# Rigorous Assessment of *P. vivax* Relapses and Primaquine Efficacy for Radical Cure

**Principal Investigator**
Dr. Lek Dysoley

**Institution**
National Centre for Parasitology Entomology and Malaria Control

NCT04706130

May 26, 2020

# APPLICATION FORM FOR SUBMIT TO ETHICS COMMITTEE

| | |
|---|---|
| PI code: ……..… <br> Submitting institution/organization: <br> National Center for Parasitology Entomology and Malaria Control (CNM) | Address: #477 Betong, Corner St92, Trapengsvay village, Sangkat Kork Khleang, Khan Sen Sok <br> City: Phnom Penh <br> Country: Cambodia |
| Protocol Title: <br> Rigorous Assessment of P. vivax Relapses and Primaquine Efficacy for Radical Cure | |
| Version Nº: 2.0 DMID number: 20-0010 (Dated: …26…/…05…../…2020……) | |
| STUDY TYPE ☑ (only one choice ): | |
| ☐ Cross Sectional study, ☐ Case-control study, ☐ Cohort, ✔ Randomized control Trial, <br> ☐ Quasi experiment, ☐ Community trials, ☐ Qualitative study, ☐ Mix methods (quant & qual) <br> ☐ Other:…….…… | |
| RESEARCH TOPIC ☑ (multi choices ): | |
| ☐ Social behaviors <br> ☐ Laboratory <br> ✔ Clinical trial: ( ☐ Phase I, ☐ Phase II, ☐ Phase III, ☐ Phase IV ✔ Other: therapeutic efficacy……..…..) <br> ☐ Genetics <br> ☐ Health system : ( ☐ Health service delivery, ☐ Human resource, ☐ Health Policy, ☐ Health economic, <br> ☐ Other:…….…… ) <br> ☐ One health ✔ Infectious diseases ☐ Non communicable disease ☐ Maternal & child health <br> ☐ Other:…… | |
| STUDY POPULATION : | |
| ☐ Healthy ✔ Patient ☐ Vulnerable groups: ……. ☐ Other……. <br> Total Participants to be included: 260……..... | |

| CHARACTERISTICS OF PARTICIPANTS PARTICIPATED : | | | | | |
|---|---|---|---|---|---|
| Age Range: Lowest: 15 , Highest: no limit | | | | | |
| Is child included? ✔ None ☐ Yes : ( ☐ < 1 yr, ☐ 1-3 yrs, ☐ > 3 yrs) | | | | | |
| Gender: ☐ Male ☐ Female ✔ Both | | | | | |
| DURATION OF THE STUDY | From: July 2020 to : July 2023 | | | | |
| MULTI-SITE COLLABORATIONS: | ☐ YES ; ✔ NO | | | | |
| FINANCIAL DISCLOSURE: | ✔ YES ; ☐ NO | | | | |
| Financial Sponsor: USA National Institutes of Health and Institut Pasteur du Cambodge<br>Country of funding: USA and Cambodia.....<br>Estimated Budget: 300,000US$…… | | | | | |
| Implementing Agencies: National Centre for Parasitology Entomology and Malaria Control (CNM) and Institut Pasteur du Cambodge | | | | | |
| PLACE OF IMPLEMENTATION: | | | | | |
| Province | Area | | Province | Area | |
| ☐ Banteay Meanchey | ☐ Rural | ☐ Urban | ☐ Pailin | ☐ Rural | ☐ Urban |
| ☐ Battambang | ☐ Rural | ☐ Urban | ☐ Phnom Penh | | ☐ Urban |
| ☐ Kampong Cham | ☐ Rural | ☐ Urban | ☐ Preah Sihanouk | ☐ Rural | ☐ Urban |
| ☐ Kampong Chhnang | ☐ Rural | ☐ Urban | ☐ Preah Vihear | ☐ Rural | ☐ Urban |
| ✔ Kampong Speu | ✔ Rural | ✔ Urban | ☐ Prey Veng | ☐ Rural | ☐ Urban |
| ☐ Kampong Thom | ☐ Rural | ☐ Urban | ☐ Pursat | ☐ Rural | ☐ Urban |
| ☐ Kampot | ☐ Rural | ☐ Urban | ☐ Ratanak kiri | ☐ Rural | ☐ Urban |
| ☐ Kandal | ☐ Rural | ☐ Urban | ☐ Siem Reap | ☐ Rural | ☐ Urban |
| ☐ Kep | ☐ Rural | ☐ Urban | ☐ Stung Treng | ☐ Rural | ☐ Urban |
| ☐ Koh Kong | ☐ Rural | ☐ Urban | ☐ Svay Rieng | ☐ Rural | ☐ Urban |

| | | | | | |
|---|---|---|---|---|---|
| ☐ Kratie | ☐ Rural | ☐ Urban | ☐ Takeo | ☐ Rural | ☐ Urban |
| ☐ Mondul Kiri | ☐ Rural | ☐ Urban | ☐ Tbong Khmum | ☐ Rural | ☐ Urban |
| ☐ Oddar Meanchey | ☐ Rural | ☐ Urban | | | |

TYPE OF DATA:

☐ Institutional Base (Ex: PHD, OD, other research institution, orphanage, NGO…);

✔ Hospital/ Health center Based (Ex: Treatment center, clinic, health post…);

☐ Public Based (Ex: Community, market, school,...)

✔ Other : Patients referred by VMWs……

| LIST OF PRINCIPAL INVESTIGATORS | | Position on the research |
|---|---|---|
| 1- Dr. Lek Dysoley, CNM, vice-director<br>2- Dr. Benoit Witkowski, Institut Pasteur du Cambodge<br>3- Dr. Jean Popovici, Institut Pasteur du Cambodge<br>4- Dr. David Serre, University of Maryland | 1- ✔ Male; ☐ Female<br>Nationality: Cambodian<br>2- ✔ Male; ☐ Female<br>Nationality: French<br>3- ✔ Male; ☐ Female<br>Nationality: French<br>4- ✔ Male; ☐ Female<br>Nationality: French | 1- PI<br>2- Co-investigator:<br>3- Co-investigator:<br>4- Co-investigator: |
| Contact of the person in charge to the study | Active Email: soleycnm@gmail.com.<br>Phone number: +855 12 523 150 | |

Submitted on……/…../……

Signature and name of applicant

The submission requirements are met

The proposal in registered on ……/…../……

Secretary of NECHR

# សង្ខេបពិធីសារ /Protocol Summary

| | | |
|---|---|---|
| | | ភាសាខ្មែរ<br>English |
| | Title<br>ចំណងជើង | Rigorous Assessment of P. vivax Relapses and Primaquine Efficacy for Radical Cure |
| 1 | Background<br>សារបាន | No study has ever evaluated the efficacy of primaquine 0.25 mg/kg/day for 14 days in Cambodia to prevent relapses while WHO recommends 0.5mg/kg/day for tropical countries. There is currently no way to identify people carrying hypnozoïtes at risk of relapses |
| 2 | Objective<br>គោលបំណងចម្បង | 1. To determine the efficacy of primaquine 0.25 mg/kg/day for 14 days and compare it to 0.5mg/kg/day.<br>2. To identify biomarker candidates of hypnozoite carriers |
| 3 | Secondary objective<br>គោលបំណងបន្ទាប់បន្សំ | 1. To assess the impact of host polymorphisms (e.g., for the CYP2D6 enzyme) on the efficacy of primaquine treatment.<br>2. To determine the number of relapses following each of the primaquine regimen administered.<br>3. To evaluate the frequency, dynamic and complexity of relapses in patients living in endemic areas.<br>4. To estimate the rate at which patients are re-infected by Plasmodium parasites. |
| 4 | Survey Population<br>ពិពណ៌នាពីក្រុមសំណាក | Individuals infected by P. vivax seeking treatment |
| 5 | Subject selection criteria<br>របៀបជ្រើសរើសក្រុមសំណាក | 1.Being aged 15 years or more and able to provide informed consent,<br>2.Presentation with acute (within 10 days), symptomatic (i.e. history of fever), uncomplicated malaria caused solely by Plasmodium vivax (verified by PCR),<br>3.Being G6PD normal as determined by quantitative spectrophotometric assay,<br>4.Written informed consent provided by the volunteer. Witnessed consent is permitted, if the individual cannot write.<br>5.Able and willing to participate based on information given to the volunteer.<br>6.Being negative for SARS-Cov-2 in nasal swab by PCR |
| 6 | Sample design<br>រៀបចំពីទម្រង់នៃការសិក្សា | The study will be an open-labelled randomized clinical trial to determine therapeutic efficacy. |

| | | |
|---|---|---|
| 7 | Data Collection<br>របៀបប្រមូលទិន្នន័យ | Data will be collected according to SOPs on a daily basis for 90 days after enrolment |
| 8 | Laboratory Procedures<br>ប្រើប្រាស់មន្ទីរពិសោធន៍ | PCR detection of parasites, G6PD quantitative analysis, pathogen serology, pathogen whole-genome or targeted sequencing, human gene sequencing, pathogen and human gene expression analysis |
| 9 | Linkage to care and treatment<br>ការថែទាំ និងព្យាបាល | This study will allow to determine the optimal dosing regimen for primaquine treatment to prevent P. vivax relapses |
| 10 | Duration of Study<br>រយៈពេលនៃការសិក្សា | 3 years |
| 11 | Exposure and Outcome<br>រៀបរាប់ពីកត្តាអ្វីដែលយកមកសិក្សា | Arm1- artesunate only, arm2- artesunate+primaquine 0.25mg/kg/day, arm3- artesunate+primaquine 0.5mg/kg/day. Outcome: proportion of patients experiencing P. vivax relapses during 90 days of follow-up |
| 12 | Statistical Methods<br>ការវិភាគស្ថិតិ | Chi-square, Fisher tests. Kaplan-Meier survival analysis, |
| 13 | Sample Size and Power Calculations<br>ការគណនារកចំនួនសំណាក<br>ឬការគណនាកំរិត power | Assuming an efficacy of 92% for the 0.5mg/kg/day and 73% for the 0.25mg/kg/day, a type I error α of 5% and a power (1-β) of 90%, a sample size of 80 individuals per arm is required (total 160). Assuming a 20% loss during follow-up, we will enroll 100 patients per arm (total 200), in line with standard antimalarial therapeutic efficacy studies. |
| 14 | Ethical considerations<br>ការពិចារណាពីក្រមសីលធម៌ | Only drugs approved by WHO will be used at dosage, regimen and for conditions approved. This is not an IND trial. Patients will be tested for SARS-CoV-2 on inclusion by PCR on nasal swabs to ensure no infected patients are included. Facial masks will be used as PPE throughout the study for all patients and study staff. Hydro alcoholic gel will be supplied to ensure regular hand disinfection. |
| 15 | Cost and compensation<br>ថវិកា<br>និងការផ្តល់ប្រាក់កំរៃដល់អ្នកចូលរួម | The overall budget is 300,000 US$. Patients will be compensated for their participation and relocation in Chbar Mon with 200 US$/month |
| 16 | Reporting of results<br>លទ្ធផលដែលអាចនិងបង្ហាញ | After the completion of all data analysis, preliminary data will be presented and discussed with the relevant Cambodian leadership, CNM, MoH, IPC. A final dissemination workshop taking place in Cambodia will be organized to present and discuss the results to a large audience involving all national and international organizations involved in malaria control and elimination in the country. |
| 17 | Limitations<br>ដែនកំណត់របស់ការសិក្សា<br>(ចំនុចខ្សោយ) | No limitations have been identified for the completion of this proposal |

# Table of Content

## Contents

# Synopsis

| | |
|---|---|
| Title: | **Rigorous Assessment of *P. vivax* Relapses and Primaquine Efficacy for Radical Cure** |
| Principal investigator: | Dr. Lek Dysoley,soleycnm@gmail.com<br>Deputy Director<br>National Center for Malaria Control, Parasitology and Entomology (CNM)<br>Ministry of Health Cambodia |
| Co-Investigators | Dr. Witkowski Benoit, bwitkowski@pasteur-kh.org and<br>Dr. Popovici Jean, jpopovici@pasteur-kh.org<br>Molecular Epidemiology Unit, Institut Pasteur du Cambodge<br>Dr. Serre David, DSerre@som.umaryland.edu<br>University of Maryland, Baltimore, USA |
| Collaborators: | Ms Kim Saorin, ksaorin@pasteur-kh.org,<br>Molecular Epidemiology Unit, Institut Pasteur du Cambodge |
| Medical Monitor | MD to be hired |
| Population: | *P. vivax* infected individuals aged >15 years old |
| Number of Sites: | Chbar Mon city, Kampong Speu province, Cambodia |
| Study Duration: | 36 months |
| Subject Duration: | 6 months |

# Proposal

# Rigorous Assessment of *P. vivax* Relapses and Primaquine Efficacy for Radical Cure

## *1. Statement of the problem: Background & Justifications*

Together, *Plasmodium falciparum* and *Plasmodium vivax* account for the vast majority of human malaria cases worldwide. *P. vivax* is notably responsible for the majority of malaria cases outside Africa, with 13 to 30 million clinical cases each year [1, 2] and more than 2.5 billion people at risk of infection [3]. While *P. vivax* kills less frequently than *P. falciparum*, several recent studies have shown that *P. vivax* parasites can cause acute malaria episodes with severe outcomes [4-6]. *P. falciparum* and *P. vivax* are often co-endemic, transmitted by the same vectors and frequently treated indiscriminately. However, they are responding very differently to on-going malaria elimination efforts: while the prevalence of falciparum malaria has decreased worldwide over several decades, the situation is much less encouraging for vivax malaria and the relative proportion of malaria attributable to *P. vivax* is increasing, including in Cambodia [7, 8]. This discrepancy is partially due to the focus on *P. falciparum* (which can easily be studied in the laboratory, while in vitro cultures of *P. vivax* are currently impossible [9-11]) but also to the very different biological characteristics of these parasites [1, 12]. In particular, the ability of *P. vivax* parasites to remain dormant in the liver for several weeks or months before causing relapse infections, greatly complicates vivax malaria elimination efforts [13].

