Chapter

Plasmodium vivax and Drug Resistance

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Abstract

Resistance to antimalarial drugs is a threat to global efforts to eliminate malaria by 2030. Currently, treatment for vivax malaria uses chloroquine or ACT for uncomplicated *P. vivax* whereas primaquine is given to eliminate latent liver stage infections (a method known as radical cure). Studies on *P. vivax* resistance to antimalarials and the molecular basis of resistance lags far behind the *P. falciparum* as *in vitro* cultivation of the *P. vivax* has not yet been established. Therefore, data on the *P. vivax* resistance to any antimalarial drugs are generated through *in vivo* studies or through monitoring of antimalarial treatments in mixed species infection. Indirect evidence through drug selective pressure on the parasites genome, as evidenced by the presence of the molecular marker(s) for drug resistance in areas where *P. falciparum* and *P. vivax* are distributed in sympatry may reflect, although require validation, the status of *P. vivax* resistance. This review focuses on the currently available data that may represent the *state-of-the art* of the *P. vivax* resistance status to antimalarial to anticipate the challenge for malaria elimination by 2030.

Keywords: *Plasmodium vivax*, antimalarials, resistance status, genetic marker(s)

1. Introduction

Plasmodium vivax presents a major challenge to achieving the global effort to eliminate malaria by 2030. The global distribution and factors that are associated with *P. vivax* occurrence in wider geographic regions in tropical, subtropical and temperate zones have extensively been reviewed recently [1, 2]. The ability of this species to undergo dormancy in the form of single-celled hypnozoites in the human liver, a safe haven from immune attack during the long mosquito-free cold seasons contributed to this phenomenon (**Figure 1**) [3]. Currently, *P. vivax* is present in 51 countries across Central and South America, the horn of Africa, Asia and the Pacific islands. Global malaria control and elimination programme successfully brought down the malaria incidence from 238 million cases in 2000 to 229 millions in 2019. The proportion of *P. vivax* cases declined from 7% in 2000 to 3% in 2019 [4]. Between 2000 and 2015, global malaria case incidence declined by 27%, and between 2015 and 2019 it declined by less than 2%, indicating a slowing of the rate of decline since 2015. Different from other human malaria, *P. vivax* uses Duffy antigen as its receptor in human to invade exclusively the young red blood cell (reticulocytes). Therefore, individuals who do not express the Duffy antigen are considered to be genetically resistant to *P. vivax* infection and this is particularly true in the majority of African sub-saharan population [5]. However, evidence for

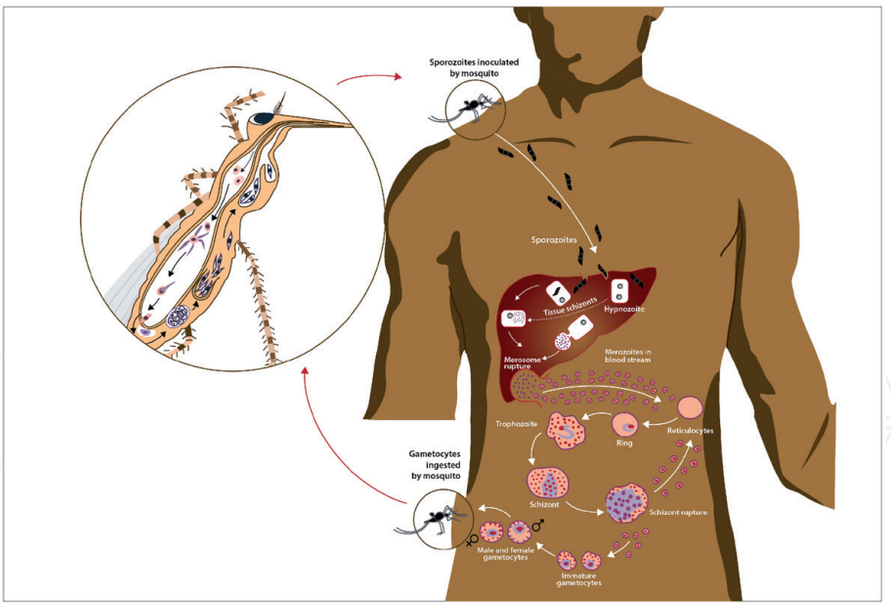

Figure 1.
Plasmodium vivax *life cycle [3].*

P. vivax infections in Duffy negative patients raise the possibility of an alternative invasion mechanism to Duffy [6, 7].

Plasmodium vivax in most geographic regions is distributed in sympatry with *P. falciparum*. This situation requires special attention during diagnosis and before prescription of drugs to the infected people. The biological characteristic of the *P. vivax* exhibit multiple exoerythrocytic cycle (relapses) from a single mosquito inoculation, and coupled with the very early emergence of gametocytes in the course of blood-stage infection, perhaps enables parasite survival and transmit to mosquito vector silently despite relatively low probability of propagation in blood. This propensity presents a unique challenge to the chemotherapeutic intervention against vivax malaria cases in which radical cure to block the reactivation of hypnozoites should be given in addition to blood schizontocide. Although *P. vivax* has been known to be less severe than *P. falciparum*, a growing body of evidence indicates that severe and fatal outcome also occurred in many *P. vivax* cases and necessitate the importance of reversing the historic neglect of this infection [8–10].

Treatment of *P. vivax* currently relies on either chloroquine or ACTs, supplemented both with 14 days primaquine as anti-hypnozoites. The only available drug for radical cure is primaquine but its use in vivax endemic region is limited by its potential serious complication among the people who inherit glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency.

The present review focuses on the recent progress that has been achieved to try to circumvent the problem of drug resistance in *P. vivax*. The global spread of chloroquine-resistant *P. vivax* has forced some countries to adopt artemisinin-based combination therapy (ACTs) to replace chloroquine as the first line drug to treat uncomplicated *P. vivax* and this policy change also impact the use of primaquine, the sole agent for anti-relapse. Many studies and reviews have been exclusively focused to elucidate the basic mechanism(s) of drug resistance in the malarial parasite using *P. falciparum* or other rodent and avian plasmodia as model while *P. vivax*, as usual, is consistently neglected. In discussing the topic, we will review all antimalarial drugs that used to be used or have been used to treat vivax malaria since the early development of quinine to date. Rapid development of resistance to

the antimalarial drug mainstays from the early days to date will also be discussed to provide better perspectives for circumventing the problem of antimalarial drug resistance. The global extent of the drug-resistant *P. vivax* will also be reviewed to determine the appropriate measures based on drug policies that have been adopted by World Health Organisation (WHO) and implemented on local setting by member countries.

2. Challenges in the validation of drug resistance in *Plasmodium vivax*

2.1 Detection of hypnozoites

Historically, until to date, very few, if any, progress that has been achieved with regard to the biology of hypnozoites. Owing to its relatively "benign" clinical manifestation, the need for developing a more effective and less side effect radical cure have been hindered by our inability to detect the presence of hypnozoites in the liver cells. The occurrence of relapses in *P. vivax* after primaquine therapy would be assumed to be the most reliable indication of resistance. Nevertheless, recurrent parasite following primary attack may originate from failed therapy against asexual blood stages (recrudescence), biting infectious anopheline mosquitoes (reinfection), and reactivation of hypnozoites (relapse).

Since parasite arising from relapse may be genetically heterologous [11–13], distinguishing these events using molecular technologies is not yet possible, and this imposes limitations in estimating primaquine efficacy against relapse or resistance. Our inability to detect the presence of hypnozoite lead us to the assumption that every acute attack of *P. vivax* shall also harbour hypnozoites in the liver cell and this is supported by the fact that without prescription of anti-relapse, almost all acute attack will experience relapse within a year [14, 15]. Recent studies have tried to identify any protein (s) that are released by hypnozoites during its dormancy and some progress have been achieved in this endeavour. Although the finding is still a proof-of-principle, the presence of human arginase-I and an uncharacterized *P. vivax* protein in plasma-derived exosomes deserves further exploration on the potential to identify biomarkers of hypnozoite infection [16].

2.2 *In vitro* cultivation of *Plasmodium vivax*

Studies on *P. vivax* resistance to drugs used either as blood schizontocide or radical cure lags far behind *P. falciparum*. The absence of *in vitro* culture system for propagating the blood stage of *P. vivax* has made the *in vivo* test is the only way to determine the *P. vivax* drug response to any antimalarial drugs [17]. Advance in molecular and cellular biology within the last few decades have contributed significantly to the progress in the establishment and refinement of ex vivo drug test and repeatable *in vitro* cultivation in *P vivax* [18–20]. It has long been known that *P. vivax* invade exclusively young erythrocyte (reticulocytes) of Duffy antigen positive vertebrate host for asexual development in human and non human primate [21]. Recent progress have identified more specifically that *Plasmodium vivax* preferentially invade stage I reticulocytes $CD71^{high}TO^{high}$) [22]. Other progress also showed the success for long term cultivation, including cryopreservation and re-cultivation of *P. vivax* using blood of certain non-human primate [23]. With the aforementioned evidence, *ex vivo* and *in vitro* drug testing platform of *P. vivax* for novel drug development is now workable.

