do so. There is no penalty for not letting us keep your/ your child's samples. We want to make sure you know what we are doing and that you agree.

Clarifications

Evandro Chagas Institute is part of the Health Surveillance Secretariat of the Ministry of Health. This is a place where we study ways to improve peoples' lives. Sometimes we discover new medicines and tests that help us know more about sicknesses and how to treat the people who have them.

You and / or your child (or person for whom you are the legal guardian) are being asked to take part in a malaria study. They and / or you have signed a consent form. After the needed tests for your/ your child's treatment or research are done, there may be some material left over. This material is usually thrown away. However, we would like to ask you / your parents to let us store to keep it in the Evandro Chagas Institute for future use. We will only do malaria studies with this material.

We will be sure that the IEC and its partners will only use this material for malaria studies. They will respect strict rules that show they respect life. We will be sure that all of your/ your child's personal information is kept private. Your/ your child's sample will be saved without your/ his/ her name on it. We will keep a list of codes so that we know which sample is yours/ your child's without using your name. Only the people in charge of the bank will be able to see this list.

If you wish, at any time, you will have access to the information generated with the use of your/ your child's samples. This includes the results from the research we have done. To do that, you need to contact the Research Ethics Committee of the Evandro Chagas Institute. The committee's contact information is listed below. You may also request that your samples and information not be stored in the Biobank anymore. If you decide to not let us use your samples anymore, you must let us know in writing. We will then destroy your/ your child's samples according to the rules of the Evandro Chagas Institute.

We remind you that your/ your child's samples can also be thrown away if it is not possible for us to use in research for quality reasons. The Instituto Evandro Chagas may also decide to end the biobank. If this happens this happens, before we throw your/ your child's sample away, we will offer it to at least two biobanks. We will ask for your permission to do this if it happens.

You will receive a copy of this consent form. You can read it at any time. You will not receive any payment. You will also not have to pay anything for your samples to be stored in the bank of samples.

When you take part in the biobank you have certain rights. You can have your questions answered and get more information about these rights through the Research Ethics Committee of the Evandro Chagas Institute. This committee is responsible for monitoring the Biobank and evaluating the future research that will be happen there.

The address of ethics committee is Rodovia BR-316, Km 07, Ananindeua, PA, Brazil. It is opened from 07:30 to 16:30. If you prefer, the service can be reached at (XX) XXXX XXXX. The person responsible is Dr. Arnaldo Jorge Martins Filho.

Declaration of Consent or Permission

Upon signing or providing a fingerprint indicating legal consent for the appropriate declaration, I agree that:

- I have read this form or the form was read to me by someone else
- I was able to ask questions about the storage of blood. My questions have been answered
- I agree in a voluntary manner to allow for the storage of my/my child's blood.

Study participant's name: _____ Date _____

_____

Guardian's name

_____

Patient's or guardian's signature

Right thumb fingerprinting

I bore witness to the informed and voluntary consent. I can also verify that the participant or responsible party was informed of the details, risks and benefits of the assessment, and had the opportunity for their questions to be answered.

_____ _____

Signature of Witness 1 Date

_____ _____

Signature of Witness 2 Date

Declaration by investigator

I have adequately read, or born witness to the exact reading of the consent form, to the participant or relative (mother or father) of the participant, or respective guardian, and the individual had the opportunity to ask questions. I confirm that the individual freely gave their consent.

Printed name of the member of the assessment team _____

Signature of the member of the assessment team _____

Annex C2. Assent Form for Storage of Remaining Samples (Participants 7 to 12 years)

Study design: Efficacy of three schemes for primaquine terminal treatment of *Plasmodium vivax* in Cruzeiro do Sul, Acre, Brazil

Invitation

You are part of a malaria study. As part of this study, we will collect some blood from you. Sometimes there is blood left over after the tests. Usually, we throw away what is left over. If there is blood left over, we want to keep it. We would keep it at Evandro Chagas Institute of the Ministry of Health.

Clarifications

At the Evandro Chagas Institute, we do studies. These studies help people live better lives.

We want to keep the remaining blood to help in other studies in malaria. We asked your parents, and they said it is okay to keep it. They know who to talk to if they have questions. We are asking you if it is also okay with you.

If you let us keep the blood, no one will know it is yours except the people responsible for the blood at Evandro Chagas. It is okay if you do not want us to keep the blood. You will be treated the same if you say yes or if you say no.

Do you understand what I said to you? You can ask your parents or me any questions.

Is it okay with you if we keep the blood that is left over from your tests?

Annex C3. Assent Form for Storage of Remaining Samples (Participants 13 to 17 years)

Study design: Efficacy of three schemes for primaquine terminal treatment for *Plasmodium vivax* in Cruzeiro do Sul, Acre, Brazil

Invitation

You are part of the malaria study. For this, you do blood tests. Sometimes there is blood left over after the tests. Usually, we throw away this blood. If there is blood left over, we want to keep it instead of throwing it away. We would keep it at Evandro Chagas Institute of the Ministry of Health.

Clarifications

At the Evandro Chagas Institute, we do studies. These studies to help people live better lives.

You are taking part in a malaria study. For this, we will collect some blood. We want to use whatever is left to use in other studies. Only studies on malaria would be done. We asked your parents, and they said it is okay to keep it. They know who to talk to if they have questions. We are asking you if it is also okay with you.

If you let us keep the blood, no one will know it is yours except the people at Evandro Chagas. It is okay if you do not want us to keep the blood. You will be treated the same if you say yes or if you say no.

Do you understand what I said to you? You can ask your parents or me any questions.

Is it okay with you if we keep the blood that is left over to do future studies?

Annex D. Antimalarial drugs that should not be used in the 30 days prior to enrolment and also during the study period

- Chloroquine (except for the prescribed period)
- Amodiaquine
- Qinino, quinidine
- Mefloquine, lumefantrine
- Artemisinin and its derivatives
- Proguanil
- Sulfadoxine, sulfamethoxazole (bactrim), dapsone
- Primaquina (except for the prescribed period)
- Atovaquone
- Antibiotics: tetracycline, doxycycline, erythromycin, azytromycin, clindamycin, rifampicin
- Pentamidine

Annex E: Patient form

Screening

Patient's name: _____

Sex: ( ) Male ( ) Female

Today's date: ____/____/_____

Screening:

Inclusion criteria :	Yes	No
1. Desire to participate in a malaria study and availability of follow-up for 6 months (patient does not intend to move or take long trips)		
2. Age ≥5 years		
3. Weight ≤120 Kg		
4. Fever (Temp ≥37.5º C) or history fo fever in the last 48 hrs. (Nurse will measure temperature)		
5. Monoinfection b *P. vivax* with 100 a 200.000 parasites/ microliter in ths slide done by the helath post		
6. Live within 30–45 min of teh sudy site		

Only continue to 'Yes' to all of the above questions. The technician takes the patient to the nurse

Screening by Nurse

Exclusion criteria:	Yes	No
1. Presence of any sign of danger: inability to drink, vomiting (more than 2 times in the previous 24 hours), seizures (one or more in the previous 24 hours), altered consciousness, inability to sit or stand		
2. Presence or suspicion of severe malaria: cerebral malaria (irreversible coma), severe anemia (extreme skin paleness, hemoglobin <8mg / ml), renal insufficiency (serum creatinine> 3mg / dl, absence of diuresis in the last 8 hours), difficulty breathing, hypoglycaemia (blood glucose <40mg / dl or clinical signs)		
3. Presence or suspicion of severe malaria (cont.): Low blood pressure (systolic blood pressure <70 mm Hg in adults, 50 mm Hg in children), spontaneous haemorrhage / disseminated intravascular coagulation, repeated generalized seizures, blood in the urine or urine red, jaundice (yellowish skin)		
4. History of chronic or serious underlying diseases (eg, heart, kidney or liver disease, HIV / AIDS, tuberculosis, malnutrition, psoriasis)		
5. Allergy to chloroquine or primaquine		

6. Use of antimalarial drugs 30 days prior to today		
7. Current pregnancy		

Only continue 'No' to all the questions in this block and sign the consent form and consent, if applicable.

Clinical Form

Identification

Patient's ID: _____

Helath center:
( ) 1. Ambulatório do Hospital Regional do Juruá
( ) 2 Centro de Cruzeirinho
( ) 3 Centro de Aeroporto Velho
( ) 4 Centor de Miritizal
( ) Other
If other, which? _____

Patient's Name: _____

(For children) Mother's Name:_____

Father's Name:_____

Address with specific directions: _____

_____

Patient's best phone number to be reached: _____

Transport cost (round trip): _____

Consent Patient

Medical history

Study group: ( ) Group 1
( ) Group 2
( ) Group 3

Today's date: ___:_____: _____
Date of birth: ____/____/_____ Sex: ( ) Male ( ) Female

Did you have fever in the last 48 hours? ( ) Yes ( ) No Duration: _____days

What other symptoms are you experiencing with your current illness? Mark all that apply

( ) Headache
( ) Chills
( ) Sweats
( ) Vomiting
( ) Diarrhea
( ) General weakness
( ) Other

Which? _____
_____
_____

Did you take any medicines for this condition or other conditions? ( ) Yes ( ) No

If yes, which ones? Dose? When? (Ask about chloroquine, Coartem, quinine, and other antimalarials)

Medicine	Dose	Date

Do you have any chronic medical condition, such as cardiac, renal, and hepatic diseases; HIV/AIDS; tuberculosis; or malnutrition)? ( ) Yes ( ) No

If yes, which one(s)?

_____
_____
_____

Do you have any allergies (especially allergies to medicines)? ( ) Yes ( ) No

If yes, which one(s)?

_____
_____
_____

Physical exam

Axillary temperature (degreesC): _____
Blood pressure: _____:_____
Weight: _____ (kg)
Heart rate: _____
Respiratory rate: _____
Mental status: ( ) Oriented in place and time
( ) Comatose _____
Chest: ( ) Difficult breathing
Abdomen: ( ) Pain at palpation
Extremities: ( ) Edema present
( ) Cianosis
Jaundice: ( ) Present

Laboratory testing (Day 0)
Species: ( ) P. vivax alone
( ) P. falciparum alone
( ) Mixed infection P. falciparum plus P. vivax
( ) Other Which? _____

For P. vivax alone cases:
Asexual parasites/leukocytes: _____/_____
Gametocytes/leukocytes: _____/_____

Hemoglobin: _____

Pregnancy test (for women 10 to 49 years old):
( ) Urine
( ) Blood
Result
( ) Negative ( ) Positive
( ) Not done
( ) Nor applicable

Treatment (Day 0)

Chloroquine dose
Day 0: _____ tablets
Time of the first try for the first dose: _____:_____
Vomiting in 30 min? ( )Yes ( ) No

Time of the second try of the first dose: (If necessary) _____
Vomiting in 30 min? ( )Yes ( ) No

Did patient receive paracetamol: Yes No Time: _____

Axillary temp after paracetamol: _____ Time: _____

For information on number of tablets for the subsequent doses only:

Chloroquine dose for Days 1 and 2: ____ tablets

G6PD result: ( ) Normal
( ) Abnormal

Section B. **Case Record Form**

Study Number: _____ Weight (kg): _____

Follow-up day	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day ____
Date (Day/Month)	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___
Chloroquine dose (____ mg per tablet) (number of tablets)												
Primaquine (____ mg per tablet) (number of tablets)												
Axillary temperature (o C)												
Since your last visit have you had: Fever? (Y/N)												
Vomiting? (Y/N)												
Diarrhea? (Y/N)												
Itching? (Y/N)												
Skin Rash? (Y/N)												
Others? (Y/N)												
Any other drug given? (Y/N)												
(If other drug given) Type and amount.												

Follow-up day	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day ____
Any other treatment? (ex., blood transfusion) (Y/N)												
Better, same or worse (B/S/W)												
Parasite species (V, F, NPF*)												
Asexual parasite density												
Gametocyte density												
Patient condition (Continue, Withdrawn, Abandoned) (C/W/A)												

**NPF = No Parasites Found.

If patient is excluded from the study or is lost to follow up, please list reasons:

_____
_____
_____

Study Number: _____ Weight (kg): _____

Follow-up day	Day 14	Day 21	Day 28	Day 56	Day 84	Day 112	Day 140	Day 168	Day 196	Day ___	Day ___	Day ____
Date (Day/Month)	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___	___/ ___
Chloroquine dose (____ mg per tablet) (number of tablets)												
Primaquine (____ mg per tablet) (number of tablets)												
Axillary temperature (o C)												
Since your last visit have you had: Fever? (Y/N)												
Vomiting? (Y/N)												
Diarrhea? (Y/N)												
Itching? (Y/N)												
Skin Rash? (Y/N)												
Any other drug given? (Y/N)												
(If other drug given) Type and amount.												
Any other treatment? (ex., blood transfusion) (Y/N)												

Follow-up day	Day 14	Day 21	Day 28	Day 56	Day 84	Day 112	Day 140	Day 168	Day 196	Day ___	Day ___	Day ____
Better, Same or Worse (B/S/W)												
Parasite species (V, F, NPF*)												
Asexual parasite density												
Gametocyte density												
Patient condition (Continue, Withdrawn, Abandoned) (C/W/A)												

**NPF = No Parasites Found.

If patient is excluded from the study or is lost to follow up, please list reasons:

_____
_____
_____

Annexx F. Adverse Events Form

Patient ID ☐☐☐

Chave para ficha de EA

Severity	1 = Mild (Grade 1): Signal or symptom awareness easily tolerated 2 = Moderate (Grade 2): Discomfort sufficient to cause interference with normal activity 3 = Severe (Grade 3): Incapacitating with incapacity for work or carrying out habitual activity 4 = At risk of death (Grade 4): Patient at risk of death at the time of the event, or requires hospitalization or prolongation of hospitalization; cause a persistent or significant disability; result in congenital anomaly or birth defect 5 = Death (Grade 5)					
Study drug related?	1=Definitly not	2=Improbably related	3=Possibly related	4= Probably related	5= Definitly related	
Outcome	1=Total recovery	2=Patient not recovered yest	3=Worsening	4=Permanet sequalae	5=Death	6=On going 7=Unknown
Action take	1=No measure	2=Dose adjustment or temporarily suspended	3=Permanent withdrawal of drugs	4=Concomitant drug given	5=No therapy given	6= Hospitalization

Number of adverse events ☐☐ Data: ☐☐ / ☐☐☐ / ☐☐

_____

Diagnosis _____

Onset date ☐☐ / ☐☐☐ / ☐☐ End date ☐☐ / ☐☐☐ / ☐☐

Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 Related to the study drug ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Result ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 Treatment ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

Treatment administered ☐ Yes ☐ No *Specify* _____

Was this a severe one? ☐ Yes ☐ No

Number of adverse event ☐☐ Reporting date ☐☐ / ☐☐☐ / ☐☐

Diagnosis _____

Onset date ☐☐ / ☐☐☐ / ☐☐ End date ☐☐ / ☐☐☐ / ☐☐

Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 Related to study drug ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 Treatment ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

Treatment administered ☐ Yes ☐ No *Specify*: _____

Was this a severe one? ☐ Yes ☐ No

Number of adverse event ☐☐ Reporting date ☐☐ / ☐☐☐ / ☐☐

Diagnosis _____

Onset date ☐☐ / ☐☐☐ / ☐☐ End date ☐☐ / ☐☐☐ / ☐☐

Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 Related to study drug ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 Treatment ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

Treatment administered	☐ Yes ☐ No	*Specify*: _____
Was this a severe one?	☐ Yes ☐ No	

Form filled by

_____ ☐☐ / ☐☐☐ / ☐☐
Name

_____
Signature

Anexx G. Randomization table.

Order	Arm
1	Grupo 3
2	Grupo 3
3	Grupo 2
4	Grupo 1
5	Grupo 2
6	Grupo 3
7	Grupo 1
8	Grupo 3
9	Grupo 2
10	Grupo 2
11	Grupo 1
12	Grupo 3
13	Grupo 1
14	Grupo 3
15	Grupo 1
16	Grupo 2
17	Grupo 2
18	Grupo 3
19	Grupo 3
20	Grupo 1
21	Grupo 2
22	Grupo 2
23	Grupo 3
24	Grupo 3
25	Grupo 2
26	Grupo 1
27	Grupo 2
28	Grupo 3
29	Grupo 2
30	Grupo 3
31	Grupo 1
32	Grupo 2
33	Grupo 1
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248	Grupo 3
249	Grupo 2
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251	Grupo 2
252	Grupo 1
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254	Grupo 2
255	Grupo 3
256	Grupo 2
257	Grupo 3

PROTOCOL AMENDMENT

Amendment

Rationale for Amendment

This protocol was amended before implementation of study.

The main three primary reasons for amendment were:

- To add/modify list of investigators to better define roles and responsibilities.
- To add details about randomization and allocation criteria.
- To add the evaluation of cytochrome P 2D6 to be added as part of the outcome analysis.
- To present a revised clinical form to collect information from patients during follow-up.

Date of amendment: March 2018

This was the only amendment of the protocol. This amendment was approved by principal investigators listed on the first page of the protocol.

Revised Protocol

Efficacy of High Total Dose of Primaquine to Prevent *Plasmodium vivax* Malaria Relapses in Cruzeiro do Sul, Brazil

Investigator(s) and Institutions

- Nathália N. Chamma Siqueira, MSc, Instituto Evandro Chagas, Brazil. Mrs. Chamma will be responsible for laboratory processing; data analysis; and coordinating manuscript writing. She will be also responsible for leading manuscript review, updating and making changes to final manuscript; and for active engagement with partner institutions.
- Dr. Suiane Negreiros do Valle, MD, PhD, Health Secretariat of Acre State (Hospital Regional do Jurua). Dr. Negreiros will be responsible for technical assistance to the protocol development, implementation; support for data cleaning and analysis; interactions with public health authorities in Brazil; ethical review process in Brazil; manuscript writing; and general oversight of the study.
- Sarah-Blythe Ballard, MD, PhD, MPH, Malaria Branch, CDC (SEV 7105). Dr. Ballard will be responsible for technical assistance to the protocol and project implementation and development. Dr. Ballard will likely interact with study subjects at the time of study implementation and supervisory visits. She will not have access to stored personal identifiers.
- Samela Farias, nurse, specialist, Health Secretariat of Acre State (Hospital Regional do Jurua). Mrs. Farias will provide technical assistance to protocol development. She will be responsible data collection, day-to-day management of study staff, leading the team of nurses, nurse assistants and microscopists involved in data collection; direct contact with participants for data collection; critical review and refinement of enrollment and follow-up standard operating procedures (SOPs); data cleaning; provision of answers to queries from CDC during data collection, data analysis, and paper writing; coordination of sample shipment.
- Sandro Patroca da Silva, PhD, Instituto Evandro Chagas. Dr Silva will provide technical assistance to protocol development of his respective area of expertise. He will be responsible for issues related to microsatellites laboratory testing, including bench work itself, result gathering, dataset generation, data interpretation, and manuscript writing.
- Stella M. Chenet, PhD, Universidad Nacional Toribio Rodriguez de Mendonza da Amazonas. Dr. Chenet will provide technical assistance to protocol development of her respective area of expertise. She will be responsible for issues related to microsatellites laboratory testing, from conception, decision on testing algorithms, analysis and interpretation, and manuscript writing.

- Flávia Póvoa da Costa, MSc, Instituto Evandro Chagas. Mrs. Costa will provide technical assistance to protocol development of her respective area of expertise. She will be responsible for issues related to CYP2D6 laboratory bench work itself, result gathering, dataset generation, and providing technical input to manuscript.
- Luann Wendel Pereira Sena, PhD, Universidade Federal do Pará. Dr. Sena will provide technical assistance to protocol development of her respective area of expertise. He will be responsible for CYP2D6 laboratory testing, including conception, laboratory bench work itself, data interpretation, and providing technical input to manuscript.
- Amanda Gabryelle Nunes Cardoso, PhD, Universidade Federal do Pará. Dr. Cardoso will provide technical assistance to protocol development of her respective area of expertise. She will be responsible for CYP2D6 laboratory testing, including algorithms testing, data interpretation, and providing technical input to manuscript.
- Eduardo Santos, PhD, Universidade Federal do Pará. Dr. Santos will provide technical assistance to protocol development of his respective area of expertise. He will be responsible for issues related to CYP2D6 laboratory testing including conception, decision on testing algorithms, data analysis and interpretation, and providing technical input to manuscript.
- Giselle Rachid Viana, PhD, Instituto Evandro Chagas. Dr. Viana will provide technical assistance to protocol development. She will be responsible for study planning, review of all laboratory tests, quality assurance and control or laboratory procedures used in this study, and critical review of manuscript.
- Paola Marchesini, MD, PhD, Grupo Técnico de Malária, Coordenação Geral de Vigilância de Zoonoses e Doenças de Transmissão Vetorial, Departamento de Imunização e Doenças Transmissíveis, Secretaria de Vigilância em Saúde (SVS), Ministério da Saúde do Brasil. Dr. Marchesini will provide technical assistance to protocol development. She will be responsible for overall project, making sure it fits into the needs of the national malaria control program in Brazil, and manuscript writing.
- Cássio Roberto Leonel Peterka, MSc., Diretoria de Vigilância Epidemiológica, Subsecretaria de Vigilância em Saúde, Secretaria Estadual de Saúde do Distrito Federal, Brasília, Distrito Federal, Brazil. Mr Peterka will provide technical assistance to protocol development. He will be responsible for overall project, making sure it fits into the needs of the national malaria control program in Brazil, and critical review of manuscript.
- Marinete Marins Povoa PhD, Instituto Evandro Chagas. Dr. Povoa will be responsible for supervising all laboratory procedures associated with this project.

- Venkatachalam (Kumar) Udhayakumar, PhD, Malaria Branch, CDC (IRB number 13427). Dr. Kumar will be responsible for supervising and troubleshooting the molecular typing to be done as part of this study. He will not have access to any personal identifier.
- Michael Green, PhD, Entomology, CDC (IRB number 17614). Dr. Green will be responsible for supervising and troubleshooting the antimalarial blood level determination to be done as part of this study. He will not have access to any personal identifier.
- Alexandre Macedo de Oliveira, MD, MSc, PhD, Malaria Branch, CDC (IRB number 9885). Dr. Macedo de Oliveira will be responsible for technical assistance to the protocol development, implementation, supervision of field work; coordination of data cleaning and analysis, interactions with all subject matter experts; manuscript writing and review; and coordination between colleagues in Brazil and at CDC. Dr. Macedo de Oliveira will likely interact with study subjects at the time of study implementation and supervisory visits. He will not have access to stored personal identifiers.

Conflict of interest

None of the authors have any conflict of interest to report.

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Summary

Background: The World Health Organization recommends that antimalarial treatment policies be evaluated every few years to check their efficacy. *P. vivax* malaria is the most common species in Brazil and cases are concentrated in the Amazon Region in Brazil.

Objectives: Assess the efficacy of 3 different regimens of chloroquine and primaquine for the treatment of *P. vivax* infections in Cruzeiro do Sul, Acre, Brazil.

Methods: An in vivo drug efficacy study will be conducted in Cruzeiro do Sul, Acre State, Brazil. A total of 257 study participants ≥5 years of age with parasitologically confirmed *P. vivax* monoinfections should be included. Patients will be divided in 3 different groups: treatment with regular dose of primaquine (0.5 mg/kg per day for 7 days) with directly observed therapy; regular dose of primaquine without directly observed therapy; and increased total dose of primaquine (0.5 mg/kg per day for14 days) with directly observed therapy. All patients will receive chloroquine (CQ) for three days at a daily dose of approximately 25 mg/Kg in accordance with the Brazilian National Malaria Control guidelines. Primaquine will be given for 7 or 14 days under supervision or not, depending on the study group. Clinical and parasitological parameters will be monitored over a 28-day follow-up period to evaluate drug efficacy and for a total period of 168 days (24 weeks) to evaluate chances of recrudescence, relapse, or reinfection. Blood samples will be taken to measure the CQ levels in blood on Day 7 and day of failure, if occurring in the initial 28 days of follow up. In addition, a blood sample will be collected on filter paper on first day and on day of suspected failure to help differentiate parasite genotypes using techniques based on polymerase chain reaction. We also intent to analyze *CYP2D6* gene polymorphisms and correlated those to metabolic activity phenotypes to correlate them with treatment response. Results from this drug efficacy study will be used to assist the Brazilian Ministry of Health in assessing their national malaria treatment policy for *P. vivax* malaria.