Unfortunately, we currently know very little about *P. vivax* relapses. To avoid the confounding effect of reinfections, *P. vivax* relapses have primarily been studied in volunteers or travelers and military personnel returning from endemic areas [14, 15]. However, these individuals are likely to have only been exposed to a handful of infectious bites and may not reflect the true burden of *P. vivax* relapses. We have recently shown that relapses are very frequent in people living in endemic areas and that dormant parasites are likely to be the main challenge of vivax malaria eradication confirming the findings of previous epidemiological studies [16, 17]. The regulation of the hypnozoites - the dormant liver-stage parasites - and the factors underlying their reactivation remain mysterious. In addition, we still do not have any molecular marker to detect patients carrying hypnozoites.

Radical cure, the complete elimination of hypnozoites from one patient, is difficult since primaquine (PQ) has side-effects in G6PD-deficient patients [18-20]. It is therefore necessary to screen patients for G6PD deficiency before prescribing PQ.

The current regimen recommended in Cambodia is 14 days of PQ at 0.25mg/kg/day. However, there is no evidence that this regimen is efficient to kill hypnozoïtes of parasites present in Cambodia. Indeed, for South East Asian strains, the WHO recommends 0.5mg of PQ per kg per day for 14 days [21]. In addition, the effect of PQ is affected by human polymorphisms underlying the metabolism of PQ into its active metabolite (most notably at the CYP2D6 gene) [22, 23].

This incomplete understanding of PQ effect on hypnozoites could have catastrophic consequences for vivax malaria elimination campaigns that would be jeopardized by emergence of PQ-resistant parasites. It is worrying that many *P. vivax* parasites might regularly be exposed to sub-therapeutic doses of PQ due to i) the unclear required dose of PQ to fully clear hypnozoites [24-26] and (ii) the heterogeneity in drug metabolism among patients and the high proportion of poor PQ metabolizers in endemic areas [19, 22, 27].
Here, we propose to **leverage the unique infrastructures and expertise of the National Centre for Parasitology Entomology, the Institut Pasteur du Cambodge and the University of Maryland** to address outstanding issues related to *P. vivax* relapses and the efficacy of PQ for radical cure treatment. We will conduct a tightly controlled clinical study designed to (i) compare the efficacies of 0.25mg/kg/day versus 0.5mg/kg/day for 14 days of primaquine to prevent relapses and (ii) characterize the mechanisms underlying hypnozoite reactivation and attempt to identify markers of hypnozoite carriage. Our findings will have immediately relevant clinical consequences and could guide the development of more efficient elimination campaigns against this important but neglected human pathogen.

## ***2. Relevance of the problem to national or local health objectives (biomedical, behavioral and health systems development)***

In Cambodia, malaria is one of the foremost public health problems and its control is a high priority for the government and NGOs. Among the estimated 13.6 million Cambodians, 2.5 million individuals live in forested areas where malaria transmission is highest compared to plains and rice field areas [8]. With the implementation of extensive control efforts, the number of reported malaria cases has globally decreased since 1997, with sporadic increases in 2003, 2006 and 2009 caused by variations in rainfall or climate change (e.g., La Niña) or sudden changes in forest-related activities (illegal woodcutting or new waves of settlements in forested areas resulting from economic downturn).
Since 2011, the number of *P. vivax* cases is higher than the number of *P. falciparum* ones. In 2014, based on malaria RDT detection (health centers and VMW data), non-*P. falciparum* infections (mostly *P. vivax*) accounted for 46% (26,183) of cases, followed by 32% (16,540) of mixed infections of *both P. falciparum* and non-*P. falciparum* and 21% (12,422) of pure *P. falciparum* cases (8). As such, *P. vivax* has become a major public health problem in Cambodia and research needs to be done specifically on this parasite if we aim to eliminate this species by 2030 [28].

With elimination showing great promise against *P. falciparum*, Cambodia now needs to address the problem of *P. vivax* and implement adequate strategies to kill hypnozoites.
The current recommended regimen of 0.25mg/kg/day of primaquine is suspected to not be enough for Southeast Asian strains, but this needs to be robustly demonstrated for parasites circulating in Cambodia.
In addition, if we could identify biomarkers of hypnozoite carriage among people, we could have tools to better target the population that needs to be treated for complete elimination.

**By providing an unbiased perspective on the efficacy of primaquine to kill hypnozoites, and possibly identifying markers of hypnozoite carriers to specifically target people infected by *P. vivax,* our studies have the potential to significantly improve current treatment and**

**elimination of vivax malaria, a major and immediate public health concern in Cambodia and Southeast Asia.**

## *3. Field(s) of application of the proposed research results*

The research proposed here will provide valuable results for better understanding and characterizing *P. vivax* relapses and response to primaquine, which will be of great interest for Cambodia and all other malaria endemic countries facing the challenge of *P. vivax* elimination.
Implementing tightly controlled clinical study will allow designing appropriate therapeutic strategies to be implemented with the objective of malaria elimination in Cambodia by 2030.

**Plan of research result dissemination**
The data from the study will be analyzed and results will be presented locally, nationally and internationally during workshops and conferences. A final report will be prepared and electronic versions will be posted on relevant websites for easy access. The team will also prepare articles for publication in peer-reviewed journals. Paper copies of reports and articles will be made available to policy makers, health administration and health scientists

**Time schedule of research implementation**
We anticipate results will be available early 2024.

## *4. Review of literature and other existing information*

In Cambodia, reductions in *P. falciparum* incidence over the last decade has been reported by the National Malaria Control Program together with an increase in the proportion of *P. vivax* cases being reported by the Health Information System data (see Figures 1A and 1B).

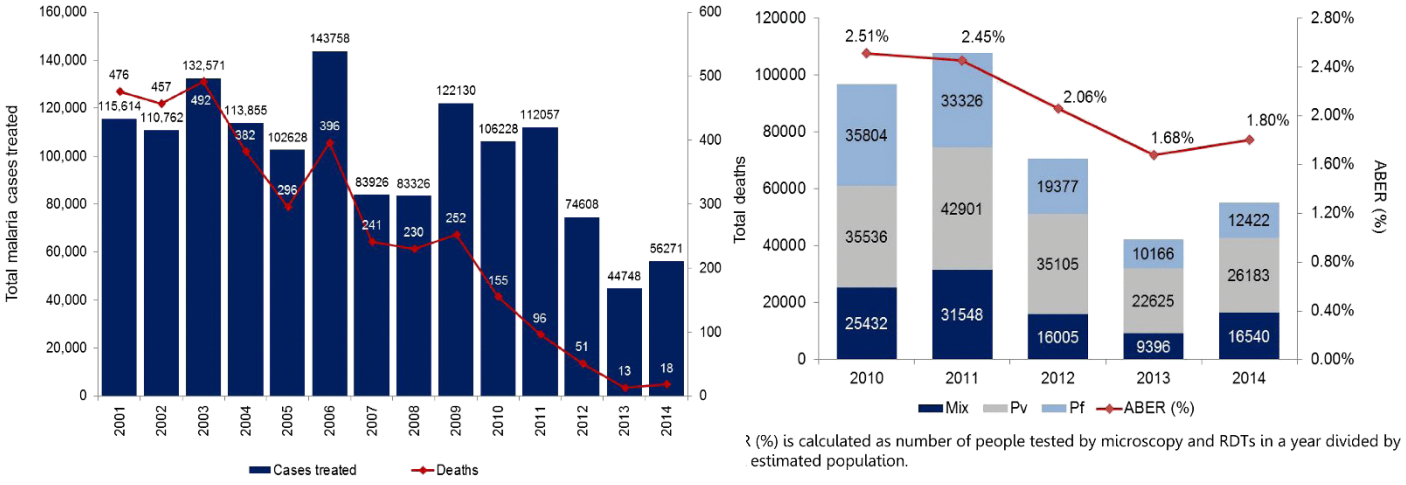


*Figure 1. A) Total treated cases (bars) and deaths (line) of malaria from 2000 to 2014; B) Proportion of Plasmodium falciparum and Plasmodium vivax cases observed by microscopy and RDT by year.*

The current first-line treatment for *P. vivax* in Cambodia is artesunate-mefloquine and primaquine at 0.25mg/kg/day for 14 days only for G6PD normal patients. The WHO recommends increasing the dose of primaquine to 0.5mg/kg/day for 14 days in South East Asia. This 0.5mg/kg/day for 14 days regimen is safe for G6PD normal patients [29, 30]. In Cambodia, no study has ever assessed the efficacy of primaquine to eliminate hypnozoites and it is therefore critical to make sure that the dose of primaquine is sufficient to avoid exposing parasites to sub-therapeutic concentrations and possibly selecting for primaquine resistance [31].
Rigorously determining the efficacy of primaquine, as well as studying relapses in general, is complicated in vivo as parasite recurrence after treatment of blood stage infections could be due to reinfection, relapses or recrudescence. We have previously designed a clinical study where patients were relocated to a no-transmission area during follow-up, which allowed us to robustly study relapses [16, 17]. Using a similar design, we will here characterize primaquine efficacy and possibly identify biomarkers of hypnozoite carriage. Indeed, there is currently no way of knowing whether an individual carries hypnozoites dormant in his liver and identifying biomarkers of hypnozoite carriage would allow us to design tools to specifically target infected individuals.
To make sure that parasite recurrences following enrollment of patients are only due to relapses (i.e., reactivation of dormant hypnozoites), we will (i) relocate patients during the entire follow-up to a no-transmission city to make sure they are not reinfected; and (ii) treat the initial infection using artesunate, a rapidly eliminated blood-stage drug highly efficient against *P. vivax*. Therefore, following this treatment and relocation, any recurrence observed will only be due to relapses from hypnozoites. We will also characterize the genotypes of all parasites (from the initial and recurrent infections) to definitely exclude the possibility of recrudescence.

**In this research study, we aim to carefully assess the efficacy of primaquine and attempt to identify biomarkers of hypnozoite carriage.**

## ***5. Statement of objectives***

The primary objectives of this research study are:

1. To determine the efficacy of primaquine 0.25 mg/kg/day for 14 days and compare it to 0.5mg/kg/day.
2. To identify biomarker candidates of hypnozoite carriers

Secondary objectives are:

1. To assess the impact of host polymorphisms (e.g., for the CYP2D6 enzyme) on the efficacy of primaquine treatment.
2. To determine the number of relapses following each of the primaquine regimen administered.
3. To evaluate the frequency, dynamic and complexity of relapses in patients living in endemic areas.
4. To estimate the rate at which patients are re-infected by *Plasmodium* parasites.