3. History of antimalarial drug use in vivax malaria

3.1 Quinine

Originally extracted from a Peruvian tree bark in South America, quinine was initially named cinchona in 1742 by Linnaeus. In 1820, two French chemists isolated quinine from the cinchona bark and this compound became a treatment of reference for intermittent fever throughout the world [24, 25] until the resistance of *P. falciparum* was first reported in Brazil in 1910 [26]. Quinine is also used for malaria prophylaxis. It was not clear as to whether the resistance rendered complete inefficacy of quinine to cure the falciparum malaria nor that it also occurred in other species, including *P. vivax* but it was evident that quinine was still widely used as the antimalarial mainstay until the second world war when the new antimalarial, chloroquine was introduced. As the advance in parasite biology provide more insight into parasite species diversity in human and other primates, the resistance phenomenon has attracted attention to investigate in animal model but the results were not conclusive [27]. At the end of 1930s, pamaquine, a new compound targeted at *P. vivax* liver stage was introduced, but 20 years later it was replaced by primaquine, a new 8-aminoquinoline compound. Primaquine is also still used until now as anti-hypnozoites for *P. vivax* [28] and malaria prophylactic [29] and anti-gametocyte drug for *P. falciparum* [30].

In response to the resistance to quinine in 1910 [26], scientists across the globe explore to find alternative treatment. Mepacrine was first synthesised in 1931 at Bayer, Germany. The product was one of the first synthetic substitutes for quinine although later superseded by chloroquine. Mepacrine (Atabrine) was used extensively during the second World War by Allied forces fighting in North Africa and the Far East to prevent malaria. This compound is also used for the treatment of giardiasis (an intestinal parasite) and has been researched as an inhibitor of phospholipase A2. Establishment of mepacrine-resistant strain in rodent plasmodia model indicated that the drug interfere with the haemozoin formation as that of quinine-resistant and primaquine-resistant strain [31]. The major breakthrough achieved during this period is the establishment of testing platform to raise drug-resistant malarial parasites in rodent model [32].

3.2 Primaquine (8-aminoquinoline)

Primaquine has long been used for radical cure to prevent relapse of malaria due to *P. vivax* and *P. ovale*. Primaquine kills hypnozoites, the dormant liver stage of the parasite. However, primaquine is known to cause severe haemolysis in patients with G6PD deficiency, a genetic disorder present in approximately 8% of the population in malaria endemic countries [33]. To prevent relapses, the World Health Organisation recommends the co-administration of CQ and standard- (total, 3.5 mg/kg) or high-dose PQ (total, 7.0 mg/kg) distributed over 14 days to patients aged >6 months with normal G6PD activity who are neither pregnant nor breast-feeding [34]. Nevertheless, adherence to such prolonged course in malaria endemic areas is poor and coupled with the fear of G6PD deficiency, prescription of this drug is mostly inadequate. Following reports of primaquine resistance in *P. vivax* in several geographic areas [35–40]. Collins and Jeffrey in1996 conducted a review and concluded that the data on the efficacy of primaquine as an anti relapse remains few and inconclusive. Therefore, the need for a standardised tool to determine primaquine resistance status should be developed [41, 42]. Primaquine is also known to possess blood schizontocidal activity as well as gametocytocidal activity in *P. falciparum.* In the context of anti-relapse activity in *P. vivax*, it is important

to distinguish as to whether the observed recurrent parasite following a radical cure indeed originates from reactivated hypnozoites or recrudescence. Attempts to prescribe safer and shorter dose of primaquine rendered several improvements but dependence on G6PD screening could not be excluded [34]. Review on primaquine use in *P. vivax* concluded that the currently suggested indications in relation to vivax malaria, namely; causal prophylaxis, terminal prophylaxis, and radical cure is still highly effective [43, 44]. With regard to primaquine treatment failure in some areas, the presence of host genetic factors, such as single nucleotide polymorphisms (SNPs) in the gene encoding enzyme involved in primaquine metabolism, CYP2D6, may also be considered before claiming primaquine resistance [45, 46].

A new compound of 8-aminoquinoline class, tafenoquine was introduced following clinical trials in several countries [47, 48]. Despite single dose prescription, tafenoquine did not show any superiority to primaquine [49]. Therefore, development of a novel compound for anti-hypnozoites that does not depend on G6PD status still has to be prioritised.

3.3 Aminoquinoline antimalarials

Chloroquine was discovered in 1934, by Hans Andersag and co-workers at the Bayer laboratories. Research by German scientists to discover a substitute for quinine led to the synthesis in 1934 of Resochin (chloroquine) and Sontochin (3-methyl-chloroquine). After the war, chloroquine have for decades been the mainstays for malaria treatment and prevention during the global malaria eradication campaign by WHO in 1950s. This safe and inexpensive 4-aminoquinoline compound is believed to exert its antimalarial property through accumulation in the food vacuole [50]. The mechanisms by which chloroquine selectively accumulates may include protonation and ion trapping of the chloroquine due to the low pH of the food vacuole, active uptake of chloroquine by a parasite transporter(s), and/or binding of chloroquine to a specific receptor in the food vacuole [51–55].

After a decade of its use, chloroquine resistant *P. falciparum* arose in four separate locations, starting with the Thai-Cambodian border around 1957; in Venezuela and parts of Colombia around 1960; in Papua New Guinea in the mid-1970s and in Africa in 1978 in Kenya and Tanzania [56]. Resistance of *P. falciparum* to chloroquine changed the treatment policy to use several drugs such as halofantrine, lumefantrine, pyronaridine, mefloquine, and sulfadoxine-pyrimethamine (SP), while chloroquine and primaquine remain effective to treat *P. vivax* until few decades.

Resistance by *P. vivax* to chloroquine was unknown until 1989, when Australians repatriated from Papua New Guinea failed routine treatment [57]. Subsequent reports affirmed that finding and CQ-resistant *P. vivax* (CRPV) was reported from Indonesia [58] and Guyana [59]. A review and meta-analytic study evaluating chloroquine clinical trials performed during the period of 1960 to 2014 found out a contrasting evidence, indicating chloroquine sensitivity as shown by elimination of the asexual parasite by day 3 [60]. Although in some studies, a high degree of resistance was confirmed, the trials exhibited heterogeneity in study design and the presence of confounding factors such as interpretation of a recurrent parasites to distinguish relapse or recrudescence. In addition, technical issues on the quality and the dose of chloroquine used may also play role as the chloroquine possesses a wide therapeutic windows that enable to increase the dose. A therapeutic efficacy study to determine the efficacy of chloroquine in uncomplicated vivax malaria was conducted in Papua, Indonesia in 2007 isolated few recurrent parasites that survive chloroquine at blood concentration ranged from 100 ng/ml to 516 ng/ml [61]. Other study performed *in vitro* chloroquine sensitivity assay on either freshly

collected or cryopreserved *P. vivax* isolates collected from Papua and Thailand [62]. The global spread of chlroquine-resistant *P. vivax* was later summarised in 2016 [63], as shown in **Figure 2**.

The absence of reliable, robust, sensitive methods for detection and monitoring of antimalarial drug efficacy in *P. vivax* has almost certainly contributed to the delayed recognition of this emerging problem [57]. Other factors include the relatively small parasite biomass in *P. vivax* infections, concomitant medication, such as primaquine to kill hypnozoites, early transmission due to the early presence of gametocytes, and high genetic diversity in natural population of *P. vivax* [64]. This delay has had important public health implications in areas where high-grade chloroquine-resistant *P. vivax* is prevalent (such as Indonesia and Oceania), partly effective drug treatments and consequent recurrent infections are an important contributing factor to severe anaemia from *P. vivax* malaria [65].

3.4 Resistance to antifolate and sulpha drugs

Proguanil, also known as chlorguanide and chloroguanide, is the first antifolate used to treat malaria. Proguanil is converted by the liver to its active metabolite, cycloguanil. The success of proguanil in treating human malaria led to further study of its chemical class and to the development of pyrimethamine in 1952. Resistance to the monotherapies of proguanil or pyrimethamine developed rapidly (within one year in the case of proguanil). A clear cut resistance to antifolate was proven in *P. falciparum, P. vivax* and *P. malariae* [66–68]. Sulfones and sulfonamides were then combined with proguanil or pyrimethamine in hopes of increasing efficacy and preventing or delaying resistance. By 1953, *P. falciparum* resistance had already been noted in Tanzania. When Sulfadoxine-pyrimethamine (SP) was introduced in Thailand in 1967, resistance appeared in the same year and spread quickly throughout South-East Asia. Resistance to SP in Africa remained low until the late 1990s but since then it has spread rapidly [69]. The SP has never been recommended for *P. vivax* treatment but evidence suggest that this compound is also effective to treat uncomplicated *P. vivax* [70, 71]. In response to resistance to SP and chloroquine, a combination of proguanil with a new class antimalarial compound, atovaquone, was introduced in 1999 by Glaxo-Smith Kline [72]. Nevertheless, prior

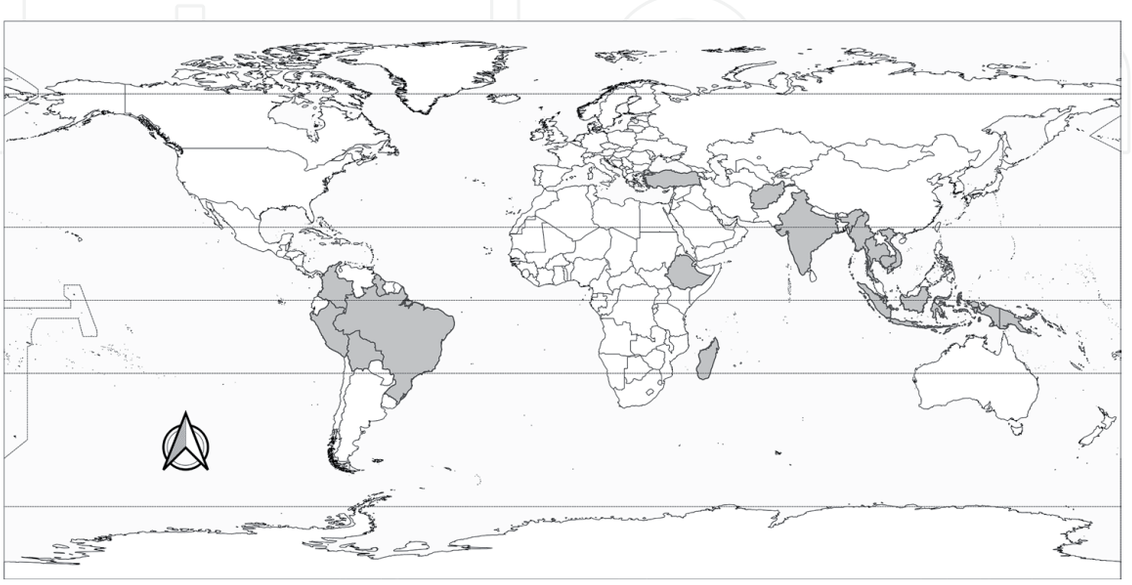

Figure 2.
Chloroquine -resistant P. vivax *infections. Source: World Wide Antimalarial Resistance Network (WWARN), available at: http://www.wwarn.org/vivax/surveyor/#0 and [64].*

to its introduction, resistance to atovaquone has been rapidly selected up in rodent plasmodia and *P. falciparum* [73, 74]. Since 2011, atovaquone-proguanil is available as a generic drug.