Background

The impact of malaria on the health and economic development of human populations is greatest in the tropics and subtropics. The World Health Organization (WHO) has estimated 212 million episodes of malaria in 2015, of which 90% of those in in Africa. There were a total of 429,000 malaria deaths worldwide, the majority in in children under 5 years of age (WHO 2015). Although the majority of deaths occur among children in sub-Saharan Africa, malaria accounts for considerable morbidity in the Americas, particularly in the Amazon Basin.

Most countries in the Americas have adopted the WHO Global Strategy for Malaria Control, which relies on prompt and effective antimalarial treatment as the major means of reducing malaria morbidity and mortality (WHO 2008). The ultimate success of this strategy rests on the ability of ministries of health to provide antimalarial drugs with proven efficacy. Although a wide variety of methods have been used to assess resistance to antimalarial drugs in vivo methods, in vitro drug sensitivity testing, and molecular analyses, most national malaria control programs rely on data from in vivo efficacy trials to assess the efficacy of the current first- and second-line drugs and to decide if charges in malaria treatment policy are needed.

The most widely used approach to conduct in vivo drug efficacy trials in the Americas follows the guidelines of the WHO (WHO 2009, WHO 2015) with the modifications recommended by the Pan American Health Organization for studies in the Americas (PAHO 2003). The goal of such studies is to assess antimalarial drugs currently being used for first-line treatment of uncomplicated malaria. Much of the effort to monitor antimalarial efficacy in the Americas has been done as part of the Amazon Network of Antimalarial Resistance Monitoring and the Amazon Malaria Initiative (PAHO 2012). This information is critical for guiding the development of rational antimalarial drug policies in endemic areas.

Chloroquine-resistant *P. vivax* was first reported from Papua New Guinea in 1989 in two Australian soldiers (Rieckmann, Davis et al. 1989). In 1995, a study in Irian Jaya, Indonesia showed resistance in at least 44% of the *P. vivax* patients treated with chloroquine (CQ) (Baird, Basri et al. 1995). Several investigators have reported cases of CQ-resistant P. vivax in South America. In 1996, in Guyana, Phillips et al. reported three patients in whom 25 mg/kg of CQ failed to eliminate parasitemia despite adequate therapeutic blood levels of CQ (Phillips, Keystone et al. 1996). Three years later, in the Brazilian Amazon region, Alecrim et al. reported a 12-year old girl with *P. vivax* malaria who continued to have parasitemia after receiving a supervised course of 25mg/kg of CQ (Alecrim, Alecrim et al. 1999). More recently, Soto et al. reported three cases of CQ-resistant *P. vivax* in Colombia (Soto, Toledo et al. 2001).

In addition, recent studies have shown high reinfection rates by *P. vivax* even after treatment with recommended dose of primaquine (0.5 mg/kg per day for 7 days) (Durand, Cabezas et al. 2014, Negreiros, Farias et al. 2016). Despite the fact that primaquine is the only drug currently available for the terminal treatment of *P. vivax* and *P. ovale* infections, variable reinfection rates have been reported in the literature (Hill, Baird et al. 2006, Durand, Cabezas et al. 2014). WHO recommends daily dose of 0.25 mg/kg/day (infections from temperate regions) and 0.50 mg/kg/day (infections from tropical regions with high rates of relapses) all over 14 days, with a total dose of 3.5 mg/kg or 7.0 mg/kg, respectively, after reports of relapses in Asia and Oceania (Collins and Jeffery 1996, Baird and Hoffman 2004, WHO 2015). This concern about frequent relapses has led CDC to change its recommendation to increase the total dose of primaquine for terminal treatment 7.0 mg/kg to be given over 14 days for terminal treatment for *P. vivax* infections from all regions of the world (Centers for Disease Control and Prevention 2015).

Because of the serious public health implications of CQ-resistant and primaquine-resistant *P. vivax* in the Americas, it is critically important to limit reports to well-confirmed cases. In most cases, this will require measurement of CQ blood levels and genotyping of parasites from the initial infection and any suspected recrudescence. *P. falciparum* in vivo trials take advantage of well-established molecular markers that help differentiate cases of recrudescence and reinfection, by polymerase chain reaction (PCR) techniques. Although no universally accepted technique for this purpose exists for *P. vivax,* microsatellites, base pair repeats in the parasite genome, described by Imwong et al (Imwong, Sudimack et al. 2006) have been used for this purpose with variable results. We believe that having PCR-corrected analysis is especially important in the context of the long follow-up period, six months, we aim for this study.

Most malaria cases in the Americas are reported in Brazil (Silveira 2001, Oliveira-Ferreira, Lacerda et al. 2010). In 2016, 151,620 malaria cases were reported to the Brazilian National Reportable Disease Information System. Most of these cases (99.7%) occurred in the Amazon region, which encompasses the states of Acre, Amazonas, Amapa, Para, Maranhao, Mato Grosso, Roraima, Rondonia, and Tocantins. In this region, socio-economic and environmental conditions, such as presence of natural breeding sites and abundance of Anopheles mosquitoes, favor malaria transmission. Amazonas, Rondonia, Para, and Acre states were responsible for 85.5% of malaria cases in 2011 according to the Brazilian National Reportable Disease Information System. As in other regions of the world, malaria is seasonal in Brazil, cases increase during or after the rainy season (Costa 2009).

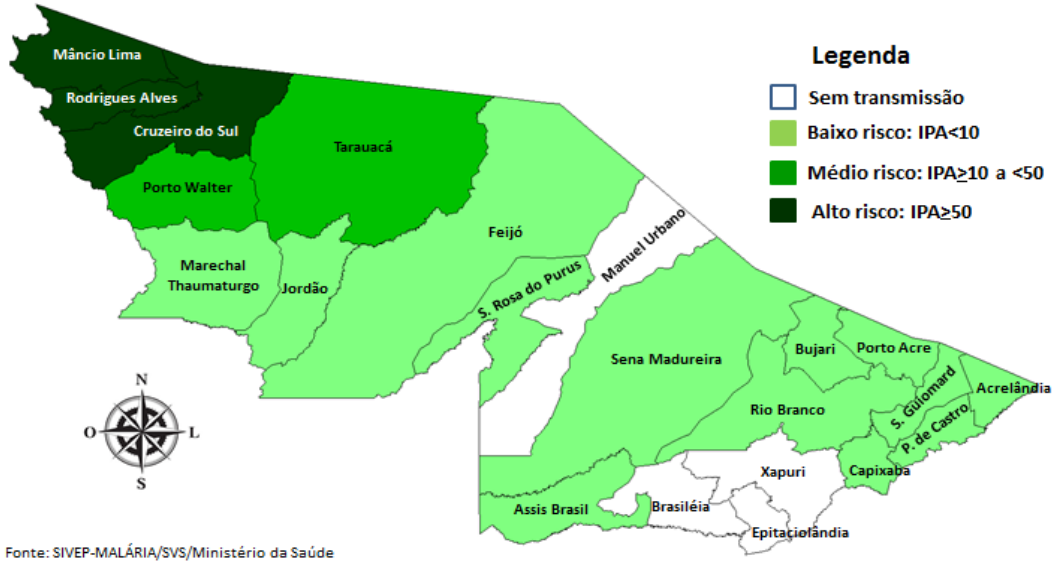
In 2016, 73.9% of malaria cases in Brazil were due to *P. vivax* alone, 9.47% to *P. falciparum* alone, and the rest due to mixed infections with these two species. *P. malariae* is rarely seen in Brazil (regular surveillance data from Brazil, SIVEP, 2017). Table 1 shows malaria cases over the last few years.

Acre state is responsible for a huge proportion of cases in the Brazilian Amazon region, especially in the municipalities of Cruzeiro do Sul, Mâncio Lima and Rodrigues Alves (Figure 1). In 2016, Acre had 35,209 cases in addition to 4,098 positive cases detected during treatment response control slides (TRCS), a total of 39,307. Cruzeiro do Sul had 20,591 cases, with additional 2,195 cases by TRCS, a total of 22,786 cases (data from the Brazilian National Surveillance System, 2017).

Table 1. Malaria cases in Brazil.

Region	2012	2013	2014	2015	2016
	Cases	Cases	Cases	Cases	Cases
Brazil	234,545	169,231	138,936	137,947	123,935
North	234,497	169,187	13,8919	137,925	123,904
Acre	27,005	33,755	30,982	26,632	34,382
Cruzeiro do Sul	16,053	19,701	17,179	13,936	19,027

Figure 1. Acre state and malaria risk by region, 2008.



Source: Acre state Health Secretariat.

Legend: Alto Risco, High risk; Medio Risco, Medium risk, and Baixo Risco, Low risk.

Although Brazil has been using primaquine for decades as part of the radical treatment for *P. vivax* malaria and currently there is no evidence of CQ-resistant or primaquine-resistant *P. vivax* in the country, there is concern about the tolerance to primaquine. The Ministry of Health would like to assess the efficacy of three regimens of CQ and primaquine for the treatment of uncomplicated P. vivax malaria as part their effort to study antimalarial drug resistance within the country.

Objectives

The objective of this study is to evaluate the therapeutic efficacy of CQ and primaquine in the treatment of *P. vivax* malaria in Cruzeiro do Sul, State of Acre, Brazil using three primaquine schemes. These are (1) routine standard dose in Brazil, WHO dose for temperate regions with supervised treatment; (2) double dose, WHO dose recommended for tropical regions with supervised treatment, and (3) standard dose treatment from Brazil without supervision.

Methods

Study site

This study will take place at the malaria treatment posts in Cruzeiro do Sul, Acre State, Brazil. Cruzeiro do Sul is a municipality with 85,000 residents in Northwest Brazil. Both *P. vivax* and *P. falciparum* malaria are endemic in the region with an annual parasite incidence (API) of 233.4/1,000 (data from the Brazilian National Surveillance System, 2017). In accordance to Brazilian national policies, malaria diagnosis and treatment are offered free of charge in the region. Road access to public health facilities is relatively easy in the urban and peri urban areas of the city. It is common practice in this region for patients to present to health facilities for diagnosis and first treatment dose. Patients could be then visited at home by health agents for directly observed therapy of the following doses.

The study will take place with patients recruited on spontaneous demand who attend the Emergency Room of the Juruá Regional Hospital (HRJ). This malaria post has a malaria diagnostic laboratory running 24 hours continuously, and it also registers the highest number of malaria cases in the city, according to statistics from the Brazilian malaria information system (SIVEP).Seven other malaria diagnostic stations will also be selected for this study, health posts located in the neighborhoods of Aeroporto Velho, Miritizal, Cruzeirinho, and others, and will be coordinated by the mobile team of the study. After the period of recruiting patients, the teams will be installed in the Clinical Research Room located at Santa Casa de Misericórdia de Cruzeiro do Sul.

Timing of study

The study will be conducted from during malaria transmission season, ideally from February 2018 to December 2018. Close communication will be maintained with regional and local health officials to determine the most appropriate time for the study to begin. Due to the 6-month follow-up period (168 days), we expect to conclude patient follow-up by December 2018 and data analysis by May 2019.

Study teams

At least two clinical teams will be required to conduct this study and interact with patients. Each team will consist of a nurse and a nursing assistant. One team will be based in the HRJ outpatient clinic and the other in the malaria research room. The team based in the clinical research room will be a mobile team responsible for admitting and following patients at least three malaria diagnostic post that are not at the HRJ (Cruzeirinho, Aeroporto Velho, Miritizal, and others). In addition to the clinical teams, one supervising physician and three microscopists will be part of the study.

Groups of study

This evaluation will have three distinct patient groups:

4. Group 1. Treatment with supervised CQ and unsupervised primaquine with usual primaquine dose of 3.5 mg/kg (0.50 mg/kg / day for 7 days).
5. Group 2. Treatments with CQ and primaquine supervised with usual primaquine dose of 3.5 mg/kg (0.50 mg/kg/day for 7 days).
6. Group 3. Treatments with supervised CQ and primaquine with increased primaquine total dose of 7.0 mg/kg (0.50 mg/kg/day for 14 days).

Group 3 will be the comparison group for efficacy and reinfection at the 24-week follow-up. Treatment response rates at Day 28 and during complete follow-up for reinfections will be compared between Groups 1 and 3, and between Groups 2 and 3.

Study procedures

The physician will act as study coordinator and will be responsible for overall study supervision, study forms, and administrative issues. The study doctor will supervise the entire clinical staff at all times and will be available to handle unexpected events.

The nurse will be responsible for evaluating the patients at the time of admission to the study, obtaining a clinical history, conducting a physical examination, administering the study medication (CQ and primaquine after confirmation of normal levels of glucose-6-phosphate dehydrogenase activity [G6PD]), visits on the first four days of follow-up (Days 0, 1, 2, and 3) and preferably Day 7, either in the study centers or at home visits (see below).

The nursing assistant will be responsible for monitoring primaquine doses for 7 or 14 days (after confirmation of normal levels of G6PD activity) depending on the study group, through visits to the patients at their homes for follow-up visits and patients' remaining check-ups. Since primaquine therapy can only be started after confirmation of normal G6PD activity, this drug will not be initiated on Day 0 when CQ will be initiated. We expect to have G6PD results within one week; therefore, primaquine will likely be initiated during the first week of follow-up. The doses of medicament, CQ and primaquine, will be administered with direct supervision, either by the nurse or by the nursing assistant, the two supervised treatment groups. In the unsupervised primaquine treatment group, only the doses of CQ will be supervised. Both the nurse and the nursing assistant can collect the blood samples, via fingerprick and venous puncture, recommended with part of this study.

As a routine practice in Ministry of Health facilities in Brazil, all febrile patients have a thick blood smear or rapid diagnostic test (RDT) for malaria examined before antimalarial treatment is administered. This usually takes place at a health facility where sick patients present. The staff of these malaria diagnostic posts, hospital and other health centers, will be informed of the study. Patients attending the study sites, HRJ or the health posts, who have a positive blood smear or RDT for *P. vivax* will be approached by a member of the study team or told by a health facility staff about this in vivo trial.

The study team nurse will assess inclusion and exclusion criteria and explain details about the study. If eligible patients agree to take part in the study, they, or the patient’s parent (in case of patient’s age < 18 years), will be asked to sign the informed consent or parental permission form (and also obtain verbal assent from children 7 through 17 years of age). After that, patient will be undergo blood collection for malaria diagnosis confirmation, medical evaluation, and all other study procedures. In case of refusal, patients will be referred back to health facility staff to receive the standard treatment for *P. vivax* malaria without any sort of penalty.

Enrolled patients will be asked to complete the CQ treatment at the health facility they were admitted at if they can. The study team nurse will schedule home visits for those patients who do not wish to come back to the health facility for follow-up during the period the patient is receiving CQ. Once a normal activity level of G6PD is documented, primaquine will be initiated and give also as supervised or not supervised doses, depending of the study group. On and after Day 3, when CQ treatment ends, arrangements will be made with the patient to be seen at home. The nurse assistant will make arrangements to visit patients at home for the remaining doses of primaquine. Study team will encourage patients to come back to health facilities, but we expect that most patients will prefer to be visited at home as this is common practice in Cruzeiro do Sul for malaria treatment and follow-up.

We believe that after CQ treatment is over and patient's health status has improved, the study team nurse assistant will be able to appropriately manage the follow-up visits. These nurse assistants will be in close contact with the nurse of their respective team and arrangements for re-evaluations will be made if necessary.

Study microscopists will be responsible for reporting blood smears results on the same day they are taken. Details on how the laboratory work will be organized are provided below. The physician will serve as a study coordinator, being responsible for the overall supervision of the study, study forms, and administrative issues. All study team personnel will be trained in their respective tasks before the start of this study. This training will take part in a 5-day period with participation of collaborators from the Centers for Disease Control and Prevention (CDC), Atlanta and Instituto Evandro Chagas, de Belem, Para. We will also invite the Unidade De Boas Praticas Clinicas da Fiocruz (Good Clinical Practices Unit at Fiocruz) to take part in the study.

In order to avoid reinfection due to malaria during the follow-up period, we will instruct patients to use malaria prevention measures. These are long-lasting insecticide-impregnated mosquito nets (ITNs).

Inclusion criteria

8. Age ≥5 years
9. Body weight <120 kg
10. Documented fever (axillary temperature ≥37.5^{o} C) or history of fever during the previous 48 hours in the absence of another obvious cause of fever, such as pneumonia, otitis media, etc
11. Monoinfection with *P. vivax* with parasitemia between 100 and 200,000 asexual parasites/µl as determined by microscopic examination of thick and thin peripheral blood smears

12. Informed consent from the patient or parent/guardian (for those <18 years), assent from child (ages 7 to 17 years inclusive), patients 5 through 6 years old will not need an assent
13. Willingness on the part of the patient to return to the clinic and/or receive home visits for regular check-ups during the 24-week (168 days) follow-up period
14. Place of residence within 30–45 minutes of study site.

Exclusion criteria

9. Presence of malaria danger signs
    a. Unable to drink
    b. Vomiting (more than twice in the previous 24 hours)
    c. Recent history of convulsions (one or more in the previous 24 hours)
    d. Impaired consciousness
    e. Unable to sit or stand
10. Presence of signs of severe malaria (WHO criteria)
    a. Cerebral malaria (unarousable coma)
    b. Severe anemia (hematocrit <15% or clinical signs) hemoglobin <5 mg/ml) (Note: we will use hemoglobin less than 8 mg/ml as exclusion criteria)
    c. Renal failure (serum creatinine >3 mg/dL or clinical signs)
    d. Pulmonary edema
    e. Hypoglycemia (blood glucose <40mg/dL or clinical signs)
    f. Shock (systolic blood pressure <70 mm Hg in adults; 50 mm Hg in children)
    g. Spontaneous bleeding/disseminate intravascular coagulation
    h. Repeated generalized convulsions
    i. Acidemia/acidosis (clinical signs)
    j. Macroscopic hemoglobinuria
    k. Jaundice
11. Self-reported presence of other underlying chronic or severe diseases (e.g., cardiac, renal, hepatic diseases, HIV/AIDS, tuberculosis, malnutrition, psoriasis)
12. History of hypersensitivity reactions to any of the drugs being tested. Mild itching with CQ is not in itself a criterion for exclusion. This occurrence will be evaluated by the study doctor before excluding the patient for this reason alone.
13. Use of drugs with antimalarial activity in the past 30 days. (Annex D)

14. Current pregnancy (either self-reported being pregnant at enrollment or a positive urine or plasma pregnancy test at time of enrollment), previous pregnancy is not an exclusion criteria
15. Hemoglobin <8 mg/mL
16. G6PD deficiency. This will be a late exclusion criteria as soon as the results of G6PD testing becomes available.

Sample size

Sample size was calculated based on the expected proportion of recurrent infection after treatment of *P. vivax* with CQ and primaquine in the study population. A previous study in Cruzeiro do Sul showed a recurrent infection rate of *P. vivax* at a 6-month follow-up of 30% and prevalence of heterologous isolates, reinfection or relapse by different genotype, from 16.6% of cases.(Negreiros, Farias et al. 2016) We estimate that the rate of recurrent infection after a double dose of primaquine (Group 3) is lower than that of 16.6% (most hypnozoites will be treated at the highest dose), and we estimate the recurrent infection rate in the dose group usual and supervised (Group 2) similar to that found by Negreiros and collaborators (30%). For Group 1, based on discussions with Brazilian Ministry of Health, we estimated that, due to the adherence of patients, this will be 40%, higher than that of Group 2.

Considering the above estimates and using Fisher Exact test, we needed 74 patients in each group to compare prevalence of 10% (Group 3) and 30% (Group 2) (power = 90% and 5% level of significance). Likewise, in order to compare difference of 10% (Group 3) and 40% (Group 1), we need 39 patients in each group. Increasing this minimum sample to 50 patients, which is recommended by the WHO as a minimum sample size for in vivo studies (WHO, 2009 # 40), and adding 30% in all groups to accommodate follow-up losses, we have sample size of 96 patients in Groups 2 and 3, and 65 patients in Group 1. The total number of patients in the study was 257 patients.

Randomization and allocation

The randomization sequence (Appendix G) present in the protocol was computer-generated by one of the investigators at the Centers for Disease Control and Prevention using SAS (SAS Institute, USA). The result of this process is a sequential enrollment list respecting the desired sample size per study groups. Since two study teams are involved in patient enrollment, one team will start from the top of the list and the other from the bottom. Regular meeting between the two nurses of the study teams will allow for checking if the total of 257 study participants in total are enrolled, so enrollment can be stopped.

The field study coordinator will assemble participant's clinical forms for both study teams and put them sequentially with a blank opaque cover on top of each one. At the time of enrollment, field study member will pick the top of their respective team pile and have the pre-selected study group for each new participant. Up to that moment, neither the field study member, nor the participant will have knowledge of study group assignment.

Informed consent

Informed consent, parental permission forms (TCLE in Portuguese acronym) and assents (TALE in Portuguese consent) are ways in which the participant confirms his or her participation in a clinical study. The TCLE (Appendices A1) is applicable for participants 18 years of age and older. In the case of participants less than age 18, the legal guardian must sign the TCLE for Parents or Legal Entities (Appendix A2), which serves as parental permission. Oral assent participants aged 7 to 17, inclusive, years will be used (Appendices B1 and B2). In the case of participants less than 18 years old at time of enrolment but who turn 18 during follow-up, we will request informed consent (TCLE) at his/her following visit after the 18th birthday. If patient denies written consent, we will withdrawal him/her at that point.

The objectives and procedures of the study and the rights of volunteers will be explained in detail to potential volunteers in Portuguese. The TCLE (Appendix A1) signed with a blue or black in three copies will be requested from all participants older than 18 years. Permission using the same forms (Appendix A2) will be asked from guardians of participants younger than 18 years of age who are willing to participate. A member of the study team should also sign this document. In addition, a TALE (Appendices B1 and B2) will be requested orally from patients 7–17, inclusive, years of age, according to the participant's age range. The practice of oral assent rather than its signature is due to the fact that the Ethics Committee of Brazil prefers only one document signed as part of the consent and consent process. Assent will not be required from patients 5 to 6, inclusive, years of age. These documents will be applied by the principal investigator or someone trained by him/her.

In the case of illiterate persons, consent forms and parental permission, as appropriate, shall be read to potential participants and obtained verbally. A digital fingerprint, which is a legal form of documentation of consent in Brazil, will be collected from each patient in the consent and parental permission, which will also be signed by two individuals not directly involved in the study as witnesses. All study participants will receive copies of the consent / assent form.

If the patient or his/her guardian refuses to grant authorization for his / her participation through the signing of the TCLE or oral assent in the case of TALE, this will not entail any detriment to patient care. All participants or their guardians may also withdraw their consent at any time. This does not cause any harm to the patient or their follow-up by the routine health service. In case of changes in the TCLE or TALE after the approval of the original protocol by the committees of ethics of law, these should be submitted to afore mentioned committees and only from then on we can be used with new participants.

A specific consent (Appendix C) will be required for patients over the age of 18 and their caregivers in cases of patients aged 5 to 17, inclusive, years for the storage of remaining samples after the study for use in malaria studies.

Patients will receive contact information cards, including phone numbers for after-hours phone calls from the study team. Consents must be filed minimum for 5 years, along with all other documents related to the clinical study. The study coordination will define where these documents will be stored after the study is completed.

Pregnancy testing

In accordance to what is considered childbearing age in Brazil, all women 10 to 49, inclusive, years old being considered for the study will undergo a plasma pregnancy test (One-Step Combo Pregnancy Test™ Markham, Canada). Female participants who become pregnant during the study will be withdrawn if the pregnancy occurs while using chloroquine or primaquine.

Enrollment procedures

The nurse in the study team will evaluate the inclusion and exclusion criteria and explain the details of the study. When an eligible patient agrees to participate in the study, he or she will be required to sign the Informed Consent Form (TCLE) and give oral assent, if applicable.

In order to expedite the patient registration procedure, one of the microscopists trained for the study will review the blade stained by the health service and estimate the parasitemia on this slide. At this moment the patient is provisionally decided to include or not a patient while waiting for the coloration and reading of the blade taken by the study team.