## *6. Statement of research hypotheses*

The first hypothesis is that 0.25mg/kg/day for 14 days is not enough to kill hypnozoites of *P. vivax* isolates circulating in Cambodia. The second hypothesis is that people carrying hypnozoites dormant in their liver (and at risk of relapses) can be identified with biomarkers, either at the gene expression (specific parasite or host gene expressed when hypnozoites are present) or serological level (specific antibodies in patients carrying hypnozoites), and specifically targeted for radical cure treatment.

## *7. Research methodology*

### 7.1 Summary of methodology (not more than 150 words)

The methodology will involve the following steps:

1- Inclusion of *P.vivax* mono-infected patients (Kampong Speu province),
2- Assessment of G6PD activity (gold standard quantitative test at IPC),
3- Enrollment of G6PD normal patients and arm allocation,
4- Follow-up while relocation in a no transmission city (Chbar Mon) during 90 days (to make sure they are not reinfected) with medical and parasitological supervision every 48h,
5- End of relocation and further monthly follow-up for 3 months,
6- Gene expression analysis (performed at University of Maryland, USA by Dr David Serre) and serological analysis by protein arrays (array probing with plasma samples performed at the Institut Pasteur du Cambodge) to identify biomarkers associated with hypnozoite carriage.

### 7.2. Study design type

The study will be an open-labelled randomized clinical trial to determine therapeutic efficacy. Note that this will ***not be an Investigational New Drug application, as only WHO-approved drugs will be used at dosage and for conditions approved***.
Eligible patients willing to participate will first be tested prior to enrolment for their G6PD status by the gold standard spectrophotometric analysis. We will exclude from the study any G6PD deficient (or intermediate females) patient. Treatment allocation will be randomized between i) 7 days of artesunate (2 mg/kg/day for 7 days) alone (Arm1), ii) same artesunate regimen + 0.25 mg/kg/day 14 days of primaquine (Arm2) and iii) same artesunate regimen + 0.5 mg/kg/day 14 days of primaquine (Arm3). All patients will be relocated to a no-transmission city (Chbar Mon) to make sure they are not reinfected during the follow-up. Follow-up will be performed every 24-48h for 90 days. At the end of the follow-up period, all patients that did not receive primaquine (arm1) will be treated according to national guidelines (14 days at 0.25mg/kg/day). Patients will additionally be followed monthly for three months after the end of the relocation. Subject participation duration will be 90 days relocated + 3 months not relocated = 6 months.

## 7.3. Study site and relocation

The study will be carried out in Kampong Speu province between July 2020 and July 2023, an area where malaria transmission is among the highest in Cambodia. The study team will set up a research office in the capital of the province, Chbar Mon city. Two mobile laboratories of Institut Pasteur du Cambodge will be setup in the office site. Chbar Mon is located one hour by car from Phnom Penh and the Institut Pasteur du Cambodge and Calmette hospital, with its ICU in case of acute medical emergency that requires ICU level management.

Additionally, a house located close to the research office will be rented during the whole duration of the study to accommodate patients during their relocation in Chbar Mon where there is no malaria transmission occurring. The house will be able to accommodate simultaneously a maximum of 30 individuals and will be comprised of separated bedrooms for male and female patients, at least two bathrooms, kitchen, living area and garden. A study staff will be hired for daily food preparation and house cleaning. We have previously successfully used a similar design with relocation of patients during their follow-up [17]. The purpose of this relocation is to make sure patients are not reinfected during the follow-up, which would affect the interpretation of primaquine efficacy data. Patients will be free to do what they want during the daytime, however, we will request that they come back to the relocation house at 4pm every day, when follow-up examination will occur (transmission occurs after sunset in forested areas). Refusal to comply, or breach in compliance will be a cause for discharge from the study. Financial compensation will be provided to patients to compensate for their likely disturbance in their source of incomes. The compensation (US$100) will be given every 15 days (total US$200 per month per participant) and in case of breach of relocation by the patient, the daily prorata will be provided and the participant will be discharged.

## 7.4. Study population, inclusion and exclusion criteria

Study subjects will be male and non-pregnant females with proven acute uncomplicated *Plasmodium vivax* malaria who meet the following inclusion and exclusion criteria.

### *7.4.1. Inclusion criteria:*

1. Being aged 15 years or more and able to provide informed consent,
2. Presentation with acute (within 10 days), symptomatic (i.e. history of fever), uncomplicated malaria caused solely by *Plasmodium vivax* (verified by PCR),
3. Being G6PD normal as determined by quantitative spectrophotometric assay,
4. Written informed consent provided by the volunteer. Witnessed consent is permitted, if the individual cannot write.
5. Able and willing to participate based on information given to the volunteer.

### *7.4.2. Exclusion criteria:*

1. Being aged 14 years or less or unable to provide informed consent,
2. Pregnant, planning to become pregnant or lactating women,
3. Having taken antimalarial drugs in the past month,
4. Being G6PD deficient or intermediate (for females),
5. Hb < 8g/dL,
6. Any clinically significant disease requiring treatment or further investigation,

7. Having any malaria danger signs: unable to swallow because of vomiting, ≥2 convulsions within previous 24 hours, reduced level of consciousness, unable to sit or walk unaided,
8. Having history of primaquine, artesunate or artesunate-mefloquine allergy or intolerance,
9. Having mixed infection with other *Plasmodium* species,
10. Neutrophils count <1500 cells/µl,
11. Being positive by PCR for SARS-CoV-2.

All eligible participants will be approached by the study staff and information about the objectives of the study will be explained. Informed consent will be obtained from each participant before inclusion.

## 7.5. Study withdrawal

### *7.5.1. Voluntary withdrawal*

Participants may withdraw from the study at any time and for any reason, and request that their blood is not be stored.

### *7.5.2. PI withdrawal*

A patient may be withdrawn by the PI from the study if:

1. The participant is not willing to comply with the study procedures,
2. Participation in the study represents a risk to the health and well-being of the participant, as judged by the PI.

### *7.5.3. Management of withdrawn subjects*

Study documentation will be completed for all withdrawn patients. Patients will still be entitled to any benefits, as outlined in the consent form, up to the point of withdrawing their consent. Withdrawn subjects will only be followed up if they have suffered an adverse event that needs follow up.

### *7.5.4. Subjects transferred to another hospital*

If a subject cannot be treated for an adverse event at the study site or local hospital, he/she will be referred to e.g. the Calmette hospital for further management of a serious adverse event. This will make completing study investigations difficult. Nevertheless, such patients are still considered to be in the study and attempts will be made to obtain relevant data and follow them up after hospital discharge in accordance with the study schedule.

## 7.6. Study treatments

*Primaquine*

Primaquine will be obtained from a WHO-qualified supplier. Primaquine will come as tablets containing 7.5 and 15 mg of primaquine base.

*Artesunate*

Artesunate will be obtained from a WHO-qualified supplier. One tablet contains 50 mg of artesunate.

*Storage and Handling*
All tablets in this study should be kept at controlled room temperature (25°C or 77°F); excursions permitted between 15°C to 30°C (59°F to 86°F).

*Drug Accountability*
The study site will maintain a record of the numbers of all artesunate and primaquine tablets obtained, used by participants and returned to the study pharmacy. On study completion, a copy of the drug accountability record will be filed in the study folder. Unused drugs will be returned to the CNM or to the local hospital pharmacy.

*Concomitant Medications*
Study patients may develop minor illnesses during follow up. The use of drugs needs to be controlled and all study patients will use the study team as their source of medical care during the study.
Drugs that may interfere with the metabolism of primaquine must be avoided.
The following drugs may interfere with the primaquine metabolism by inhibiting the cytochrome (CYP) 450 especially CYP 3A4:

1. Ketoconazole, itraconazole
2. Cimetidine
3. Erythromycin
4. Ritonivir

The following drugs may interfere with the primaquine metabolism by inducing CYP 450, especially CYP 3A4:

1. Barbiturates
2. Carbamazepine
3. Phenytoin
4. Rifampicin
5. Macrolide antibiotics
6. Glucocorticoids

*Antipyretic*
If an antipyretic is needed, paracetamol is recommended and will be supplied under the medical monitor supervision.

*Antiemetic*
An antiemetic may be used for nausea and vomiting, if clinically indicated and under medical supervision.

## 7.7. Sample size

Based on our previous experience and results from the literature, we estimate that at least 50% of patients will have *P. vivax* relapses during the 90 days follow-up in absence of any radical cure regimen [16, 17]. To ensure a high enough number of relapsing and non-relapsing patients in arm1 (no primaquine) to allow the identification of biomarkers associated with hypnozoite presence, we will enroll 60 patients in this arm (expected ~ 30 patients with relapses and ~30 patients without). Note that this arm is not intended as therapy for practice,

but aims to determine the patterns and dynamics of *P. vivax* relapses as well as to identify much needed biomarkers of hypnozoite carriers.
Concerning the comparison of the two regimens of primaquine, this is a typical therapeutic efficacy study as performed routinely for antimalarial drug surveillance and a sample size of 100 individuals per arm is standard. Defining robust sample size is complicated and the number of relapses expected in each arm is difficult to even crudely estimate since most previous studies did not correct for confounding factors (e.g., reinfection or poor treatment adherence). Assuming an efficacy of 92% for the 0.5mg/kg/day [15] and 73% for the 0.25mg/kg/day [32], a type I error α of 5% and a power (1-β) of 90%, a sample size of 80 individuals per arm is required (total 160). Assuming a 20% loss during follow-up, we will enroll 100 patients per arm (total 200), in line with standard antimalarial therapeutic efficacy studies.

## 7.8. Procedures, sample and data collection

### *7.8.1. Pre-study training*

Pre-study training will consist of the following elements:

1. Aim and objectives of the study.
2. Study procedures.
3. Informed consent, with an emphasis on the free giving of consent and the need for the investigators to be confident that the subjects have a good understanding of what the study involves.
4. Data accuracy and integrity, including internal monitoring of data.
5. The principles of Good Clinical Practice.
6. How and when to report adverse events (AE) and serious AEs.
7. What to do if unsure of how to manage an adverse event.
8. Subject follow up and communication.
9. The treatment of medical emergencies, notably acute anaphylaxis.
10. How to manage hemolysis.

### *7.8.2. Pre-study sensitization*

After setting up the research office in Chbar Mon, members of the research team will visit surrounding villages to meet with villagers and village elders to explain the nature of the study.
Staff involved in this research study will be composed of scientists, medical doctors and technicians from the CNM and IPC. Additional collaborators (Reference hospital, Health centers and VMW staff from the Kampong Speu province) will also be involved in the project.

### *7.8.3. Subject registration, consent and numbering*

Patients with fever who attend the local clinic will be managed according to local practice. This usually involves either the preparation of a blood film or use of a rapid diagnostic test (or both) to determine if the patient has malaria. Pre-study training will be given on slide preparation.
If a subject has vivax malaria, he or she will be asked if they would like to be part of a research study. If the answer is yes, a copy of the consent form in Khmer will be given to them to read or the consent form will be read to them. A trained study nurse may assist the research physicians in the consent process. Any questions that may arise will be answered by one of

the study doctors. We will make sure they understand that, if they refuse to participate, we will still provide free malaria treatment for them (standard artesunate-mefloquine 3-day treatment).
If the potential study subjects / guardians volunteer to join the study, they will be asked to sign the consent form. A screening number will be assigned to them starting at S001.
After the consent form has been signed, all mandated study investigations to determine eligibility to the study will be performed.
The screening numbers will be used on all relevant labels, case report forms, the database and stored samples.

*P. falciparum* malaria cases will be managed by VMW and Health Centers as usually according to Cambodian National guidelines.

### *7.8.4. Assessments for study entry*

After obtaining informed consent, 2 heparin tubes (2 x 10 mL) and two EDTA tube (2 x 5 mL) will be collected for:

1. Hb (HemoCue),
2. Free (plasma) Hb (HemoCue)
3. Full Blood Count
4. G6PD activity
5. parasite diagnostics confirmation by qPCR and quantification

A nasal swab will be collected for SARS-CoV-2 PCR diagnostic.
A physical examination will be performed by the medical monitor.
Temperature will be measured and recorded as well as sex, age, weight, height, self-reported prior history of malaria infections and medication used in the past four weeks.
Female patients will perform a urine β-human chorionic gonadotropin pregnancy test.