3.5 Artemisinin-based combination therapy (ACTs)

Artemisinin is a sesquiterpene lactone, containing the peroxide group, extracted and isolated from the leaves of *Artemisia annua.* by Chinese scientists in 1972 [75]. The drug and its derivatives play a role in killing *Plasmodium falciparum* by inhibiting the activity of phosphatidylinositol-3-kinase (PfPI3K) [76]. Initially, it was used as monotherapy to treat uncomplicated malaria but due to high recrudescence rate, a combination therapy was advised. Artemisinin-based Combination Therapy (ACTs), particularly artesunate-mefloquine, was introduced in Thailand during the early 1990s [77]. Since 2001, artemisinin (ART) combination therapy (ACT) has been recommended as the first-line treatment in the national treatment guidelines of most malaria endemic countries and have played an important role in reducing global malaria-associated mortality and morbidity [78].

Resistance to artemisinin was first detected in the Greater Mekong Subregion (GMS) region in 2008 [79]. Since then, ART resistance has spread and/or emerged in other areas of the GMS [80–83]. Exposure of the parasite population to artemisinin monotherapies in subtherapeutic doses for over 30 years, and the availability of substandard artemisinin, have probably been the main driving force in the selection of the resistant phenotype in the region. ART resistance is defined as the parasite clearance half-life of >5 h or presence of parasites in patients 3 days after treatment but has been more challenging to define, mostly because artemisinin act potently and rapidly clear parasites from the bloodstream by a unique mechanism involving the spleen [84, 85].

Currently, several drugs have been recommended (**Table 1**) for the treatment of severe and uncomplicated vivax malaria [34, 63, 86–90] and WHO is considering

	Drugs
Severe	
1	Artesunate
2	Artemether
3	Quinine
Uncomplicated	
1	Artesunate - Amodiaquine
2	Artemether - Lumefantrine
3	Artesunate - Mefloquine
4	Artesunate - Pyronaridine
5	Artesunate – Sulfadoxine/Pyrimethamine
6	Dihydroartemisinin - Piperaquine
7	Chloroquine
Antirelapse	
1	Primaquine
2	Tafenoquine

Table 1.
Antimalarial drugs for the treatment of Plasmodium vivax *malaria.*

the use of artesunate-pyronaridine, in areas where other ACTs are failing. In the absence of resistance, all six partner drugs would be highly efficacious as monotherapies at the dose used in the ACTs. Two injectable treatments, artesunate and artemether, are recommended for the treatment of severe malaria and should be followed by an ACTs once the patient can tolerate oral therapy [34].

Studies to monitor the efficacy of the ACTs on both *P. falciparum* and *P. vivax* have been conducted since the introduction of this drug in 2001. Evidence to date revealed that resistance of *P. falciparum* to artemisinin so far is not only confined to the Greater Mekong Subregion (GMS). Recent evidence indicated that *P. falciparum* isolates carrying the kelch13 C580Y mutation has been found in Papua New Guinea [91]. The finding is quite worrying as both PNG and Indonesia shared terrestrial border and the mutations may have spread to Indonesia. Therapeutic efficacy studies (TES) conducted during the period of 2009–2018 in various sites in Indonesia, including the Indonesia-PNG border documented no cases of either *P. falciparum and P. vivax* resistance nor treatment failure associated with artemisinin in Indonesia [92–96]. Nevertheless, recurrent parasite at late observation day was reported and this recurrence certainly nothing to do with artemisinin but rather with partner drug.

4. Molecular basis of *P. vivax* resistance to antimalarial drugs

The advent of molecular and cellular parasitology within the last 4 decades have brought along a lot of substantial innovations in the antimalarial drug testing platforms, molecular assays to phenotype as well as genotype the malarial parasite, although it mainly attributed to *P. falciparum*. In *P. vivax*, attempts to develop a repeatable *in vitro* drug resistance test continue to elude us, although certain progress has been achieved [23]. As a consequence, progress on the studies to elucidate the molecular basis of the *P. vivax* resistance to antimalarial drugs, particularly chloroquine and artemisinin is lagged far behind *P. falciparum*. While studies on molecular basis of resistance to chloroquine and artemisinin successfully identified candidate gene (s) through a clear phenotypic and genotypic assay, similar progress in *P. vivax* could not be achieved. The molecular basis of *Plasmodium* resistance to antifolates and sulpha drugs had been well described [97–99]. This evidence also applies to *P. vivax*, and the underlying genetic polymorphisms in dhfr and dhps genes, conferring resistance to antifolates and sulpha drugs, respectively. Likewise, resistance to atovaquone, a partner drug of proguanil has been associated genetic polymorphisms in the c*ytb* gene of the malarial parasite [73, 74].

Resistance to chloroquine, has long been subject for research in many laboratories around the globe. A yet unclear mechanism of action of this compound making it more attractive for elucidation using molecular tool. Initially the role of *Plasmodium falciparum* multidrug resistance 1 (pfmdr1), homologous to the mammalian multiple drug resistance (MDR) gene were incriminated [100–102]. The product of thePfmdr1gene, P-glycoprotein homolog 1 *(Pgh1)* has been localized to the membrane of the digestive vacuole of mature blood stage parasites. This model predicted that thePfmdr1gene would be amplified and/or over expressed in CQ-resistant isolates. Further study, however identified different mechanism for chloroquine resistance but support for the role of this pfmdr1 in other antimalarials such as mefloquine, halofantrine and quinine [103–106]. Chloroquine-resistant parasites pump chloroquine out at 40 times the rate of chloroquine-sensitive parasites; the pump is coded by the *P. falciparum* chloroquine resistance transporter (*PfCRT*) gene [107, 108]. The natural function of the chloroquine pump is to transport peptides: mutations to the pump that allow it to pump chloroquine out impairs its function as a peptide pump and comes at a cost to the parasite, making it less fit.

Several genetic polymorphisms at the PfCRT gene have been associated with resistance to chloroquine in a wide geographic regions of malaria endemic areas [108]. Nevertheless, attempts to prove this finding in CRPV still fail, primarily because the technical difficulties in proving the resistant phenotype in *P. vivax*. Molecular analysis of the *P. vivax* isolates that have been phenotypically determined to be resistant in a rigorous *in vivo* and limited in vitro tests did not reveal any polymorphisms in the PvCRT gene as that of PfCRT. Instead, amplification of Pvmdr1 and several SNPs in the pvmdr1 was found to associate with CRPV [62]. Recent evidence found out that increases in PvCRT copy number associated with the *P. vivax* resistance to chloroquine [109–113].

The molecular basis for artemisinin resistance in the malarial parasite have also been described recently. Since mammalian kelch proteins can detect oxidants and other stressors, mutations in K13-propeller were reasonably implicated in mediating resistance to artemisinin and have been proposed as molecular marker [114–117]. Subsequent studies provided a more detail biochemical impact of the *PfKelch13* mutations on the decreased abundance of PfKelch13 protein, decreased haemoglobin digestion, and enhanced glutathione production [118]. However, the finding on the interaction of dihydroartemisinin with phosphatidylinositol-3-phosphate kinase, and that elevated phosphatidyl-inositol-3 phosphate can be associated with resistance in the absence of PfK13 mutations suggested for other mechanism [119]. In line with this evidence, Tyagi *et al* [120] raised a clear-cut artemisinin resistant isolates of *P. falciparum* following artesunate drug pressure in humanised mouse and the molecular analyses of the ART-resistant isolates revealed no mutations in PfK13 gene. Instead, an obvious selective pressure on RAD5 gene. Interestingly, the ART-resistant isolates also exhibited concomitant resistance to quinine, a second line drug used for treating severe malaria cases. The association between mutations in RAD5 gene and the resistance to artemisinin require further confirmation through either reverse genetics or genetic gross in mosquito.

Resistance of *P. vivax* to artemisinin so far has never been reported in areas where ACTs have long been used as first line drug for *P. vivax* malaria in South and Southeast Asia and the Pacific islands to replace chloroquine. This evidence, however, has to be carefully considered as *P. vivax* perhaps has long experienced with artemisinin pressure as that of *P. falciparum*, particularly in the GMS region where both species are distributed in sympatry and undetectable mixed species infection are common [121]. In support of this assumption, molecular analysis of *P. vivax* isolate from the GMS region revealed a high diversity and *ex vivo* analysis indicate reduced sensitivity to chloroquine, mefloquine, pyronaridine, piperaquine, quinine, artesunate and dihydroartemisinin [122, 123]. In this context, regular monitoring of the antimalarial treatment as well as genomic surveillance of the PvK12 gene, orthologues of the PfK13, in *P. vivax* and other relevant gene (s) should be conducted to monitor the emergence of artemisinin-resistant *P. vivax* and to contain the spread of the resistance to other regions [124, 125].