Study steps are the following:

- Following the randomization table (Annex G), allocate each patient to one of the study groups using a prepopulated randomization table obtained with SAS (SAS Institute, NC) or statistical software. Therefore, patient allocation will be determined in advance to the patient encounter itself.
- A Case Report Form, consisting of demographic and clinical information (Appendix E).
- Patient's body weight and axillary temperature will be measured. Results will be recorded on the Case Report Form.
- On Day 0 (day of admission), all blood samples will be collected by one single venous drawing of approximately 7 ml in a dry collection tub. The nurse, nurse assistant, or the laboratory technician will use the drawing to perform two sets of thick and thin smears to confirm malaria diagnosis and estimate parasitemia, he or she will put one drop of blood in each of the four circles of the Whatman 903 Protein Saver Cards™ (Sigma Aldrich, St. Louis, USA, lot number: 7097017), transfer 5.0 ml to a tube with EDTA for G6PD activity determination, and also hemoglobin level determination.
- Women aged 10 to 49, inclusive, years old, will also receive a pregnancy test, using a 0.5 mL aliquot of the venous blood.
- We will rely on the microscopist hired for this study to provide the first reading, allowing for patient enrollment and treatment initiation. This professional will undergo a refresher training and competency evaluation by laboratorians from Instituto Evandro Chagas before study initiation. Within 24 hours after the first reading, a second microscopist also trained for this study will read slides, blinded to the first reading results (see below).
- Four drops of blood will be collected on a filter paper, Whatman 903 Protein Saver Cards™ (Sigma Aldrich, St. Louis, USA, lot number: 7097017),. This sample will be used for differentiation of reinfection vs. recrudescence and relapse during the follow-up period (on or after Day 4). This collection will be repeated in case of parasite reappearance on or after Day 4.
- Five mL of blood will be collected in an EDTA tube on Day 0 to allow for G6PD activity determination (Kit NeoLISA™ G6PD, Intercientífica, São José dos Campos, Brazil).
- One drop of blood will be used to determine the hemoglobin level on Days 0, 14, and 28. Blood for hemoglobin on Days 14 and 28 will be obtained by fingerprick. Hemoglobim determination will be conduct using HemoCue ABTM, Ängelholm, Sweden, lot number: 171252.
- 2 mL of blood will be collected in an EDTA tube on Day 7 to allow for chloroquine level determination. This collection will be repeated in case of parasite reappearance from Day 7 through Day 28.

Schedule of study procedures

The following chart outlines the procedures and treatment for study subjects:

Tasks	Day 0 (Enrollment)	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28 to Day 168 (monthly, 6 times)	Any other day (after Day 3, if fever)	Day of any recurrent parasitemia (on or after Day 4)
Blood smear	X		X	X	X	X	X	X	X	X
Axillary temperature	X	X	X	X	X	X	X	X	X	X
Focused history and physical exam	X									
Review of symptoms and physical exam	X	X	X	X	X	X	X	X	X	X
Blood for PCR in filter paper	X									X

Blood for G6PD (EDTA-coated tube)	X									
Blood for CQ levels (EDTA-coated tube)					X					X[1]
Hemoglobin level	X					X		X, on Day 28 only, not monthly afterwards		
Check G6PD result		X	X	X	X					
Treatment										
CQ[2] dose	X	X	X							
Primaquine[3] dose										

1. Blood for chloroquine level will only be taken during the first 28 days of follow up.
2. See chloroquine doses on Tables 2 and 3.
3. Primaquine should be initiated as soon as result of G6PD activity is available. Primaquine course should be of at least seven or 14 consecutive days, depending on the study group of the patient with doses adjusted for body weight as recommended (Table 4a and 4b).

Blood smear

A Giemsa-stained thick blood film will be examined at 1,000x to identify parasite species and determine the parasite density. Laboratory refresher training to be provided by colleagues from Instituto Evandro Chagas will cover these procedures. Thick smears should be stained with diluted Giemsa stain (1:50, vol/vol) (or another) for 20 minutes. Thin smears should be stained with diluted Giemsa stain (1:50, vol/vol) (or another) for 30 minutes. Parasite density will be calculated by counting the number of asexual parasites using a hand tally counter against 200 white blood cells (WBCs) in the thick and thin smear, based on a WBC count of 6,000 WBC/µl. The parasite density per microliter will be calculated using the following formula:

$$\text{Parasite density/}\mu\text{l} = \frac{\text{number of parasites counted} \times 6{,}000}{\text{number of WBC counted}}$$

If the parasite count is <100 parasites/200 WBC, counting will be continued until 500 WBCs have been counted. If no asexual parasites are found after counting 500 WBCs, the count will continue until 1,000 WBCs are counted. In other words, a total of 1,000 WBCs will be examined before a blood smear is considered negative. Gametocytes will also be counted and number of gametocytes per microliter will be estimated on the same manner.

All blood smears will be examined by two independent microscopists. The first microscopist will be based at the clinic at the Hospital Regional do Jurua and the second at the malaria office at the Santa Casa de Cruzeiro do Sul. This way the two initial readings will be done by professionals in Cruzeiro do Sul. Blood smears with differences in species diagnosis or asexual parasite density of >50% between the two microscopists will be re-examined by a third, independent microscopist at Instituto Evandro Chagas or at the reference laboratory in Cruzeiro do Sul. Asexual parasite density will be calculated by averaging the counts of the two concordant microscopists using geometric mean.

Asexual parasite densities will be calculated by averaging the results of the two concordant microscopists using geometric mean. Gametocytemia will be the arithmetic means of the same two readings. The use of geometric and arithmetic means is decided based on the expected distribution of these values in previous similar studies.

Antimalarial therapy

All treatments will be provided free of charge to patients. We will use the medication procured by the Brazilian Ministry of Health, which undergo regular quality control testing. Additionally, these medications

had their quality confirmed by trials carried out at the Centers for Disease Control and Prevention (CDC). CQ (Fundação Oswaldo Cruz/Farmanguinhos, Rio de Janeiro, Brazil, 150mg per pill, lot number:1700388) and primaquine (Medopharm, Asuncion, Paraguay, lot numbers: 7E254 [15mg per pill] and S12 [5 mg per pill]) tablets were obtained from reputable sources to guarantee quality. All doses of CQ and primaquine will be administered under supervision of a study team member for Groups 2 and 3. Only CQ doses will be overserved for Group 1. Primaquine treatment will only be started for patients with documented normal activity of G6PD and will be given for seven or 14 consecutive days. Patients with abnormal G6PD activity level will not undergo primaquine treatment, will be excluded from the study and referred for follow up at health facility. These patients will need to be replaced to reach sample size.

Patients will be observed for 30 minutes after treatment for adverse reactions or vomiting. Any subject who vomits during this period will be retreated with the same dose of CQ or primaquine and observed for an additional 30 minutes. If a subject vomits a second time, he/she will be referred for alternative treatment respecting referral policies in Cruzeiro do Sul. Patient will be withdrawn from the study.

Following national recommendations of Brazil, dosing of both CQ and primaquine will be adjusted by weight according to Tables 2 (a and b) and 3 (a and b) for the treatment of 7 or 14 days.

Table 2a. Dosing of CQ and primaquine for *P vivax* malaria treatment (Groups for 7 days) for patients ≤24 Kg.

Weight (kg)	Number of pills per day						
	Day 0		Day 1		Day 2		Additional 4 days
	CQ	Primaquine (5 mg tablet)*	CQ	Primaquine (5 mg tablet)*	CQ	Primaquine (5 mg tablet)*	Primaquine (5 mg tablet)*
5–9 Kg	1/2	1	1/4	1	1/4	1	1/2
10–14Kg	1	2	1/2	1	1/2	1	1
15–24 Kg	1	2	1	2	1	2	2

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.

Table 2b. Dosing of CQ and primaquine for *P vivax* malaria treatment (Groups for 14 days) for patients ≤24 Kg.

Weight (kg)	Peso (kg)	Número de comprimidos por dia							
		Day 0		Day 1		Day 2		Additional 4 days	7 additional days
		CQ	Primaquine (5 mg tablet)*	CQ	Primaquine (5 mg tablet)*	CQ	Primaquine (5 mg tablet)*	Primaquine (5 mg tablet)*	Primaquine (5 mg tablet)*
5–9 Kg	5 a 9 kg	1/2	1/4	1/4	1/4	1/4	1/4	1/4	1
10–14Kg	10 a 14 kg	1	1	1/2	1	1/2	1	1	1 and 1/2
15–24 Kg	15 a 24 kg	1	2	1	2	1	2	2	2

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.

Table 3a. Dosing of CQ and primaquine for *P vivax* malaria treatment for patients >25 kg (Groups for 7 days).

Weight (kg)	Number of pills per day						
	Day 0		Day 1		Day 2		Additional 4 days
	CQ	Primaquine (15 mg tablet)*	CQ	Primaquine (15 mg tablet)*	CQ	Primaquine (15 mg tablet)*	Primaquine (15 mg tablet)*
25–34 Kg	2	1	2	1	2	1	1
35–49 Kg	3	2	2	2	2	2	1
≥50 Kg**	4	2**	3	2**	3	2**	2**

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.
** Patients ≥70 Kgs will have to have the primaquine dose corrected by weight as shown in Tables 4a and 4b.

Table 3b. Dosing of CQ and primaquine for *P vivax* malaria treatment for patients >25 kg (Groups for 14 days).

Weight (kg)	Peso (kg)	Número de comprimidos por dia						
		Day 0		Day 1		Day 2		11 additional days
		CQ	Primaquine (15 mg tablet)*	CQ	CQ	Primaquine (15 mg tablet)*	CQ	Primaquine (15 mg tablet)*
25–34 Kg	25 a 34 kg	2	1	2	1	1	1	1
35–49 Kg	35 a 49 kg	3	1 1/2	2	1 1/2	1 1/2	1 1/2	1 1/2
≥50 Kg**	≥ 50 kg**	4.	2**	3	2**	2**	2**	2**

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.
** Patients ≥70 Kgs will have to have the primaquine dose corrected by weight as shown in Tables 4a and 4b.

In case of patients 70–120 Kgs, primaquine should be taken as a daily dose of 30 mg (2 adult pills) for a number of days according to patient weight (Tables 4a and 4b).

Table 4a. Dosing of primaquine for *P vivax* malaria treatment for patients >70 kg (Groups 1 and 2).

Weight	Number of treatment days with primaquine (two 15 mg tablets per day)*	Total dose (mg)**
70–79 Kg	8	240
80–89 Kg	9	272
90–99 Kg	10	304
100–109 Kg	11	336
110–119 Kg	12	368

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.

** Total dose estimated.

Table 4b. Dosing of primaquine for *P vivax* malaria treatment for patients >70 kg for groups taking 14 days of primaquine (Group 3).

Weight	Number of treatment days with primaquine (two 15 mg tablets per day)*	Total dose (mg)**
70–79 Kg	17	510
80–89 Kg	18	540
90–99 Kg	21	630
100–109 Kg	23	690
110–119 Kg	26	780

* Attention: Primaquine not to be initiated until a normal value of G6PD activity is documented for the patient.

** Total dose estimated.

Concomitant treatment

Using standard healthcare practices in Brazil, study team members or clinic personnel will administer supportive treatment to subjects as necessary. This include paracetamol, which will be given for axillary temperatures >38.0^{o} C and parents/guardians will be instructed in the use of tepid sponging for children. Other conditions that may require additional treatment or evaluation will be referred to the reference center for specific follow up.

Follow-up procedures

Subjects will be asked to return to each one of the study sites on Days 1, 2, 3, 7, 14, 21, 28, and every four weeks until Day 168 (24 weeks mark). On those visits a clinical assessment and blood collection via finger prick (except for Day 7, when venous puncture will be made) will take place. We will not collect blood on Day 1. In case patients cannot or do not wish to return to the health facility, arrangements will be made for patients to be visited at home by a study team member: the nurse during the period he/she has to take CQ, or the nurse assistant afterwards. All CQ doses will be given as directly observed therapy. Doses of primaquine will be observed only in patients in Groups 2 and 3. Tolerance of +/- 1 day will be allowed for visits on Day 14 or after.

At all times, patients and any member of the study staff can count on the physician part of this study to evaluate patients and manage medical situations that might arise. If unexpected situations arise, patients will be referred for treatment at routine services in Cruzeiro do Sul, including emergency services and hospitalizations if needed. A schedule with dates of follow-up visits and contact information of study staff will be provided to participants.

Since only ambulatory patients with uncomplicated malaria and drugs with well-known safety profile is being assessed as part of this protocol, there is no need for daily follow-up after treatment is finished. Subjects and parents/ guardians (in the case of participants less than 18 years old) will be encouraged to reach study team for further assessment and/or treatment at any point during follow-up at which the subject is perceived to be ill. Telephone numbers and address of key study personnel will be provided to all participants at the time of enrollment. Compensation for transport to and from the clinic can also be provided, for those patients that incur on transportation costs to come for the visits, or we can arrange a study staff to visit you at your home or pick up you up for the visit.

When the research subject is female and pregnancy occurs before the end of the treatment with chloroquine and primaquine, there will be interruption of their participation in the research and it will be referred to the assistance of the municipality regarding the care of pregnant women with malaria. If a patient becomes pregnant after treatment with chloroquine and primaquine, the participant will remain in the study.

During the consent procedure, we will ask patients and their guardians to keep samples for future malaria studies (Annexes C1, C2, C3). The sample collected represents the minimum necessary for the purposes described in this study and the remaining aliquots will be minimal. We will not increase volumes of samples in order to have leftovers for stocking. In the case of non-concurrence with leftover storage, any leftover filter paper will be destroyed 2 years after the study and even during this period will only be used for assays related to this study and already described in this protocol, such as in need of repeating assays to confirm results.

Chloroquine blood levels

To aid in the interpretation of treatment failures during the first 28 days of follow-up (reappearance of parasitemia between Days 4 and 28), blood levels of CQ and its major metabolite desethylchloroquine (DCQ) will be measured on Day 7 and at the time of any reappearance of parasitemia from Day 7 to Day 28. A blood level of CQ plus DCQ ≥100 mg/ml (equivalent to ≥15ng/ml in plasma), in the presence of *P. vivax* parasites in the blood is highly suggestive of CQ resistance.

To permit this assessment, 2 ml of blood will be taken by venipuncture from all patients on Day 7 and again at the time of any recurrent parasitemia on or before Day 28, in an heparin tube and stored in a cold chamber at 4° C. Chloroquine and DCQ levels will be measured by high-performance liquid chromatography (HPLC) by reference laboratories at laboratories in Brazil.(WHO 2009, Miranda, Silva et al. 2015)

Molecular analysis

Microsatellite genotyping

We plan to use the microsatellite markers described by Imwong et al. for *P. vivax* in this study.(Imwong, Sudimack et al. 2006) In brief, in case of parasitemia appearing on or after Day 4, blood collected on the day of the parasite reappearance will be processed in pair with sample collected on Day 0. We will extract DNA from DBSs using commercial kits (QIAamp DNA Mini Kit, ™ Qiagen, Valencia, California, USA). Polymerase chain reaction (PCR) aiming to amplify the microsatellites previously described will be conducted. We will analyze microsatellite fluorescent-labeled PCR products using Applied Biosystems 3130

Capillary Sequencer™ and determine allele sizes with Gene Marker™ v1.95. Fluorescent-labeled PCR products will be analyzed and when the size of an allele was at least different for more than two base pairs from other alleles, it will be considered a different allele. Multiplicity of infection (MOI) and haplotype frequencies will be estimated by maximum-likelihood using day 0 samples of patients with recurrences and randomly selected day 0 samples to reach 128 samples.

This work will be conducted by collaborators at Instituto Evandro Chagas in Brazil with support from CDC/Atlanta, but we can extract the DNA from the parasite contained in the collected material and send this aliquot to the CDC laboratories in Atlanta if necessary for processing and quality control.

***CYP2D6* genotyping and metabolic activity phenotyping**

We also plan to analyze *CYP2D6* gene polymorphisms and to correlate those to metabolic activity phenotypes.(Gaedigk, Dinh et al. 2018, Caudle, Sangkuhl et al. 2020) Seven single nucleotide polymorphisms; C-1584G (rs1080985), C100T (rs1065852), C1023T (rs28371706), G1846A (rs3892097), G31A (rs769258), G3183A (rs59421388), and G4180C (rs1135840); and one deletion, 2615_2617delAAG (rs5030656), will be genotyped using all day 0 samples by TaqMan™ SNP Genotyping Assays (Thermo Fisher Scientific, Waltham, Massachusetts, USA). We will perform amplifications, fluorescence detection, and analysis using StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Then we will determine enzyme metabolic activity scores (ASs) for each haplotype: poor metabolizer (AS= 0), intermediate metabolizer (AS= 0.25–1.00), normal metabolizer (AS= 1.25–2.25), and ultrarapid metabolizer (AS >2.25)(Gaedigk, Simon et al. 2008, Friedrich, Genro et al. 2014, Caudle, Sangkuhl et al. 2020). This work will be conducted by collaborators at Instituto Evandro Chagas with support from Federal University of Pará State in Brazil.

Outcome measures

The efficacy and lack of efficacy of the drug being evaluated will be based on an assessment of the parasitologic and clinical outcomes recommended by WHO (WHO 2009). These definitions are valid only for the initial 28 days of follow-up. These are:

- Early Treatment Failure (ETF), if patient develops one of the following conditions during the first three days of follow-up:

  - Development of danger signs or severe malaria on Days 1, 2, or 3, in the presence of asexual parasitemia;
  - Asexual parasitemia on Day 2 >Day 0 count in disregard to presence of fever;
  - Asexual parasitemia on Day 3 in the presence of axillary temperature ≥37.5°C;
  - Asexual parasitemia on Day 3 >25% of Day 0 count.
- Late Treatment Failure (LTF), if patient develops one of the following conditions during the follow-up period from Day 4 to 28:
  - Late clinical failure (LCF)
    - Development of danger signs or severe malaria in the presence of asexual parasitemia on any day from Day 4 to Day 28, without previously meeting any of the criteria of ETF, or
    - Axillary temperature ≥37.5°C (or history of fever), in the presence of asexual parasitemia on any day from Day 4 to 28, without previously meeting any of the criteria of ETF.
  - Late parasitological failure (LPF)
    - Presence of asexual parasitemia on any day between Days 7 and 28 and axillary temperature <37.5ºC without previously meeting any of the criteria of ETF or late clinical failure.
- Adequate Clinical and Parasitological Response (ACPR), if patient shows the following conditions during the 28-day follow-up period:
  - Absence of asexual parasitemia between Days 14 and 28 without previously meeting any of the criteria of ETF or LTF.

The resurgence of malaria symptoms with proven parasitemia between Day 28 and Day 168 will be classified as Failure in Extended Follow-up.

Besides that, we will also consider a simplified outcome algorithm recommended by WHO as follows: Day 28 treatment failure defined as (1) clinical deterioration due to *P. vivax* illness requiring hospitalization in the presence of parasitemia, (2) presence of parasitemia and fever (axillary temperature ≥37.5 °C) on any day between days 3 and 28; or (3) presence of parasitemia on any day between days 7 and 28, irrespective of clinical condition. The resurgence of parasitemia between days 28 and 168, with or without fever, will be classified as extended follow-up failure. A day 28 adequate clinical and parasitological response (ACPR) will include participants who will not meet criteria for day 28 treatment failure; we also ascertained day 168

recurrence-free proportion as patients who will not meet any failure criteria during the entire follow-up. Participants with treatment failure will be withdrawn and referred for rescue treatment.

PCR results will be used to elucidate whether these cases are due to homologous isolates (same genotypic profile between Day 0 isolate and day of reinfection) or heterologous (different genotypic profiles between Day 0 isolate and day of reinfection).

Freedom from recurrence at day 28 and day 168 are the primary outcomes of this study. We will perform intention to treat and per-protocol analyses, and plan to report both on study reports. We will compare day 28 ACPR and day 168 recurrence-free proportions for groups 1 and 3, and groups 2 and 3 using adequate statistical methods (e.g., logrank test, and Wald test) and appropriate correction factors to account for multiplicity of comparison (e.g., Bonferroni adjustment) depending on the analyses done. We will also repeat intention-to-treat analysis of day 168 recurrence-free proportions considering only homologous recurrences, using genotyping results.

In all cases of therapeutic failure, patients will be withdrawn from the study and referred to the routine treatment service according to Brazilian Health Ministry regulations.

Loss to follow-up

Loss to follow-up occurs when, despite all reasonable efforts, an enrolled patient cannot be found. Include failure to find a patient in the community after he or she misses a scheduled follow-up visit. Except for the period patient is taking CQ, it is acceptable for patients to be seen on the day before or day after the schedule visit before he/she is considered loss of follow-up. In addition, any patient who leaves the study area is also considered loss of follow-up. Patients who are lost to follow-up should be distinguished from those who voluntarily leave the study. If a patient is lost to follow up during the period she/he is receiving medications, all efforts will be made by the study team to locate patient and guarantee he/she takes all doses.

Withdrawal from study

A study patient (or a study patient's parent or guardian) who decides not to participate any further in the study is referred to as a voluntary withdrawal. An example of a cause for involuntary withdrawal would development of a concomitant illness that would interfere with the clear interpretation of study outcomes.

Participation interruption

In some instances, a participant may be removed from the study because of an event that does not allow for continued accurate interpretation of response to treatment. Examples include missed treatment dose, detection of a mixed infection during follow-up, or a credible report of additional antimalarial drug use outside the study protocol (such as self-medication).

Subjects meeting any of the following criteria will be withdrawn from follow-up (include reasons for voluntary and involuntary withdrawal):

1. Withdrawal of consent
2. Failure to complete the CQ treatment as prescribed. We will tolerate one day for the primaquine doses
3. Low levels of G6PD activity, these patients will require another patient to be admitted as a replacement
4. Persistent vomiting during the treatment with either CQ or primaquine
5. Erroneous inclusion of a patient outside of the inclusion/exclusion criteria
6. Severe side-effects (see below)
7. Occurrence during the follow-up of concomitant disease that would interfere with a clear classification of the treatment outcome
8. Need for or receipt of blood transfusion
9. Detection of another malaria species infection during the follow-up. These patients will be referred for appropriate treatment.
10. Antimalarial (or antibiotics with antimalarial activity) treatment administered by a third party or self-medication with antimalarial (or antibiotics with antimalarial activity) (doxycycline, clindamycin, etc)
11. Failure to attend scheduled visits within the +/- 1 day of tolerance on or after Day 14 Severe malaria occurring after enrollment
12. Misclassification of a patient due to a laboratory error (parasitemia) leading to the administration of the rescue treatment

The cause of withdrawal from the study or loss to follow-up will be recorded in the Case Report Form. In case of items from 1 to 6 listed above, patients will be excluded from all analysis in the other instances, we will censor patient data until the day of the event.

Adverse events

Occurrence of side-effects associated with the study drugs or procedures will be closely monitored and recorded on appropriate form (Annex F). Patients with side-effects necessitating medical treatment or hospitalization will be referred for appropriate treatment. Study participation may be discontinued if it is established that study procedures cannot be safely continued, such as study drug allergic events during treatment or G6PD deficiency, for that study participant. Of note, patients with skin rash or any severe allergic reaction will be withdrawn from the study, while patients experiencing only itching will not be withdrawn from the study. In addition, adverse events that are unexpected, likely related to the research study, and that place the participants at greater risk of harm will be reported to the local and CDC IRBs in accordance with required reporting schedules after their occurrence, i. e., in no later than 2 working days of CDC awareness.

Alternative treatment

Patients with intolerance to the therapy, therapeutic failure, or reinfection with *P. viva*x infection will be referred to reference health facility with alternative treatment. Alternative treatment will be decided by attending physician.

Limitations

The findings of this study are limited to the catchment area of the study health facilities in Northwest Acre state. We acknowledge that results may not be representative of the whole country. In addition, only consenting participants will be included, which may introduce selection bias in the study. These points will be taken into consideration for interpretation of results.

Ethical considerations

Consent procedure: Informed consent or permission will be asked from adult participants and child's parents or guardian (in case of <18 years old) for those who meet the study inclusion criteria. The age of majority in Brazil is 18 years (Appendices A1 and A2). For participants 7 to 17, inclusive, years old, we will also ask for his/her verbal assent (Appendices B1 and B2); assent will not be asked for children 5 through 6 years old. In case of illiterate participants, we will read the consent/ assent and two witnesses not associated with the study will be asked to sign the forms; and the participant's or guardian's fingerprint, which is a legal form of documentation of consent in Brazil, will be added to the respective forms. Signed consent forms will be kept separate from case report forms. The study details, participant benefits, and possible risks will

be explained in Portuguese, the national language in Brazil. All patient information will be kept confidential. Unique numerical identifiers, and not personal identifiers, will be used for data entry and analysis.

