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Review

Challenges in Antimalarial Drug Treatment for Vivax Malaria Control

Jean Popovici[1,*] and Didier Ménard[1,*]

***Plasmodium vivax* is the most widespread human malaria parasite, but has received much less attention than *Plasmodium falciparum* during the past 50 years of research. *Plasmodium vivax* was historically seen as causing only benign disease, but this view has recently changed, with increased recognition of the burden of vivax malaria, as well as numerous case reports of severe malaria or death caused by this parasite. The complexity of *P. vivax* biology is characteristic of specific features of the parasite, and recent years have seen major progress in our understanding of this complexity. In this review, we analyze the latest advances in the field, describing the constraints that the unique features of *P. vivax* place on drug treatments aimed at controlling or eliminating it.**

Vivax Malaria: A Neglected Disease Despite Its Global Spread

Plasmodium falciparum is the human malaria parasite inflicting the highest burden in Africa, but *Plasmodium vivax* is the most widespread of the five species infecting humans around the world, with a higher incidence of illness cases relative to *P. falciparum* in many regions. Up to 2.5 billion people are at risk of vivax malaria in more than 90 countries. Endemic *P. vivax* transmission is thought to occur over one-third of the land surface of the Earth, mostly in rural areas [1–3]. The global public health costs associated with vivax malaria have been estimated at US$1.4–4.0 billion annually [1]. In 2013, the WHO estimated the number of cases of vivax malaria to be 12–22 million [4]. Despite its global distribution and the burden that it inflicts on many communities, *P. vivax* has been largely neglected over the past 50 years. Between 2007 and 2009, only approximately 3% of the malaria research budget was devoted to studies on *P. vivax* [5]. In 2009, the Wellcome Trust and the US National Institutes of Health allocated around 9% and 5%, respectively, of their malaria research budget to grants focusing on *P. vivax* [2,6]. As a consequence, our understanding of the biology and epidemiology of *P. vivax* lags far behind that for *P. falciparum*. Furthermore, although it is tempting to transpose the knowledge gained from *P. falciparum* to *P. vivax*, this approach can have serious pitfalls as the two parasites are fundamentally different (Table 1).

We describe here the latest progress made towards understanding the biology of this parasite and its impact on the antimalarial drugs used to treat vivax malaria. The restricted tropism of *P. vivax* for **reticulocytes** (see Glossary) and the occurrence of liver **hypnozoites** inducing relapses, are the two main biological factors rendering vivax control challenging. Both these aspects have been the focus of significant research in recent years, potentially improving the options for killing this parasite. Currently, physicians face a dilemma when attempting to kill the concealed parasite hypnozoites in the liver. Primaquine, the only available antihypnozoite drug, can be prescribed, but with a risk of causing acute hemolysis in glucose-6-phosphate

Trends

The cell tropism of *Plasmodium vivax* is narrower than previously thought, being restricted to young reticulocytes displaying high levels of CD71 expression, and rendering the establishment of continuous *in vitro* parasite cultures difficult.

Primaquine, the only licensed antihypnozoite drug capable of preventing relapses of *P. vivax* malaria, may cause acute hemolysis in G6PD-deficient patients and its efficacy is also compromised by *CYP2D6* polymorphisms, an enzyme that normally transforms the drug into its active metabolites in humans.

Failure of chloroquine treatment for *P. vivax* infections is no longer limited to the Papua-Indonesia area and is prevalent in many endemic areas, including Southeast Asia and South America. However, the mechanisms of drug resistance in vivax remain unknown.

Despite the lack of *in vitro* culture methods for *P. vivax*, short-term cultures established with *ex vivo* parasites and reticulocyte-rich preparations from human cord blood can be used to evaluate the efficacy of blood-stage parasite vaccines.

Humanized mouse models and *in vitro* hepatocyte cultures are paving the way for *P. vivax* liver-stage drug and vaccine discovery.

[1]Malaria Molecular Epidemiology Unit, Institut Pasteur in Cambodia, Phnom Penh, Cambodia

*Correspondence: jpopovici@pasteur-kh.org (J. Popovici) and dmenard@pasteur-kh.org (D. Ménard).

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Table 1. Key Differences between *Plasmodium vivax* and *Plasmodium falciparum*[a]

