# A Liver Model for Chemoprotection Against Malaria

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## ***Problem Overview:***

To develop a mechanistic PKPD model to evaluate the chemo-protective capabilities of an anti-malarial drug candidate.

## ***Background:***

Malaria is an infectious disease and a global health problem as it puts about half of the world's population at risk. It mostly affects pregnant women and children under 5 in developing countries. According to the World Health Organization's World Malaria Report 2016 [1], an estimated 212 million cases of malaria and 429,000 deaths were reported in 2015 worldwide, out of which 90% were in Africa and 70 % were children. The great effort directed at reducing the global burden of malaria between 2000 and 2015 resulted in a 41% fall in the incidence rate and 60% fall in malaria mortality. However, further progress in the development of new malaria treatment and chemoprotection is required in the drive towards elimination and eradication.

A first attempt to eradicate malaria was conducted in the late 1940s, which led to the National and Global Malaria Eradication Program. By 1955, house spraying with insecticides and antimalarial drug treatment achieved successful eradication in many nations with temperate climates and seasonal malaria transmission including the United States and Europe, but did not achieve similar success in sub-Saharan Africa. In 1969, due to technical challenges and failure in defining an executable strategy, the eradication program was abandoned. It was only in 1998, under the WHO flag, that the Roll Back Malaria Partnership was launched to coordinate a global response to the disease. This partnership was followed by the Abuja Declaration in 2000 [2], where the objectives were reemphasized and the global strategy defined for the following decade.

Before suggesting any strategy to eradicate malaria, it is important to understand its life cycle to identify the stages that can be disrupted (Figure 1). The protozoan parasite *Plasmodium spp.* is the causative agent of malaria and there are five identified species that pose a threat for humans, namely *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* [3,4]. *P. falciparum* is the most prevalent and deadliest parasite in sub-Saharan Africa, whereas in South America and South-East Asia both *P. falciparum* and *P. vivax* cause most malaria cases. *Plasmodium spp.* has a complex life cycle alternating between the female *Anopheles* mosquito and the human host [5]. Once bitten by an infected mosquito, around 100 parasites which are referred to as sporozoites at this life stage, travel through the dermis into the bloodstream. They then escape through endothelia and Kupffer cells to ultimately infect hepatocytes. During this exo-erythrocytic phase, which lasts approximately one week depending on the species, the parasite replicates and develops into schizonts containing merozoites. At time of maturation, the hepatocytes rupture and tens of thousands of merozoites are released into the bloodstream, ready to infect red blood cells. Additionally, in the case of *P. vivax* and *P. ovale* some

sporozoites develop into hypnozoites, the dormant forms of the parasite, which can remain in hepatocytes for months or years until they cause a relapsed malaria infection.

Malaria associated symptoms, *e.g.*, anaemia and fever, are the result of the fast-asexual multiplication of merozoites in erythrocytes. Once released in the hepatic circulation, single merozoites quickly invade erythrocytes and replicate via schizogony within 48h for *P. falciparum*, *P. vivax* and *P. ovale*, 72h for *P. malariae* and 24h for *P. knowlesi*. After completion of cell division, the merozoites egress from the cells resulting in the destruction of the erythrocyte membrane and followed by re-invasion. In parallel, a small proportion of the merozoites will transform into male and female gametocytes. These are sexual forms, which will be taken up by mosquitoes, completing the cycle.

This overview of the malaria life cycle gives is an idea of possible strategies to eradicate this disease. The current strategies consist of (i) Reducing transmission from mosquito to human by, *e.g.*, insecticide spraying and/or providing insecticide-treated bed nets (ii) Using antimalarial drugs with causal prophylactic activity against the liver-stages, known as chemoprotection, to disrupt the cycle prior to the blood-stage infection (iii) Treating patients with clinical symptoms using drugs that target the erythrocytic life stages (iv) Treating patients to reduce parasite transmission from human to mosquito with drugs that target the gametocytes. The WHO and its partners deploy all these strategies to maximize the chance of success.

Medicines for Malaria Venture (MMV) is a not-for-profit public-private partnership, that focuses on developing drugs and combinations that can help to prevent, cure and/or block transmission of malaria. Historically, the drugs such as quinine, chloroquine, mefloquine or artemisinin focused on reducing clinical symptoms and therefore targeted the erythrocytic life cycle. Traditionally, malaria is monitored by measuring the blood-stage parasitemia (*i.e.* total number of parasites in the blood), and there is little, if any, data for liver-stage parasitemia due to the impossibility of obtaining data on the liver-stage from a biopsy on patients for ethical reasons. Therefore, it is difficult to deconvolute the effect on the liver-stage and blood-stage. Consequently, the first mathematical models focused on understanding the dynamic of blood-stage parasitemia [6–8] (*i.e.* total number of parasites in the blood) and quantifying how this dynamic is affected by antimalarial drugs [9–11]. However, they do not provide any information on how efficient the drug is to kill liver-stage parasites.