5. Conclusion

Reports of *P. vivax* resistance to primaquine and chloroquine have been well documented. Nevertheless, attempts to validate the resistance status of primaquine rendered an equivocal results. With the current limitation in testing platform both *in vivo* and *in vitro*, the use of primaquine as anti-relaps compound is still recommended. Therefore, factors that may limit its use in *P. vivax* endemic setting such as G5PD deficiency should be excluded by deploying a cheap, easy to use Point-of-Care (PoC) G6PD test.

Plasmodium vivax resistance to chloroquine present different burden to each geographic areas. Therefore, the use of alternative drug ACTs should be tailored following the degree of resistance to chloroquine, as well as therapeutic response to any available ACTs.

Plasmodium vivax resistance to artemisinin has never been found in any of the *P. vivax* isolates examined from different geographic regions but resistance to partner drug such as amodiaquine, piperaquine, lumefantrine, mefloquine and pyronaridine should be regularly monitored to safeguard our arsenal for achieving malaria elimination by 2030.

6. Future perspectives

Resistance of *P. vivax* to the antimalarial drug mainstays, chloroquine and primaquine poses a serious challenge to achieving the global malaria elimination that has been set up in 2030. Despite ambiguous evidence on both of this drug, chloroquine and primaquine deserve further exploration on its efficacy in different geographic setting before being side lined. To ensure the safe provision of primaquine treatment in *P. vivax,* local capacity to determine the existence host genetic factors such as G6PD deficiency as well as CYP2D6 allelic frequency should be established to mitigate the treatment failure that potentially increasing the risk of severe and fatal outcome.

Recent progress on the *in vitro* cultivation of *P. vivax* renew our interest to carefully validate the clinical phenotype of *P. vivax* isolates to the antimalarial drug mainstays, chloroquine, ACT and primaquine as well as the association with the genotype through genome-wide association study. In this context, progress achieved in *P. falciparum* certainly provide guidance to circumvent the limitations in *P. vivax*.

The proven efficacy of ACTs to vivax malaria in general and CRPV in particular, also support for our readiness to circumvent the problem of *P. vivax* resistance toward the remaining years ahead. Although the ACT is hastily paired with primaquine, evidence to date is still supportive.

Apart from our readiness to cope in turn the chemotherapeutic issue in combating *P. vivax,* efforts to mitigate the transmission through vector control should also be encouraged. A regular vector surveillance and control around the dwelling areas should be promoted to prevent the silent transmission of the parasite to Anopheles vector.

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List of acronyms

ACT	Artemisinin-based combination therapy
ART	Artemisinin

CQ	Chloroquine
CRPV	Chloroquine Resistant *Plasmodium vivax*
Cyp2D6	Cytochrome P450 2D6
G6PD	Glucose-6-phospate dehydrogenase
PfCRT	*P. falciparum* chloroquine resistance transporter
PfMDR	*P. falciparum* multidrug resistance
Pfdhfr	*Plasmodium falciparum* dihydrofolate reductase
Pfdhps	*Plasmodium falciparum* dihydropteroate synthetase
SNPs	Single nucleotide polymorphisms
SP	Sulfadoxine/Pyrimethamine
TES	Therapeutic efficacy studies

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References

[1] Howes RE, Battle KE, Mendis KN, Smith DL, Cibulskis RE, Baird JK, Hay SI. Global Epidemiology of *Plasmodium vivax*. The American Journal Tropical Medicine and Hygiene. 2016; 95(6). DOI: 10.4269/ajtmh.16-0141

[2] Battle KE, Lucas TCD, Nguyen M, Howes RE, Nandi AK, Twohig KA, Pfeffer DA, Cameron E, Rao PC, Casey D, Gibson HS, Rozier JA, Dalrymple U, Keddie SH, Collins EL, Harris JR, Guerra CA, Thorn MP, Bisanzio D, Fullman N, Huynh CK, Kulikoff X, Kutz MJ, Lopez AD, Mokdad AH, Naghavi M, Nguyen G, Shackelford KA, Vos T, Wang H, Lim SS, Murray CLJ, Price RN, Baird JK, Smith DL, Bhatt S, Weiss DJ, Hay SI, Gething PW. Mapping the global endemicity and clinical burden of P*lasmodium vivax*, 2000-17: a spatial and temporal modelling study. Lancet. 2019;394(10195):332-343. DOI: 10.1016/S0140-6736(19)31096-7

[3] Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar DK, Alonso PL, del Portillo HA. Key gaps in the knowledge of *Plasmodium vivax*, a neglected human malaria parasite. Lancet Infectious Diseases. 2009;(9):555-566. DOI: 10.1016/S1473-3099(09)70177-X

[4] World Health Organization. World Malaria Report. 2020. ISBN 978-92-4-001579-1

[5] Howes RE, Patil AP, Piel FB, Nyangiri OA, Kabaria CW, Gething PW, Zimmerman PA, Barnadas C, Beall CM, Gebremedhin A, Menard D, Williams TN, Weatherall DJ, Hay SI. 2011. The global distribution of the Duffy blood group. Nature Communication. 2011;2:266. DOI: 10.1038/ncomms1265

[6] Ménard D, Barnadas C, Bouchier C, Henry-Halldin C, Gray LR, Ratsimbasoa A, Thonier V, Carod JF, Domarle O, Colin Y, Bertrand O, Picot J, King CL, Grimberg BT, Mercereau-Puijalon O, Zimmerman PA. *Plasmodium vivax* clinical malaria is commonly observed in Duffy-negative Malagasy people. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(13):5967-5971. DOI: 10.1073/pnas.0912496107

[7] Zimmerman PA, Ferreira MU, Howes RE, Mercereau-Puijalon O. Red blood cell polymorphism and susceptibility to *Plasmodium vivax*. Advances in Parasitology. 2013;81:27-76. DOI: 10.1016/B978-0-12-407826-0.00002-3

[8] Rahimi BA, Thakkinstian A, White NJ, Sirivichayakul C, Dondorp AM, Chokejindachai W. Severe vivax malaria: A systematic review and meta-analysis of clinical studies since 1900. Malaria Journal. 2014;13: 481. DOI: 10.1186/1475-2875-13-481

[9] Baird JK, 2013. Evidence and implications of mortality associated with acute *Plasmodium vivax* malaria. Clinical Microbiology Review. 2013;26(1):36-57. DOI: 10.1128/CMR.00074-12

[10] Nicholas M Anstey, Nicholas M Douglas, Jeanne R Poespoprodjo, Ric N Price *Plasmodium vivax*: Clinical spectrum, risk factors and pathogenesis. Advance Parasitology. 2012; 80:151-201. DOI: 10.1016/B978-0-12-397900-1.00003-7

[11] Chen N, Auliff A, Rieckmann K, Gatton M, Cheng Q. Relapses of *Plasmodium vivax* infection result from clonal hypnozoites activated at predetermined intervals. The Journal Infectious Diseases. 2007; 195(7):934-941. DOI: 10.1086/512242

[12] Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, Guthmann JP, Nosten F, Carlton J, Looareesuwan S, Nair S, Sudimack D, Day NP, Anderson TJC, White NJ. Relapses of *Plasmodium vivax* infection usually result from activation of heterologous hypnozoites. The Journal Infectious Diseases. 2007;195(7):927-933. DOI: 10.1086/512241

[13] Imwong M, Boel M, Pagornrat W, Pimanpanarak M, McGready R, Day NPJ, Nosten F, White NJ. The first *Plasmodium vivax* relapses of life are usually genetically homologous. The Journal Infectious Diseases. 2012;205(4):680-683. DOI: 10.1093/infdis/jir806

[14] Baird JK, Hoffman SL. Primaquine therapy for malaria. Clinical Infectious Diseases. 2004; ;39(9):1336-45. DOI: 10.1086/424663

[15] Thomas D, Tazerouni H, Sundararaj KGS, Cooper JC. Therapeutic failure of primaquine and need for new medicines in radical cure of plasmodium vivax. Acta Tropica. 2016;160:35-38. DOI: 10.1016/j.actatropica.2016.04.009

[16] Gualdrón-López M, Flannery EL, Kangwanrangsan N, Chuenchob V, Fernandez-Orth D, Segui-Barber J, Royo F, Falcón-Pérez JM, Fernandez-Becerra C, Lacerda MVG, Kappe SHI, Sattabongkot J, Gonzalez JR, Mikolajczak SA, Del-Portillo HA. Characterization of plasmodium vivax proteins in plasma-derived exosomes from malaria-infected liver-chimeric humanized mice. Front Microbiology. 2018;9:1271. DOI: 10.3389/fmicb.2018.01271

[17] Udomsangpetch R, Kaneko O, Chotivanich K, Sattabongkot J. Cultivation of *Plasmodium vivax*. Trends Parasitology. 2008;24(2):85-88. DOI: 10.1016/j.pt.2007.09.010

[18] Shaw-Saliba K, Clarke D, Santos JM, Menezes MJ, Lim C, Mascarenhas A, Chery L, Gomes E, March S, Bhatia SN, Rathod PK, Ferreira MU, Catteruccia F, Duraisingh MT. Infection of laboratory colonies of anopheles mosquitoes with plasmodium vivax from cryopreserved clinical isolates. International Journal Parasitololy. 2016;46(11):679-683. DOI: 10.1016/j.ijpara.2016.06.003.