Patients will be relocated from the day consent is given to our study site where food, beverage and accommodation will be provided until the end of the 90-days follow-up. Facial masks will be supplied and all staff and participant will be required to wear one at all time. Hydro-alcoholic gel will be supplied and participants will be encouraged to disinfect their hands regularly. Study staff will always wear single-use gloves during interventions.

Blood and nasal swab analysis to make sure patients fill inclusion criteria will be performed immediately and results will be available within 48h-72h after the enrollment.

Patients meeting any of the following exclusion criteria based on biological analysis

1. Hb<8g/L
2. G6PD activity< 80% normal
3. positive pregnancy test
4. *P. falciparum* detection by qPCR
5. neutrophils <1500/µl
6. PCR positive for SARS-CoV-2

will be excluded from the study and antimalarial medication will be provided as per National Guidelines (standard artesunate-mefloquine 3-day treatment). SARS-CoV-2 positive patients will be treated with antimalarial drug and immediately referred to the referral hospital for

case management of COVID-19 patients following guidelines of the Cambodian Ministry of Health.

### *7.8.5. Drug administration, procedure and follow-up*

All treatments will be given supervised and will be signed for by the study team member giving the drugs.
All patients will receive 7 doses of artesunate at 2 mg/kg per day every 24h (total, 7 doses at D0, D1, D2, D3, D4, D5, D6).
Eligible patients will be randomly assigned to one of the three arms (arm1: no primaquine, arm2: primaquine 0.25mg/kg/day for 14 days, arm3 primaquine 0.5mg/kg/day for 14 days). Randomization will be computer generated in blocks of 6 and assigned by phone call to the study staff.
Primaquine treatment will be supervised and will start on D7.
Participants will be observed for one hour after each drug administration. If vomiting occurs within 30 minutes after artesunate or primaquine administration, a repeat dose of artesunate or primaquine, will be administered. If vomiting occurs between 30 and 60 minutes, half the dose will be administered. This will be recorded on the drug treatment CRF.

Fingerprick capillary blood collection (~ 250µL) will be performed at 1h, 2h, 4h, 8h, 16h, 24h (D1), 48h (D2) and 72h (D3) after the first dose of artesunate for measuring parasite clearance (by microscopy, PCR and genotyping), parasite response to artesunate, and drug pharmacokinetics.

From D3 to D21, every 24h a fingerprick capillary blood collection (~ 250 µL) will be performed to determine parasite presence by PCR and microscopy, reticulocytemia, EPO, CRP, hemoglobin concentration (HemoCue).
Clinical examination, temperature measure and oximeter data (Massimo: SpO2, SpCO, SpMet, pulse rate and perfusion index) will be recorded every 24h until D21.

From D22 until D90, clinical examination, temperature measure and oximeter data (Massimo: SpO2, SpCO, SpMet, pulse rate and perfusion index) will be recorded every 48h along with collection of a fingerprick capillary blood collection (~ 250 µL) for parasite diagnostic by PCR and microscopy, reticulocytemia, EPO, CRP, hemoglobin concentration and hematocrit, serology and gene expression.

In case of parasite recurrence during the follow-up (detected by microscopy and mono-infection confirmed by PCR), 2 heparin tubes (2x10 ml) and 1 EDTA tube (1x5ml) of venous blood will be collected for culture of parasite, parasite and host gene expression analysis and parasite genome sequencing. A full blood count will be performed and if neutrophils counts are above 1,500/µL and patients did not show any adverse event during the initial artesunate course, enrollment procedures will be repeated, and patients re-treated with the same 7-day regimen of artesunate (2 mg/kg/day for 7 days). Follow-up visits and procedures will be restarted as if newly recruited until the end of the initial 90-day follow-up. If patients had adverse events during the initial artesunate treatment, or if their neutrophils counts are below 1,500/µL, they will be discharged from the study and treated with the standard 3-day regimen of artesunate-mefloquine + 14 days 0.25 mg/kg/day of primaquine as per Cambodian guidelines.

At the end of the follow-up, any patient positive for *P. vivax* will be treated with a standard 3-day regimen of artesunate-mefloquine + 14 days 0.25mg/kg/day of primaquine as per Cambodian guidelines. In addition, all patients in arm1 (who had artesunate only) will receive primaquine following Cambodian recommendations (0.25mg/kg/day for 14 days).

In case of *P. falciparum* detection during the follow-up, patients will be treated following Cambodian treatment guidelines (3-day artesunate-mefloquine + single low dose primaquine) and discharged from the study.

In case of respiratory infection symptoms during the follow-up, a nasal swab will be performed for SARS-CoV-2 PCR diagnostic. In case of positive PCR, the patient will be discharged from the study and referred to the referral hospital to be managed following the guidelines of the Cambodian Ministry of Health.

Upon completion of the 90-day relocation, patients will be allowed to go back to their daily activities in transmission areas. They will be followed monthly for the next three months for determining the re-infection rate. At each of the three monthly visits, a fingerprick capillary blood will be collected for parasite detection by RDT, microscopy and PCR.

Blood samples collected at enrolment or during the follow-up will be used for the following investigations:

1. EPO and CRP quantification
2. Reticulocytemia determination
3. Sequencing of host genes possibly involved in drug efficacy, pathogens invasion and development
4. Serology analysis of antibodies against pathogens
5. Whole-Genome and targeted sequencing of pathogens
6. Parasite culture
7. RNA-Seq analysis of host and pathogen gene expression.

## ***8. Safety assessment and reporting***

For reference, this study will use the Division of Microbiology and Infectious Diseases (DMID) toxicity table available from the NIAID web site:
https://www.niaid.nih.gov/sites/default/files/dmidadulttox.pdf

### 8.1. Definition of adverse events (AE)

An AE is any undesirable event that occurs to a study participant during the course of the study; that is, from the time of the first dose of study drug(s) until the last follow up visit, whether or not the AE is related to the study drug, a concomitant drug, a procedure or an intercurrent illness. Therefore, an AE can be absent at baseline but newly develops or was present at baseline and worsens. An AE may be one of the following: a symptom, a physical sign, an abnormal laboratory result, a new illness. Any new clinical sign or clinical deterioration that occurs between signing the consent form and the administration of study drugs is not an AE. This information will be captured when the physical examination is done.

We will use the DMID Toxicity Table as a reference for grading adverse events. For AEs that are not specifically listed in the toxicity table, we will use the category "Estimating Severity Grade" located at the top of page 2 of the toxicity table.

## 8.2. Definition of serious adverse events (SAE)

In this study, an AE is a serious AE if it results in any of the following outcomes: (i) death, (ii) life-threatening event (this means that the participant was at immediate risk of death at the time of the event and required immediate medical intervention. It does not refer to an event which might have caused death if it were more severe), (iii) requires admission to hospital for treatment, (iv) persistent or significant disability/incapacity (a substantial disruption of a person's ability to conduct normal life functions), (v) a congenital abnormality or (vi) an important but not life threatening event e.g. a medical or surgical illness requiring acute treatment.

## 8.3. Reporting procedures

### *8.3.1. Relatedness of adverse event to study drugs*

In this study, the relationship between AEs and artesunate or primaquine or both drugs will be determined as: (i) Unrelated: clearly not related to the study agent, (ii) unlikely related: doubtfully related to the study agent (iii) possibly related: may be related to the study agent, (iv) Probably related: likely related to the study agent (v) definitely related: clearly related to the study agent.
The relationship of the AE to study drugs will be recorded on the AE CRF.

### *8.3.2 Adverse event reporting*

This study is more interested in objective laboratory measurements of haematological adverse events and other drug related AEs that are the known expected risks associated to artesunate and primaquine treatments. Note that in this study, **we will only use those drugs at the dosage and for the conditions approved by the WHO and therefore the risks are minimal**.
Subjects will answer a symptom questionnaire at baseline and be asked during each follow up visit how they are. Symptoms will be recorded at the 24-48h follow up visits. Vital sign and eye signs will also be recorded. If the subject requires any further assessments, these will be done.
Only the following clinical AEs will be recorded as AEs on the AE CRF:

1. All SAEs
2. All rashes
3. Itching without a rash
4. Haemoglobinuria
5. Persistent vomiting requiring a change of treatment
6. Clinically significant illnesses requiring treatment

The following laboratory parameters will be recorded in the study:

Hb, $SpO_2$, SpCO, SpMet, pulse rate and perfusion index, FBC, Reticulocytemia,

Many of the changes in these parameters will be due to treated malaria and trends in these parameters will be analyzed.
All laboratory results will be reviewed by the clinicians of the research team to be graded using the toxicity table and determine their clinical significance.

Only grade 3 and 4 laboratory AEs will be recorded on the AE CRF. All other laboratory AEs will be determined at the time of data analysis and will not be recorded on the AE form. The following laboratory AEs will be recorded: grade 3 or 4 values for:

1. Hb
2. Low total WCC
3. neutropenia
4. lymphopenia
5. decreased platelet counts
6. rise in metHb

If study consent is withdrawn, AE recording will cease but existing AEs will be followed up, as clinically indicated.

***8.3.3. Serious Adverse Event reporting***

SAEs will be reported to the groups who oversee the trial: the CNM and the Institut Pasteur du Cambodge.
The PI will inform, within 24 hours of any SAE, the Cambodian National Ethics Committee and the DMID Pharmacovigilance Group. The report will consist of a summary of the clinical information on the SAE form. Follow up reports will be sent as needed but in any case a minimum of one follow up report will be sent within 7 days. These reports will be sent by email to:

Cambodian National Ethics Committee secretary: Dr. Saphonn Vonthanak.
Tel: +855-12280790
E mail: research03@nchads.org

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

***8.3.4. Patient Management of Adverse Events***

All AEs will be treated as clinically indicated and if concomitant treatment is given this will be recorded on the Concomitant treatment CRF. The medical doctor in charge of medical monitoring will be responsible for evaluating the clinical condition of patients. Study staff will be trained in simple procedures to assist participants feeling faint and to achieve temporary hemostasis in the event of continued bleeding. Study staff will also be trained to recognize signs/symptoms of more serious adverse events such as hemolytic anemia and soft tissue infection. If necessary, participants will be referred for specialist care at the Calmette hospital

in Phnom Penh. Any relevant clinical information from a hospital admission will be obtained from the hospital physicians by the PI or her designee.
The participant will be followed and treated by the research team until the clinical or laboratory AE has resolved or stabilized. The physician should perform any tests that are clinically indicated.
All medical costs, including transportation costs, incurred as a result of study participation will be paid from the study budget.

## *9. Quality Control*

### 9.1 Training and supervision of field teams and quality management

A field study supervisor will be dedicated to ongoing supervision and monitoring of study implementation. Clinical procedures and data collection will be evaluated regularly by the field study supervisor. Other quality control measures will include daily review of patient records, observation of participant interactions and clinical procedures, adherence to the approved protocol and Standard Operating Procedures (SOPs) and ongoing evaluation of malaria laboratory procedures according to standardized checklists. Refresher training will be provided as necessary.
The PI and co-investigators will lead the preparation for the study, together with the field team. Collaborative involvement of the PI and co-investigators will be present prior to study implementation, as well as throughout the study.
Monitoring of data and clinical specimen quality will occur throughout the study. Constructive feedback for field study staff, as well as additional training, will be provided as needed based on the real-time assessments of data and specimen quality.

### 9.2 Diagnostics/Clinical Assays

Routine quality control procedures will include monitoring and supervision of assay performance against standardized monitoring checklists on a monthly basis. Laboratory assay performance will be assessed by Institut Pasteur du Cambodge standards guidelines. All blood analysis will be performed in an ISO 15189 laboratory. Appropriate laboratory controls will be included with performance of laboratory assays. Laboratory instrument validation will be conducted daily. All drugs will be sourced from WHO-qualified suppliers.

### 9.3 Data monitoring

Each subject will have a study folder which will contain all the case record forms (CRFs) and the laboratory reports. The study folder will be the main source document. The PI is responsible for maintaining accurate, complete and up to date CRFs and for checking the laboratory reports. The CRFs are to be completed on an ongoing basis during the course of the study. All subject CRFs will be reviewed by the Principal Investigator and signed as required. Data will be doubled entered by two different data entry clerks from the study folder into a suitable software package. Data will be compared and discrepancies will be resolved by examining the CRFs and laboratory reports. Once all the data have been entered,

internal checks on the database will be done and any discrepancies corrected. Once the database is considered clean, the data will be analysed by the team statisticians.