Features	*Plasmodium vivax*	*Plasmodium falciparum*
Red blood cell tropism	$CD71^{high}$ reticulocytes	All erythrocytes
Red blood cell invasion pathways	Mostly, but not exclusively dependent on PvDBPII–DARC interactions	Multiple pathways
Continuous *in vitro* culture and genetic transformation	No	Both
Occurrence of dormant forms responsible for subsequent relapses	Yes	No
Dynamics of transmission to mosquitoes	Short-lived gametocytes produced concomitantly to erythrocytic cycle initiation	Long-lived gametocytes produced after onset of clinical symptoms, with multiple erythrocytic cycles
Drugs for which resistance has been described	Chloroquine, sulfadoxine, and pyrimethamine	Chloroquine, amodiaquine, quinine, sulfadoxine, pyrimethamine, mefloquine, artemisinin derivatives, piperaquine, atovaquone, and proguanil
Molecular mechanisms and markers of drug resistance	*Pvdhfr*, *Pvdhps*	Multiple mechanisms described, including mutations in genes relating to parasite targets of the drug (*Pfdhfr*, *Pfdhps*, and *Pfcytb*), influx/efflux pumps affecting intraparasitic concentrations of drugs (*Pfcrt*, *Pfmdr1*, *Pfmrp*, and *Pfnhe*), as well as protein–protein interactions (*K13*)

[a]Abbreviations: crt, chloroquine resistance transporter; cytb, cytochome b; DARC, Duffy antigen receptor for chemokines; DBPII, Duffy binding protein region II.

dehydrogenase (G6PD)-deficient patients. Alternatively, no treatment can be offered, leaving the patient with the burden of subsequent relapses. Recent reports of the emergence of chloroquine resistance across the world have added to the difficulties of vivax malaria control. Finally, we conclude with an assessment of current developments for drug treatment alternatives, in an attempt to control and eliminate vivax malaria.

Key Biological Considerations Rendering the Pharmacological Treatment of Vivax Malaria Challenging

Tropism for Reticulocytes: Keeping a Low Profile

Clinically, malaria occurs when the parasites invade and replicate in red blood cells during their erythrocytic cycle (Figure 1). *Plasmodium falciparum* can invade almost any red blood cell, whereas *P. vivax* invades only reticulocytes, the precursors of red blood cells [7]. Furthermore, reticulocytes form a heterogeneous subpopulation of cells at various stages of differentiation, released from the bone marrow to complete their maturation into **normocytes** in the bloodstream. During this maturation process, they undergo major biochemical and biophysical changes, including the exosome-mediated loss of reticular content and of membrane proteins, such as the transferrin receptor (CD71) [8]. Recent biological investigations with *P. vivax*-infected blood samples collected from patients, and reticulocytes from human cord blood, have shown that the parasite preferentially invades young reticulocytes with high levels of CD71 protein expression [9]. Reticulocytes constitute a minor fraction of circulating red blood cells in human adult peripheral blood (typically 0.5–1.5%), and the percentage of $CD71^{high}$ reticulocytes is small; therefore, this tropism keeps *P. vivax* parasitemia levels low [10]. This has two implications in terms of clinical manifestations and epidemiology. First, it provides some explanation as to why *P. vivax* directly kills fewer people than *P. falciparum* (Boxes 1 and 2). The pyrogenic threshold is reached at low parasitemia in *P. vivax* infections, so parasitemia is rarely life threatening (usually less than 10 000 parasites μl^{-1} blood), or at least less than for *P. falciparum* (often >50 000–100 000 parasites μl^{-1}) [11]. However, a significant proportion of the *P. vivax* biomass can be

Glossary

Duffy antigen receptor for chemokines (DARC): a membrane protein present on the surface of erythrocytes and key to the invasion of *Plasmodium vivax* through interaction with the *P. vivax* Duffy-binding protein region II (DBPII). Until recently, individuals lacking DARC receptor expression on their red blood cells (Duffy-negative individuals, frequent in African populations) were considered to be naturally resistant to *P. vivax* infections. However, this paradigm has been called into question by the description in recent years of *P. vivax* infections in Duffy-negative individuals, demonstrating the existence of alternative invasion mechanisms.

Gametocytes (male and female): intracellular sexual stages of the parasite found in the bloodstream after the differentiation of merozoites in infected reticulocytes. These stages are responsible for transmission to mosquitoes. In *P. vivax* infections, gametocyte production is concomitant with the first erythrocytic cycles, resulting in transmission before the onset of clinical symptoms.

Giemsa stain: a classic blood-film stain for the gold standard diagnosis of malaria by light microscopy.

Hypnozoites and relapses: dormant intracellular hepatic forms of *P. vivax* parasites, but not produced by *Plasmodium falciparum*. Upon reactivation, they induce subsequent malaria attacks, called relapses, which may occur weeks, months or even years after the initial infection.

Merozoites: extracellular blood stage of the parasite able to invade reticulocytes, released from hepatic or erythrocytic schizonts.

Parasitemia: the percentage of circulating red blood cells infected with erythrocytic stages of the parasite. This percentage is low in *P. vivax* infections, partly due to the restricted tropism of the parasite, which infects only $CD71^{high}$ reticulocytes.

Rings, trophozoites and schizonts: intracellular asexual blood stages of the parasite within infected reticulocytes. Following invasion of the reticulocyte, the parasite initiates its erythrocytic cycle, developing from ring-stages to trophozoites and then replicating its DNA and multiplying to form a schizont that bursts, releasing