[19] Rangel GW. Empowering the Experimental Biology of Plasmodium Vivax Through Elucidating Requirements for Ex Vivo Culture. 2019. Doctoral Disertation, Harvard university, Graduare School of Arts and Sciences.

[20] Rangel GW, Clark MA, Kanjee U, Lim C, Shaw-Saliba K, Menezes MJ, Mascarenhas A, Chery L, Gomes E, Rathod PK, Ferreira MU, Duraisingh MT. Enhanced ex vivo plasmodium vivax Intraerythrocytic enrichment and maturation for rapid and sensitive parasite growth assays. Antimicrobial Agents Chemotherapy. 2018;62(4):e02519-e02517. DOI: 10.1128/AAC.02519-17. Print 2018 Apr.

[21] King CL, Adams JH, Xianli J, Grimberg BT, McHenry AM, Greenberg LJ, Siddiqui A, Howes RE, da Silva-Nunes M, Ferreira MU, Zimmerman PA. Fy(a)/Fy(b) antigen polymorphism in human erythrocyte Duffyantigen affects susceptibility to *Plasmodium vivax* malaria. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(50):20113-20118. DOI: 10.1073/pnas.1109621108

[22] Bermúdez M, Moreno-Pérez DA, Arévalo-Pinzón G, Curtidor H, Patarroyo MA. *Plasmodium vivax in vitro* continuous culture: The spoke in the wheel. Malaria Journal. 2018; 17:301. DOI: https://doi.org/10.1186/s12936-018-2456-5

[23] Mehlotra RK, Blankenship D, Howes RE, Rakotomanga TA,

Ramiranirina B, Ramboarina S, Franchard T, Linger MH, Zikursh-Blood M, Ratsimbasoa AC, Zimmerman PA, Grimberg BT. Long term in vitro culture of plasmodium vivax isolates from Madagascar maintained in *Saimiri boliviensis* blood. Malaria Journal. 2017;16:442. DOI: 10.1186/s12936-017-2090-7

[24] Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, Baliraine FN, Rosenthal PJ, D'Alessandro U. Quinine, an old anti-malarial drug in a modern world: Role in the treatment of malaria. Malaria journal. 2011;10:144. DOI: https://doi.org/10.1186/1475-2875-10-144

[25] Bunnag D, Karbwang J, Na-Bangchang K, Thanavibul A, Chittamas S, Harinasuta T. Quinine-tetracycline for multidrug resistant falciparum malaria. The Southeast Asian Journal of Tropical Medicine and Public Health. 1996;27:15-18. PMID: 9031393

[26] Da Silva AF, Benchimol F. Malaria and quinine resistance: A medical and scientific issue between Brazil and Germany, (1907-1919). Medical history. 2014:58(1):1-26. DOI: 10.1017/mdh.2013.69

[27] Peters W. Plasmodium: Resistance To Antimalarial Drugs. Annales de Parasitologie Humaine et Comparee. 1990; 65:603-606

[28] John GK, Douglas NM, von Seidlein L, Nosten F, Baird JK, White NJ, Price RN. Primaquine radical cure of *Plasmodium vivax*: A critical review of the literature. Malaria Journal. 2012; 11:280. DOI: 10.1186/1475-2875-11-280

[29] Hill DR, Baird JK, Parise ME, Lewis LS, Ryan ET, Magill AJ. Primaquine: Report from CDC expert meeting on malaria chemoprophylaxis I. The American Journal Tropical Medicine and Hygiene. 2006;75:402-415. PMID: 16968913

[30] World Health Organization: Single dose primaquine as a gametocytocide in Plasmodium falciparum malaria. Geneva, Switzerland: October 2012. Archived from the original on 2 January 2014

[31] Peters W. Mepacrine- and Primaquine-resistant strains of plasmodium berghei, Vincke and lips, 1948, 1964. Nature. 208:1290. DOI: https://doi.org/10.1038/208693a0

[32] Peters W. Chemotherapy and Drug Resistance in Malaria. 2nd ed. *Academic Press,* London, 1987.

[33] Howes RE, Piel FB, Patil AP, Nyangiri OA, Gething PW, Dewi M, Hogg MM, Battle KE, Padilla CD, Baird JK, Hay SI. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: A geostatistical model-based map. PLoS Medicine. 2012;9: e1001339. DOI: doi: 10.1371/journal.pmed.1001339

[34] World Health Organization: Guidelines for the treatment of malaria. 3rd ed. Geneva. 2015 (http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf. opens in new tab)

[35] Krotoski WA, 1980. Frequency of relapse and primaquine resistance in southeast Asian vivax malaria. The New England journal of medicine. 303: 587. DOI: 10.1056/NEJM198009043031022

[36] Rombo L, Edwards G, Ward SA, Eriksson G, Lindquist L, Lindberg A, Runehagen A, Bjorkman A, Hylander NO. Seven patients with relapses of *Plasmodium vivax* or *P. ovale* despite primaquine treatment. Tropical Medicine and Parasitology. 1987;38(1):49-50. PMID: 3299660

[37] Cabezos J, Duran E, Tomas D, Bada JL. Resistencia de *Plasmodium vivax* a Ia primaquina. Medicina Clinica. 1994; 103. PMID: 8072336

[38] Luzzi GA, Warrell DA, Barnes AJ, Dunbar EM. Treatment of primaquine-resistant plasmodium vivax malaria. Lancet. 1992; 340: 310. DOI: 10.1016/0140-6736(92)92404-4

[39] Charoenlarp P. Harinasuta T, 1973. Relapses of vivax malaria after a conventional course of primaquine and chloroquine: Report of 2 cases. The Southeast Asian Journal of Tropical Medicine and Public Health. 1973;4(1):135-137. PMID: 4577922

[40] Bunnag D, Karbwang J, Thanavibul A, Chittamas S. Ratana pongse Y, Chalermrut K, Bangshang KN, Harinasuta T. High dose of primaquine in primaquine resistant vivax malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1994;88: 2 18-219. DOI: 10.1016/0035-9203(94)90305-0

[41] Collins WE, Jeffrey GM. Primaquine resistance in *Plasmodium vivax*. The American Journal Tropical Medicine and Hygiene. 1996;55(3)341-349. DOI: 10.4269/ajtmh.1996.55.243

[42] Fernando D, Rodrigo C, Rajapakse S. Primaquine in vivax malaria: An update and review on management issues. Malaria Journal. 2011;10-15. DOI: 10.1186/1475-2875-10-351

[43] Baird JK. Therapeutic principles of primaquine against relapse of *Plasmodium vivax* malaria. IOP Conf. Series: Earth and Environmental Science 12132455(627081980) 012098. DOI: 10.1088/1755-1315/125/1/012098

[44] Dijanic C, Nickerson J, Shakya S, Dijanic A, Fabbri M. "Relapsing malaria: A case report of Primaquine resistance", *Case Reports in Infectious Diseases*, vol. 2018, Article ID 9720823, 3 Pages. DOI: https://doi.org/10.1155/2018/9720823

[45] Bennett JW, Pybus BS, Yadava A, Tosh A, Jason RN, Sousa C, McCarthy WF, Deye G, Melendez V, Ockenhouse CF. Primaquine failure and cytochrome P-450 2D6 in *Plasmodium vivax* malaria. The New England Journal of Medicine. 2013;369(14):1381-1382. DOI: 10.1056/NEJMc1301936

[46] Baird JK, Louisa M, Noviyanti R, Ekawati L, Elyazar I, Subekti D, Chand K, Gayatri A, Instiaty, Soebianto S, Crenna-Darusallam C, Djoko D, Hasto BD, Meriyenes D, Wesche D, Nelwan EJ, Sutanto I, Sudoyo H, Setiabudy R. Association of Impaired Cytochrome P450 2D6 Activity Genotype and Phenotype With Therapeutic Efficacy of Primaquine Treatment for Latent Plasmodium vivax Malaria. Journal of the American Medical Association Network Open. 2018;1(4):e181449. DOI: 10.1001/jamanetworkopen.2018.1449

[47] Peters W. The evolution of tafenoquine--antimalarial for a new millennium?". Journal of the Royal Society of Medicine. 1999; 92(7):345-352. DOI: 10.1177/014107689909200705

[48] Haston JC, Hwang J, Tan KR. Guidance for Using Tafenoquine for Prevention and Antirelapse Therapy for Malaria — United States. "Morbidity and Mortality Weekly Report. 2019;68 (46): 1062-1068. DOI: doi:10.15585/mmwr.mm6846a4

[49] Quinn JC, McCarthy S. Tafenoquine versus Primaquine to prevent relapse of *Plasmodium vivax* malaria. The New England journal of medicine. 2019 May 9;380(19):1875. DOI: 10.1056/NEJMc1902327

[50] Homewood CA, Warhurst DC, Peters W, Baggaley VC. Lysosomes, pH and the anti-malarial action of chloroquine. Nature. 1972; 235: 50-52. DOI: 10.1038/235050a0

[51] Yayon A, Cabantchik ZI, Ginsburg H. Identification of the acidic compartment of *Plasmodium*

falciparum-infected human erythrocytes as the target of the antimalarial drug chloroquine. European Molecular Biology Organization. 1984;3: 2695-2700. PMCID: PMC557751

[52] Bray PG, Janneh O, Raynes KJ, Mungthin M, Ginsburg H, Ward SA. Cellular uptake of chloroquine is dependent on binding to ferriprotoporphyrin IX and is independent of NHE activity in *Plasmodium falciparum*. Journal of Cell Biology. 1999;145: 363-376. DOI: 10.1083/jcb.145.2.363

[53] Bray PG, Mungthin M, Ridley RG, Ward SA. Access to hematin: The basis of chloroquine resistance. Molecular pharmacology. 1998;54: 170-179. DOI: https://doi.org/10.1124/mol.54.1.170

[54] Ridley RG. Malaria: Dissecting chloroquine resistance. 1998. Current Biology. 1998; 8:8346-8349. DOI: 10.1016/s0960-9822(98)70218-0

[55] Peters W. Resistance in human malaria IV: 4-aminoquinolines and multiple resistance. In: Chemotherapy and Drug Resistance in Malaria. Vol 2. London: Academic Press, 1987:659-786.