Studying parasites in the liver is more challenging than in the blood. Less is known and fewer models have been developed. Fortunately, during the last couple of years, experimental protocols were developed to assess the efficacy of drugs on the liver-stage *in vitro* and *in vivo* models and therefore to define their chemo-protective capabilities. However, finding the dosing regimen that could protect people from malaria is still empirical. If a drug shows some efficacy in killing liver-stage parasites *in vitro* and *in vivo* models, a dosing regimen is tested in a challenge study. Sporozoites are inoculated in healthy volunteers and the dosing regimen is considered successful if no parasites appear in the blood one month after inoculation. If unsuccessful, the dosing regimen is adjusted and tested again. It is hoped that a mathematical model can make use of the *in vitro* and *in vivo* data to help select and find the most effective dosing regimen in humans quicker and at a lower cost.

***Available Data:***

The data of one compound under development will be made available.

Liver-stage experiments:

1. *P. falciparum* liver-stage *in vitro* model:
   Primary hepatocytes from 2 different donors are infected with *P. falciparum*. The candidate drug is added post infection at different concentrations and replaced daily. The read out is done using staining of the parasite with a specific antibody to determine the parasite number and size [12].

2. *P. falciparum* prophylaxis *in vivo* model:
   In this experiment, human liver-chimeric mice are infected with *P. falciparum.* The parasite line is genetically modified to express Luciferase similarly to the *in vitro* assay. The mice are inoculated by the bites of infected mosquitoes. As read out the infection is imaged and the mice are sacrificed to determine the parasitemia by PCR and microscopy [13,14].

3. Controlled Human Malaria Infection (Figure 2):
   In a Phase I clinical trial, healthy volunteers receive the candidate drug or placebo (negative control) and they are challenged either with five infectious bites in a single episode from *P. falciparum*-infected female *Anopheles stephensi* mosquitoes or by direct intravenous inoculation of *P. falciparum* sporozoites. The efficacy against the *Plasmodium* liver-stage is assessed by monitoring blood-stage parasitemia (*i.e.* breakthrough from the liver into the blood). Rescue medication is given after the subjects become blood-stage positive above a pre-defined threshold [15–17].

There are also blood-stage experiments (*in vitro*, *in vivo* and Human Challenges [18]) that focus on the blood-stages where merozoites are used, which does not affect the liver. Therefore, those experiments quantify the real effect on the blood-stage and could potentially be used to deconvolute liver and blood stages for the liver-stage experiments where the read out is the blood-stage parasitemia.

***Question to be addressed:***

1. How would you choose the most effective dosing regimen (dose, frequency, interval) for chemoprevention from *in vitro* and animal data?
2. If not effective in the Controlled Human Malaria Infection trial, how would you refine the dosing regimen? Which method would you use and how would you design the study to get the effective dosing regimen with a minimal number of subjects?
3. How can the liver-stage be deconvoluted from the blood-stage?