### 9.4. Laboratory Specimens

Study samples, including stored samples, will be labelled with:

1. Participant study number
2. Subject initials
3. Date of specimen
4. Type of specimen, if applicable.

The study site will follow the principles of good laboratory practice for clinical trials and local standard operating procedures (SOPs) and SOPs specifically for this study for the collection, processing, labelling, transport and storage of all specimens.

Study site staff will store specimens in appropriate, fully functioning freezers (e.g. DNA can be stored at -20°C or -80°C) so that the protocol related analyses can be performed. After these tests have been performed, blood or extracted nucleic acids will be kept for long term storage if participants agreed for it.

The PI will inform the ECs when specimens have been destroyed and if specimens are lost or thrown away e.g. due to a power failure.

For those specimens that need to be transported outside of Cambodia, a Material Transfer Agreement (MTA) will also be agreed upon by all the relevant parties, following Cambodian law. Specimens will be transported by air in accordance with the necessary IATA regulations. Transport of specimens between the research clinic and the Institut Pasteur du Cambodge will follow Pasteur SOPs. Prior to shipping, all samples will be anonymized and each patient sample will be recoded using a unique patient identifier. No patient personal information will be shared with coinvestigators outside of Cambodia and released genetic information through protected databases (e.g., dbGaP) will not be linked to any personal information.

## ***10. Statistical Considerations and Data Analysis***

### 10.1 Study outcome measures

Primaquine efficacy:

Efficacy of the primaquine regimen will be determined by the percentage of patients experiencing at least one microscopically detected and PCR confirmed *P. vivax* recurrence during the 90-day follow-up while being relocated in the no-transmission area. The total number of recurrences experienced by each participant in all arms will also be determined. The time between each recurrence will be determined. The protein-coding sequence of genes involved in primaquine metabolism (including CYP2D6) will be determined for each participant by DNA sequencing.

Reinfection rates:
The frequency of participants diagnosed with *Plasmodium* infection (any species) after the end of the 90-day relocation will be determined.
Relapse frequency, dynamic, complexity and biomarkers:
The time to the first recurrence and the total number of recurrences experienced during the monitoring period by participants not treated with primaquine (arm1) will be determined. The whole genome sequences of parasites at each recurrence will be determined. The serological and host gene expression profiles of each participant will be evaluated by protein microarray during and prior to the recurrence. Serological data will be expressed as fluorescence intensity for each antigen. The gene expression profiles of patients and parasites will be determined by RNA sequencing and measured as normalized read counts for each human or *Plasmodium* gene. Quantitative biological data will be recorded (EPO, Hb concentrations, reticulocytemia).

## 10.2. Sample size calculation

See 7.7

## 10.3. Analytic Plan

Data will be collected using paper collection forms and subsequently digitized using Excel spreadsheet with appropriate validation rules to promote accurate transcription. Paper forms will be archived in a locked storage container within the Institut Pasteur du Cambodge office for reference in the event electronic data entry is called into question. All statistical analyses will be performed using R version 3.5.0 or Graphpad Prism version 7, following direct importation of electronic databases lacking personal identifiers.
Primaquine efficacy between the two regimens will be characterized by 2 x 2 tables classifying the results for each regimen (parasite-free participant, participant with at least one recurrence). Chi-square or Fisher tests will test for differences between the two regimens.
Kaplan-Meier survival analysis will test for differences between the two regimens to prevent all relapses over the 90-day follow-up. Additional analyses might be performed to take into account the effect of patients' polymorphisms at metabolic genes. Patients will be stratified based on the validated or predicted effects of the polymorphisms of enzyme activity (from high metabolizer to low metabolized and null) and the primaquine efficacy data analyzed using this classification as a covariate (e.g., using Cochran–Mantel–Haenszel test).
Reinfection rates will be estimated by expressing the number of patients with *Plasmodium* parasite detected within three months after the end of the 90-day relocation as incidence rates (recurrences per person-year).
Associations between *P. vivax* relapses in the no-primaquine arm and variables measured will be evaluated by univariate descriptive analysis that will include t-tests and nonparametric methods for continuous variables and chi-square test for comparison of categorical variables. All statistical tests will be performed with a two-sided significance level of $\alpha = 0.05$. Gene expression analyses will be conducted using the statistical framework implemented in the EdgeR package and corrected for multiple testing using false discovery rates.

## *11. Ethical considerations*

The study will be performed in accordance with ethical principles based on the Declaration of Helsinki and International Conference on Harmonization (ICH)/Good Clinical Practice (GCP) and all applicable regulatory requirements.

### 11.1. Informed Consent/Assent

The principles of research ethics as described in the current edition of the Declaration of Helsinki will be applied, and protocol-specified procedures will be initiated only after obtaining informed consent or assent. The written consent documents will embody the elements of informed consent as described in the current edition of the Declaration of Helsinki, will adhere to the ICH Harmonized Tripartite Guideline for Good Clinical Practice and 45CFR46 and 21CFR50, and will also comply with applicable Cambodian regulations.

Information about the study will be given to prospective participants, in a local language (Khmer), in both oral and written forms whenever possible. Independent witnesses will be used to attest that illiterate potential participants have understood the contents of the informed consent document.

All informed consent will be sought in the local language (Khmer). The written forms will be translated into Khmer and then translated back to English by a second translator to verify accuracy of information or certified by an official translator. These will be provided to participants and/or their caretakers in their respective local language. If a participant or parent/guardian is illiterate, the consent form will be read to them in their respective local language and a thumbprint will be accepted as a legally effective signature, which is accepted in the Cambodian context. In such situations, a witness signature will be sought. Consenting participants and/or their caretakers will be advised that they are free to decline any question or procedure and that they may terminate their participation at any time.

All participants will be asked to provide informed consent and/or assent.
The study investigators and staff will closely work with the CNM and other relevant local research staff at the study sites, providing training, and re-training if necessary, in Protection of Human Subjects, Good Clinical Practice, and responsible conducts of research to assure study information and informed consent procedures meet requirements of the community, sponsor and all relevant ethic review oversight committees. During the consent process, emphasis will be made to ensure that the participants understand that the study participation is entirely voluntary, refusal to participate will not have any negative consequences or repercussions and that they are free to withdraw from the study at any time. We consider informed consent to be a dynamic, ongoing process, with continuous availability of investigators to answer any questions that arise in the course of the study and to ensure that participants and their parents/guardians understand study procedures.

### 11.2. Confidentiality

All study related information will be stored securely at the study site. All participant information will be stored in locked filing cabinets in areas with access limited to study staff. All laboratory specimens, including stored specimens, reports, study data collection, process,

and administrative forms will be identified by a coded number. Names will not be used. Forms for all research tests will have the subject initials but not their name. This is to allow the running of routine blood tests through the usual hospital system.
Forms, lists, logbooks, appointment books, consent forms, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.
All local databases will be secured with password-protected access systems.
Participant's study information will not be released without the written permission of the participant, except as necessary for the independent monitoring. Representatives of the Cambodian MOH and regulatory authorities, the ECs can request to see the source documents.
For genetic information: potential harm or risk from the release of this information is minimal as study participants' information will be coded in password-protected databases and the genetic data physically separated from the encoded personal information. No genetic information will be released to study participants to minimize any potential social harm that might arise. Genomic data and associated metadata will be uploaded on secured databases (dbGaP) without any information enabling identification of patients.

## 11.3. Potential risks

-Fingerprick capillary blood collection: mild, temporary discomfort and a minimal risk of presyncope/syncope accompany the procedure; though rare subsequent bruising, bleeding, and infection at the puncture site also may occur. The volume of blood collected will not adversely affect participant health. Mitigation: proper use of antiseptic skin cleanser at the site of blood collection prior to skin puncture.

-Venous blood collection: mild, temporary discomfort is associated with the procedure. Certain individuals may experience presyncope or syncope. Additionally, venipuncture may result in subsequent bleeding, nerve damage, or the development of hematoma and/or infection at the site of the blood draw. Mitigation: utilization of study staff trained in phlebotomy and by proper use of antiseptic skin cleanser at the site of blood collection prior to venipuncture.

-Primaquine administration: The United States Food and Drug Administration (FDA) lists the following potential adverse effects associated with primaquine when used at dosages appropriate for radical cure of *P. vivax* malaria: nausea, vomiting, epigastric distress, abdominal cramps, cardiac arrhythmia, dizziness, rash, pruritus, leukopenia, methemoglobinemia in nicotinamide adenine dinucleotide (NADH) methemoglobin reductase deficient individuals, and hemolytic anemia in G6PD deficient individuals. Adverse effects are more commonly seen in association with overdosage of medication. Certain side effects, including acute hemolysis in G6PD deficient individuals, could subside following discontinuation of primaquine. Mitigation: only G6PD normal individuals will be enrolled and all individuals receiving primaquine for radical cure of *P. vivax* malaria will be closely monitored for the development of adverse effects, both by patient report of symptoms and routine assessment of changes in hemoglobin concentration over time.

-Artesunate treatment: artesunate is among the safest blood-stage antimalarial available. In some rare cases, at high doses (6mg/kg/day) it has been associated with self-limiting neutropenia [33]. Mitigation: a full blood count will be performed within 48h after initiation of the 7-day course of artesunate. The treatment will be interrupted if neutrophil counts are below 1,500/µl.

-Financial risk for being relocated 90 days: participants might not be able to continue their activities to comply with the relocation. Mitigation: we will provide financial compensation for their time. Accommodation, food, beverage will be provided and paid from the study budget.

-Potential risks to study personnel: Main risks to study personnel are associated with exposure to/handling of blood collected from study participants. Mitigation: standard operating procedure to enhance worker safety during the acquisition, transport, and laboratory manipulation of participant specimens will be followed. Adequate puncture-proof containers for the disposal of used clinical materials (e.g. needles, lancets, specimen tubes) will be provided. Universal precautions will be applied when handling all patient specimens.

### 11.4. Potential benefits:

All individuals infected with *Plasmodium* parasites (any species) will be treated for free regardless if they accept to participate or not in the study thereby providing them a direct benefit. Individuals enrolled in the study will benefit from early diagnostics and treatment of recurrent *P. vivax*.
All participants will be informed of the results of their G6PD status potentially providing future benefits to study participants. A booklet in Khmer language and verbal information will be provided to all G6PD deficient individuals to explain the risks associated with G6PD deficiency and drugs they should avoid.
More generally, the study will provide benefits to the community in Cambodia by determining the optimal primaquine regimen for *P. vivax* radical cure.
This study has the potential to identify biomarkers of hypnozoite carriage, which could assist in developing diagnostic tests to identify patients at risk of relapses.

### 11.5. Result Dissemination, Data Publications and Authorship

After the completion of all data analysis, preliminary data will be presented and discussed with the relevant Cambodian leadership, CNM, MoH, IPC. A final dissemination workshop taking place in Cambodia will be organized to present and discuss the results to a large audience involving all national and international organizations involved in malaria control and elimination in the country.

Findings and data will be used to indicate the most appropriate primaquine regimen for *P. vivax* radical cure.
Publication of the results in a peer-reviewed journal will be considered.

Data generated from this study are highly valuable for the whole public health community, particularly for those involved in malaria control and elimination from the Greater Mekong

Subregion, and prompt dissemination of data is critical. After proper review, discussion and approval from country leadership and partners, we will share the results and supporting data in several venues that are highly accessible to a large public audience (e.g. Institut Pasteur International Network, web-based media), as well as with international agencies such as WHO and USAID/CDC. Together with IPC support, the CNM team will provide a lead role in data analysis, interpretation of results, and dissemination of findings (in an appropriate peer-reviewed scientific journal as well as international public health assemblies, such as the annual meeting of American Society of Tropical Medicine and Hygiene).

Authorship of resulting publications and presentations will be discussed and decided collectively based on the multiple means of contribution to the success of the study.

### 11.6. Special considerations to mitigate any risk related to SARS-CoV-2

Because of the particular situation due to the global pandemic of SARS-CoV-2, we will ensure the following points to make sure none of the participants nor the study staff get infected during the study:

-All participants will be screened on inclusion for the detection of SARS-CoV-2 by qPCR from nasal swabs. Positive individuals will not be included in the study and will be managed as per Cambodian guidelines in SARS-CoV-2 dedicated referral hospitals.