[56] Payne D. Spread of chloroquine resistance in *Plasmodium falciparum*. Parasitology Today. 1987;3:241-246. DOI: 10.1016/0169-4758(87)90147-5

[57] Rieckmann KH, Davis DR, Hutton DC. *Plasmodium vivax* resistance to chloroquine? Lancet. 1989;2:1183-1184. DOI: 10.1016/s0140-6736(89)91792-3

[58] Baird JK, Basri H, Purnomo Bangs MJ, Subianto B, Patchen LC. Resistance to chloroquine by *Plasmodium vivax* in Irian Jaya, Indonesia. The American Journal of Tropical Medicine and Hygiene. 1991;44:547-552. DOI: 10.4269/ajtmh.1991.44.547

[59] Phillips EJ, Keystone JS, Kain KC 1996. Failure of combined chloro- quine and high-dose primaquine therapy for *Plasmodium vivax* malaria acquired in Guyana, South America. Clin Infect Dis *23*: 1171-1173.

[60] Price RN, Seidlein LV, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant *Plasmodium vivax*: A systematic review and meta-analysis. Lancet Infectious Diseases. 2014;14(10):982-991. DOI: 10.1016/S1473-3099(14)70855-2

[61] Asih PBS, Syafruddin D, Leake J, Sorontou Y, Sadikin M, Sauerwein RW, Vinetz J, Baird JK. Phenotyping clinical resistance to chloroquine in *Plasmodium vivax* in northeastern Papua, Indonesia. International Journal for Parasitology: Drugs and Drug Resistance, 2011;1:28-32. DOI: 10.1016/j.ijpddr.2011.08.001

[62] Suwanarusk R, Chavchich M, Russell B, Jaidee A, Chalfein F, Barends M, et al. Amplification of pvmdr1 associated with multidrug-resistant Plasmodium vivax. J Infect Dis. 2008; 198(10):1558-64. https://doi.org/10.1086/592451 PMID: 18808339; PubMed Central PMCID: PMC4337975

[63] Baird JK, Valecha N, Duparc S, White NJ and Price RN. Diagnosis and treatment of plasmodium vivax malaria. Am. J. Trop. Med. Hyg., 95(Suppl 6), 2016, pp. 35-51 doi:10.4269/ajtmh.16-0171

[64] Ferreira MU, de Sousa TN, Rangel GW, Johansen IC, Corder RM, Ladeia-Andrade S, Gilf JP. Monitoring plasmodium vivax resistance to antimalarials: Persisting challenges and future directions. International Journal Parasitology Drugs and Drug Resistance. 2021;15:9-24.DOI: 10.1016/j.ijpddr.2020.12.001

[65] Price RN, Simpson JA, Nosten F, Luxemburger C, Hkirjaroen L, ter Kuile F, Chongsuphajaisiddhi T,

White NJ. Factors contributing to anemia after uncomplicated falciparum malaria. The American Journal of Tropical Medicine and Hygiene. 2001; 65(5): 614-22. DOI: https://doi.org/10.4269/ajtmh.2001.65.614

[66] Cowman AF, Morry MJ, Biggs BA, Cross GA, Foote SJ. Amino acid changes linked to pyrimethamine resistance in the dihydrofolate reductase-thymidylate synthase gene of *Plasmodium falciparum*. Proceedings of the National Academy of Sciences of the United States of America. 1988;85:9109-9113. DOI: 10.1073/pnas.85.23.9109.

[67] Peterson DS, Walliker D, Wellems TE. Evidence that a point mutation in dihydrofolate reductase-thymidylate synthase confers resistance to pyrimethamine in falciparum malaria. Proceedings of the National Academy of Sciences of the United States of America. 1988;85: 9114-9118. DOI: 10.1073/pnas.85.23.9114

[68] Foote SJ, Galatis D, Cowman AF. Amino acids in the dihydrofolate reductase-thymidylate synthase gene of *Plasmodium falciparum* involved in cycloguanil resistance differ from those involved in pyrimethamine resistance. Proceedings of the National Academy of Sciences of the United States of America. 1990;87: 3014-3017. DOI: 10.1073/pnas.87.8.3014

[69] Amimo F, Lambert B, Magit A, Sacarlal J, Hashizume M. Shibuya K. Plasmodium falciparum resistance to Sulfadoxine-Pyrimethamine in Africa: A systematic analysis of national trend. British Medical Journal Global Health. 2020;5(11):e003217. DOI: 10.1136/bmjgh-2020-003217

[70] Doberstyn EB, Teerakiartkamjorn C, Andre RG, Phintuyothin P and Noeypatimanondh. Treatment of vivax malaria with sulfadoxine-pyrimethamine and with pyrimethamine alone. Transactions of the Royal Society of Tropical Medicine and Hygiene, 1979; 73, 1

[71] Asih,PBS, Marantina SS, Nababan R, Lobo NF, Rozi, mIR, Sumarto W, Dewi RM, Sekartuti, Taufik AS, Mulyanto, Sauerwein RS and Din Syafruddin. Distribution of Plasmodium vivax pvdhfr and pvdhps alleles and their association with sulfadoxine–pyrimethamine treatment outcomes in Indonesia. Malar J 2015;14:365 DOI 10.1186/s12936-015-0903-0

[72] Nakato H, Vivancos R, Hunter PR. A systematic review and meta-analysis of the effectiveness and safety of atovaquone–proguanil (Malarone) for chemoprophylaxis against malaria. Journal of Antimicrobial Chemotherapy. 2007;60(5):929-936. DOI: 10.1093/jac/dkm337

[73] Syafruddin, Siregar JE, Marzuki S. Mutations in the *cytochrome b* gene of *Plasmodium berghei* conferring resistance to atovaquone. Molecular and Biochemical Parasitology. 1999;104:185-194. DOI: 10.1016/s0166-6851(99)00148-6

[74] Korsinczky M, Chen N, Kotecka B, Saul A, Rieckmann K, Cheng Q. Mutations in *Plasmodium falciparum* cytochrome b that are associated with atovaquone resistance are located at a putative drug-binding site. Antimicrobial Agents and Chemotherapy, 2000;44(8):2100-2108. DOI: 10.1128/aac.44.8.2100-2108

[75] Tu Y. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nature Medicine. 2011;17(10):1217-1220. DOI: 10.1038/nm.2471

[76] Mok S, Ashley EA, Ferreira PE, Zhu L, Lin Z, Yeo T, Chotivanich K, Imwong M, Pukrittayakamee S, Dhorda M, Nguon C, Lim P, Amaratunga C, Suon S, Hien TT, Htut Y,

Faiz MA, Onyamboko MA, Mayxay M, Newton PN, Tripura R, Woodrow CJ, Miotto O, Kwiatkowski DP, Nosten F, Day NPJ, Preiser PR, White NJ, Dondorp AM, Fairhurst RM, Bozdech B. Drug resistance: Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science. 2015 ;347(6220):431-435. DOI: 10.1126/ science.1260403

[77] Nosten F, Luxemburger C, ter Kuile FO, Woodrow C, Eh JP, Chongsuphajaisiddhi T, White NJ. Treatment Of Multidrug-Resistant *Plasmodium falcipa*rum Malaria With 3-Day Artesunate-Mefloquine Combination. The Journal of Infectious Diseases. 1994;170(4):971-977. DOI: https: //doi.org/10.1093/infdis/170.4.971

[78] Eastman RT, Fidock DA. Artemisinin-based combination therapies: A vital tool in efforts to eliminate malaria. Nature Reviews Microbiology. 2009;7(12):864-874. DOI: 10.1038/nrmicro2239

[79] Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM, Artemisinin resistance in Cambodia 1 (ARC1) study consortium. Evidence of artemisinin-resistant malaria in western Cambodia. The New England Journal of Medicine. 2008;359(24):2619-2620. DOI: 10.1056/NEJMc0805011

[80] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut S, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Pratap Singhasivanon, Nicholas P J Day, Niklas Lindegardh, Socheat D, White NJ. Artemisinin-resistant plasmodium falciparum malaria. The New England Journal of Medicine. 2009;361(5):455-467. DOI: 10.1056/ NEJMoa0808859.