Any patients who decide not to enter the protocol will be evaluated as usual by the healthcare facility personnel as customary with no prejudice to the patient or his/her family. In case of confirmed malaria, the patient will be treated in accordance to the guidelines from the National Malaria program in Brazil.

Procedure and risks: There is an inherent minimal risk associated with the finger sticks and venipunctures, which are to be performed as part of this study. The patient may experience a brief moment of discomfort and/or fear during the finger stick or venipuncture and the puncture area may get infected or bruised. We estimate that a total of 10 finger pricks will be needed for each study participant, in addition two blood drawing, one of 7 ml on Day 0 and the other of 2 ml on Day 7, will be taken from the patients. It may be required to collect additional blood in case of suspicion of recurrent infection. Blood collections were reduced to the minimal necessary and we will make sure trained phlebotomists and/or laboratory technicians perform those to minimize risks and discomfort. In addition, to reduce risks, we tried to combine blood collections to be done on the same day in a way to collect blood with just one of the procedures. Separate consent/assent (Annexes C1, C2, C3) will be required for storage of remaining material from the samples. If no authorization is given, all material from that patient will be destroyed upon completion of the analysis of the study, estimated at two years after its closure. This material will only be used for malaria assessments.

A minority of patients may experience drug side effects. The side effects most commonly associated with CQ are nausea, vomiting, rash, headache, dizziness, urticaria and abdominal pain. On rare occasions, higher doses of CQ have been associated with aplastic anemia. Commonly reported side effects of primaquine include hemolysis in patients with G6PD deficiency and gastrointestinal symptoms. We note that the 14-day primaquine group is receiving a higher dose of the drug than recommended by the Brazilian malaria co-ordination, but it is within the WHO recommendations.(WHO 2015)

Benefits: The participants in this study have some direct and indirect benefits. Patients will receive supervised treatment for malaria and will be closely monitored to check the efficacy of it. In addition, participants will be examined and referred for treatment of other concurrent illnesses. At each visit, the patient or his/her guardian will be informed of his/her physical status and the medical procedures that will take place. The patient's health condition will be closely monitored during the duration of the study. Patients will be encouraged to return for the follow-up visit and in case they do not return, a member of the

investigation team will perform a home visit. Indirectly, this study will also allow a better understanding of the efficacy of *P. vivax* malaria treatment in the Amazon region of Brazil. This will ensure policies in Brazil are in place to reduce the morbidity and mortality of malaria.

Patient selection: Malaria affects both children and adults in Brazil. The methods to monitor malaria and evaluate national policies published by the WHO recommends enrollment of patients that are representative of the population (WHO 2009). For this reason, adults and children ≥5 years old will be enrolled in the present study.

Confidentiality: All study forms and materials will be kept in locked cabinets and locked rooms. Patients' identifiers will be available to Cruzeiro do Sul study personnel only to allow for patient follow-up. Electronic databases will be password protected and will not contain personal identifiers. In spite of all of these precautions to maintain confidentiality of the data, it is possible that the confidentiality could be compromised. We will make our best efforts to avoid dissemination of any personal information.

Experimental products or procedures: No new therapies, products, or procedures will be used in this study. The use of CQ and primaquine is a practice in Brazil and routinely recommended for treatment of *P. vivax* infections. We will, however, evaluate a higher dose of primaquine that than the one recommended in Brazil, but this dose is already recommended by WHO.(WHO 2015, Negreiros, Farias et al. 2016)

Data management

All forms and files will be kept at a safe place in Cruzeiro do Sul, Acre state, Brazil. File cabinets and rooms where data are stored will be protected by keys and locks and only study investigators will have access to them. Personal identifiers are collected mainly for follow-up reasons, i.e. to allow for patient to be located in case of no show to follow up appointments. This information will not be entered on electronic databases, nor will it be used during the analysis phase.

Case report forms and other study information will be entered in an electronic database in Microsoft Access or equivalent, which will be under responsibility and ownership of the field investigators in Cruzeiro do Sul. No personal identifier will be entered in the electronic databases. A copy of the database, which by design will not contain any personal identifier, will be shared with co-investigators at CDC and IEC for assistance with analysis. Statistical programs, such as Epi-Info (Centers for Disease Control and Prevention, Atlanta, GA) and SAS (SAS Institute, NC), will be used for data management and analysis.

Statistical analysis

Data will be analyzed using two methods: first, by survival analysis where all enrolled patients (including those who were withdrawn from the study or who were lost to follow-up) will be included; second, by the traditional method where patients excluded from the study or lost-to-follow-up during the course of the project are not included.

The number and proportion of subjects who have treatment failures and ACPRs, as defined above, by Day 28 and Day 168 will be calculated. These are the main and primary outcome measures for treatment efficacy. Potential risk factors (e.g., age, initial parasite density, etc.) will be assessed for an association with the probability of a therapeutic failure and the time to therapeutic failure. Percentages of *P vivax* infections from Day 29 to Day 168 will be evaluated.

Dissemination of results

All results will be shared with the Ministry of Health of Brazil and its partners. The Ministry of Health will make the final determination as to how the results should be presented to the regional and local health officers. Results will also be submitted to peer-reviewed scientific journals and presented at national and international meetings.

At the time participants complete the study (Day 168), or earlier if they have had a therapeutic failure, they will be informed verbally by study staff of the outcome/results of their treatment. At enrollment and at the end the study, the investigation team will inform patients about points of contact at the healthcare facility and Ministry of Health levels to obtain the study findings. The Ministry of Health will determine such points of contact.

Budget

The funds for this study will come from CDC as part of AMI/RAVREDA and will be used to hire project coordinator, nurses, nurse assistants, and microscopists. The budget also accounts for laboratory testing to take place in Cruzeiro do Sul. Other study-related costs, such as study drugs, sample collection supplies, and office supplies, are not included because those refer mainly to materials and consumables that will be purchased directly by partner institutions and shipped to study site.

Table 5. Budget for field costs

Expense	Cost (R$)	Number of months	Total Cost (R$)	Total Cost (US$)	Justification
Principal investigator	1,000.00 per month	12	12,000.00	4,000.00	Study coordination
Nurse	3,200.00 per month	12	38,400.00	12,800.00	This professional will have direct oversight of teams
Nurse	2,600.00 per month	12	31,200.00	10,400.00	This professional will have direct oversight of teams
Nurse assistant	1,000.00 per month	12	12,000.00	4,000.00	Follow up visits
Nurse assistant	1,000.00 per month	12	12,000.00	4,000.00	Follow up visits during enrolment period (8 months only)
Microscopist	1,000.00	12	12,000.00	4,000.00	
Microscopist	1,000.00	12	12,000.00	4,000.00	Part-time
G6PD testing	100.00 per sample	250	25,000.00	8,333.33	
Laboratory supplies (needles, syringes, tubes, etc)	12,000.00	NA	12,000.00	4,000.00	This will be provided at no cost to study team by partner organization.
Total			164,250.00	56,393.67	

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Appendix A1: Consent Form. Informed Consent Form for Patients Aged 18 years and older. (Flesh-Kincaid 7.6)

Project: Efficacy of High Total Dose of Primaquine to Prevent *Plasmodium vivax* Malaria Relapses in Cruzeiro do Sul, Brazil

Purpose

The Brazilian Ministry of Health is working with the Municipal Health Secretariat, State Department of Health of Acre, the Evandro Chagas Institute and the Centers for Disease Control and Prevention (CDC) in the United States of America. They are working on an evaluation of the malaria treatment in Cruzeiro do Sul, Acre. Malaria is a disease caused by a parasite that infects cells in your blood.

The goal of this research is to see if the drugs used to treat malaria are working. We will evaluate treatment with chloroquine and primaquine for vivax malaria. This is the kind of malaria most common in Brazil.

This evaluation is being coordinated by Dr. Suiane Negreiros do Valle. She is a physician at the State Department of Health of Acre, and she works at the Hospital Regional do Jurua.

We intend to include a total of 257 people aged 5 years and over in this assessment.

If you agree, we intend to follow you during the malaria treatment and for six months after the malaria treatment. We will collect blood samples during this time. This is to know if you are free of malaria. If you choose not to take part, you will be treated in the same place with the same medications without any loss or penalty.

Getting Started

After the positive result for malaria vivax, we will place you in one of three groups in this study. The decision to which group you will enter will be random, which means it is by chance like flipping a coin. One group will receive the three-day treatment with chloroquine under supervision and will take primaquine treatment at home for seven days. This is the recommended practice by the Ministry of Health. Another group will receive chloroquine treatment for three days and primaquine for seven days under supervision. The third group will also receive complete treatment under supervision but primaquine will be given for a

total of 14 days under supervision. In this last group, the total dose of primaquine is higher than the one currently recommended in Brazil, but it is within the recommendations by the World Health Organization.

We will ask you some questions to know your general health and condition of your current illness. We will ask you some questions to know your age and for how long you have been sick. You do not have to answer any questions you do not want to answer. In addition, you can decide at any time that you no longer want to take part in this survey without penalty. Your care at the health clinic will not change in quality if you choose not to respond or do not want to take part in this study. We also want to examine you and collect blood samples to confirm that you have malaria. We will collect a small amount of blood (about 7 ml, a tea spoon and a half) of blood from the vein in your arm. This blood will be used to confirm the diagnosis of malaria. It will also confirm you have an active substance in your blood to be able to tolerate the treatment with primaquine. We will do these tests right here in Cruzeiro do Sul. After these initial tests, the blood will be stored for other laboratory tests to compare the response to the treatment in case you have another malaria episode during the follow-up. These new tests will be conducted at the Evandro Chagas Institute in Belém do Pará. A small part of the parasite material may be sent to the Centers for Disease Control and Prevention in the USA.

After blood collection and confirmation of the diagnosis, we will immediately start treatment with chloroquine. The dose of medication and the number of days of treatment depend on your weight. For the chloroquine dose, we follow the guidelines and recommendations of the Ministry of Health of Brazil. This means this medication is the same one that you would be receiving even if you did not take part in this study. Chloroquine should be taken for a total of three days. We will ask you to come to this place to receive treatment from our hands. This is true for all three study groups. We can go to your home if you prefer. Chloroquine is well tolerated. It can cause nausea and some pain in the belly within the first few hours after taking in some cases. There is also the risk of allergy to the medicines. At each of these visits, three more visits after today, we need to collect four drops of blood from the tip of your finger and also give you chloroquine for two more days. We will not need to collect blood from you tomorrow.

When we get the results of your laboratory tests, we will start primaquine treatment. This drug is complementary to chloroquine treatment. It is also routinely indicated by the Brazilian Ministry of Health. It is usually taken for a total of seven days. As part of this study, you may be asked to take it for 14 days. This , which is different than the recommendation in Brazil, but within the limits recommended by the World Health Organization. Depending on your weight, you may need to take primaquine for a few more days. You

will need to take the medicine either in front of the health professional or at home, depending on the study group. Since it will not be necessary to collect blood all these days, you can decide if you prefer to come here. We can also come to your house to give the drug.

Treatment and evaluation will be free of charge. The interview, examination, and blood collection will take about 30 minutes on the first visit. It will take about 15 minutes on the next visits. We recommend you sleep under mosquito net with insecticide. This will protect you from malaria.

Follow-Up

After treatment of malaria, we would like to visit you on a weekly basis for the first 28 days after malaria diagnosis. Afterwards, we would like to visit you once a month until six months are complete. It is important that you can take part in those visits. Please let us know if you think that would not be possible. During those visits, we will check your health and collect two drops of blood from your finger. This is to see whether the malaria has been treated. If the malaria comes back, we will refer you for treatment. The results of those tests are available the same day. We will let you know the results. On the seventh day of follow-up, we need to collect a blood sample of more or less 2 mL (less than a teaspoon) from the vein in your arm. This is to measure the levels of chloroquine. In total, there will be 10 samples from the fingertips and two blood samples from the vein in your arm. We will give you a card with the visit scheduling.

For women, we will perform a urine or blood pregnancy test at the beginning of the study for women less than 50 years old. If you are a woman of any age and become pregnant while taking the medication, we will need to stop your participation. You will be referred for treatment of malaria by a reference service here in Cruzeiro do Sul. If you become pregnant after you have finished the drugs, we will follow you as planned.

If you have any health problems or have any questions, please contact Dr. Suiane at the Hospital do Jurua in Cruzeiro do Sul. You can also contact the person who is seen you today here or at the Sala de Estudos em Malaria na Santa Casa. A member of the team will be able to assess you even if it is not the day of your visit at the clinic. The address of the hospital and sala de Estudos are:

Hospital Regional do Juruá	Santa Casa de Misericórdia
Rua 25 de agosto, 5121	Rua Lauro Muller, 473
BairroAeroporto Velho	Bairro Manoel Terças
	Sala ao lado da CERIMAGEM
Phone: (XX) XXXX XXXX	(XX) XXXX XXXX

Risks and Discomfort

There is some discomfort and a small risk if you decide to take part in this study. You may have minimal pain when the blood is collected. Our team is well trained to avoid this. In rare cases, there occurs light bleeding or bruising. If any injury occurs, we will take steps for you to receive the proper care. We think it is very unlikely you will have any harm.

Benefits

There will be direct and indirect benefit for you upon taking part in this study. As part of this study, we will closely monitor you with respect to your disease. So, we will be able to offer you any additional treatment very quickly if you need it. Your participation will also assist the Brazilian malaria program to know if the malaria drugs are still working.

Compensation

You will not receive any compensation for participating in this study. However, we can reimburse you for food and transportation costs to attend the clinic visits. If you need to be hospitalized, this will be done at a facility of the public health service in Brazil free of charge for you. Under the care of the study physician.

Confidentiality

We will keep your information confidential to the full extent permitted by the law. Your name will be used only during the time you come for your appointment or we visit your house. We will not share your name with people outside of the study group or outside Cruzeiro do Sul. None of the reports from this study will contain your name. Patient's forms will be kept in locked cabinets. Your personal information will not be used for any purpose during this study.

Contact Information

If you have any questions or feel you have been harmed by being in the study, contact:

- Dr. Suiane Negreiros do Valle, general coordinator of the project in Cruzeiro do Sul, phone (XX) XXXX XXXX.
- Dr. Paola Marchesini, researcher, responsible for the follow-up of this study with the coordination of the Brazilian Malaria Program in Brasilia, phone (XX) XXXX XXXX.

You can contact Dr. Arnaldo (information below) if you have any questions. She is available to answer questions about your rights as a participant. She is able to explain your rights as a patient in this study. She is not directly involved in this study, but her role is to make sure your, rights are respected.

Arnaldo Jorge Martins Filho
Coordenador do Comitê de Ética em Pesquisa do Instituto Evandro Chagas - CEP/IEC
Rodovia Br - 316, km 7 s/n
67.030-000 Levilandia
Ananindeua, Pará, Brasil
Phone: (XX) XXXX XXXX

Voluntary participation, refusal and withdrawal

Several aspects of this study are very important. Remember that:

- You are free to decide if you want to take part in this study or not. If you decide not to take part, you will receive the necessary free routine care and treatment, including for malaria. You will not incur any penalty or loss for not taking part.
- You are free to withdraw from this study at any time. That fact will not affect your treatment or the care received. You will still receive the routine free treatment for malaria.
- In some situations, if you develop side effects to the treatment or you get malaria, we may need to withdraw you from the study.
- There is no cost to you for taking part in this study.
- You may let me know now if you have any questions with respect to the study. You can also ask questions about your participation.

Declaration of Consent

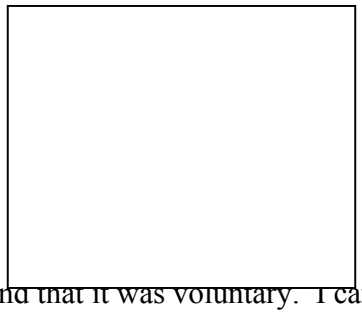
Upon signing or providing a fingerprint indicating legal consent for the appropriate declaration, I agree that:

- I have read this form or the form was read to me by someone else
- I was able to ask questions about the study. My questions have been answered
- I agree in a voluntary manner to participate in this study
- I was informed that I have the right to withdraw from the study at any time. That will not affect the care given to me.

Study participant's name: _____ Date _____

_____

Participant's signature

Right thumb fingerprinting 

I bore witness to the informed consent and that it was voluntary. I can also verify that the participant or responsible party was informed of the details, risks and benefits of the assessment, and had the opportunity for their questions to be answered.

_____ _____

Signature of Witness 1 Date

_____ _____

Signature of Witness 2 Date

Declaration by investigator

I have adequately read, or born witness to the exact reading of the consent form, to the participant and the individual had the opportunity to ask questions. I confirm that the individual freely gave their consent.

Printed name of the member of the assessment team _____

Signature of the member of the assessment team _____

Note: Three copies will be made of this consent. One will remain with the patient or individual responsible for him or her, another will remain on file at the institution where the research takes place, and the third will remain with the coordinator of this assessment.

Appendix A2: Parental Permission Form for Parents of Patients Aged 5 Through 17 years. (Flesh-Kincaid 7.6)

Project: Efficacy of High Total Dose of Primaquine to Prevent *Plasmodium vivax* Malaria Relapses in Cruzeiro do Sul, Brazil

Purpose

The Brazilian Ministry of Health is working with the Municipal Health Secretariat, State Department of Health of Acre, the Evandro Chagas Institute and the Centers for Disease Control and Prevention (CDC) in the United States of America. They are working on an evaluation on malaria treatment in Cruzeiro do Sul, Acre. Malaria is a disease caused by a parasite that infects cells in your blood.

The goal of this research is to see if the drugs used to treat malaria are working. We will evaluate treatment with chloroquine and primaquine for vivax malaria. This is the kind of malaria most common in Brazil.

This evaluation is being coordinated by Dr. Suiane Negreiros do Valle. She is a physician at the State Department of Health of Acre, works at the Hospital Regional do Jurua.

We intend to include a total of 257 people aged 5 years and over in this assessment.

If you agree, we intend to follow your child during the malaria treatment and for six months after the malaria treatment. We will collect blood samples during this time. This is to know if your child is free of malaria. If you choose not to take part, your child will be treated in the same place with the same medications without any loss or penalty.

Getting Started

After the positive result for malaria vivax, we will place your child in one of three groups in this study. The decision to which group your child will enter will be random, which means it is by chance like flipping a coin. One group will receive the three-day treatment with chloroquine under supervision and will take primaquine treatment at home for seven days. This is the recommended practice by the Ministry of Health. Another group will receive chloroquine treatment for three days and primaquine for seven days under supervision. The third group will also receive complete treatment under supervision but primaquine will be given for a total of 14 days under supervision. . In this last group, the total dose of primaquine is higher than

the one currently recommended in Brazil, but it is within the recommendations by the World Health Organization.

We will ask you some questions to know his/ her general health and condition of his/her current illness. We will ask you some questions to know his/her age and for how long your child has been sick. You do not have to answer any questions you do not want. In addition, you can decide at any time that you no longer want to take part in this survey.

Your child's care at the health clinic will not change if you choose not to respond or do not want to take part in this survey. We also want to examine your child and collect blood samples to confirm that he/ she has malaria. We will collect a small amount of blood (about 7 ml, a tea spoon and a half) of blood from the vein in your child's arm. This blood will be used to confirm the diagnosis of malaria. It will also confirm your child has an active substance in his/ her blood to be able to tolerate the treatment with primaquine. We will do these tests right here in Cruzeiro do Sul. After these initial tests, the blood will be stored for other laboratory tests to compare the response to the treatment in case your child has another malaria episode during the follow-up. These new tests will be conducted at the Evandro Chagas Institute in Belém do Pará. A small part of the parasite material may be sent to the Centers for Disease Control and Prevention in the USA.

After blood collection and confirmation of the diagnosis, we will immediately start treatment with chloroquine. The dose of medication and the number of days of treatment depend on your child's weight. For the chloroquine dose, we follow the guidelines and recommendations of the Ministry of Health of Brazil. This means this medication is the same one that your child would be receiving even if she/ he did not take part in this evaluation. Chloroquine should be taken for a total of three days. We will ask you to come to this place to receive treatment from our hands. This is valid for all three study groups. We can go to his/ her home if you prefer. Chloroquine is well tolerated. It can cause nausea and some pain in the belly within the first few hours after taking it in some cases. There is also the risk of allergy to the medicines. At each of these visits, three more visits after today, we need to collect four drops of blood from the tip of your child's finger and also give him/ her chloroquine for two more days. We will not need to collect blood from him/ her you tomorrow.

When we get the results of your child's laboratory tests, we will start primaquine treatment. This drug is complementary to chloroquine treatment. It is also routinely indicated by the Brazilian Ministry of Health.

It is usually taken for a total of seven days. As part of this study, your child may be asked to take it for 14 days. This, which is different than the recommendation in Brazil, is within the limits recommended by the World Health Organization. Depending on his/ her weight, she/ he may need to take primaquine for a few more days. He/ she will need to take the medicine either in front of the health professional or at home, depending on the study group. Since it will not be necessary to collect blood all these days, you can decide if you prefer to come here. We can also come to your child's house to give the drug.

Treatment and evaluation will be free of charge. The interview, examination, and blood collection will take about 30 minutes on the first visit. It will take about 15 minutes on the next visits. We recommend your child sleep under mosquito net with insecticide. This will protect him/ her from malaria.

Follow-Up

After treatment of the malaria, we would like to visit your child on a weekly basis for the first 28 days after malaria diagnosis. Afterwards, we would like to visit your child once a month until six months are complete. It is important that you can take part in those visits. Please let us know if you think that would not be possible. During those visits, we will check his/ her health and collect two drops of blood from his/ her finger. This is to see whether the malaria has been treated. If the malaria comes back, we will refer your child for treatment. The results of those tests are available the same day. We will let you know the results. On the seventh day of follow-up, we need to collect a blood sample of more or less 2 mL (less than a teaspoon) from the vein in his/her arm. This is to measure the levels of chloroquine. In total, there will be 10 samples from the fingertips and two blood samples from the vein in your arm. We will give you a card with the visit scheduling.

If your child is a girl aged more than 10 years old, inclusive, we will perform a urine or blood pregnancy test at the beginning of the study. If your child of any age becomes pregnant while taking the medication, we will need to interrupt your participation. Your child will be referred for treatment of malaria by a reference service here in Cruzeiro do Sul. If she becomes pregnant after you have finished the drugs, we will follow your child as planned.

If you or your child have any health problems or have any questions, please contact Dr. Suiane at the Hospital do Jurua in Cruzeiro do Sul. You can also contact the person who is seen you today here or at the Sala de Estudos em Malaria na Santa Casa. A member of the team will be able to assess you and your child

even if it is not the day of your child's visit at the clinic. The address of the hospital and sala de pesquisa are:

Hospital Regional do Juruá	Santa Casa de Misericórdia
Rua 25 de agosto, 5121	Rua Lauro Muller, 473
BairroAeroporto Velho	Bairro Manoel Terças
	Sala ao lado da CERIMAGEM
Phone: (XX) XXXX XXXX	(XX) XXXX XXXX

Risks and Discomfort

There is some discomfort and a small risk if you decide to take part in this study. Your child may have minimal pain when the blood is collected. Our team is well trained to avoid this. In rare cases, there occurs light bleeding or bruising. If any injury occurs, we will take steps for your child to receive the proper care. We think it is very unlikely your child will have any harm.

Benefits

There will be direct and indirect benefit for your child upon taking part in this study. As part of this study, we will closely monitor your child with respect to his/ her disease. So, we will be able to offer your child any additional treatment very soon. His/ her participation will also assist the Brazilian malaria program to know if the malaria drugs are still working.

Compensation

You will not receive any compensation for participating in this study. However, we can reimburse you for food and transportation costs to attend the clinic visits. If your child needs to be hospitalized, this will be done at a facility of the public health service in Brazil free of charge for you and your child. Under the care of the study physician.