-In case of respiratory infection symptoms during the follow-up, a nasal swab will be performed for SARS-CoV-2 qPCR diagnostic. In case of positive qPCR, the participant will be discharged from the study and referred to the referral hospital to be managed following the guidelines of the Cambodian Ministry of Health

-Facial masks will be worn at all time by participants and study staff. Hand sanitizers and soap will be made available at all time.

## *12. References*


1. Price, R.N., et al., *Vivax Malaria: Neglected and Not Benign.* The American Journal of Tropical Medicine and Hygiene, 2007. **77**(6 Suppl): p. 79-87.
2. Mendis, K., et al., *The neglected burden of Plasmodium vivax malaria.* The American journal of tropical medicine and hygiene, 2001. **64**(1 suppl): p. 97-106.
3. Battle, K.E., et al., *Mapping the global endemicity and clinical burden of Plasmodium vivax, 2000–17: a spatial and temporal modelling study.* The Lancet, 2019.
4. Baird, J.K., *Evidence and Implications of Mortality Associated with Acute Plasmodium vivax Malaria.* Clinical Microbiology Reviews, 2013. **26**(1): p. 36-57.
5. Genton, B., et al., *Plasmodium vivax and Mixed Infections Are Associated with Severe Malaria in Children: A Prospective Cohort Study from Papua New Guinea.* PLoS Med, 2008. **5**(6): p. e127.
6. Douglas, N., et al., *Mortality attributable to Plasmodium vivax malaria: a clinical audit from Papua, Indonesia.* BMC Medicine, 2014. **12**(1): p. 217.
7. Popovici, J. and D. Menard, *Challenges in Antimalarial Drug Treatment for Vivax Malaria Control.* Trends Mol Med, 2015. **21**(12): p. 776-88.
8. Siv, S., et al., *Plasmodium vivax Malaria in Cambodia.* The American Journal of Tropical Medicine and Hygiene, 2016. **95**(6_Suppl): p. 97-107.
9. Thomson-Luque, R., et al., *From marginal to essential: the golden thread between nutrient sensing, medium composition and Plasmodium vivax maturation in in vitro culture.* Malaria journal, 2019. **18**(1): p. 344-344.
10. Bermúdez, M., et al., *Plasmodium vivax in vitro continuous culture: the spoke in the wheel.* Malaria journal, 2018. **17**(1): p. 301-301.
11. Noulin, F., et al., *1912–2012: a century of research on Plasmodium vivax in vitro culture.* Trends in Parasitology, 2013. **29**(6): p. 286-294.
12. Mueller, I., et al., *Key gaps in the knowledge of Plasmodium vivax, a neglected human malaria parasite.* The Lancet Infectious Diseases, 2009. **9**(9): p. 555-566.
13. Robinson, L.J., et al., *Strategies for Understanding and Reducing the Plasmodium vivax and Plasmodium ovale Hypnozoite Reservoir in Papua New Guinean Children: A Randomised Placebo-Controlled Trial and Mathematical Model.* PLoS Medicine, 2015. **12**(10): p. e1001891.
14. White, N.J., *Determinants of relapse periodicity in Plasmodium vivax malaria.* Malaria Journal, 2011. **10**(1): p. 297.
15. Chu, C.S., et al., *Comparison of the Cumulative Efficacy and Safety of Chloroquine, Artesunate, and Chloroquine-Primaquine in Plasmodium vivax Malaria.* Clinical Infectious Diseases, 2018. **67**(10): p. 1543-1549.
16. Popovici, J., et al., *Genomic Analyses Reveal the Common Occurrence and Complexity of Plasmodium vivax Relapses in Cambodia.* mBio, 2018. **9**(1).
17. Popovici, J., et al., *Recrudescence, reinfection or relapse? A more rigorous framework to assess chloroquine efficacy for vivax malaria.* The Journal of Infectious Diseases, 2018: p. jiy484-jiy484.
18. Wells, T.N.C., J.N. Burrows, and J.K. Baird, *Targeting the hypnozoite reservoir of Plasmodium vivax: the hidden obstacle to malaria elimination.* Trends in Parasitology, 2010. **26**(3): p. 145-151.
19. Baird, J.K., K.E. Battle, and R.E. Howes, *Primaquine ineligibility in anti-relapse therapy of Plasmodium vivax malaria: the problem of G6PD deficiency and cytochrome P-450 2D6 polymorphisms.* Malaria Journal, 2018. **17**: p. 42.
20. Baird, K., *Origins and implications of neglect of G6PD deficiency and primaquine toxicity in Plasmodium vivax malaria.* Pathogens and Global Health, 2015. **109**(3): p. 93-106.
21. WHO, *Guidelines for the treatment of malaria – 3rd edition.* 2015.

22. Baird, J., et al., *Association of impaired cytochrome p450 2d6 activity genotype and phenotype with therapeutic efficacy of primaquine treatment for latent plasmodium vivax malaria.* JAMA Network Open, 2018. **1**(4): p. e181449.
23. Bennett, J.W., et al., *Primaquine Failure and Cytochrome P-450 2D6 in Plasmodium vivax Malaria.* New England Journal of Medicine, 2013. **369**(14): p. 1381-1382.
24. Recht, J., E.A. Ashley, and N.J. White, *Use of primaquine and glucose-6-phosphate dehydrogenase deficiency testing: Divergent policies and practices in malaria endemic countries.* PLoS neglected tropical diseases, 2018. **12**(4): p. e0006230-e0006230.
25. Commons, R.J., et al., *The effect of chloroquine dose and primaquine on Plasmodium vivax recurrence: a WorldWide Antimalarial Resistance Network systematic review and individual patient pooled meta-analysis.* The Lancet Infectious Diseases, 2018.
26. Valdes, A., et al., *Primaquine 30 mg/day versus 15 mg/day during 14 days for the prevention of Plasmodium vivax relapses in adults in French Guiana: a historical comparison.* Malaria Journal, 2018. **17**(1): p. 237.
27. Marcsisin, S.R., G. Reichard, and B.S. Pybus, *Primaquine pharmacology in the context of CYP 2D6 pharmacogenomics: Current state of the art.* Pharmacology & Therapeutics, 2016. **161**: p. 1-10.
28. Lover, A.A., et al., *Malaria Elimination: Time to Target All Species.* The American Journal of Tropical Medicine and Hygiene, 2018. **99**(1): p. 17-23.
29. Taylor, W.R.J., et al., *Short-course primaquine for the radical cure of Plasmodium vivax malaria: a multicentre, randomised, placebo-controlled non-inferiority trial.* The Lancet, 2019. **394**(10202): p. 929-938.
30. Fryauff, D., et al., *Randomised placebo-controlled trial of primaquine for prophylaxis of falciparum and vivax malaria.* The Lancet, 1995. **346**(8984): p. 1190-1193.
31. Collins, W.E. and G.M. Jeffery, *Primaquine Resistance in Plasmodium vivax.* The American Journal of Tropical Medicine and Hygiene, 1996. **55**(3): p. 243-249.
32. Llanos-Cuentas, A., et al., *Tafenoquine versus Primaquine to Prevent Relapse of Plasmodium vivax Malaria.* New England Journal of Medicine, 2019. **380**(3): p. 229-241.
33. Bethell, D., et al., *Dose-Dependent Risk of Neutropenia after 7-Day Courses of Artesunate Monotherapy in Cambodian Patients with Acute Plasmodium falciparum Malaria.* Clinical Infectious Diseases, 2010. **51**(12): p. e105-e114.

## *13. Curriculum Vitae of applicants*

**Principal Investigator**


Family Name: LEK
First Names: DYSOLEY
Date of Birth: JAN05-1972
Sex: Male
Marital Status: Married
Nationality: Cambodian
Official address: National Center for Parasitology Entomology and Malaria Programme (CNM). Corner St.92, TrapaingSvay Village, Sangkat PhnomPenh Thmey, Khan SenSok, PhnomPenh, Cambodia
Emailaddress: soleycnm@gmail.com
HP: (855-12)523-150
Presently position: Deputy Director of CNM


**1-RECORD OF EDUCATIONAL QUALIFICATIONS AND PROFESSIONAL TRAININGS**

**2- SPECIAL EDUCATION RECEIVED**

- 1998-1998: National Research Design and Methodology. Phnom Penh, Kingdom of Cambodia.
- 1999-1999: Second Regional training on Geographic Information System(GIS), Bangkok, Thailand
- 1999-1999: Food Safety and Food Control training.Bangkok,Thailand.
- 2000-2000: The Specialized Regional Field-Based Training Programme in Epidemiology and Control of Tropical Disease. Bangkok, Thailand
- 2001-2001: The Fifth Advanced Asian Course in Tropical Epidemiology. Kuala Lumpur, Malaysia.
- 2010-2010: Regional GMS Monitoring and Evaluation Training, Vientiane, Laos PRD.
- 2013-2013: The Science of Malaria Eradication in Barcelona, Spain

| **Name of degree/diploma** | **Institution (name,city, country)** | **Completion date (month and year)** |
|---|---|---|
| PhD | Tokyo Women's Medical University, Tokyo, Japan | 20March,2007 |
| Master | Faculty of Tropical Medicine Mahidol University, Bangkok, Thailand | 30June,2001 |
| Medical Doctor | University of Health Science, Phnom Penh, Cambodia | 15October,1996 |

**3- EMPLOYMENT HISTORY**

| **Year(s)** | **Institution/Organisation, City,** | **PositionHeld** |
|---|---|---|
| 2013 | CNM, Phnom Penh,Cambodia | Deputy Director |
| 2010 | CNM, Phnom Penh,Cambodia | Deputy chief of Technical Bureau |
| 2008 | CNM, Phnom Penh,Cambodia | Assistant Manager of National Dengue Control Programme |
| 1997 | CNM, Phnom Penh,Cambodia | Technical Officer |

## 4- LIST OF AWARDS/ RESEARCH & PUBLICATIONS

| D | Name of Honours/Awards/Scholarships/Fellowships received |
|---|---|
| 2004 | Scholarship from JICA to studyin Japan |
| 1999 | Scholarship from World Bank to study in Thailand |

- Significant efficacy of single low dose primaquine compared to stand alone artemisinin combination therapy in reducing gametocyte carriage in Cambodian patients with uncomplicated multidrug resistant Plasmodium falciparum malaria. Co-Author: Lek Dysoley, Antimicrob. Agents Chemother. doi:10.1128/AAC.02108-19, 16 March 2020
- Impact of the first-line treatment shift from dihydroartemisinin/piperaquine to artesunate/mefloquine on Plasmodium vivax drug susceptibility in Cambodia, Co-Author: Dysoley, Antimicrob Chemother, 20 February 2020.
- Evaluation of the CareStart™ glucose-6-phosphate dehydrogenase (G6PD) rapid diagnostic test in the field settings and assessment of perceived risk from primaquine at the community level in Cambodia. Co-Author: Lek Dysoley, PLOS/ONE January 10, 2020
- Recrudescence, reinfection or relapse? A more rigorous framework to assess chloroquine efficacy for vivax malaria. Co-Author: Lek Dysoley, J Infect Dis. 2019 Jan 15; 219(2): 315–322. 2018 Aug 9.
- Therapeutic and Transmission-Blocking Efficacy of Dihydroartemisinin/Piperaquine and Chloroquine against Plasmodium vivax Malaria, Cambodia. Co-Author: Lek Dysoley, Emerg Infect Dis. 2018 Aug; 24(8): 1516–1519.
- Contribution to Malaria Transmission of Symptomatic and Asymptomatic Parasite Carriers in Cambodia. Co-Author: Lek Dysoley, *The Journal of Infectious Diseases*, Volume 217, Issue 10, 15 May 2018, Pages 1561–1568,
- Genomic Analyses Reveal the Common Occurrence and Complexity of Plasmodium vivax Relapses in Cambodia. Co-Author: Lek Dysoley, mBio. 2018 Jan-Feb; 9(1): e01888-17.
- Barriers to routine G6PD testing prior to treatment with primaquine. Co-Author: Lek Dysoley, *Malar J (2017) 16:329* Published**:** 10 August 2017.
- Policy and Practice: Implementation of G6PD testing and primaquine for *P. vivax* radical cure: operational perspectives from Thailand and Cambodia. Co-Author: Lek Dysoley, WHO South-East Asia Journal of Public Health | September 2017 | 6(2)
- Challenges for achieving safe and effective radical cure of Plasmodium vivax a round table discussion of the APMEN Vivax Working Group. Co-Author: Lek Dysoley, Malar J 2017 Apr 5;16(1):141. Epub 2017 Apr 5.
- Plasmodium vivax Malaria in Cambodia. Co-Author: Lek Dysoley, Am J Trop Med Hyg 2016 Dec 5;95(6 Suppl):97-107. Epub 2016 Oct 5.