## ***Figures:***

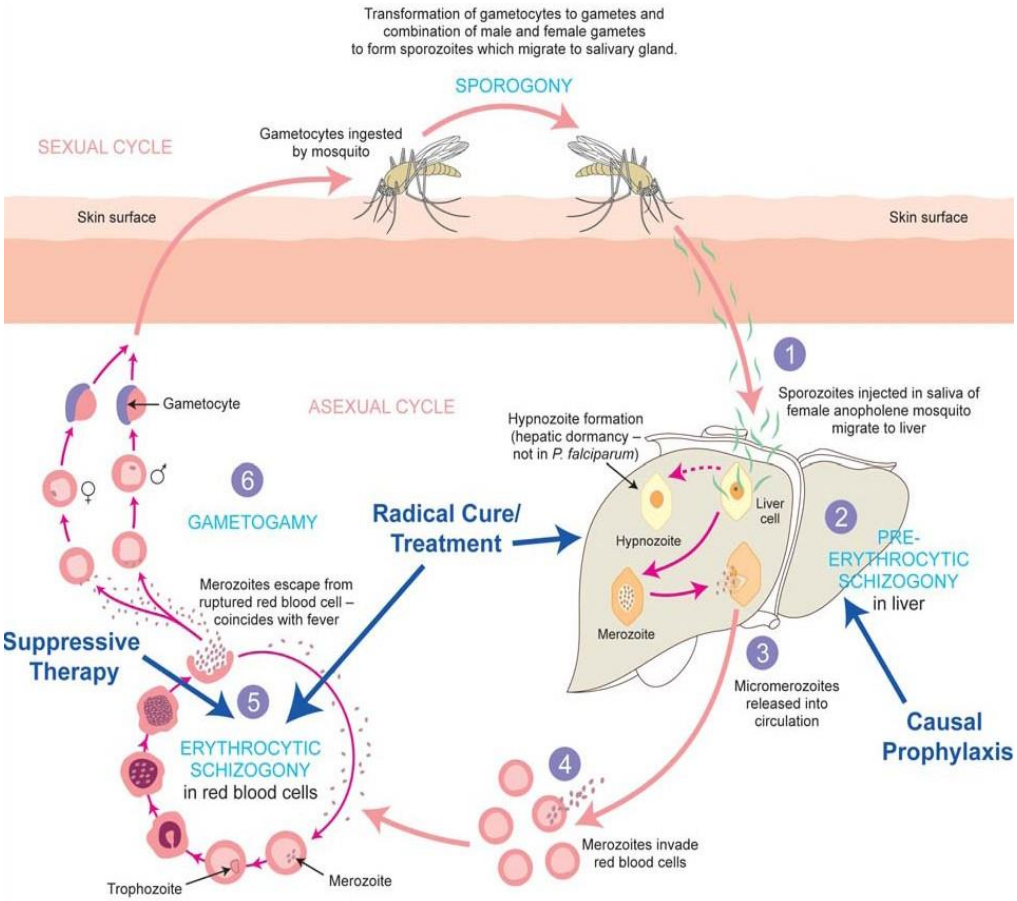


*Figure 1 : Malaria Life Cycle with the different possible treatment.*

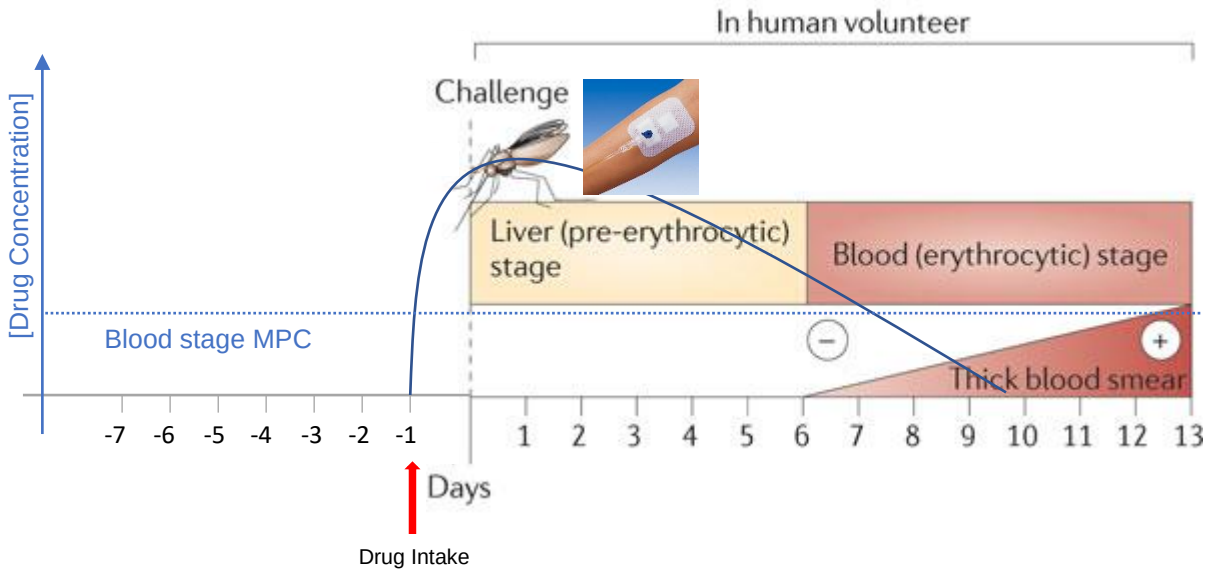


*Figure 2 : Illustration of a controlled human malaria infection for chemoprotection.*

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