Confidentiality

We will keep your child's information confidential to the full extent permitted by the law. His/ her name will be used only during the time she/he comes for her/his appointment or we visit your child's house. We will not share your child's name with people outside of the study group or outside Cruzeiro do Sul. None of the reports from this study will contain your child's name. Patient's forms will be kept in locked cabinets. Her/ his personal information will not be used for any purpose during this study.

Contact Information

If you have any questions or feel your child has been harmed by being in this study, contact:

- Dr. Suiane Negreiros do Valle, general coordinator of the project in Cruzeiro do Sul, phone (XX) XXXX XXXX.
- Dr. Paola Marchesini, researcher, responsible for the follow-up of this study with the coordination of the Brazilian Malaria Program in Brasilia, (XX) XXXX XXXX.

You can contact Dr. Arnaldo (information below) if you have any questions. She is available to answer questions about your child's rights as a participant. She is able to explain your rights as a patient in this study. She is not directly involved in this study, but her role is to make sure your and your child's rights are respected.

Arnaldo Jorge Martins Filho
Coordenador do Comitê de Ética em Pesquisa do Instituto Evandro Chagas - CEP/IEC
Rodovia Br - 316, km 7 s/n
67.030-000 Levilandia
Ananindeua, Pará, Brasil

Phone: (XX) XXXX XXXX

Voluntary participation, refusal and withdrawal

Several aspects of this study are very important. Remember that:

- You are free to decide if you want your child to take part in this study or not. If you decide not to take part, he/ she will receive the necessary free routine care and treatment, including for malaria. You or your child will not incur any penalty or loss for not taking part.
- You are free to withdraw from this study at any time. That fact will not affect your child's treatment or the care received. He/ she will still receive the routine free treatment for malaria.
- In some situations, if your child develops side effects to the treatment or she/he gets malaria, we may need to withdraw your child from the study.
- There is no cost to you or your child for taking part in this study.
- You may let me know now if you have any questions with respect to the study. You can also ask questions about your child's participation.

Declaration of Consent

Upon signing or providing a fingerprint indicating legal consent for the appropriate declaration, I agree that:

- I have read this form or the form was read to me by someone else
- I was able to ask questions about the study. My questions have been answered
- I agree in a voluntary manner to participate in this study
- I was informed that I have the right to withdraw from the study at any time. That will not affect the care given to me.

Study participant's name: _____ Date _____

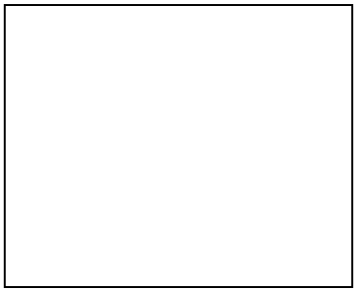
_____

Guardian's name

_____

Guardian's signature

Right thumb fingerprinting



I bore witness to the informed parental permission and that it was voluntary. I can also verify that the participant or responsible party was informed of the details, risks and benefits of the assessment, and had the opportunity for their questions to be answered.

_____ _____

Signature of Witness 1 Date

_____ _____

Signature of Witness 2 Date

Declaration by investigator

I have adequately read, or born witness to the exact reading of the parental permission to the relative (mother or father) of the participant, or respective guardian, and the individual had the opportunity to ask questions. I confirm that the individual freely gave their consent.

Printed name of the member of the assessment team _____

Signature of the member of the assessment team _____

Note: Three copies will be made of this consent. One will remain with the patient or individual responsible for him or her, another will remain on file at the institution where the research takes place, and the third will remain with the coordinator of this assessment.

Appendix B1: Assent Form (for participants aged 7–12, inclusive) (Flesh-Kincaid 3.8)

Project: Efficacy of High Total Dose of Primaquine to Prevent *Plasmodium vivax* Malaria Relapses in Cruzeiro do Sul, Brazil

The Secretariat that takes care of the health of the people in Cruzeiro do Sul will conduct a study. This is to know if the drugs that are used to treat malaria are being good.

Your parents / guardian let you take part. I am asking you now if you want to take part. You are not required to take part. Only if you want.

As part of this study, you will be visited more often by a member of our team than if you were not part of it. You will receive the same routine malaria drugs.

If you agree, you will receive two medications, one white and one orange. They are not in liquid but in pill. Today we will also take a small amount of blood from your arm. This is to do some tests. When we visit you to find out how you are. We will also conduct further tests to see if you are still sick with malaria. We will also want to know if the medicine is still in your blood, and how your body is reacting to the medicine. If you are a woman 10 years of age or older, we will do a pregnancy test. This is done in blood or urine. We will inform your guardian of these results.

You do not have to be afraid to take the sample. The people who will take your blood are already used to doing this. But it may hurt a little. We will also take some blood from your fingers when you come to see us. In one of these days, we will need to take blood from your arm again. If you see or feel anything different after you take medicine tell mom or dad. You can also tell the person who comes to see you. If you already know how to call, you can even call us. We are here to help you.

If you no longer want to receive our visits, you can tell us. This will not cause you any trouble.

Do you understand what I said to you? You can ask your parents or me any questions.

Want to be part of the project?

Appendix B2: Assent Form (for participants aged 13–17, inclusive) (Flesh-Kincaid 4.6)

Project: Efficacy of High Total Dose of Primaquine to Prevent *Plasmodium vivax* Malaria Relapses in Cruzeiro do Sul, Brazil

The Acre Health Department is working with the Ministry of Health of Brazil and with international partners. We are conducting a study to evaluate the treatment of malaria here in Cruzeiro do Sul.

Your parents / guardian agreed for to you take part. I am asking you now if you want to take part. You are not required to take part if you do not want to.

This study will offer you the same malaria medications you would receive if you were not in the study. However, you will be evaluated by our team more often than if you were not part of it.

If you agree, you will receive the two drugs. They are called chloroquine and primaquine. You will need to come back here or we will come to you 13 more times in the next 6 months. This is to see how you are feeling. If you are not feeling well or have a fever, you may need to return more often. At each visit, we will collect a small amount of blood from your finger. This is to check if you are still sick and what type of malaria you have.

Today and on the seventh day, we will also collect a small amount of blood from your arm. This is to check the medicine in your blood. It will also help us know how your body is reacting to the medicine. If you are a woman, we will do a pregnancy test. This is done in blood or urine. We will inform your parents of the result. We will also ask your parents to save any left-over blood for future studies in malaria.

It can hurt when we stick your finger or arm with the needle. An infection may occur. To prevent this, trained people will do the blood collection. Chloroquine can cause nausea and itchiness. Primaquine can cause heartburn, vomiting and diarrhea. These symptoms are mild. You may have severe symptoms, which is rare.

There will be a person here every day to help you.

You may decide not to continue the study at any time and this will not cause you any problem.

Do you have any questions regarding the study? You can ask your parents or me any questions.

Do you want to be part of the project?

Annex C1. Authorization for storage of remaining samples (Participants aged 18 years and older and Parents of Participants aged five through 17 years) (Flesh-Kinkaid level 7.6)

Invitation

We would like to store what is left of the samples we collect during this study. We will keep these samples at the Institutional biobank of the Evandro Chagas Institute. This is part of the Brazilian Ministry of Health. A biobank is a place where we keep samples from people. We respect people and the samples they give us to keep. We need your free and informed consent to keep your/ your child's samples. We say the following things to make sure that you are taking part because you want to do so. There is no penalty for not letting us keep your/ your child's samples. We want to make sure you know what we are doing and that you agree.

Clarifications

Evandro Chagas Institute is part of the Health Surveillance Secretariat of the Ministry of Health. This is a place where we study ways to improve peoples' lives. Sometimes we discover new medicines and tests that help us know more about sicknesses and how to treat the people who have them.

You and / or your child (or person for whom you are the legal guardian) are being asked to take part in a malaria study. They and / or you have signed a consent form. After the needed tests for your/ your child's treatment or research are done, there may be some material left over. This material is usually thrown away. However, we would like to ask you / your parents to let us store to keep it in the Evandro Chagas Institute for future use. We will only do malaria studies with this material.

We will be sure that the IEC and its partners will only use this material for malaria studies. They will respect strict rules that show they respect life. We will be sure that all of your/ your child's personal information is kept private. Your/ your child's sample will be saved without your/ his/ her name on it. We will keep a list of codes so that we know which sample is yours/ your child's without using your name. Only the people in charge of the bank will be able to see this list.

If you wish, at any time, you will have access to the information generated with the use of your/ your child's samples. This includes the results from the research we have done. To do that, you need to contact the Research Ethics Committee of the Evandro Chagas Institute. The committee's contact information is listed below. You may also request that your samples and information not be stored in the Biobank anymore. If

you decide to not let us use your samples anymore, you must let us know in writing. We will then destroy your/ your child's samples according to the rules of the Evandro Chagas Institute.

We remind you that your/ your child's samples can also be thrown away if it is not possible for us to use in research for quality reasons. The Instituto Evandro Chagas may also decide to end the biobank. If this happens this happens, before we throw your/ your child's sample away, we will offer it to at least two biobanks. We will ask for your permission to do this if it happens.

You will receive a copy of this consent form. You can read it at any time. You will not receive any payment. You will also not have to pay anything for your samples to be stored in the bank of samples.

When you take part in the biobank you have certain rights. You can have your questions answered and get more information about these rights through the Research Ethics Committee of the Evandro Chagas Institute. This committee is responsible for monitoring the Biobank and evaluating the future research that will be happen there.

The address of ethics committee is Rodovia BR-316, Km 07, Ananindeua, PA, Brazil. It is opened from 07:30 to 16:30. If you prefer, the service can be reached at (68) 3214 2237. The person responsible is Dr. Arnaldo Jorge Martins Filho.

Declaration of Consent or Permission

Upon signing or providing a fingerprint indicating legal consent for the appropriate declaration, I agree that:

- I have read this form or the form was read to me by someone else
- I was able to ask questions about the storage of blood. My questions have been answered
- I agree in a voluntary manner to allow for the storage of my/my child's blood.

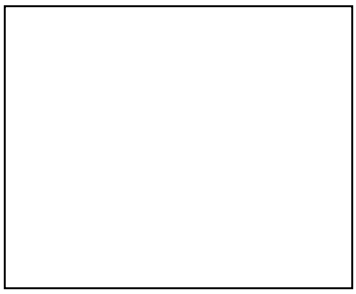
Study participant's name: _____ Date _____

_____

Guardian's name

_____

Patient's or guardian's signature

Right thumb fingerprinting 

I bore witness to the informed and voluntary consent. I can also verify that the participant or responsible party was informed of the details, risks and benefits of the assessment, and had the opportunity for their questions to be answered.

_____ _____

Signature of Witness 1 Date

_____ _____

Signature of Witness 2 Date

Declaration by investigator

I have adequately read, or born witness to the exact reading of the consent form, to the participant or relative (mother or father) of the participant, or respective guardian, and the individual had the opportunity to ask questions. I confirm that the individual freely gave their consent.

Printed name of the member of the assessment team _____

Signature of the member of the assessment team _____

Annex C2. Assent Form for Storage of Remaining Samples (Participants 7 to 12 years)

Study design: **Efficacy of High Total Dose of Primaquine to Prevent *Plasmodium vivax* Malaria Relapses in Cruzeiro do Sul, Brazil**

Invitation

You are part of a malaria study. As part of this study, we will collect some blood from you. Sometimes there is blood left over after the tests. Usually, we throw away what is left over. If there is blood left over, we want to keep it. We would keep it at Evandro Chagas Institute of the Ministry of Health.

Clarifications

At the Evandro Chagas Institute, we do studies. These studies help people live better lives.

We want to keep the remaining blood to help in other studies in malaria. We asked your parents, and they said it is okay to keep it. They know who to talk to if they have questions. We are asking you if it is also okay with you.

If you let us keep the blood, no one will know it is yours except the people responsible for the blood at Evandro Chagas. It is okay if you do not want us to keep the blood. You will be treated the same if you say yes or if you say no.

Do you understand what I said to you? You can ask your parents or me any questions.

Is it okay with you if we keep the blood that is left over from your tests?

Annex C3. Assent Form for Storage of Remaining Samples (Participants 13 to 17 years)

Study design: **Efficacy of High Total Dose of Primaquine to Prevent *Plasmodium vivax* Malaria Relapses in Cruzeiro do Sul, Brazil**

Invitation

You are part of the malaria study. For this, you do blood tests. Sometimes there is blood left over after the tests. Usually, we throw away this blood. If there is blood left over, we want to keep it instead of throwing it away. We would keep it at Evandro Chagas Institute of the Ministry of Health.

Clarifications

At the Evandro Chagas Institute, we do studies. These studies to help people live better lives.

You are taking part in a malaria study. For this, we will collect some blood. We want to use whatever is left to use in other studies. Only studies on malaria would be done. We asked your parents, and they said it is okay to keep it. They know who to talk to if they have questions. We are asking you if it is also okay with you.

If you let us keep the blood, no one will know it is yours except the people at Evandro Chagas. It is okay if you do not want us to keep the blood. You will be treated the same if you say yes or if you say no.

Do you understand what I said to you? You can ask your parents or me any questions.

Is it okay with you if we keep the blood that is left over to do future studies?

Annex D. Antimalarial drugs that should not be used in the 30 days prior to enrolment and also during the study period

- Chloroquine (except for the prescribed period)
- Amodiaquine
- Qinino, quinidine
- Mefloquine, lumefantrine
- Artemisinin and its derivatives
- Proguanil
- Sulfadoxine, sulfamethoxazole (bactrim), dapsone
- Primaquina (except for the prescribed period)
- Atovaquone
- Antibiotics: tetracycline, doxycycline, erythromycin, azytromycin, clindamycin, rifampicin
- Pentamidine

Annex E: Patient form

IDENTIFICATION *(This page is not typed into the database.)*	
Patient number	*Necklace label*
Patient name	
If child, <18 years	
Mother's name	
Father's name	
Name of the person in charge	
Address with reference points how to get	
Best(s) phone number(s) to talk to the patient	
Cost of transportation per day (round trip)	(real)

Reminder (mark the steps taken):

☐ ***Sorting sheet ok***

☐ ***Consent to the patient***

☐ ***Harvest the blood of Day 0 (admission)***.

Clinical Form

CLINICAL HISTORICAL	
Patient number	*Necklace label*
Date (dd/mm/aaa) and time **(e.g., 09:00, 15:00) of admission**	__ __ /__ __ /2018 __ __: __ __ hours
Treatment group	☐Group 1 (7 days, **patient** unsupervised) ☐Group 2 (7 days, **patient** with supervision) ☐Group 3 (14 days, **patient** with supervision)
Health center	☐1. Hospital Regional do Juruá ☐2. Posto 25 de Agosto ☐ 3. Aeroporto Velho ☐ 4. Cruzeirinho ☐ 5. Other If another, which one? _____
Sex	☐ 1. Male ☐ 2. Female
Date of birth (dd/mm/aaaa)	__ __ /__ __ /__ __ __ __
Consent and assents	☐ Informed consent of the study delivered ☐ Informed consent for sample storage delivered Study assent ☐Does not apply (adult patient) ☐ Delivered Assent for sample storage ☐Does not apply (adult patient) ☐ Delivered

Continue on the next page*

CLINICAL HISTORICAL

When did you start showing symptoms of the current illness?	_____/_____/2018
Have you had a fever in the last 48 hours?	☐ Yes If yes, fever duration: _____ days ☐ No
What other symptoms do you experience during your current illness? (*Mark all possible.)*	☐ Headache ☐ Vomiting ☐Calf pain ☐ Chills ☐ Diarrhea ☐ Dark urine ☐ Sweats ☐ General weakness ☐ Other *If others, which ones?* _____
Have you taken any medication for this disease or taken any medication in the last 30 days? ***If the patient has been in use or recent use of antimalarials (see list of antimalarials),*** ***terminate.***	☐ Yes *If so, what medications? Dose? When is it?* ☐ No

Medication	Dose	Start and end

Do you suffer from the following chronic diseases: kidneys diseases, HIV or AIDS, tuberculosis and or malnutrition?	☐ Yes *If so, which ones?* _____ _____ _____ ☐ No ***If yes, terminate***
Do you have any allergies (especially allergies to medications)?	☐ Yes *If so, which ones?* _____ _____ _____ ☐ No ***If allergy to chloroquine or primaquine (severe allergy), END. In the case of only mild itching (itching) with the use of chloroquine, discuss with Dr. Suiane).***

Continue on the next page

PHYSICAL EXAMINATION (DAY 0)	
Axillar temperature	_____
Systolic blood pressure	_____ mmHg
Diastolic blood pressure	_____ mmHg
Weight	_____
Heart rate	_____ beats per minute
Respiratory rate	_____

	Normal	**Abnormal**
Mental state	☐ Awake and oriented	☐ Sleepy, but oriented ☐ Disoriented ☐ Comatose
Chest	☐ Normal breathing	☐ Breathing difficulty ☐Increased breathing (>20 movements per minute)
Abdomen	☐ Not distended and pain-without on palpation	☐ Pain on palpation ☐ Distended ☐ Painful and distended
Extremities (lower)	☐ No cyanosis and/or edema	☐ Cyanosis ☐ Edema ☐ Cyanosis and edema
Jaundice	☐ Absent	☐ This

*****In the event of any marking on the right column (abnormal), discuss with Dr. Suiane patient inclusion****

Hemoglobin	_____ *mg*/dl

Pregnancy test performed in women aged 10 to 49 years	☐It does not apply	**Man; woman <10 or >49 years*
	☐ Blood	
	☐ Urine	
	☐They have not	*******If positive or not performed in women aged 10 to 49 years,* ***terminate***
Pregnancy test result	☐ Negative	
	☐ Positive	*******END**
	☐ Refused	****END****

G6PD	_____ U/g Hb	**If less than 7.0 UI/g Hb,* ***shut down***

****Continue on the next page****

LABORATORY TEST (DAY 0)	
First blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ***END*** ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ***END*** ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > $\frac{\text{Minor parasitemia?}}{2}$ Or species discrepancy?	☒ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ***END*** ☐Another Which? _____ ***Close***
Third reading **asexual parasitemia**	_____ /microliter
Third reading **gametocytemia**	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Write All the Doses of Medication (Chloroquine and Primaquine) For Information Only	
Group number _____	
Patient weight	_____ kg
Chloroquine (150 mg per tablet)	D0: _____ tablets D1: _____ tablets D2: _____ tablets
Primaquine (_____mg per tablet)	_____ treatment days
Primaquine	1st dose _____ tablets 2nd dose _____ tablets 3rd dose _____ tablets 4th to 7th doses _____ tablets ≥8th doses _____ tablets

Note to supervising nurse.
Check this information.

Treatment	
Day 0 (Chloroquine, hemoglobin level)	
1st dose of chloroquine	_____ 150 mg tablet (s)
Time of first attempt for 1st dose*	__ __ __ __ __ (ex. 09:00, 18:00)
Vomit in 30 minutes?	☐ Yes ☐ No
If vomiting in the first 30 min, time of the second attempt for the 1st dose*	__ __ : __ __
Vomit in 30 minutes? ***If vomiting on the second attempt, END***	☐ Yes ☐ No
Did the patient receive paracetamol in this consultation?	☐ Yes ☐ No
Observation	_____ _____ _____ _____
Responsible for the visit:	_____

Reminder (mark the steps taken):

☐***Slides***

☐ ***Filter paper***

☐ ***Hemoglobin***

☐ ***Testes G6PD (EDTA* tube*)***

☐***Pregnancy test (leave blank if not applicable)***

☐ ***Schedule return***

☐ ***Fill in the returns on the patient card (pencil)***

Return **NURSE DAY 1 (Chloroquine)**	
Date of visit	__ __ /__ __ /2018
Axillary temperature (°C)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continues next page

2nd dose of chloroquine	_____ 150 mg tablet (s)
Time of first attempt for dose	__ __ : __ __
Vomit in 30 minutes?	☐ Yes ☐ No
If vomiting in the first 30 min, time of the second attempt for the dose	__ __ : __ __
Vomit in 30 minutes? ***If vomiting on the second attempt, END***	☐ Yes ☐ No

Day 1 condition of the patient	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____
Observation	_____ _____ _____ _____ _____ _____ _____

Reminder (mark the steps taken):

☐ ***Check G6PD (give primaquine if normal)***

☐ ***Schedule return***

Return **NURSE DAY 2 (Chloroquine)**	
Date of visit	__ __ /__ __ /2018
Axillary temperature (°C)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 2)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > Minor parasitemia? / 2 Or species discrepancy?	☐ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading asexual parasitemia	_____ /microliter
Third reading gametocytemia	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Treatment on the next page

3rd dose of chloroquine	_____ 150 mg tablet (s)
Time of first attempt for dose	__ __ : __ __
Vomit in 30 minutes?	☐ Yes ☐ No
If vomiting in the first 30 min, time of the second attempt for the dose	__ __ : __ __
Vomit in 30 minutes? ***If vomiting on the second attempt, END***	☐ Yes ☐ No

Day 2 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____
Observation	_____ _____ _____ _____ _____ _____ _____

Reminder (mark the steps taken):

☐ ***Slides***

☐ ***Check G6PD (give primaquine if normal)***

☐ ***Schedule return***

Return **NURSE DAY 3**	
Date of visit	__ __ /__ __ /2018
Axillary temperature (°C)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 3)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading asexual parasitemia	_____ /microliter
First reading gametocytemia	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > Minor parasitemia? / 2 Or species discrepancy?	☐ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading asexual parasitemia	_____ /microliter
Third reading gametocytemia	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Continue on the next page

Day 3 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____

Observation	

Reminder (mark the steps taken):

☐ ***Slides***

☐ ***Check G6PD (give primaquine if normal)***

☐ ***Schedule return***

Return **DAY 7 (Chloroquine level*)***	
Date of visit	__ __ /__ __ /2018
Axillary temperature (°C)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 7)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > Minor parasitemia? / 2 Or species discrepancy?	☐ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading asexual parasitemia	_____ /microliter
Third reading gametocytemia	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Continue on the next page

Day 7 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____
Chloroquine	_____ mg/dl
Observation	

Reminder (mark the steps taken):

☐ ***Chloroquine level (heparin tube)***
☐ ***Slides***
☐ ***Check G6PD (give primaquine if normal)***

☐***Schedule return***

☐ ***Schedule doses of Primaquine***

Return **DAY 14(Hemoglobin level)**	
Date of visit	__ __ /__ __ /2018
Axillary temperature (°C)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 14)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading asexual parasitemia	_____ /microliter
First reading gametocytemia	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* *END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > Minor parasitemia? / 2 Or species discrepancy?	☐ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading **asexual parasitemia**	_____ /microliter
Third reading **gametocytemia**	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Continue on the next page*

Day 14 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____

Hemoglobin	_____ mg/dl

Observation	

Reminder (mark the steps taken):

☐ ***Blood for hemoglobin level***
☐ ***Blood for microscopy (slides)***
☐ ***Check G6PD (give primaquine if normal)***
☐***Schedule return***

☐ ***Schedule doses of Primaquine***

Return DAY 21	
Date of visit	__ __ /__ __ /2018
Axillary temperature (°C)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 21)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > Minor parasitemia? / 2 Or species discrepancy?	☐ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading **asexual parasitemia**	_____ /microliter
Third reading **gametocytemia**	_____ /microliter
Mean geomistrica of the mostclose values ofasexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Continue on the next page*

Day 21 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____

Observation	

Reminder (mark the steps taken):

☐ ***Slides)***
☐ ***Check G6PD (give primaquine is normal)***
☐ ***Schedule return Schedule primaquine doses***
☐

Return **DAY 28 (Hemoglobin*level)***	
Date of visit	__ __ /__ __ /2018
Axillary temperature(theC)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 28)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END***** ☐*P falciparum* and *mixed vivax P* ***END***** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* ***END***** ☐*P falciparum* and *mixed vivax P* ***END***** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > Minor parasitemia? / 2 ☐ Yes ☐ No Or species discrepancy? ***If 'yes', ask for third reading***	
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END***** ☐*P falciparum* and *mixed vivax P* ***END***** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading **sexual parasitemia**	_____ /microliter
Third reading **gametocytemia**	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Continue on the next page*

Hemoglobin	_____ mg/dl

Have you finished all doses of your treatment with primaquine (7 days) ? (Group 1 only)	☐ Yes ☐ No ☐ Does not apply (Groups 2 and 3)