## Co-Investigators

| NAME & SURNAME | CONTACT | POSITION TITLE | BIRTHDATE & PLACE |
|---|---|---|---|
| Witkowski Benoit | +33607513062<br>bwitkowski@pasteur-kh.org | Head of Unit - Malaria Molecular Epidemiology Unit<br>Institut Pasteur du Cambodge | 06/08/1980,<br>MONTAUBAN (82)<br>FRANCE |

**EDUCATION/TRAINING**

| INSTITUTION & location | DEGREE - HDR | YEAR(s) | FIELD OF STUDIES |
|---|---|---|---|
| Lycée Institut Familial, Montauban | Baccalauréat S | 1997-1998 | Scientific-chemistry |
| UNIVERSITE DE TOULOUSE III | DEUG | 2000-2002 | biology |
| UNIVERSITE DE TOULOUSE III | License (BSc) | 2002-2003 | cell biology and physiology |
| UNIVERSITE DE TOULOUSE III | Maitrise | 2003-2004 | cell biology and microbiology |
| UNIVERSITE DE RENNES I | DEA (MSc) | 2004-2005 | bacteriology |
| CHU de MONTREAL | internship | 2005-2006 | virology-HIV |
| UNIVERSITE DE TOULOUSE III | PhD | 2006-2010 | parasitology-malaria |
| INSTITUT PASTEUR DU CAMBODGE | Post Doc | 2010-2013 | parasitology-malaria |

**POSITIONS & HONORS.**

**2006-2010:** LCC-CNRS/Service de Parasitologie-Mycologie, CHU de Toulouse, PhD candidate under co-supervision of F. Benoit-Vical and A. Berry

**2010-2013:** Institut Pasteur du Cambodge, Post doctoral fellow

**2013-2014**: Institut Pasteur du Cambodge, Research Assistant, Scientific coordinator of WWARN in vitro group

**2014-2017 :** Institut Pasteur du Cambodge, Researcher (Chargé de Recherche Institut Pasteur, permanent position). Deputy Head

**2017-now:** Institut Pasteur du Cambodge, Researcher (Chargé de Recherche Institut Pasteur, permanent position). Head of Unit

### Selected Publications

1. Berry A, Deymier C, Sertorio M, Witkowski B, Benoit-Vical F. Pfs 16 pivotal role in Plasmodium falciparum gametocytogenesis: a potential antiplasmodial drug target. Exp Parasitol 2009;121(2):189-92.

2. Soh PN, Witkowski B, Olagnier D, et al. In vitro and in vivo properties of ellagic acid in malaria treatment. Antimicrob Agents Chemother 2009;53(3):1100-6.

3. Witkowski B, Berry A, Benoit-Vical F. Resistance to antimalarial compounds: methods and applications. Drug Resist Updat 2009;12(1-2):42-50.

4. Iriart X, Witkowski B, Courtais C, et al. Cellular and cytokine changes in the alveolar environment among immunocompromised patients during Pneumocystis jirovecii infection. Med Mycol 2010;48(8):1075-87.
5. Witkowski B, Iriart X, Soh PN, et al. pfmdr1 amplification associated with clinical resistance to mefloquine in West Africa: implications for efficacy of artemisinin combination therapies. J Clin Microbiol 2010;48(10):3797

6. Witkowski B, Lelievre J, Barragan MJ, et al. Increased tolerance to artemisinin in Plasmodium falciparum is mediated by a quiescence mechanism. Antimicrob Agents Chemother 2010;54(5):1872-7.

7. Witkowski B, Nicolau ML, Soh PN, et al. Plasmodium falciparum isolates with increased pfmdr1 copy number circulate in West Africa. Antimicrob Agents Chemother 2010;54(7):3049-51.

8. Berry A, Iriart X, Wilhelm N, et al. Imported Plasmodium knowlesi malaria in a French tourist returning from Thailand. Am J Trop Med Hyg 2011;84(4):535-8.

9. Iriart X, Witkowski B, Cassaing S, et al. Alveolar and blood T lymphocyte profiles in Pneumocystis jirovecii-positive patients: effects of HIV status. J Infect Dis 2011;204(4):544-53.

10. Chan ER, Menard D, David PH, et al. Whole genome sequencing of field isolates provides robust characterization of genetic diversity in Plasmodium vivax. PLoS Negl Trop Dis 2012;6(9):e1811.

11. Hoyer S, Nguon S, Kim S, et al. Focused Screening and Treatment (FSAT): a PCR-based strategy to detect malaria parasite carriers and contain drug resistant P. falciparum, Pailin, Cambodia. PLoS One 2012;7(10):e45797.

12. Njomnang Soh P, Witkowski B, Gales A, et al. Implication of glutathione in the in vitro antiplasmodial mechanism of action of ellagic acid. PLoS One 2012;7(9):e45906.

13. Witkowski B, Lelievre J, Nicolau-Travers ML, et al. Evidence for the contribution of the hemozoin synthesis pathway of the murine Plasmodium yoelii to the resistance to artemisinin-related drugs. PLoS One 2012;7(3):e32620.

14. Andriantsoanirina V, Khim N, Ratsimbasoa A, et al. Plasmodium falciparum Na+/H+ exchanger (pfnhe-1) genetic polymorphism in Indian Ocean malaria-endemic areas. Am J Trop Med Hyg 2013;88(1):37-42.

15. Bouillon A, Giganti D, Benedet C, et al. In Silico screening on the three-dimensional model of the Plasmodium vivax SUB1 protease leads to the validation of a novel anti-parasite compound. J Biol Chem 2013;288(25):18561-73.

16. Khim N, Benedet C, Kim S, et al. G6PD deficiency in Plasmodium falciparum and Plasmodium vivax malaria-infected Cambodian patients. Malar J 2013;12(1):171.

17. Menard D, Chan ER, Benedet C, et al. Whole Genome Sequencing of Field Isolates Reveals a Common Duplication of the Duffy Binding Protein Gene in Malagasy Plasmodium vivax Strains. PLoS Negl Trop Dis 2013;7(11):e2489.

18. Robert A, Claparols C, Witkowski B, Benoit-Vical F. Correlation between Plasmodium yoelii nigeriensis susceptibility to artemisinin and alkylation of heme by the drug. Antimicrob Agents Chemother 2013;57(8):3998-4000.

19. Witkowski B, Amaratunga C, Khim N, et al. Novel phenotypic assays for the detection of artemisinin-resistant Plasmodium falciparum malaria in Cambodia: in-vitro and ex-vivo drug-response studies. Lancet Infect Dis 2013;13(12):1043-9.

20. Witkowski B, Khim N, Chim P, et al. Reduced artemisinin susceptibility of Plasmodium falciparum ring stages in western Cambodia. Antimicrob Agents Chemother 2013;57(2):914-23.

21. Amaratunga C, Witkowski B, Dek D, et al. Plasmodium falciparum founder populations in western Cambodia have reduced artemisinin sensitivity in vitro. Antimicrob Agents Chemother 2014;58(8):4935-7.

22. Amaratunga C, Witkowski B, Khim N, Menard D, Fairhurst RM. Artemisinin resistance in Plasmodium falciparum. Lancet Infect Dis 2014;14(6):449-50.

23. Ariey F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature 2014;505(7481):50-5.

24. Duru V, Khim N, Leang R, et al. Plasmodium falciparum dihydroartemisinin-piperaquine failures in Cambodia are associated with mutant K13 parasites presenting high survival rates in novel piperaquine in vitro assays: retrospective and prospective investigations. BMC Med 2015;13:305.

25. Leang R, Taylor WR, Bouth DM, et al. Evidence of Plasmodium falciparum Malaria Multidrug Resistance to Artemisinin and Piperaquine in Western Cambodia: Dihydroartemisinin-Piperaquine Open-Label Multicenter Clinical Assessment. Antimicrob Agents Chemother 2015;59(8):4719-26.

26. Leba LJ, Musset L, Pelleau S, et al. Use of Plasmodium falciparum culture-adapted field isolates for in vitro exflagellation-blocking assay. Malar J 2015;14:234.

27. Malmquist NA, Sundriyal S, Caron J, et al. Histone methyltransferase inhibitors are orally bioavailable, fast-acting molecules with activity against different species causing malaria in humans. Antimicrob Agents Chemother 2015;59(2):950-9.

28. Menard S, Ben Haddou T, Ramadani AP, et al. Induction of Multidrug Tolerance in Plasmodium falciparum by Extended Artemisinin Pressure. Emerg Infect Dis 2015;21(10):1733-41.

29. Straimer J, Gnadig NF, Witkowski B, et al. Drug resistance. K13-propeller mutations confer artemisinin resistance in Plasmodium falciparum clinical isolates. Science 2015;347(6220):428-31.

30. Beghain J, Langlois AC, Legrand E, et al. Plasmodium copy number variation scan: gene copy numbers evaluation in haploid genomes. Malar J 2016;15(1):206.

31. Boullé M, Witkowski B, Menard D, Nosten F. Phenotype profiles of K13 mutant alleles. Emerg Infect Dis 2016

32. Witkowski B, Khim N, Kim S, Domergue A, Duru V, Menard D. [Multiple and successive treatment failures in a patient infected by Plasmodium falciparum in Cambodia and treated by dihydroartemisinin-piperaquine]. Bull Soc Pathol Exot 2016;109(2):87-90.

33. Menard D, Khim N, Adegnika A, et al. A Worldwide map of Plasmodium falciparum K13-Propeller Polymorphism. New England Journal of Medicine 2016

34. Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, Chy S, Kim S, Ke S, Kloeung N, Eam R, Khean C, Ken M, Loch K, Bouillon A, Domergue A, Ma L, Bouchier C, Leang R, Huy R, Nuel G, Barale JC, Legrand E, Ringwald P, Fidock DA, Mercereau-Puijalon O, Ariey F, Ménard D. A surrogate marker of piperaquine-resistant Plasmodium falciparum malaria: a phenotype-genotype association study. Lancet Infect Dis. 2017 Feb;17(2):174-183.

**Awards**

Prix Robert Deschiens 2014 from ‘’Société de Pathologie Exotique’’

**Patents**

A molecular marker for detecting Plasmodium falciparum artemisinin resistance (US61/904651 and US62/062439)

| NAME & SURNAME | CONTACT | POSITION TITLE | BIRTHDATE & PLACE |
|---|---|---|---|
| POPOVICI Jean | Tel +855 89 851 806<br>jpopovici@pasteur-kh.org | Contractual Researcher - Malaria Molecular Epidemiology Unit<br>Institut Pasteur du Cambodge | November 8, 1980<br>Rillieux-la-Pape (France) |

**EDUCATION/TRAINING**

| INSTITUTION & location | DEGREE - HDR | YEAR(s) | FIELD OF STUDIES |
|---|---|---|---|
| LycéeDescartes - Antony | BaccalauréatS | 1998 | General |
| Université Lyon 1 | DEUG | 2001 | General |
| Université Lyon 1 | BSc_Maitrise Sciences et Techniques « Chimie Biologie Vegetale» | 2003 | Analytical Chemistry and Biology |
| Université Lyon 1 | MSc_Diplome d'Etudes Approfondies « Ecologie Microbienne » | 2004 | Host-microbe interactions |
| Université Lyon 1 | PhD_Diplome de doctorat «Ecologie Microbienne » | 2008 | Host-microbe interactions |
| Université Lyon 1 | HDR | 2017 | Host-microbe interactions |

**POSITIONS & HONORS.**

**2009-2012**: Postdoc researcher. University of Queensland, Brisbane and Monash University, Melbourne, Australia. Scott O'Neill lab. «Implementation of lab work into field trials of the innovative approach using Wolbachia bacterium to control dengue transmission by Aedes aegypti and study of its molecular mechanisms»

**2013**: Postdoc researcher. Institut Pasteur de Guyane, /UMR CNRS ECoFoG, Cayenne Guyane francaise. Christophe Duplais lab. «Setting in vitro exflagellation-blocking bioassays for transmission-blocking antimalarial discovery from Amazonian natural extracts»

**Since 2014**: Contractual researcher. Institut Pasteur in Cambodia. Malaria Molecular Epidemiology Unit, Phnom Penh, Cambodia.