[81] Phyo AP, Nkhoma S, Stepniewska S, Ashley EA, Nair S, McGready R, Moo CL, Al-Saai S, Dondorp AM, Lwin KM, Singhasivanon P, Day NPJ, White NJ, Anderson TJM, Nosten F. Emergence of artemisinin-resistant malaria on the western border of Thailand: A longitudinal study. Lancet. 2012 ;379(9830):1960-1966. DOI: 10.1016/S0140-6736(12)60484-X

[82] Hien TT, Thuy-Nhien NY, Phu NH, Boni MF, Thanh NV, Nha-Ca NT, Thai LH, Thai LQ, Toi PV, Thuan PD, Long LT, Dong LT, Merson L, Dolecek C, Stepniewska K, Ringwald P, White NJ, Farrar J, Wolbers M. *In vivo* susceptibility of *Plasmodium falciparum* to artesunate in Binh Phuoc Province Vietnam. Malaria journal. 2012;11:355. DOI: https://doi. org/10.1186/1475-2875-11-355

[83] Kyaw MP, Nyunt MH, Chit K, Aye MM, Aye KH, Aye MM, Lindegardh N, Tarning J, Imwong M, Jacob CG, Rasmussen C, Perin J, Ringwald P, Nyunt MM. Reduced susceptibility of *Plasmodium falciparum* to artesunate in southern Myanmar. PLoS ONE. 2013;8(3):e57689. DOI: 10.1371/journal.pone.0057689

[84] Chotivanich K, Udomsangpetch R, Dondorp A, Williams T, Angus B, Simpson JA, Pukrittayakamee S, Looareesuwan S, Newbold CI, White NJ. The mechanisms of parasite clearance after antimalarial treatment of *Plasmodium falciparum* malaria. Journal Infectious Diseases. 2000; 182(2):629-33. DOI: https://doi. org/10.1086/315718

[85] Buffet PA, Milon G, Brousse V, Correas JM, Dousset B, Couvelard A, Kianmanesh R, Farges O, Sauvanet A, Paye F, Ungeheuer MN, Ottone C, Khun H, Fiette L, Guigon G, Huerre M, Mercereau-Puijalon O, David PH. *Ex vivo* perfusion of human spleens maintains clearing and processing functions. Blood. 2006;107(9):3745-3752. DOI: 10.1182/ blood-2005-10-4094

[86] Commons RJ, Simpson JA, Thriemer K, Abreha T, Adam I, Anstey NM, Assefa A, Awab GR, Baird JK, Barber BE, Chu CS, Dahal P, Daher A, Davis TME, Dondorp AM, Grigg MJ, Humphreys GS, Hwang J, Karunajeewa H, Laman M, Lidia K, Moore BR, Mueller I, Nosten F, Pasaribu AP, Pereira DB, Phyo AP, Poespoprodjo JR, Sibley CH, Stepniewska K, Sutanto I, Thwaites G, Hien TT, White NJ, William T, Woodrow CJ, Guerin PJ, Price RN. The efficacy of dihydroartemisinin-piperaquine and artemether-lumefantrine with and without primaquine on *Plasmodium vivax* recurrence: A systematic review and individual patient data meta-analysis. PLoS Medicine. 2019; 16(10): e1002928. DOI: https://doi.org/10.1371/journal.pmed.1002928

[87] Ratcliff A, Siswantoro H, Kenangalem E, Maristela R, Wuwung RM, Laihad F, Ebsworth EP, Anstey NM, Tjitra E, Price RN. Two fixed-dose artemisinin combinations for drug-resistant falciparum and vivax malaria in Papua, Indonesia: an open-label randomised comparison. Lancet. 2007; 369(9563): 757-765. DOI: https://doi.org/10.1016/S0140-6736(07)60160-3

[88] Hasugian AR, Purba HL, Kenangalem E, Wuwung RM, Ebsworth EP, Maristela R, Penttinen PMP, Laihad F, Anstey NM, Tjitra E, Price RN. Dihydroartemisinin-piperaquine versus artesunate-amodiaquine: superior efficacy and posttreatment prophylaxis against multidrug-resistant *Plasmodium falciparum* and *Plasmodium vivax* malaria. Clinical Infectious Diseases. 2007; 44(8): 1067-74. DOI: https://doi.org/10.1086/512677

[89] Smithuis F, Kyaw MK, Phe O, Win T, Aung PP, Oo APP, Naing AL, Nyo MY, Myint NZH, Imwong M, Ashley E, Lee SJ, White NJ. Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: An open-label randomised trial. Lancet Infectious Diseases. 2010; 10(10): 673-81. DOI: https://doi.org/10.1016/S1473-3099(10)70187-0

[90] Gogtay N, Kannan S, Thatte UM, Olliaro PL, Sinclair D. Artemisinin-based combination therapy for treating uncomplicated *Plasmodium vivax* malaria. Cochrane Database Systematic Review. 2013;10 CD008492. DOI: 10.1002/14651858.CD008492

[91] Miotto O, Sekihara M, Tachibana SI, Yamauchi M, Pearson RD, Amato R, Gonçalves S, Mehra S, Noviyanti R, Marfurt J, Auburn S, Price RN, Mueller I, Ikeda M, Mori T, Hirai M, Tavul L, Hetzel MW, Laman M, Barry AE, Ringwald P, Ohashi J, Hombhanje F, Kwiatkowski DP, Mita T. Emergence of artemisinin-resistant *Plasmodium falciparum* with kelch13 C580Y mutations on the island of New Guinea. PLoS Pathogen. 2020;16(12):e1009133. DOI: 10.1371/journal.ppat.1009133

[92] Asih PBS, Dewi RM, Tuti S, Sadikin M, Sumarto W, Sinaga BN, van der Ven AJAM, Sauerwein RW, Syafruddin D. Efficacy of artemisinin-based combination therapy for treatment of persons with uncomplicated *Plasmodium falciparum* malaria in west Sumba District, East Nusa Tenggara Province, Indonesia, and genotypic profiles of the parasite. The American Journal of Tropical Medicine and Hygiene. 2009;80(6):914-918. PMID: 19478248

[93] Syafruddin D. Evaluation of the parasite clearance day following treatment with artesunate-amodiaquine in subjects uncomplicated *Plasmodium falciparum* malaria in Indonesia. 2012. Technical Report TES Indonesia

[94] Syafruddin D. Efficacy and safety of dihydroartemisinin-piperaquine for the treatment of uncomplicated *Plasmodium falciparum* and *Plasmodium vivax* malaria in 5 sentinel sites in Indonesia . 2016. Technical Report TES Indonesia

[95] Poespoprodjo JR, Kenangalem E, Wafom J, Chandrawati F, Puspitasari AM, Ley B, Trianty L, Korten Z, Surya A, Syafruddin D, Anstey NM, Marfurt J, Noviyanti R, Price RN. Therapeutic Response to Dihydroartemisinin-Piperaquine for *P. falciparum* and *P. vivax* Nine Years after Its Introduction in Southern Papua, Indonesia. The American Society of Tropical Medicine and Hygiene. 2018; 98(3):677-82. DOI: https://doi.org/10.4269/ajtmh.17-0662

[96] Asih PB, Rozi IE, Dewayanti FK, Wangsamuda S, Zulfah S, Robaha M, Hutahaean J, Anggraeni ND, Kusumaningsih M, Mulyani PS, Sariwati E, Basri HH, Bustos MDG, Syafruddin D. fficacy and safety of dihydroartemisinin-piperaquine for the treatment of uncomplicated *Plasmodium falciparum* and *Plasmodium vivax* malaria in Northern Papua and Jambi, Indonesia. 2020; medRXiv. DOI: https://doi.org/10.1101/2020.09.04.20188706

[97] Peterson DS, Milhous WK, Wellems TE. Molecular basis of differential resistance to cycloguanil and pyrimethamine in *Plasmodium falciparum* malaria. Proceedings of the National Academy of Sciences of the United States of America 1990;87: 3018-3022. DOI: 10.1073/pnas.87.8.3018

[98] Triglia T, Wang P, Sims PF, Hyde JE, Cowman AF, 1998. Allelic exchange at the endogenous genomic locus in *Plasmodium falciparum* proves the role of dihydropteroate synthase in sulfadoxine-resistant malaria. European Molecular Biology Organization Journal. 17: 3807-3815. DOI: 10.1093/emboj/17.14.3807

[99] Triglia T, Cowman AF. The mechanism of resistance to sulfa drugs in *Plasmodium falciparum*. Drug Resistance Update. 1999;2: 15-19. DOI: doi: 10.1054/drup.1998.0060

[100] Foote SJ, Thompson JK, Cowman AF, Kemp DJ. Amplification of the multidrug resistance gene in some chloroquine-resistant isolates of *P. falciparum*. Cell 1989;57:921-931. DOI: 10.1128/mcb.11.10.5244

[101] Foote SJ, Kyle DE, Martin RK, Oduola AMJ, Forsyth K, KempDJ, Cowman AF. Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*. Nature 1990;345:255-258. DOI: 10.1038/345255a0

[102] Cowman AF, Karcz S, Galatis D, Culvenor JG. A P-glycoprotein homologue of *Plasmodium falciparum* is localized on the digestive vacuole. J Cell Biol 1991;113:1033-1042. DOI: 10.1083/jcb.113.5.1033

[103] Ekong R, Robson KJH, Baker DA, Warhurst DC. Transcripts of the multidrug resistance genes in chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*. Parasitology 1993;106:107-115. DOI: 10.1017/s0031182000074904

[104] Peel SA, Bright P, Yount B, Handy J, Baric RS. A strong association between mefloquine and halofantrine resistance and amplification, overexpression, and mutation in the P-glycoprotein gene homologue (pfmdr) of *Plasmodium falciparum* in vitro. Am J Trop Med Hyg 1994;51:648-658. DOI: 10.4269/ajtmh.1994.51.648

[105] Barnes DA, Foote SJ, Galatis D, Kemp DJ, Cowman AF. Selection for high-level chloroquine resistance results indeamplification of thepfmdr1gene and increased sensitivityto mefloquine in *Plasmodium falciparum*. EMBO J 1992;11:3067-3075. PMID: 1353446:

[106] Cowman AF, Galatis D, Thompson JK. Selection for mefloquine resistance in *Plasmodium falciparum* is linked to amplification of the pfmdr1 gene and cross-resistance to halofantrine and quinine. Proc Natl Acad Sci USA 1994;91:1143-1147. DOI: 10.1073/pnas.91.3.1143