Day 28 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____

Observation	_____ _____ _____ _____ _____ _____ _____ _____ _____

Reminder (mark the steps taken):

☐ ***Blood for hemoglobin level***
☐ ***Slides***
☐ ***Check G6PD (give primaquine is normal)***
☐ ***Schedule return***

☐***Check end of primaquina***

Return DAY 56 / MONTH 2	
Date of visit	__ __ /__ __ /2018
Axillary temperature(theC)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 56 / MES 2)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END* ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* *END* ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > Minor parasitemia? / 2 Or species discrepancy?	☐ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END* ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading **asexual parasitemia**	_____ /microliter
Third reading **gametocytemia**	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Continue on the next page*

Day 56 / month 2 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____

Observation	

Reminder (mark the steps taken):

☐ ***Slides***
☐ ***Check that the primaquine doses are complete (if G6PD normal)***
☐ ***Schedule return***

Return DAY 84 / MONTH 3	
Date of visit	__ __ /__ __ /2018
Axillary temperature(theC)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 84 / MES 3)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END* ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* *END* ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > $\frac{\text{Minor parasitemia?}}{2}$ Or species discrepancy?	☐ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END* ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading **asexual parasitemia**	_____ /microliter
Third reading **gametocytemia**	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Continue on the next page*

Day 84 / month 3 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____

Observation	_____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____

Reminder (mark the steps taken):

☐ ***Slides***

☐ ***Schedule return***

Return DAY 112 / MONTH 4	
Date of visit	__ __ /__ __ /20____
Axillary temperature(theC)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 112 / MONTH 4)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > $\frac{\text{Minor parasitemia?}}{2}$ Or species discrepancy?	☐ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading **asexual parasitemia**	_____ /microliter
Third reading **gametocytemia**	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive (_____ and _____)	_____ /microliter

Continue on the next page*

Day 112 / month 4 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____

Observation	_____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____

Reminder (mark the steps taken):

☐ ***Slides***

☐***Schedule Return***

Return **DAY 140 / MONTH 5**	
Date of visit	__ __ /__ __ /20____
Axillary temperature (°C)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 140 / MONTH 5)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > $\frac{\text{Minor parasitemia?}}{2}$ Or species discrepancy?	☐ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading **asexual parasitemia**	_____ /microliter
Third reading **gametocytemia**	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive (_____ and _____)	_____ /microliter

Continue on the next page*

Day 140 / month 5 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____

Observation	

Reminder (mark the steps taken):

☐ ***Slides***

☐ ***Schedule return***

Return **DAY 168 / MONTH 6**	
Date of visit	__ __ /__ __ /20____
Axillary temperature (°C)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

LABORATORY TEST (DAY 168 / MONTH 6)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > Minor parasitemia? / 2 Or species discrepancy?	☐ Yes ☐ No ***If 'yes', ask for third reading***
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading **asexual parasitemia**	_____ /microliter
Third reading **gametocytemia**	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Continue on the next page*

Day 168 / month 6 patient condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination
Responsible for the visit:	_____

Observation	_____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____

***Reminder** (mark the steps taken):*

☐ ***Slides***
☐ ***Schedule return***

FINAL PATIENT OUTCOME

Outcome date _____/_____/20____

Outcome follow-up day _____

☐ Completed 168 days of follow-up

☐ Discontinued follow-up before 168 days with parasitemia or other therapeutic failure

☐ Revoked consent or nod

☐ Loss of follow-up

☐ Interrupted medication due to adverse effects

☐ Other. What? _____

OBSERVATION OF THE OUTCOME (FILLED OUT BY NURSE)

Responsible for the visit:	_____

EXTRA DAY FOR SYMPTOM AFTER DAY 3	
Study day	_____ DAY
Date of visit	__ __ /__ __ /20___
Axillary temperature(theC)	_____
Since your last appointment you had:	
Fever?	☐ Yes ☐ No
Headache?	☐ Yes ☐ No
Calf pain?	☐ Yes ☐ No
Dark urine?	☐ Yes ☐ No
Vomiting?	☐ Yes ☐ No
Diarrhea?	☐ Yes ☐ No
Itching?	☐ Yes ☐ No
Red spots on the body (cutaneous erythema)?	☐ Yes ☐ No
Menstrual delay (women 10 to 49 years)? If yes, pregnancy test result	☐ Yes ☐No☐ Does not apply ☐ Negative ☐ Positive ☐ Refused ***END*** ***END***
Any other symptoms? If so, which one?	☐ Yes ☐ No _____
Have you taken any other medication besides the treatment given here?	☐ Yes ☐ No
In the case of another medicinal product, name and quantity and start and end date	
If you experience any symptoms, treatment, or use of medication, warn Dr. Suiane***	
Patient felt better, equal, or worse?	☐ Better ☐ Equal ☐ Worse

Continue on the next page*

EXTRA DAY LABORATORY EXAMINATION SYMPTOM (____DAY)	
First blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* ***END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
First reading **asexual parasitemia**	_____ /microliter
First reading **gametocytemia**	_____ /microliter
Second blade reading (Mark only one response)	☐Only *P. vivax* ☐Only *P. falciparum* *END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Second **reading asexual parasitemia**	_____ /microliter
Second reading **gametocytemia**	_____ /microliter
Comparison between first and second readings	
Difference= _____ (Higher asexual parasitemia) - _____ (Smallest asexual parasitemia) = _____	
Difference > Minor parasitemia? / 2 ☐ Yes ☐ No Or species discrepancy? ***If 'yes', ask for third reading***	
Third blade reading (Mark only one response)	☐Only *P. Vivax* ☐Only *P. falciparum* *END*** ☐*P falciparum* and *mixed vivax P* ***END*** ☐ No parasites found ☐Another Which? _____ ***Close***
Third reading **asexual parasitemia**	_____ /microliter
Third reading **gametocytemia**	_____ /microliter
Geometric mean of the closest values of asexual parasitemia (if positive) (_____ and _____)	_____ /microliter

Continue on the next page*

Patient's condition	☐ Continue ☐ Exclude ☐ Left ☐ Abandoned ☐ Follow-up termination

Chloroquine (if visited between Day 8 and Day 28only for special **reasons)**	_____ mg/dl

Observation	_____ _____ _____ _____ _____ _____ _____ _____ _____

Conduct	_____ _____ _____ _____ _____

Responsible for the visit:	_____

Reminder (mark the steps taken):

☐ ***Spoon filter paper if positive blade***
☐ ***Collect blood (heparin tube, green) for chloroquine if Day 28 or earlier***
☒ ***Schedule return***

Annex F. Adverse Events Form

Patient ID | | | |

Chave para ficha de EA

Severity	1 = Mild (Grade 1): Signal or symptom awareness easily tolerated 2 = Moderate (Grade 2): Discomfort sufficient to cause interference with normal activity 3 = Severe (Grade 3): Incapacitating with incapacity for work or carrying out habitual activity 4 = At risk of death (Grade 4): Patient at risk of death at the time of the event, or requires hospitalization or prolongation of hospitalization; cause a persistent or significant disability; result in congenital anomaly or birth defect 5 = Death (Grade 5)					
Study drug related?	1=Definitly not	2=Improbably related	3=Possibly related	4= Probably related	5= Definitly related	
Outcome	1=Total recovery	2=Patient not recovered yest	3=Worsening	4=Permanet sequalae	5=Death	6=On going 7=Unknown
Action take	1=No measure	2=Dose adjustment or temporarily suspended	3=Permanent withdrawal of drugs	4=Concomitant drug given	5=No therapy given	6= Hospitalization

Number of adverse events | | | Data: | | / | | | | / | | |

_____

Diagnosis _____

Onset date | | / | | | | / | | End date | | / | | | | / | |

Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 Related to the study drug ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Result ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 Treatment ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

Treatment administered ☐ Yes ☐ No *Specify* _____

Was this a severe one? ☐ Yes ☐ No

Number of adverse event ☐☐ Reporting date ☐☐ / ☐☐☐ / ☐☐

Diagnosis _____

_____

Onset date ☐☐ / ☐☐☐ / ☐☐ End date ☐☐ / ☐☐☐ / ☐☐

Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 Related to study drug ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 Treatment ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

Treatment administered ☐ Yes ☐ No *Specify*: _____

Was this a severe one? ☐ Yes ☐ No

Number of adverse event ☐☐ Reporting date ☐☐ / ☐☐☐ / ☐☐

Diagnosis _____

_____

Onset date ☐☐ / ☐☐☐ / ☐☐ End date ☐☐ / ☐☐☐ / ☐☐

Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 Related to study drug ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Outcome	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	Treatment	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6
Treatment administered	☐ Yes ☐ No	*Specify*: _____	
Was this a severe one?	☐ Yes ☐ No		

Form filled by

_____ ☐☐ / ☐☐☐ / ☐☐

Name

_____

Signature

Annex G. Randomization table.

Order	Arm
1	Grupo 3
2	Grupo 3
3	Grupo 2
4	Grupo 1
5	Grupo 2
6	Grupo 3
7	Grupo 1
8	Grupo 3
9	Grupo 2
10	Grupo 2
11	Grupo 1
12	Grupo 3
13	Grupo 1
14	Grupo 3
15	Grupo 1
16	Grupo 2
17	Grupo 2
18	Grupo 3
19	Grupo 3
20	Grupo 1
21	Grupo 2
22	Grupo 2
23	Grupo 3
24	Grupo 3
25	Grupo 2
26	Grupo 1
27	Grupo 2
28	Grupo 3
29	Grupo 2
30	Grupo 3
31	Grupo 1
32	Grupo 2
33	Grupo 1
34	Grupo 3
35	Grupo 3
36	Grupo 2
37	Grupo 2
38	Grupo 2
39	Grupo 3
40	Grupo 2
41	Grupo 3
42	Grupo 1
43	Grupo 3
44	Grupo 1
45	Grupo 3
46	Grupo 2
47	Grupo 2
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49	Grupo 3
50	Grupo 1
51	Grupo 3
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53	Grupo 2
54	Grupo 2
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57	Grupo 2
58	Grupo 3
59	Grupo 3
60	Grupo 3
61	Grupo 2
62	Grupo 1
63	Grupo 2
64	Grupo 2
65	Grupo 3
66	Grupo 1
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68	Grupo 2
69	Grupo 2
70	Grupo 2
71	Grupo 3
72	Grupo 1
73	Grupo 3
74	Grupo 1
75	Grupo 2
76	Grupo 3
77	Grupo 3
78	Grupo 2
79	Grupo 3
80	Grupo 3
81	Grupo 2
82	Grupo 1
83	Grupo 2
84	Grupo 1
85	Grupo 1
86	Grupo 2
87	Grupo 2
88	Grupo 3
89	Grupo 3
90	Grupo 1
91	Grupo 1
92	Grupo 2
93	Grupo 3
94	Grupo 2
95	Grupo 3
96	Grupo 2
97	Grupo 1
98	Grupo 3
99	Grupo 3
100	Grupo 2
101	Grupo 1
102	Grupo 3
103	Grupo 3
104	Grupo 2
105	Grupo 2
106	Grupo 1
107	Grupo 1
108	Grupo 2
109	Grupo 3
110	Grupo 2
111	Grupo 3

112	Grupo 3	155	Grupo 2	198	Grupo 1
113	Grupo 2	156	Grupo 3	199	Grupo 3
114	Grupo 1	157	Grupo 2	200	Grupo 2
115	Grupo 1	158	Grupo 1	201	Grupo 3
116	Grupo 3	159	Grupo 2	202	Grupo 1
117	Grupo 2	160	Grupo 3	203	Grupo 2
118	Grupo 3	161	Grupo 1	204	Grupo 2
119	Grupo 1	162	Grupo 2	205	Grupo 1
120	Grupo 2	163	Grupo 3	206	Grupo 2
121	Grupo 2	164	Grupo 3	207	Grupo 1
122	Grupo 3	165	Grupo 2	208	Grupo 3
123	Grupo 1	166	Grupo 1	209	Grupo 3
124	Grupo 1	167	Grupo 2	210	Grupo 1
125	Grupo 3	168	Grupo 1	211	Grupo 2
126	Grupo 2	169	Grupo 2	212	Grupo 3
127	Grupo 2	170	Grupo 3	213	Grupo 3
128	Grupo 3	171	Grupo 3	214	Grupo 2
129	Grupo 1	172	Grupo 1	215	Grupo 3
130	Grupo 2	173	Grupo 3	216	Grupo 2
131	Grupo 2	174	Grupo 2	217	Grupo 3
132	Grupo 3	175	Grupo 2	218	Grupo 1
133	Grupo 3	176	Grupo 3	219	Grupo 2
134	Grupo 2	177	Grupo 3	220	Grupo 2
135	Grupo 2	178	Grupo 3	221	Grupo 2
136	Grupo 3	179	Grupo 2	222	Grupo 3
137	Grupo 1	180	Grupo 1	223	Grupo 1
138	Grupo 3	181	Grupo 2	224	Grupo 3
139	Grupo 3	182	Grupo 1	225	Grupo 2
140	Grupo 2	183	Grupo 2	226	Grupo 2
141	Grupo 3	184	Grupo 3	227	Grupo 3
142	Grupo 2	185	Grupo 2	228	Grupo 1
143	Grupo 1	186	Grupo 3	229	Grupo 3
144	Grupo 1	187	Grupo 1	230	Grupo 3
145	Grupo 2	188	Grupo 1	231	Grupo 2
146	Grupo 3	189	Grupo 2	232	Grupo 3
147	Grupo 2	190	Grupo 2	233	Grupo 2
148	Grupo 1	191	Grupo 3	234	Grupo 1
149	Grupo 3	192	Grupo 3	235	Grupo 1
150	Grupo 3	193	Grupo 3	236	Grupo 3
151	Grupo 1	194	Grupo 1	237	Grupo 2
152	Grupo 2	195	Grupo 3	238	Grupo 1
153	Grupo 3	196	Grupo 2	239	Grupo 3
154	Grupo 1	197	Grupo 2	240	Grupo 2

241	Grupo 3
242	Grupo 1
243	Grupo 2
244	Grupo 2
245	Grupo 3
246	Grupo 1
247	Grupo 1
248	Grupo 3
249	Grupo 2
250	Grupo 3
251	Grupo 2
252	Grupo 1
253	Grupo 1
254	Grupo 2
255	Grupo 3
256	Grupo 2
257	Grupo 3

Original Analysis Plan

Efficacy of High Total Dose of Primaquine to Prevent *Plasmodium vivax* Malaria Relapses in Cruzeiro do Sul, Brazil

Statistical Analysis Plan

Principal investigators: Suiane da Costa Negreiros, Giselle Maria Rachid Viana, Alexandre Macedo de Oliveira

Co-investigators: Nathália Nogueira Chamma-Siqueira, Sarah-Blythe Ballard, Samela Farias, Sandro Patroca da Silva, Stella M. Chenet, Eduardo José Melo dos Santos, Luann Wendel Pereira de Sena, Flávia Póvoa da Costa, Amanda Gabryelle Nunes Cardoso Mello, Paola Barbosa Marchesini, Cassio Roberto Leonel Peterka

Analysis plan developed by Alexandre Macedo de Oliveira and Nathália Nogueira Chamma-Siqueira, study investigators

Date: March 9, 2020, before data analysis was initiated.

CDC IRB: #7061.0
Clinicaltrials.gov registration number: NCT03610399

Funder: Latin America and Caribbean Regional Malaria Program

Table of contents

Introduction

Design overview

This is a three-arm randomized clinical trial to evaluate the therapeutic efficacy of chloroquine (CQ) and primaquine in the treatment of *Plasmodium vivax* malaria in Cruzeiro do Sul, State of Acre, Brazil using three primaquine schemes. Patients in group 1 are instructed to take primaquine as non-observed therapy for a total dose of 3.5 mg/kg over seven days (0.5 mg/kg/day), while those in group 2 receive the same regimen, but under directly observed therapy. Participants in group 3 receive directly observed primaquine therapy for 14 days (0.5 mg/kg/day) aiming for a total dose of 7.0 mg/kg. We will compare treatment response between groups 1 and 3, and groups 2 and 3.

Sample size

Seventy-four participants in groups 2 and 3 are needed to compare an expected and estimated day 168 recurrence-free proportion of 70% in group 2 and 90% in group 3 (power= 90% and level of significance= 5%); estimates based on prior studies in the region.[1,2] Similarly, to compare a day 168 recurrence-free proportion of 90% (group 3) and 60% (group 1), we need 39 participants in each group, which will be increased to 50 participants per prior recommendations.[3] We will add 30% in each study group to account for loss to follow-up and reached a sample size of 96 participants in groups 2 and 3, and 65 in group 1; 257 participants in total.

Randomization and allocation

The randomization sequence (Appendix A) present in the protocol was computer-generated by one of the investigators at the Centers for Disease Control and Prevention using SAS. The result of this process is a sequential enrollment list respecting the desired sample size per study groups. Since two study teams are involved in patient enrollment, one team will start from the top of the list and the other from the bottom. Regular meeting between the two nurses of the study teams will allow for checking if the total of 257 study participants in total are enrolled, so enrollment can be stopped.

The field study coordinator will assemble participant's clinical forms for both study teams and put them sequentially with a blank opaque cover on top of each one. At the time of enrollment, field study member will pick the top of their respective team pile and have the pre-selected study group for each new participant.

Up to that moment, neither the field study member, nor the participant will have knowledge of study group assignment.

Purpose of the analysis plan

The purpose of this document is to outline the statistical analysis plan for this study, which is primarily meant to compare the efficacy of the higher dose of primaquine to prevent *P. vivax* recurrences during a 168-day follow-up. Additionally, we will also evaluate co-variables and the outcome.

Definitions and variables of interest

Definitions

***P. vivax* infection**

P. vivax infection will be defined as presence of *P. vivax* parasites in peripheral blood collected at protocol scheduled visits or in case of symptomatic patients using microscopic examination. In case of positive samples, malaria species will be identified (*P. vivax, P. falciparum,* others) and quantified according to parasite characteristics and laboratory standard operating procedures developed by study investigators.

Molecular genotyping

We will use microsatellite genotyping to differentiate homologous day 0–day of recurrence parasites from heterologous ones as homologous recurrences most likely reflect relapses or recrudescences and, therefore, more accurately relate to drug efficacy. Day 0 and day of recurrence samples processed in parallel will allow investigators to classify their haplotypes profiles; paired samples with at least one microsatellite difference are considered heterologous haplotypes, while all others homologous.[2,4] In addition to the main outcome of interest, we will evaluate treatment efficacy and comparisons considering homologous recurrences only.

Variables of interest

We plan to collect demographic (e.g., sex, age, place of residence, etc), clinical (presence of fever at enrolment, etc), and parasitological (parasite density at enrollment, time for parasite clearance, etc) variables in the course of this study using standard clinical forms.

We will evaluate different variables for their role as confounders and effect modifiers in our analysis and use appropriate statistical methods to account for those. These variables are primaquine initiation date, glucose

6-phosphate dehydrogenase (G6PD) activity, sex, place of residence (rural vs. urban), and cytochrome 2PD6 phenotype, for example.

Morbidity endpoint definitions

Outcome definitions

Treatment efficacy will be evaluated using a combination of clinical and laboratory information.[3] There are different approaches to determine outcomes in antimalarial efficacy trials. We chose the simpler classification adapted for *P. vivax* studies. Day 28 treatment failure is defined as (1) clinical deterioration due to *P. vivax* illness requiring hospitalization in the presence of parasitemia, (2) presence of parasitemia and fever (axillary temperature ≥37.5 °C) on any day between days 3 and 28; or (3) presence of parasitemia on any day between days 7 and 28, irrespective of clinical condition.

Since we are also interested in recurrences that occur as a result of failure to the primaquine component, we will also monitor the resurgence of parasitemia between days 28 and 168, with or without fever. Those cases will be classified as extended follow-up failure. A day 28 adequate clinical and parasitological response (ACPR) includes participants who do not meet criteria for day 28 treatment failure. We will also determine day 168 ACPR as patients who do not meet any failure criteria during the entire follow-up (168 days).

The clinical and parasitological diagnosis of malaria is the main focus of this study and the main outcome of interest. We will, however, replicate the per-protocol analysis (details below) considering genotyping information comparing the genotype of parasites from day 0 (enrollment) and day of failure considering only homologous recurrences as failures. The secondary analysis will be done in the same way as for the primary one.

Study endpoints

Primary efficacy outcome

The primary outcome is development of clinical or parasitological *P. vivax* infection during the follow-up period (168 days).

Secondary efficacy outcomes

The secondary outcome is the molecular-corrected *P. vivax* infection (homologous recurrences) during follow-up (168 days).

Statistical methods

Data cleaning and descriptive statistics

Clinical forms will be double entered in databases. Data will be compared and cleaned before proceeding to data analysis. Variables will be checked for the presence of outliers, using tabulation, frequency distribution, and box plots. We will provide and display descriptive summaries for each of the primary and secondary variables. In general, tables will summarize data by study group. Continuous, quantitative, variable summaries will include the number of subjects (n) with non-missing values, mean, standard deviation (SD), median, and range (minimum and maximum), unless otherwise specified. Categorical, qualitative, variable summaries will include the frequency and percentage of participants in each category per study group.

Means, SD, and any other statistics other than quartiles will be reported to one decimal place greater than the original unit of measure. Quartiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g., regression coefficients) will be reported to three significant figures.

In general, we will consider the total number of participants in the analysis set for the study groups as the denominator for the percentage calculation. All statistical tests will be based on a 2-sided test at the significance level of 0.05 and 95% confidence intervals, unless otherwise specified. Finally, we will use graphical and table-format presentation to show results from the statistical analyses.

We will use SAS™ version 9.4 (SAS Institute, Cary, North Carolina, USA) for data cleaning and analysis. We will follow the Consolidated Standards of Reporting Trials (CONSORT) guidelines for reporting of clinical trials (http://www.consort-statement.org/).

Analytical statistics

The number and proportion of subjects who have treatment failures and ACPRs, as defined above, by day 28 and 168 will be calculated. We will also assess the potential risk factors (e.g., age, initial parasite density,

etc.) for an association with the probability of a therapeutic failure and the time to therapeutic failure using Chi squared tests for dichotomous variables and Student's t-tests for continuous variables.

Proportion of *P. vivax* infections during follow-up will be evaluated. We will determine the proportion of participants with ACPR in a per-protocol analysis approach, excluding participants with loss to follow-up, malaria infections other than *P. vivax* or withdrawal for other reasons. We will compare these proportions between groups 1 and 3, and groups 2 and 3 using Pearson chi-square test for the per-protocol analysis. We will repeat this analysis considering only homologous recurrences as failures.

A list of intended tables of anticipated descriptive and analytical tables is presented in Appendix B. Appendix C shows the Consort check list intended for use at time of report and manuscript writing.

Adverse events

Occurrence of side-effects and adverse events (AEs) associated with the study drugs or procedures will be monitored and recorded on an appropriate form. An AE is defined as treatment emergent if the first onset or worsening is after the first administration of study medication and not more than 7 days after the last administration of a study medication. AEs will be investigated and classified as associated or not with study participation. Patients with side-effects necessitating medical treatment or hospitalization will be referred for appropriate treatment. Study participation may be discontinued if it is established that study procedures cannot be safely continued, such as study drug allergic events during treatment or G6PD deficiency, for that study participant.

Number and frequency of AEs leading to withdrawal of study, by study group and specific medication or procedure associated with them, will be produced and reported. A data listing of AEs leading to withdrawal of study medication will also be provided, displaying details of the event(s) captured on the clinical reporting form.

Patients with skin rash or any severe allergic reaction will be withdrawn from the study, while patients experiencing only itching will not be withdrawn from the study. In addition, adverse events that are unexpected, likely related to the research study, and that place the participants at greater risk of harm will be

reported to the local and CDC institutional review board in accordance with required reporting schedules after their occurrence, i. e., in no later than 2 working days of CDC awareness.

Limitations

Findings will be restricted to this region of Brazil and, until information from other parts of Brazil and the Amazon region can be collected, they should not be generalized. We will adopt a 6-month follow up period as an attempt to detect relapses, although relapses can occur up to 1 year or longer. An even longer duration of follow-up is recommended to comprehensively estimate relapse rates in *P. vivax* studies. However, longer studies would increase the likelihood of reinfections, and until methodological consensus exists on differentiating relapses from reinfection, we felt this would introduce undesired bias.