### Other Experiences and Professional Memberships

2014. Supervision of *P. vivax* field studies in Rattanakiri, Cambodia.
2013. Visiting researcher "Plasmodium falciparum gametocyte culture and in vitro transmission-blocking bioassay" Hosts: Michael Delves and Robert Sinden. Imperial College, London, UK.
Reviewer for PLoS ONE, BioMed Research International. Molecules, Malaria Journal, Infection Genetics and Evolution, Int j parasitol drugs & drug resistance

### Selected Publications


1- Amplification of Duffy Binding Protein-encoding gene allows Plasmodium vivax to evade host anti-DBP humoral immunity. 2020. **Nature Communications**. **J Popovici**, C Roesch, LL Carias, N Khim, S Kim, A Vantaux, I Mueller, CE Chitnis, CL King, B Witkowski.

2- The enigmatic mechanisms by which Plasmodium vivax infects Duffy-negative individuals. 2020. **PLoS Pathogens. J Popovici**, C Roesch, V Rougeron.

3- Structural basis for neutralization of P. vivax by naturally-acquired human antibodies that target DBP. 2019. **Nature Microbiology**. D Urusova, L Carias, Y Huang, V Nicholete, **J Popovici**, C Roesch, N D. Salinas, B Witkowski, M Ferreira, J H. Adams, M L. Gross, C L. King, N H. Tolia.

4- Identification and Characterization of Functional Human Monoclonal Antibodies to Plasmodium vivax Duffy Binding Protein. 2019. **Journal of Immunology**. L Carias, S Dechavanne, V Nicolete, S Sreng, S Suon, C Amaratunga, R Fairhurst, C Dechavanne, S Barnes, B Witkowski, **J Popovici**, C Roesch, E Chen, M Ferreira, N Tolia, J Adams, C King.

5- Plasmodium vivax transcriptomes reveal stage-specific chloroquine response and differential regulation of male and female gametocytes. 2019. **Nature Communications**. A Kim, **J Popovici**, D Menard, D Serre.

6- Targeting a Reticulocyte Binding Protein and Duffy Binding Protein to Inhibit Reticulocyte Invasion by Plasmodium vivax. 2018. **Scientific Reports**. S Gupta, S Singh, **J Popovici**, C Roesch, A Rushdi Shakri, M Guilotte-Blisnick, C Huon, D Menard, CE Chitnis.

7- Genetic diversity in two Plasmodium vivax protein ligands for reticulocyte invasion. 2018. **PLoS Neglected Tropical Diseases.** C Roesch, **J Popovici**, S Bin, V Run, S Kim, S Ramboarina, E Rakotomalala, R Rakotoarison, T Rasoloharimanana, Z Andrianamanantena, A Kumar, M Guillotte-Blisnick, C Huon, D Serre, C Chitnis, I Vigan-Womas, D Menard

8- Recrudescence, reinfection or relapse? A more rigorous framework to assess chloroquine efficacy for vivax malaria. 2018. **The Journal of Infectious Diseases. J Popovici**, L Friedrich, S Kim, S Bin, V Run, D Lek, H K Hor, L L Soon-U, M Cannon, D Serre, D Menard.

9- Therapeutic and transmission-blocking efficacy of dihydroartemisinin-piperaquine and chloroquine in Plasmodium vivax malaria, Cambodia. 2018. **Emerging Infectious Diseases. J Popovici**, A Vantaux, L Primault, R Samreth, E Piv, S Bin, S Kim, D Lek, D Serre, D Menard.

10- Genomic Analyses Reveal the Common Occurrence and Complexity of Plasmodium vivax Relapses in Cambodia. 2018**. mBio. J Popovici**, L Friedrich, S Kim, S Bin, V Run, D Lek, M V.Cannon, D Menard, D Serre.

11- Complexity of infection and genetic diversity in Cambodian Plasmodium vivax. 2016. **PLoS Neglected Tropical Diseases**. Lindsey Friedrich, **Jean Popovici**, Saorin Kim, Dysoley Lek, Peter Zimmerman, Didier Menard, and David Serre

12- National Malaria Prevalence in Cambodia: Microscopy versus Polymerase Chain Reaction estimates. 2016. **American Journal of Tropical Medicine & Hygiene.** Dysoley Lek, **Jean Popovici**, Frederic Ariey, Seshu Babu Vinjamuri, Sylvia Meek, Jan Bruce, Walter RJ Taylor, Duong Socheat, Didier Menard,William O. Rogers

13- Challenges in Antimalarial Drug Treatment for Vivax Malaria Control. 2015. **Trends in Molecular Medicine. J Popovici,** D Menard

14- Use of Plasmodium falciparum culture-adapted field isolates for in vitro exflagellation-blocking assay. 2015. **Malaria Journal.** LJ Leba, L Musset, S Pelleau, Y Estevez, C Birer, S Briolant, B Witkowski, D Menard, M Delves, E Legrand, C Duplais, **J Popovici**

15- Reduced polymorphism in P. vivax kelch-propeller domain in Cambodian isolates. 2014. **Antimicrobial Agents and Chemotherapy**. J **Popovici**, S Kao, L Eal, S Bin, S Kim, D Menard.

16- Effects of Mefloquine Use on Plasmodium vivax Multidrug Resistance. 2014. **Emerging Infectious Disease**. Kim, Andrianaranjaka, **Popovici**, Kim, Ratsimbasoa, Benedet, Barnadas, Durand, Thellier, Legrand, Musset, Menegon, Severini, Nour, Tichit, Bouchier, Mercereau-Puijalon, Ménard

| NAME & SURNAME | CONTACT | POSITION TITLE | BIRTHDATE & PLACE |
|---|---|---|---|
| SERRE David | Tel +1 (410) 706-798<br>dserre@som.umaryland.edu | Associate Professor – Institute for Genome Sciences & Department of Microbiology and Immunology, University of Maryland School of Medicine | September 22, 1976<br>Echirolles (France) |

**EDUCATION/TRAINING**

| INSTITUTION & location | DEGREE - HDR | YEAR(s) | FIELD OF STUDIES |
|---|---|---|---|
| Lycée E. Mounier – Grenoble | Baccalauréat S | 1994 | General |
| Ecole Nationale Superieure de Chimie de Montpellier | Engineering degree | 2000 | Chemistry |
| Leipzig University | PhD | 2004 | Biology |

**POSITIONS & HONORS.**

**2004-2007**: Postdoc researcher. McGill University and Genome Quebec Innovation Centre, Montreal, Canada

**2007-2014**: Assistant Professor, Cleveland Clinic Lerner College of Medicine of CWRU, Cleveland, USA

**2015-2016**: Associate Professor, Cleveland Clinic Lerner College of Medicine of CWRU, Cleveland, USA

**2016-now**: Associate Professor, Dept. of Microbiology and Immunology, University of Maryland School of Medicine

**Selected Publications**

1-Kim A, Popovici J, Menard D, **Serre D** (2019) Plasmodium vivax transcriptomes reveal stage-specific chloroquine response and differential regulation of male and female gametocytes. **Nature Communications**. 10: 371.

2-Roesch C, Popovici J, Bin S, Run V, Kim S, Ramboarina S, Rakotomalala E, Rakotoarison RL, Rasoloharimanana T, Andriamanantena Z, Kumar A, Guillotte-Blisnick M, Huon C, **Serre D**, Chitnis CE, Vigan-Womas I, Menard D (2018) Genetic diversity in two Plasmodium vivax protein ligands for reticulocyte invasion. **PLoS Negl Trop Dis.** 12(10):e0006555.

3-Popovici J, Pierce-Friedrich L, Kim S, Bin S, Run V, Lek D, Hor Daryl HK, Lee Soon-U L, Cannon MV, **Serre D**, Menard D (2018) Recrudescence, reinfection or relapse? A more rigorous framework to assess chloroquine efficacy for vivax malaria. **J Infect Dis.** 219(7):315-322.

4-Popovici J, Vantaux A, Primault L, Samreth R, Piv EP, Bin S, Kim S, Lek D, **Serre D**, Menard D (2018) Therapeutic and Transmission-Blocking Efficacy of Dihydroartemisinin-Piperaquine and Chloroquine against Plasmodium vivax Malaria, Cambodia. **Emerg Infect Dis.** 24(8):1516-1519.

5-Popovici J, Friedrich LR, Kim S, Bin S, Run V, Lek D, Cannon MV, Menard D, **Serre D** (2018) Genomic Analyses Reveal the Common Occurrence and Complexity of Plasmodium vivax Relapses in Cambodia. **MBio.** 9(1): e01888-17.

6-Kim A, Popovici J, Vantaux A, Samreth R, Bin S, Kim S, Roesch C, Liang L, Davies H, Felgner P, Herrera S, Arévalo-Herrera M, Ménard D, **Serre D** (2017) Characterization of P. vivax blood stage transcriptomes from field isolates reveals similarities among infections and complex gene isoforms. **Sci Rep.** 7(1):7761.

7-Auburn S, **Serre D**, Pearson RD, Amato R, Sriprawat K, To S, Handayuni I, Suwanarusk R, Russell B, Drury E, Stalker J, Miotto O, Kwiatkowski DP, Nosten F, Price RN (2016) Genomic Analysis Reveals a Common Breakpoint in Amplifications of the Plasmodium vivax Multidrug Resistance 1 Locus in Thailand. **J Infect Dis.** 214(8):1235-42.

8-Friedrich LR, Popovici J, Kim S, Dysoley L, Zimmerman PA, Menard D and **Serre D** (2016) Complexity of infection and genetic diversity in Cambodian Plasmodium vivax. **PLoS Negl Trop Dis.** 10(3):e0004526.

9-Baniecki ML, Faust AL, Schaffner SF, Park DJ, Galinsky K, Daniels RF, Hamilton E, Ferreira MU, Karunaweera ND, **Serre D**, Zimmerman PA, Sá JM, Wellems TE, Musset L, Legrand E, Melnikov A, Neafsey DE, Volkman SK, Wirth DF, Sabeti PC (2015) Development of a Single Nucleotide Polymorphism Barcode to Genotype Plasmodium vivax Infections. **PLoS Negl Trop Dis.** 9(3):e0003539.

10-Chan ER, Barnwell J, Zimmerman PA and **Serre D** (2015) Comparative analysis of field-isolates and monkey-adapted Plasmodium vivax genomes. **PLoS Negl Trop Dis.** 9(3):e0003566.

11-Nair S, Nkhoma SC, **Serre D**, Zimmerman PA, Gorena K, Daniel BJ, Nosten F, Anderson TJ and Cheeseman IH (2014) Single-cell genomics for dissection of complex malaria infections. **Genome Res.** 24(6):1028-38.

12-Hester J, Chan R, Menard D, Mercereau-Puijalon O, Barnwell J, Zimmerman PA and **Serre D** (2013) De Novo Assembly of a field isolate Genome Reveals Novel Plasmodium vivax Erythrocyte Invasion Genes. **PLoS Negl Trop Dis.** 7:e2569.

13-Menard D, Chan R, Benedet C, Ratsimbasoa A, Kim S, Chim P, Do C, Witkowski B, Durand R, Thellier M, Severini C, Legrand E, Musset L, Nour B, Mercereau-Puijalon O, **Serre D** and Zimmerman PA (2013) Whole Genome Sequencing of Field Isolates Reveals a Common Duplication of the Duffy Binding Protein Gene in Malagasy Plasmodium vivax Strains. **PLoS Negl Trop Dis**. 7:e2489.

14-Chan ER, Menard D, David PH, Ratsimbasoa A, Kim S, Chim P, Do C, Witkowski B, Mercereau-Puijalon O, Zimmerman PA and **Serre D** (2012) Whole genome sequencing of field isolates provides robust characterization of genetic diversity in Plasmodium vivax. **PLoS Negl Trop Dis.** 6(9): e1811.