[107] Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, Ursos LM, Sidhu AB, Naude B, Deitsch KW, Su XZ, Wootton JC, Roepe PD, Wellems TE. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Molecular Cell. 2000; 6: 861-871. DOI: 10.1016/s1097-2765(05)00077-8

[108] Sidhu AB, Verdier-Pinard D, Fidock DA, 2002. Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by *pfcrt* mutations. Science. 2002;298: 210-213. DOI: 10.1126/science.1074045

[109] Nomura T, Carlton JM, Baird JK, del Portillo HA, Fryauff DJ, Rathore D, Fidock DA, Su X, Collins WE, McCutchan TF, Wootton JC, Wellems TE. Evidence for different mechanisms of chloroquine resistance in 2 plasmodium species that cause human malaria. The Journal of infectious diseases. 2001;183:1653-1661. DOI: 10.1086/320707

[110] Asih PBS, Sadikin M, Baird JK, Leake J, Sorontou Y, Sauerwein RW, Vinetz J, and, Syafruddin D. Polymorphisms of Pvmdr1 gene associated with Chloroquine Resistance Phenotype among *Plasmodium vivax* isolates in Indonesia. Proceeding International Conges for Parasitology. 2010;199- 204

[111] Melo GC, Monteiro WM, Siqueira AM, Silva SR, Magalhaes BM, Alencar AC, Kuehn A, del Portillo HA, Fernandez-Becerra C, Lacerda MVG. Expression levels of *pvcrt-o* and *pvmdr-1* are associated with chloroquine resistance and severe *Plasmodium vivax* malaria in patients of the Brazilian Amazon. PLoS ONE. 2014;9:e105922. DOI: 10.1371/journal.pone.0105922

[112] Lu F, Lim CS, Nam DH, Kim K, Lin K, Kim TS, Lee HW, Chen JH, Wang Y, Sattabongkot J, Han ET. Genetic polymorphism in *pvmdr1* and *pvcrt-o* genes in relation to *in vitro* drug susceptibility of *Plasmodium vivax* isolates from malaria-endemic countries. Acta Tropica. 2011;117:69-75. DOI: 10.1016/j.actatropica.2010.08.011

[113] Imwong M, Pukrittayakamee S, Pongtavornpinyo W, Nakeesathit S, Nair S, Newton P, Nosten F, AndersonTJC, Dondorp a, Day NPJ, White NJ. Gene amplification of the multidrug resistance 1 gene of *Plasmodium vivax* isolates from Thailand, Laos, and Myanmar. Antimicrobial Agents and Chemotherapy. 2008;52:2657-2659. DOI: 10.1128/AAC.01459-07

[114] Silva SR, Almeida ACG, da Silva GAV, Ramasawmy R, Lopes SCV, Siqueira AM, Costa GL, Sousa TN, Vieira JLF, Lacerda MVG, Monteiro WM, de Melo GC. Chloroquine resistance is associated to multi-copy pvcrt-o gene in *Plasmodium vivax* malaria in the Brazilian Amazon. Malaria Journal. 2018;17:267. DOI: 10.1186/s12936-018-2411-5

[115] Sá JM, Kaslow SR, Barro RRM, Brazeau NF, Parobek CM, Tao D, Salzman RE, Gibson TJ, Velmurugan S, Krause MA, Melendez-Muniz V, Kite WA, Han PK, Eastman RT, Kim A, Kessler EG, Abebe Y, James ER, Chakravarty S, Orr-Gonzalez S, Lambert LE, Engels T, Thomas ML, Fasinu PS, Serre D, Gwadz RW, Walker L, DeConti DK, Mu J, Bailey JA, Sim BKL, Hoffman S, Fay MP, Dinglasan RR, Juliano JJ, Wellems TE. *Plasmodium vivax* chloroquine

resistance links to pvcrt transcription in a genetic cross. Nature Communication. 2019;10:4300. DOI: https://doi.org/10.1038/s41467-019-1225

[116] Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, Kim S, Duru V, Bouchier M, Ma L, Lim P, Leang R, Duong S, Sreng S, Suon S, Chuor CM, Bout DM, Ménard S, Rogers WO, Genton B, Fandeur T, Miotto O, Ringwald P, Bras JL, Berry L, Barale JC, Fairhurst RM, Benoit-Vical F, Mercereau-Puijalon O, Ménard D. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;505(7481):50-55. DOI: 10.1038/nature12876

[117] Takala-Harrison S, Clark TG, Jacob CG, Cummings MP, Miotto O, Dondorp AM, Fukuda MM, Nosten F, Noedl H, Imwong M, Bethell D, Se Y, Lon C, Tyner SD, Saunders DL, Socheat D, Ariey F, Phyo AP, Starzengruber P, Fuehrer HP, Swoboda P, Stepniewska K, Flegg J, Arze C, Cerqueira GC, Silva JC, Ricklefs SM, Porcella SF, Stephens RM, Adams M, Kenefic LJ, Campino S, Auburn S, MacInnis B, Kwiatkowski DP, Su X, White NJ, Ringwald P, Plowe CV. Genetic loci associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment in Southeast Asia. Proceedings of the National Academy of Sciences of the United States of America. 2013;110:240-5. DOI: 10.1073/pnas.1211205110

[118] Miotto O, Amato R, Ashley EA, MacInnis B, Almagro-Garcia J, Amaratunga C, Lim P, Mead D, Oyola SO, Dhorda M, Imwong M, Woodrow C, Manske M, Stalker J, Drury E, Campino S, Amenga-Etego L, Thanh TN, Tran HT, Ringwald P, Bethell D, Nosten F, Phyo AP, Pukrittayakamee S, Chotivanich K, Chuor CM, Nguon C, Suon S, Sreng S, Newton PN, Mayxay N, Khanthavong M, Hongvanthong B, Htut Y, Han KT, Kyaw MP, Faiz MA, Fanello CI, Onyamboko M, Mokuolu OA, Jacob CG, Takala-Harrison S, Plowe CV, Day NP, Dondorp AM, Spencer CAC, McVean G, Fairhurst RM, White NJ, Kwiatkowski DP. Genetic architecture of artemisinin-resistant *Plasmodium falciparum*. Nature Genetics. 2015;47:226-34. DOI: doi: 10.1038/ng.3189

[119] Siddiqui G, Srivastava A, Russell AS, Creek DJ. Multi-omics based identification of specific biochemical changes associated with PfKelch13-mutant artemisinin-resistant *Plasmodium falciparum*. J Infect Dis. 2017; 215(9):1435-1444. doi: 10.1093/infdis/jix156

[120] Mbengue A, Bhattacharjee S, Pandharkar T, Liu H, Estiu G, Stahelin RV, Rizk SS, Njimoh DL, Ryan Y, Chotivanich K, Nguon C, Ghorbal M, Lopez-Rubio J, Pfrender M, Emrich S, Mohandas N, Dondorp A, Wiest O, Haldar K. A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria. Nature. 2015; 520(7549):683-687. doi: 10.1038/nature14412

[121] Tyagi RK, Gleeson PI, Pérignon JL, Olliaro P, Arnold L, Tahar R, Prieur E, Decorsterd L, Pengnon JL, Druilhe P. High-level artemisinin-resistance with quinine co-resistance emerges in *P. falciparum* malaria under in vivo artesunate pressure. BMC Medicine (2018) 16:181 https://doi.org/10.1186/s12916-018-1156-x

[122] Hossain MS, Commons RJ, Douglas NM, Thriemer K, Alemayehu BH, Amaratunga C, Anvikar AR, Ashley EA, Asih PBS, Carrara VI, Lon C D'Alessandro U, Davis TME, Dondorp AM, Edstein MD, Fairhurst RM, Ferreira MU, Hwang J, Janssens B, Karunajeewa H, Kiechel JR, Ladeia-Andrade S, Laman M, Mayxay M, McGready R, Moore BR, Mueller I, Newton PN, Thuy-Nhien NT,

Noedl H, Nosten F, Phyo AP, Poespoprodjo JR, Saunders DL, Smithuis F, Spring MD, Stepniewska K, Suon S, Suputtamongkol Y, Syafruddin D, Tran HT, Valecha N, Herp MV, Vugt MV, White NJ, Guerin PJ, Simpson JA, Price RN. The risk of *Plasmodium vivax* parasitaemia after *P. falciparum* malaria: An individual patient data meta-analysis from the World Wid6 Antimalarial Resistance Network. 2020. PloS Medicine 2020;17(11):e1003393. DOI: 10.1371/journal.pmed.1003393

[123] Li J, Zhang J, Li Q, Hu Y, Ruan Y, Tao Z, Xia H, Qiao J, Meng L, Zeng W, Li C, He X, Zhao L, Siddiqui FA, Miao J, Yang Z, Fang Q, Cui L. Ex vivo susceptibilities of *Plasmodium vivax* isolates from the China-Myanmar border to antimalarial drugs and association with polymorphisms in Pvmdr1 and Pvcrt-o genes. PLoS Neglected Tropical Diseases. 2020;14(6):e0008255. DOI: 10.1371/journal.pntd.0008255.

[124] Brashear AM, Fan Q, Hu Y, Li Y, Zhao Y, Wang Z, Cao Y, Miao J, Barry A, Cui L. Population genomics identifies a distinct *Plasmodium vivax* population on the China-Myanmar border of Southeast Asia. PLoS Neglected Tropical Diseases. 2020;14(8):e0008506. DOI: 10.1371/journal.pntd.0008506.

[125] Wang M, Siddiqui FA, Qi Fan, Luo E, Cao Y, Cui L. Limited genetic diversity in the PvK12 Kelch protein in *Plasmodium vivax* isolates from Southeast Asia. Malaria Journal. 2016;15:537. DOI 10.1186/s12936-016-1583-0.