Primaquine dosing will not be initiated at the same time for all participants due to variation in wait times for G6PD study results. Ideally, we would like to administer primaquine at the same time for all patients in order to prevent differences in treatment of hypnozoites and, therefore, differential impact on the likelihood of relapse. However, given our need to start primaquine as soon as possible and delays in G6PD determination, strict timing is not feasible.

Our background information about the clonal variability of *P. vivax* microsatellite regions is derived from the variability of these regions in the 257 day 0 samples only. This approach may not detect alleles in lower frequency in the population. However, resource limitations do not allow for more accurate methods, such as deep sequencing, and chose to use microsatellite genotyping.

There is no consensus on how to differentiate relapse and reinfection when recurrent *P. vivax* infection. Our analysis will focus on any *P. vivax* recurrence during follow up period. We will, however, also determine ACPR proportions considering only homologous recurrences since we believe those more directly relate to treatment response.

References

1. Lacerda MVG, Llanos-Cuentas A, Krudsood S, et al. Single-Dose Tafenoquine to Prevent Relapse of Plasmodium vivax Malaria. *The New England journal of medicine* 2019; **380**(3): 215-28.

2. Negreiros S, Farias S, Viana GM, et al. Efficacy of Chloroquine and Primaquine for the Treatment of Uncomplicated Plasmodium vivax Malaria in Cruzeiro do Sul, Brazil. *Am J Trop Med Hyg* 2016; **95**(5): 1061-8.

3. WHO. Methods for Surveillance of Antimalarial Drug Efficacy; 2009.

4. Beck HP, Wampfler R, Carter N, et al. Estimation of the Antirelapse Efficacy of Tafenoquine, Using Plasmodium vivax Genotyping. *J Infect Dis* 2016; **213**(5): 794-9.

Appendix A. Randomization table

Order	Arm	42	Grupo 1	84	Grupo 1
1	Grupo 3	43	Grupo 3	85	Grupo 1
2	Grupo 3	44	Grupo 1	86	Grupo 2
3	Grupo 2	45	Grupo 3	87	Grupo 2
4	Grupo 1	46	Grupo 2	88	Grupo 3
5	Grupo 2	47	Grupo 2	89	Grupo 3
6	Grupo 3	48	Grupo 2	90	Grupo 1
7	Grupo 1	49	Grupo 3	91	Grupo 1
8	Grupo 3	50	Grupo 1	92	Grupo 2
9	Grupo 2	51	Grupo 3	93	Grupo 3
10	Grupo 2	52	Grupo 1	94	Grupo 2
11	Grupo 1	53	Grupo 2	95	Grupo 3
12	Grupo 3	54	Grupo 2	96	Grupo 2
13	Grupo 1	55	Grupo 3	97	Grupo 1
14	Grupo 3	56	Grupo 1	98	Grupo 3
15	Grupo 1	57	Grupo 2	99	Grupo 3
16	Grupo 2	58	Grupo 3	100	Grupo 2
17	Grupo 2	59	Grupo 3	101	Grupo 1
18	Grupo 3	60	Grupo 3	102	Grupo 3
19	Grupo 3	61	Grupo 2	103	Grupo 3
20	Grupo 1	62	Grupo 1	104	Grupo 2
21	Grupo 2	63	Grupo 2	105	Grupo 2
22	Grupo 2	64	Grupo 2	106	Grupo 1
23	Grupo 3	65	Grupo 3	107	Grupo 1
24	Grupo 3	66	Grupo 1	108	Grupo 2
25	Grupo 2	67	Grupo 3	109	Grupo 3
26	Grupo 1	68	Grupo 2	110	Grupo 2
27	Grupo 2	69	Grupo 2	111	Grupo 3
28	Grupo 3	70	Grupo 2	112	Grupo 3
29	Grupo 2	71	Grupo 3	113	Grupo 2
30	Grupo 3	72	Grupo 1	114	Grupo 1
31	Grupo 1	73	Grupo 3	115	Grupo 1
32	Grupo 2	74	Grupo 1	116	Grupo 3
33	Grupo 1	75	Grupo 2	117	Grupo 2
34	Grupo 3	76	Grupo 3	118	Grupo 3
35	Grupo 3	77	Grupo 3	119	Grupo 1
36	Grupo 2	78	Grupo 2	120	Grupo 2
37	Grupo 2	79	Grupo 3	121	Grupo 2
38	Grupo 2	80	Grupo 3	122	Grupo 3
39	Grupo 3	81	Grupo 2	123	Grupo 1
40	Grupo 2	82	Grupo 1	124	Grupo 1
41	Grupo 3	83	Grupo 2	125	Grupo 3

126	Grupo 2	169	Grupo 2	212	Grupo 3
127	Grupo 2	170	Grupo 3	213	Grupo 3
128	Grupo 3	171	Grupo 3	214	Grupo 2
129	Grupo 1	172	Grupo 1	215	Grupo 3
130	Grupo 2	173	Grupo 3	216	Grupo 2
131	Grupo 2	174	Grupo 2	217	Grupo 3
132	Grupo 3	175	Grupo 2	218	Grupo 1
133	Grupo 3	176	Grupo 3	219	Grupo 2
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135	Grupo 2	178	Grupo 3	221	Grupo 2
136	Grupo 3	179	Grupo 2	222	Grupo 3
137	Grupo 1	180	Grupo 1	223	Grupo 1
138	Grupo 3	181	Grupo 2	224	Grupo 3
139	Grupo 3	182	Grupo 1	225	Grupo 2
140	Grupo 2	183	Grupo 2	226	Grupo 2
141	Grupo 3	184	Grupo 3	227	Grupo 3
142	Grupo 2	185	Grupo 2	228	Grupo 1
143	Grupo 1	186	Grupo 3	229	Grupo 3
144	Grupo 1	187	Grupo 1	230	Grupo 3
145	Grupo 2	188	Grupo 1	231	Grupo 2
146	Grupo 3	189	Grupo 2	232	Grupo 3
147	Grupo 2	190	Grupo 2	233	Grupo 2
148	Grupo 1	191	Grupo 3	234	Grupo 1
149	Grupo 3	192	Grupo 3	235	Grupo 1
150	Grupo 3	193	Grupo 3	236	Grupo 3
151	Grupo 1	194	Grupo 1	237	Grupo 2
152	Grupo 2	195	Grupo 3	238	Grupo 1
153	Grupo 3	196	Grupo 2	239	Grupo 3
154	Grupo 1	197	Grupo 2	240	Grupo 2
155	Grupo 2	198	Grupo 1	241	Grupo 3
156	Grupo 3	199	Grupo 3	242	Grupo 1
157	Grupo 2	200	Grupo 2	243	Grupo 2
158	Grupo 1	201	Grupo 3	244	Grupo 2
159	Grupo 2	202	Grupo 1	245	Grupo 3
160	Grupo 3	203	Grupo 2	246	Grupo 1
161	Grupo 1	204	Grupo 2	247	Grupo 1
162	Grupo 2	205	Grupo 1	248	Grupo 3
163	Grupo 3	206	Grupo 2	249	Grupo 2
164	Grupo 3	207	Grupo 1	250	Grupo 3
165	Grupo 2	208	Grupo 3	251	Grupo 2
166	Grupo 1	209	Grupo 3	252	Grupo 1
167	Grupo 2	210	Grupo 1	253	Grupo 1
168	Grupo 1	211	Grupo 2	254	Grupo 2

255 Grupo 3
256 Grupo 2
257 Grupo 3

Appendix B. List of anticipated tables and figures for data analysis.

1. Study design and randomization into treatment groups.

2. Participant's characteristics at enrollment and during follow-up per study group, Cruzeiro do Sul, 2018.

3. Common symptoms at enrollment per study group.

4. Asexual parasite density clearance per study day per study group.

5. Participant's outcome in the per-protocol analysis per study group at day 28 and day 168, Cruzeiro do Sul, 2018.

6. Haplotypes classification of paired samples *Plasmodium vivax* recurrences with valid microsatellite results, Cruzeiro do Sul, 2018.

7. Participant's CYP2D6 metabolic phenotypes profiles correlated with *Plasmodium vivax* recurrences, Cruzeiro do Sul, 2018.

8. Geometric mean of asexual parasitemia of *Plasmodium vivax* among participants per treatment group during first 28 days of follow-up, Cruzeiro do Sul, 2018.

Appendix C. CONSORT checklist of information to include when reporting a trial

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomized trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	

Randomization:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomization; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	
	13b	For each group, losses and exclusions after randomization, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	

Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomized trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

STATISTICAL PLAN AMENDMENT SUMMARY

Amendment

Rationale for Amendment I

This statistical plan was amended for two main reasons:

- To add the survival curves analysis and comparison methods.
- To provide details on the multivariate analysis for outcome determination.

There was just one amendment of this statistical plan, which was approved by principal investigators with support from CDC statistician.

Revised Statistical Plan I

Efficacy of High Total Dose of Primaquine to Prevent *Plasmodium vivax* Malaria Relapses in Cruzeiro do Sul, Brazil

Statistical Analysis Plan

Principal investigators: Suiane da Costa Negreiros, Giselle Maria Rachid Viana, Alexandre Macedo de Oliveira

Co-investigators: Nathália Nogueira Chamma-Siqueira, Sarah-Blythe Ballard, Samela Farias, Sandro Patroca da Silva, Stella M. Chenet, Eduardo José Melo dos Santos, Luann Wendel Pereira de Sena, Flávia Póvoa da Costa, Amanda Gabryelle Nunes Cardoso Mello, Paola Barbosa Marchesini, Cassio Roberto Leonel Peterka

Analysis plan developed by Alexandre Macedo de Oliveira and Nathália Nogueira Chamma-Siqueira, study investigators

Analysis plan revision supported by John Williamson, statistician, Malaria Branch, Division of Parasitic Diseases and Malaria, CDC.

Date: April 2020

CDC IRB: #7061.0
Clinicaltrials.gov registration number: NCT03610399

Funder: Latin America and Caribbean Regional Malaria Program

Table of contents

Introduction

Design overview

This is a three-arm randomized clinical trial to evaluate the therapeutic efficacy of chloroquine (CQ) and primaquine in the treatment of *Plasmodium vivax* malaria in Cruzeiro do Sul, State of Acre, Brazil using three primaquine schemes. Patients in group 1 are instructed to take primaquine as non-observed therapy for a total dose of 3.5 mg/kg over seven days (0.5 mg/kg/day), while those in group 2 receive the same regimen, but under directly observed therapy. Participants in group 3 receive directly observed primaquine therapy for 14 days (0.5 mg/kg/day) aiming for a total dose of 7.0 mg/kg. We will compare treatment response between groups 1 and 3, and groups 2 and 3.

Sample size

Seventy-four participants in groups 2 and 3 are needed to compare an expected and estimated day 168 recurrence-free proportion of 70% in group 2 and 90% in group 3 (power= 90% and level of significance= 5%); estimates based on prior studies in the region.[1,2] Similarly, to compare a day 168 recurrence-free proportion of 90% (group 3) and 60% (group 1), we need 39 participants in each group, which will be increased to 50 participants per prior recommendations.[3] We will add 30% in each study group to account for loss to follow-up and reached a sample size of 96 participants in groups 2 and 3, and 65 in group 1; 257 participants in total.

Randomization and allocation

The randomization sequence (Appendix A) present in the protocol was computer-generated by one of the investigators at the Centers for Disease Control and Prevention using SAS. The result of this process is a sequential enrollment list respecting the desired sample size per study groups. Since two study teams are involved in patient enrollment, one team will start from the top of the list and the other from the bottom. Regular meeting between the two nurses of the study teams will allow for checking if the total of 257 study participants in total are enrolled, so enrollment can be stopped.

The field study coordinator will assemble participant's clinical forms for both study teams and put them sequentially with a blank opaque cover on top of each one. At the time of enrollment, field study member will pick the top of their respective team pile and have the pre-selected study group for each new participant.

Up to that moment, neither the field study member, nor the participant will have knowledge of study group assignment.

Purpose of the analysis plan

The purpose of this document is to outline the statistical analysis plan for this study, which is primarily meant to compare the efficacy of the higher dose of primaquine to prevent *P. vivax* recurrences during a 168-day follow-up. Additionally, we will also evaluate co-variables and the outcome.

Definitions and variables of interest

Definitions

***P. vivax* infection**

P. vivax infection will be defined as presence of *P. vivax* parasites in peripheral blood collected at protocol scheduled visits or in case of symptomatic patients using microscopic examination. In case of positive samples, malaria species will be identified (*P. vivax, P. falciparum,* others) and quantified according to parasite characteristics and laboratory standard operating procedures developed by study investigators.

Molecular genotyping

We will use microsatellite genotyping to differentiate homologous day 0–day of recurrence parasites from heterologous ones as homologous recurrences most likely reflect relapses or recrudescences and, therefore, more accurately relate to drug efficacy. Day 0 and day of recurrence samples processed in parallel will allow investigators to classify their haplotypes profiles; paired samples with at least one microsatellite difference are considered heterologous haplotypes, while all others homologous.[2,4] In addition to the main outcome of interest, we will evaluate treatment efficacy and comparisons considering homologous recurrences only.

Variables of interest

We plan to collect demographic (e.g., sex, age, place of residence, etc), clinical (presence of fever at enrolment, etc), and parasitological (parasite density at enrollment, time for parasite clearance, etc) variables in the course of this study using standard clinical forms.

We will evaluate different variables for their role as confounders and effect modifiers in our analysis and use appropriate statistical methods to account for those. These variables are primaquine initiation date, glucose

6-phosphate dehydrogenase (G6PD) activity, sex, place of residence (rural vs. urban), and cytochrome 2PD6 phenotype, for example.

Morbidity endpoint definitions

Outcome definitions

Treatment efficacy will be evaluated using a combination of clinical and laboratory information.[3] There are different approaches to determine outcomes in antimalarial efficacy trials. We chose the simpler classification adapted for *P. vivax* studies. Day 28 treatment failure is defined as (1) clinical deterioration due to *P. vivax* illness requiring hospitalization in the presence of parasitemia, (2) presence of parasitemia and fever (axillary temperature ≥37.5 °C) on any day between days 3 and 28; or (3) presence of parasitemia on any day between days 7 and 28, irrespective of clinical condition.

Since we are also interested in recurrences that occur as a result of failure to the primaquine component, we will also monitor the resurgence of parasitemia between days 28 and 168, with or without fever. Those cases will be classified as extended follow-up failure. A day 28 adequate clinical and parasitological response (ACPR) includes participants who do not meet criteria for day 28 treatment failure. We will also determine day 168 ACPR as patients who do not meet any failure criteria during the entire follow-up (168 days).

The clinical and parasitological diagnosis of malaria is the main focus of this study and the main outcome of interest. We will, however, replicate the per-protocol analysis (details below) considering genotyping information comparing the genotype of parasites from day 0 (enrollment) and day of failure considering only homologous recurrences as failures. The secondary analysis will be done in the same way as for the primary one.

Study endpoints

Primary efficacy outcome

The primary outcome is development of clinical or parasitological *P. vivax* infection during the follow-up period (168 days).

Secondary efficacy outcomes

The secondary outcome is the molecular-corrected *P. vivax* infection (homologous recurrences) during follow-up (168 days).

Statistical methods

Data cleaning and descriptive statistics

Clinical forms will be double entered in databases. Data will be compared and cleaned before proceeding to data analysis. Variables will be checked for the presence of outliers, using tabulation, frequency distribution, and box plots. We will provide and display descriptive summaries for each of the primary and secondary variables. In general, tables will summarize data by study group. Continuous, quantitative, variable summaries will include the number of subjects (n) with non-missing values, mean, standard deviation (SD), median, and range (minimum and maximum), unless otherwise specified. Categorical, qualitative, variable summaries will include the frequency and percentage of participants in each category per study group.

Means, SD, and any other statistics other than quartiles will be reported to one decimal place greater than the original unit of measure. Quartiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g., regression coefficients) will be reported to three significant figures.

In general, we will consider the total number of participants in the analysis set for the study groups as the denominator for the percentage calculation. All statistical tests will be based on a 2-sided test at the significance level of 0.05 and 95% confidence intervals, unless otherwise specified. Finally, we will use graphical and table-format presentation to show results from the statistical analyses.

We will use SAS™ version 9.4 (SAS Institute, Cary, North Carolina, USA) for data cleaning and analysis. We will follow the Consolidated Standards of Reporting Trials (CONSORT) guidelines for reporting of clinical trials (http://www.consort-statement.org/).

Analytical statistics

The number and proportion of subjects who have treatment failures and ACPRs, as defined above, by day 28 and 168 will be calculated. We will also assess the potential risk factors (e.g., age, initial parasite density,

etc.) for an association with the probability of a therapeutic failure and the time to therapeutic failure using Chi squared tests for dichotomous variables and Student's t-tests for continuous variables.

Proportion of *P. vivax* infections during follow-up will be evaluated. We will determine the proportion of participants with ACPR in a per-protocol analysis approach, excluding participants with loss to follow-up, malaria infections other than *P. vivax* or withdrawal for other reasons. We will compare these proportions between groups 1 and 3, and groups 2 and 3 using Pearson chi-square test for the per-protocol analysis. We will repeat this analysis considering only homologous recurrences as failures.

We will perform a survival analysis the Kaplan-Meier method, considering patients for as long as they stayed in the study. Similarly, to the per-protocol analysis, we will stratify these curves by study group. The log-rank test will be used to compare survival times across treatment groups. Cox proportional hazard regression will be used to evaluate potential confounders and effect modifiers (primaquine initiation date, G6PD activity, etc) on treatment efficacy response.

A list of intended tables of anticipated descriptive and analytical tables is presented in Appendix B. Appendix C shows the Consort check list intended for use at time of report and manuscript writing.

Adverse events

Occurrence of side-effects and adverse events (AEs) associated with the study drugs or procedures will be monitored and recorded on an appropriate form. An AE is defined as treatment emergent if the first onset or worsening is after the first administration of study medication and not more than 7 days after the last administration of a study medication. AEs will be investigated and classified as associated or not with study participation. Patients with side-effects necessitating medical treatment or hospitalization will be referred for appropriate treatment. Study participation may be discontinued if it is established that study procedures cannot be safely continued, such as study drug allergic events during treatment or G6PD deficiency, for that study participant.

Number and frequency of AEs leading to withdrawal of study, by study group and specific medication or procedure associated with them, will be produced and reported. A data listing of AEs leading to withdrawal

of study medication will also be provided, displaying details of the event(s) captured on the clinical reporting form.

Patients with skin rash or any severe allergic reaction will be withdrawn from the study, while patients experiencing only itching will not be withdrawn from the study. In addition, adverse events that are unexpected, likely related to the research study, and that place the participants at greater risk of harm will be reported to the local and CDC institutional review board in accordance with required reporting schedules after their occurrence, i. e., in no later than 2 working days of CDC awareness.

Limitations

Findings will be restricted to this region of Brazil and, until information from other parts of Brazil and the Amazon region can be collected, they should not be generalized. We will adopt a 6-month follow up period as an attempt to detect relapses, although relapses can occur up to 1 year or longer. An even longer duration of follow-up is recommended to comprehensively estimate relapse rates in *P. vivax* studies. However, longer studies would increase the likelihood of reinfections, and until methodological consensus exists on differentiating relapses from reinfection, we felt this would introduce undesired bias.

Primaquine dosing will not be initiated at the same time for all participants due to variation in wait times for G6PD study results. Ideally, we would like to administer primaquine at the same time for all patients in order to prevent differences in treatment of hypnozoites and, therefore, differential impact on the likelihood of relapse. However, given our need to start primaquine as soon as possible and delays in G6PD determination, strict timing is not feasible.

Our background information about the clonal variability of *P. vivax* microsatellite regions is derived from the variability of these regions in the 257 day 0 samples only. This approach may not detect alleles in lower frequency in the population. However, resource limitations do not allow for more accurate methods, such as deep sequencing, and chose to use microsatellite genotyping.

There is no consensus on how to differentiate relapse and reinfection when recurrent *P. vivax* infection. Our analysis will focus on any *P. vivax* recurrence during follow up period. We will, however, also determine

ACPR proportions considering only homologous recurrences since we believe those more directly relate to treatment response.

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1. Lacerda MVG, Llanos-Cuentas A, Krudsood S, et al. Single-Dose Tafenoquine to Prevent Relapse of Plasmodium vivax Malaria. *The New England journal of medicine* 2019; **380**(3): 215-28.

2. Negreiros S, Farias S, Viana GM, et al. Efficacy of Chloroquine and Primaquine for the Treatment of Uncomplicated Plasmodium vivax Malaria in Cruzeiro do Sul, Brazil. *Am J Trop Med Hyg* 2016; **95**(5): 1061-8.

3. WHO. Methods for Surveillance of Antimalarial Drug Efficacy; 2009.

4. Beck HP, Wampfler R, Carter N, et al. Estimation of the Antirelapse Efficacy of Tafenoquine, Using Plasmodium vivax Genotyping. *J Infect Dis* 2016; **213**(5): 794-9.

Appendix A. Randomization table

Order	Arm
1	Grupo 3
2	Grupo 3
3	Grupo 2
4	Grupo 1
5	Grupo 2
6	Grupo 3
7	Grupo 1
8	Grupo 3
9	Grupo 2
10	Grupo 2
11	Grupo 1
12	Grupo 3
13	Grupo 1
14	Grupo 3
15	Grupo 1
16	Grupo 2
17	Grupo 2
18	Grupo 3
19	Grupo 3
20	Grupo 1
21	Grupo 2
22	Grupo 2
23	Grupo 3
24	Grupo 3
25	Grupo 2
26	Grupo 1
27	Grupo 2
28	Grupo 3
29	Grupo 2
30	Grupo 3
31	Grupo 1
32	Grupo 2
33	Grupo 1
34	Grupo 3
35	Grupo 3
36	Grupo 2
37	Grupo 2
38	Grupo 2
39	Grupo 3
40	Grupo 2
41	Grupo 3
42	Grupo 1
43	Grupo 3
44	Grupo 1
45	Grupo 3
46	Grupo 2
47	Grupo 2
48	Grupo 2
49	Grupo 3
50	Grupo 1
51	Grupo 3
52	Grupo 1
53	Grupo 2
54	Grupo 2
55	Grupo 3
56	Grupo 1
57	Grupo 2
58	Grupo 3
59	Grupo 3
60	Grupo 3
61	Grupo 2
62	Grupo 1
63	Grupo 2
64	Grupo 2
65	Grupo 3
66	Grupo 1
67	Grupo 3
68	Grupo 2
69	Grupo 2
70	Grupo 2
71	Grupo 3
72	Grupo 1
73	Grupo 3
74	Grupo 1
75	Grupo 2
76	Grupo 3
77	Grupo 3
78	Grupo 2
79	Grupo 3
80	Grupo 3
81	Grupo 2
82	Grupo 1
83	Grupo 2
84	Grupo 1
85	Grupo 1
86	Grupo 2
87	Grupo 2
88	Grupo 3
89	Grupo 3
90	Grupo 1
91	Grupo 1
92	Grupo 2
93	Grupo 3
94	Grupo 2
95	Grupo 3
96	Grupo 2
97	Grupo 1
98	Grupo 3
99	Grupo 3
100	Grupo 2
101	Grupo 1
102	Grupo 3
103	Grupo 3
104	Grupo 2
105	Grupo 2
106	Grupo 1
107	Grupo 1
108	Grupo 2
109	Grupo 3
110	Grupo 2
111	Grupo 3
112	Grupo 3
113	Grupo 2
114	Grupo 1
115	Grupo 1
116	Grupo 3
117	Grupo 2
118	Grupo 3
119	Grupo 1
120	Grupo 2
121	Grupo 2
122	Grupo 3

123	Grupo 1	166	Grupo 1	209	Grupo 3
124	Grupo 1	167	Grupo 2	210	Grupo 1
125	Grupo 3	168	Grupo 1	211	Grupo 2
126	Grupo 2	169	Grupo 2	212	Grupo 3
127	Grupo 2	170	Grupo 3	213	Grupo 3
128	Grupo 3	171	Grupo 3	214	Grupo 2
129	Grupo 1	172	Grupo 1	215	Grupo 3
130	Grupo 2	173	Grupo 3	216	Grupo 2
131	Grupo 2	174	Grupo 2	217	Grupo 3
132	Grupo 3	175	Grupo 2	218	Grupo 1
133	Grupo 3	176	Grupo 3	219	Grupo 2
134	Grupo 2	177	Grupo 3	220	Grupo 2
135	Grupo 2	178	Grupo 3	221	Grupo 2
136	Grupo 3	179	Grupo 2	222	Grupo 3
137	Grupo 1	180	Grupo 1	223	Grupo 1
138	Grupo 3	181	Grupo 2	224	Grupo 3
139	Grupo 3	182	Grupo 1	225	Grupo 2
140	Grupo 2	183	Grupo 2	226	Grupo 2
141	Grupo 3	184	Grupo 3	227	Grupo 3
142	Grupo 2	185	Grupo 2	228	Grupo 1
143	Grupo 1	186	Grupo 3	229	Grupo 3
144	Grupo 1	187	Grupo 1	230	Grupo 3
145	Grupo 2	188	Grupo 1	231	Grupo 2
146	Grupo 3	189	Grupo 2	232	Grupo 3
147	Grupo 2	190	Grupo 2	233	Grupo 2
148	Grupo 1	191	Grupo 3	234	Grupo 1
149	Grupo 3	192	Grupo 3	235	Grupo 1
150	Grupo 3	193	Grupo 3	236	Grupo 3
151	Grupo 1	194	Grupo 1	237	Grupo 2
152	Grupo 2	195	Grupo 3	238	Grupo 1
153	Grupo 3	196	Grupo 2	239	Grupo 3
154	Grupo 1	197	Grupo 2	240	Grupo 2
155	Grupo 2	198	Grupo 1	241	Grupo 3
156	Grupo 3	199	Grupo 3	242	Grupo 1
157	Grupo 2	200	Grupo 2	243	Grupo 2
158	Grupo 1	201	Grupo 3	244	Grupo 2
159	Grupo 2	202	Grupo 1	245	Grupo 3
160	Grupo 3	203	Grupo 2	246	Grupo 1
161	Grupo 1	204	Grupo 2	247	Grupo 1
162	Grupo 2	205	Grupo 1	248	Grupo 3
163	Grupo 3	206	Grupo 2	249	Grupo 2
164	Grupo 3	207	Grupo 1	250	Grupo 3
165	Grupo 2	208	Grupo 3	251	Grupo 2

252 Grupo 1
253 Grupo 1
254 Grupo 2
255 Grupo 3
256 Grupo 2
257 Grupo 3

Appendix B. List of anticipated tables and figures for data analysis.

1. Study design and randomization into treatment groups.

2. Participant's characteristics at enrollment and during follow-up per study group, Cruzeiro do Sul, 2018.

3. Common symptoms at enrollment per study group.

4. Asexual parasite density clearance per study day per study group.

5. Participant's outcome in the per-protocol analysis per study group at day 28 and day 168, Cruzeiro do Sul, 2018.

6. Haplotypes classification of paired samples *Plasmodium vivax* recurrences with valid microsatellite results, Cruzeiro do Sul, 2018.

7. Participant's CYP2D6 metabolic phenotypes profiles correlated with *Plasmodium vivax* recurrences, Cruzeiro do Sul, 2018.

8. Cox regression model for freedom from recurrence at day 168, Cruzeiro do Sul.

9. Geometric mean of asexual parasitemia of *Plasmodium vivax* among participants per treatment group during first 28 days of follow-up, Cruzeiro do Sul, 2018.

Appendix C. CONSORT checklist of information to include when reporting a trial

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomized trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	

Randomization:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomization; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	
	13b	For each group, losses and exclusions after randomization, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	

Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomized trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

STATISTICAL PLAN AMENDMENT SUMMARY

Amendment 2

Rationale for Amendment II

This statistical plan was amended:

- To update the primary outcome of interest and also the methods to estimate response rates and comparison between study groups.
- To update the multivariate analysis model.

There were two amendments to this statistical plan, which was approved by principal investigators with support from CDC statistician.

Revised Statistical Plan II

Efficacy of High Total Dose of Primaquine to Prevent *Plasmodium vivax* Malaria Relapses in Cruzeiro do Sul, Brazil

Statistical Analysis Plan

Principal investigators: Suiane da Costa Negreiros, Giselle Maria Rachid Viana, Alexandre Macedo de Oliveira

Co-investigators: Nathália Nogueira Chamma-Siqueira, Sarah-Blythe Ballard, Samela Farias, Sandro Patroca da Silva, Stella M. Chenet, Eduardo José Melo dos Santos, Luann Wendel Pereira de Sena, Flávia Póvoa da Costa, Amanda Gabryelle Nunes Cardoso Mello, Paola Barbosa Marchesini, Cassio Roberto Leonel Peterka

Analysis plan developed by Alexandre Macedo de Oliveira and Nathália Nogueira Chamma-Siqueira, study investigators

Analysis plan revision supported by John Williamson, statistician, Malaria Branch, Division of Parasitic Diseases and Malaria, CDC.

Date: May 2021

CDC IRB: #7061.0
Clinicaltrials.gov registration number: NCT03610399

Funder: Latin America and Caribbean Regional Malaria Program

Table of contents

Introduction

Design overview

This is a three-arm randomized clinical trial to evaluate the therapeutic efficacy of chloroquine (CQ) and primaquine in the treatment of *Plasmodium vivax* malaria in Cruzeiro do Sul, State of Acre, Brazil using three primaquine schemes. Patients in group 1 are instructed to take primaquine as non-observed therapy for a total dose of 3.5 mg/kg over seven days (0.5 mg/kg/day), while those in group 2 receive the same regimen, but under directly observed therapy. Participants in group 3 receive directly observed primaquine therapy for 14 days (0.5 mg/kg/day) aiming for a total dose of 7.0 mg/kg. We will compare treatment response between groups 1 and 3, and groups 2 and 3.

Sample size

Seventy-four participants in groups 2 and 3 are needed to compare an expected and estimated day 168 recurrence-free proportion of 70% in group 2 and 90% in group 3 (power= 90% and level of significance= 5%); estimates based on prior studies in the region.[1,2] Similarly, to compare a day 168 recurrence-free proportion of 90% (group 3) and 60% (group 1), we need 39 participants in each group, which will be increased to 50 participants per prior recommendations.[3] We will add 30% in each study group to account for loss to follow-up and reached a sample size of 96 participants in groups 2 and 3, and 65 in group 1; 257 participants in total.

Randomization and allocation

The randomization sequence (Appendix A) present in the protocol was computer-generated by one of the investigators at the Centers for Disease Control and Prevention using SAS. The result of this process is a sequential enrollment list respecting the desired sample size per study groups. Since two study teams are involved in patient enrollment, one team will start from the top of the list and the other from the bottom. Regular meetings between the two nurses of the study teams will allow for checking if the total of 257 study participants in total are enrolled, so enrollment can be stopped.

The field study coordinator will assemble participant's clinical forms for both study teams and put them sequentially with a blank opaque cover on top of each one. At the time of enrollment, field study member will pick the top of their respective team pile and have the pre-selected study group for each new participant.

Up to that moment, neither the field study member, nor the participant will have knowledge of study group assignment.

Purpose of the analysis plan

The purpose of this document is to outline the statistical analysis plan for this study, which is primarily meant to compare the efficacy of the higher dose of primaquine to prevent *P. vivax* recurrences during a 168-day follow-up. Additionally, we will also evaluate co-variables and the outcome.

Definitions and variables of interest

Definitions

***P. vivax* infection**

P. vivax infection will be defined as presence of *P. vivax* parasites in peripheral blood collected at scheduled visits listed in the protocol or in case of symptomatic patients using microscopic examination. In case of positive samples, malaria species will be identified (*P. vivax, P. falciparum,* others) and quantified according to parasite characteristics and laboratory standard operating procedures developed by study investigators as delineated in the protocol.

Molecular genotyping

We will use microsatellite genotyping to differentiate homologous day 0–day of recurrence parasites from heterologous ones as homologous recurrences most likely reflect relapses or recrudescences and, therefore, more accurately relate to drug efficacy. Day 0 and day of recurrence samples processed in parallel will allow investigators to classify their haplotypes profiles; paired samples with at least one microsatellite difference are considered heterologous haplotypes, while all others homologous.[2,4] Molecular typing is detailed in the protocol. In addition to the main outcomes of interest, freedom from recurrence at day 28 and day 168, we will evaluate treatment efficacy and comparisons considering homologous recurrences only.

Variables of interest

We plan to collect demographic (e.g., sex, age, place of residence, etc), clinical (presence of fever at enrolment, etc), and parasitological (parasite density at enrollment, time for parasite clearance, etc) variables in the course of this study using standard clinical forms.

We will evaluate different variables for their role as confounders and effect modifiers in our analysis and use appropriate statistical methods to account for those. These variables are patient's weight, primaquine initiation date, glucose 6-phosphate dehydrogenase (G6PD) activity, sex, place of residence (rural vs. urban), and cytochrome 2PD6 phenotype, for example.

Morbidity endpoint definitions

Outcome definitions

Treatment efficacy will be evaluated using a combination of clinical and laboratory information.[3] There are different approaches to determine outcomes in antimalarial efficacy trials. We chose the simpler classification adapted for *P. vivax* studies as stated in guidance by the World Health Organization (WHO). Day 28 treatment failure is defined as (1) clinical deterioration due to *P. vivax* illness requiring hospitalization in the presence of parasitemia, (2) presence of parasitemia and fever (axillary temperature ≥37.5 °C) on any day between days 3 and 28; or (3) presence of parasitemia on any day between days 7 and 28, irrespective of clinical condition.

Since we are also interested in recurrences that occur as a result of failure to the primaquine component, we will monitor the resurgence of parasitemia between days 28 and 168, with or without fever. Those cases will be classified as extended follow-up failure. A day 28 adequate clinical and parasitological response (ACPR) includes participants who do not meet criteria for day 28 treatment failure. We will also determine day 168 ACPR as patients who do not meet any failure criteria during the entire follow-up (168 days).

Freedom from recurrence at day 28 and day 168 are the primary outcomes of this study. The primary analyses are day 28 ACPR and day 168 recurrence-free proportions estimated per an intention-to-treat analysis using Kaplan–Meier survival. We will compare day 28 ACPR and day 168 recurrence-free proportions for groups 1 and 3, and groups 2 and 3 using adequate statistical methods (e.g., logrank test, and Wald test) and appropriate correction factors to account for multiplicity of comparison (e.g., Bonferroni adjustment). We will also repeat intention-to-treat analysis of day 168recurrence-free proportions considering only homologous recurrences, using genotyping results (consult protocol for details), and also compare groups 1 and 3, and groups 2 and 3 proportions.

To provide estimates comparable to what WHO recommends for in vivo trials, we will provide per-protocol analyses of day 28 ACPR and day 168 recurrence-free proportions. The secondary analysis will be done in the same way as for the primary one.

Study endpoints

Primary efficacy outcome

The primary outcome is freedom from *P. vivax* recurrence at day 28 and day 168.

Secondary efficacy outcomes

The secondary outcome is freedom from homologous *P. vivax* recurrence (genotyping-corrected) at day 168.

Statistical methods

Data cleaning and descriptive statistics

Clinical forms will be double entered in databases. Data will be compared and cleaned before proceeding to data analysis. Variables will be checked for the presence of outliers, using tabulation, frequency distribution, and box plots. We will provide and display descriptive summaries for each of the primary and secondary variables. In general, tables will summarize data by study group. Continuous, quantitative, variable summaries will include the number of subjects (n) with non-missing values, mean, standard deviation (SD), median, and range (minimum and maximum), unless otherwise specified. Categorical, qualitative, variable summaries will include the frequency and percentage of participants in each category per study group.

Means, SD, and any other statistics other than quartiles will be reported to one decimal place greater than the original unit of measure. Quartiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g., regression coefficients) will be reported to three significant figures.

In general, we will consider the total number of participants in the analysis set for the study groups as the denominator for the freedom-from-recurrence proportion calculation. We will report 95% confidence intervals for those estimates. We will use a Bonferroni correction to account for multiple testing when conducting the two pairwise hypothesis tests (group 1 versus group 3 and group 2 versus group 3), and thus only considered p-values <0.025 to be statistically significant. In other cases, statistical tests will be based

on a 2-sided test at the significance level of 0.05 and 95% confidence intervals, unless otherwise specified. Finally, we will use graphical and table-format presentation to show results from the statistical analyses.

We will use SAS™ version 9.4 (SAS Institute, Cary, North Carolina, USA) for data cleaning and analysis. We will follow the Consolidated Standards of Reporting Trials (CONSORT) guidelines for reporting of clinical trials (http://www.consort-statement.org/).

Analytical statistics

The number and proportion of subjects who have treatment failures and freedom-from-recurrence proportions, as defined above, by day 28 and 168 will be calculated. We will also assess the potential risk factors (e.g., age, initial parasite density, etc.) for an association with the probability of a therapeutic failure and the time to therapeutic failure using Chi squared tests for dichotomous variables and Student's t-tests for continuous variables.

Proportion of *P. vivax* infections during follow-up will be evaluated. We will stratify curves of freedom-from-recurrence curves by study group as the primary method for outcome comparisons. The log-rank and Wald tests will be used to compare survival times across treatment groups at day 28 and day 168. Cox proportional hazard regression will be used to evaluate potential confounders and effect modifiers (primaquine initiation date, G6PD activity, patient's weight, etc) on treatment efficacy response.

To allow for comparison with prior publications, we will also determine the proportion of participants with ACPR at days 28 and 168 in a per-protocol analysis approach, i.e., excluding participants with loss to follow-up, malaria infections other than *P. vivax* or withdrawal for other reasons. We will compare these proportions between groups 1 and 3, and groups 2 and 3, using appropriate statistical tests (e.g., chi square test). We will repeat this analysis considering only homologous recurrences as failures.

A list of intended tables of anticipated descriptive and analytical tables is presented in Appendix B. Appendix C shows the Consort check list intended for use at time of report and manuscript writing.

Adverse events

Considering the long use of the drugs used in this study in Brazil without G6PD testing, and the fact that all study participants will undergo G6PD testing prior to primaquine use, occurrence of side-effects and adverse events (AEs) associated with the study drugs or procedures will not be systematically monitored. AEs voluntarily reported by patients or noticed by study staff at scheduled visits will be recorded on an appropriate form. An AE is defined as treatment emergent if the first onset or worsening is after the first administration of study medication and not more than 7 days after the last administration of a study medication. AEs will be investigated and classified as associated or not with study participation. Patients with side-effects necessitating medical treatment or hospitalization will be referred for appropriate treatment. Study participation may be discontinued if it is established that study procedures cannot be safely continued, such as study drug allergic events during treatment or G6PD deficiency, for that study participant.

Number and frequency of AEs leading to withdrawal of study, by study group and specific medication or procedure associated with them, will be produced and reported, if they occur. In these cases, a line listing of AEs leading to withdrawal of study medication will also be provided, displaying details of the event(s) captured on the clinical reporting form.

Patients with skin rash or any severe allergic reaction will be withdrawn from the study, while patients experiencing only itching will not be withdrawn from the study. In addition, AEs that are unexpected, likely related to the research study, and that place the participants at greater risk of harm will be reported to the local and CDC institutional review board in accordance with required reporting schedules after their occurrence, i. e., in no later than 2 working days of CDC awareness.

Limitations

Findings will be restricted to this region of Brazil and, until information from other parts of Brazil and the Amazon region can be collected, they should not be generalized. We will adopt a 6-month follow up period as an attempt to detect relapses, although relapses can occur up to 1 year or longer. An even longer duration of follow-up is recommended to comprehensively estimate relapse rates in *P. vivax* studies. However, longer studies would increase the likelihood of reinfections, and until methodological consensus exists on differentiating relapses from reinfection, we felt this would introduce undesired bias.

Primaquine dosing will not be initiated at the same time for all participants due to variation in wait times for G6PD study results. Ideally, we would like to administer primaquine at the same time for all patients in order to prevent differences in treatment of hypnozoites and, therefore, differential impact on the likelihood of relapse. However, given our need to start primaquine as soon as possible and delays in G6PD determination, strict timing is not feasible.

Our background information about the clonal variability of *P. vivax* microsatellite regions is derived from the variability of these regions in the 257 day 0 samples only. This approach may not detect alleles in lower frequency in the population. However, resource limitations do not allow for more accurate methods, such as deep sequencing, and chose to use microsatellite genotyping.

There is no consensus on how to differentiate relapses and reinfections among *P. vivax* recurrences. Our analysis will focus on any *P. vivax* recurrence during follow up period. We will, however, also determine ACPR proportions considering only homologous recurrences since we believe those more directly relate to treatment response. This view is shared by other authors as well.

References

1. Lacerda MVG, Llanos-Cuentas A, Krudsood S, et al. Single-Dose Tafenoquine to Prevent Relapse of Plasmodium vivax Malaria. *The New England journal of medicine* 2019; **380**(3): 215-28.

2. Negreiros S, Farias S, Viana GM, et al. Efficacy of Chloroquine and Primaquine for the Treatment of Uncomplicated Plasmodium vivax Malaria in Cruzeiro do Sul, Brazil. *Am J Trop Med Hyg* 2016; **95**(5): 1061-8.

3. WHO. Methods for Surveillance of Antimalarial Drug Efficacy; 2009.

4. Beck HP, Wampfler R, Carter N, et al. Estimation of the Antirelapse Efficacy of Tafenoquine, Using Plasmodium vivax Genotyping. *J Infect Dis* 2016; **213**(5): 794-9.

Appendix A. Randomization table

Order	Arm
1	Grupo 3
2	Grupo 3
3	Grupo 2
4	Grupo 1
5	Grupo 2
6	Grupo 3
7	Grupo 1
8	Grupo 3
9	Grupo 2
10	Grupo 2
11	Grupo 1
12	Grupo 3
13	Grupo 1
14	Grupo 3
15	Grupo 1
16	Grupo 2
17	Grupo 2
18	Grupo 3
19	Grupo 3
20	Grupo 1
21	Grupo 2
22	Grupo 2
23	Grupo 3
24	Grupo 3
25	Grupo 2
26	Grupo 1
27	Grupo 2
28	Grupo 3
29	Grupo 2
30	Grupo 3
31	Grupo 1
32	Grupo 2
33	Grupo 1
34	Grupo 3
35	Grupo 3
36	Grupo 2
37	Grupo 2
38	Grupo 2
39	Grupo 3
40	Grupo 2
41	Grupo 3
42	Grupo 1
43	Grupo 3
44	Grupo 1
45	Grupo 3
46	Grupo 2
47	Grupo 2
48	Grupo 2
49	Grupo 3
50	Grupo 1
51	Grupo 3
52	Grupo 1
53	Grupo 2
54	Grupo 2
55	Grupo 3
56	Grupo 1
57	Grupo 2
58	Grupo 3
59	Grupo 3
60	Grupo 3
61	Grupo 2
62	Grupo 1
63	Grupo 2
64	Grupo 2
65	Grupo 3
66	Grupo 1
67	Grupo 3
68	Grupo 2
69	Grupo 2
70	Grupo 2
71	Grupo 3
72	Grupo 1
73	Grupo 3
74	Grupo 1
75	Grupo 2
76	Grupo 3
77	Grupo 3
78	Grupo 2
79	Grupo 3
80	Grupo 3
81	Grupo 2
82	Grupo 1
83	Grupo 2
84	Grupo 1
85	Grupo 1
86	Grupo 2
87	Grupo 2
88	Grupo 3
89	Grupo 3
90	Grupo 1
91	Grupo 1
92	Grupo 2
93	Grupo 3
94	Grupo 2
95	Grupo 3
96	Grupo 2
97	Grupo 1
98	Grupo 3
99	Grupo 3
100	Grupo 2
101	Grupo 1
102	Grupo 3
103	Grupo 3
104	Grupo 2
105	Grupo 2
106	Grupo 1
107	Grupo 1
108	Grupo 2
109	Grupo 3
110	Grupo 2
111	Grupo 3
112	Grupo 3
113	Grupo 2
114	Grupo 1
115	Grupo 1
116	Grupo 3
117	Grupo 2
118	Grupo 3
119	Grupo 1

120	Grupo 2	162	Grupo 2	204	Grupo 2
121	Grupo 2	163	Grupo 3	205	Grupo 1
122	Grupo 3	164	Grupo 3	206	Grupo 2
123	Grupo 1	165	Grupo 2	207	Grupo 1
124	Grupo 1	166	Grupo 1	208	Grupo 3
125	Grupo 3	167	Grupo 2	209	Grupo 3
126	Grupo 2	168	Grupo 1	210	Grupo 1
127	Grupo 2	169	Grupo 2	211	Grupo 2
128	Grupo 3	170	Grupo 3	212	Grupo 3
129	Grupo 1	171	Grupo 3	213	Grupo 3
130	Grupo 2	172	Grupo 1	214	Grupo 2
131	Grupo 2	173	Grupo 3	215	Grupo 3
132	Grupo 3	174	Grupo 2	216	Grupo 2
133	Grupo 3	175	Grupo 2	217	Grupo 3
134	Grupo 2	176	Grupo 3	218	Grupo 1
135	Grupo 2	177	Grupo 3	219	Grupo 2
136	Grupo 3	178	Grupo 3	220	Grupo 2
137	Grupo 1	179	Grupo 2	221	Grupo 2
138	Grupo 3	180	Grupo 1	222	Grupo 3
139	Grupo 3	181	Grupo 2	223	Grupo 1
140	Grupo 2	182	Grupo 1	224	Grupo 3
141	Grupo 3	183	Grupo 2	225	Grupo 2
142	Grupo 2	184	Grupo 3	226	Grupo 2
143	Grupo 1	185	Grupo 2	227	Grupo 3
144	Grupo 1	186	Grupo 3	228	Grupo 1
145	Grupo 2	187	Grupo 1	229	Grupo 3
146	Grupo 3	188	Grupo 1	230	Grupo 3
147	Grupo 2	189	Grupo 2	231	Grupo 2
148	Grupo 1	190	Grupo 2	232	Grupo 3
149	Grupo 3	191	Grupo 3	233	Grupo 2
150	Grupo 3	192	Grupo 3	234	Grupo 1
151	Grupo 1	193	Grupo 3	235	Grupo 1
152	Grupo 2	194	Grupo 1	236	Grupo 3
153	Grupo 3	195	Grupo 3	237	Grupo 2
154	Grupo 1	196	Grupo 2	238	Grupo 1
155	Grupo 2	197	Grupo 2	239	Grupo 3
156	Grupo 3	198	Grupo 1	240	Grupo 2
157	Grupo 2	199	Grupo 3	241	Grupo 3
158	Grupo 1	200	Grupo 2	242	Grupo 1
159	Grupo 2	201	Grupo 3	243	Grupo 2
160	Grupo 3	202	Grupo 1	244	Grupo 2
161	Grupo 1	203	Grupo 2	245	Grupo 3

246 Grupo 1
247 Grupo 1
248 Grupo 3
249 Grupo 2
250 Grupo 3
251 Grupo 2
252 Grupo 1
253 Grupo 1
254 Grupo 2
255 Grupo 3
256 Grupo 2
257 Grupo 3

Appendix B. List of anticipated tables and figures for data analysis.

10. Study design and randomization into treatment groups.

11. Participant's characteristics at enrollment and during follow-up per study group, Cruzeiro do Sul, 2018.

12. Common symptoms at enrollment per study group.

13. Asexual parasite density clearance per study day per study group.

14. Participant's outcome in the per-protocol analysis per study group at day 28 and day 168, Cruzeiro do Sul, 2018.

15. Haplotypes classification of paired samples *Plasmodium vivax* recurrences with valid microsatellite results, Cruzeiro do Sul, 2018.

16. Participant's CYP2D6 metabolic phenotypes profiles correlated with *Plasmodium vivax* recurrences, Cruzeiro do Sul, 2018.

17. Cox regression model for freedom from recurrence at day 168, Cruzeiro do Sul.

18. Geometric mean of asexual parasitemia of *Plasmodium vivax* among participants per treatment group during first 28 days of follow-up, Cruzeiro do Sul, 2018.

Appendix C. CONSORT checklist of information to include when reporting a trial

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomized trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	

Randomization:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomization; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	
	13b	For each group, losses and exclusions after randomization, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	

Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomized trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.