

REVIEW

Open Access



# How has mass drug administration with dihydroartemisinin-piperaquine impacted molecular markers of drug resistance? A systematic review

Sophie Moss<sup>1\*</sup> , Emilia Mańko<sup>1</sup>, Sanjeev Krishna<sup>2</sup>, Susana Campino<sup>1†</sup>, Taane G. Clark<sup>1,3†</sup> and Anna Last<sup>1\*†</sup>

## Abstract

The World Health Organization (WHO) recommends surveillance of molecular markers of resistance to anti-malarial drugs. This is particularly important in the case of mass drug administration (MDA), which is endorsed by the WHO in some settings to combat malaria. Dihydroartemisinin-piperaquine (DHA-PPQ) is an artemisinin-based combination therapy which has been used in MDA. This review analyses the impact of MDA with DHA-PPQ on the evolution of molecular markers of drug resistance. The review is split into two parts. Section I reviews the current evidence for different molecular markers of resistance to DHA-PPQ. This includes an overview of the prevalence of these molecular markers in *Plasmodium falciparum* Whole Genome Sequence data from the MalariaGEN Pf3k project. Section II is a systematic literature review of the impact that MDA with DHA-PPQ has had on the evolution of molecular markers of resistance. This systematic review followed PRISMA guidelines. This review found that despite being a recognised surveillance tool by the WHO, the surveillance of molecular markers of resistance following MDA with DHA-PPQ was not commonly performed. Of the total 96 papers screened for eligibility in this review, only 20 analysed molecular markers of drug resistance. The molecular markers published were also not standardized. Overall, this warrants greater reporting of molecular marker prevalence following MDA implementation. This should include putative *pfcrt* mutations which have been found to convey resistance to DHA-PPQ in vitro.

**Keywords:** Antimalarial resistance, Dihydroartemisinin-piperaquine, Mass drug administration, Molecular markers

## Background

The evolution of anti-malarial drug resistance presents an alarming threat to eliminating malaria; a disease which causes over 500,000 deaths every year [1]. Malaria is caused by the protozoan parasite *Plasmodium*, with most fatal cases caused by *Plasmodium falciparum* [1]. The role of Mass Drug Administration (MDA) in the

evolution of anti-malarial resistance is not well understood. MDA is defined by the WHO as the mass treatment of all, or a section of, the population, whether or not symptoms are present [2]. Historical MDA practices, such as the addition of the anti-malarial chloroquine to table salt, have been correlated with a subsequent rise in chloroquine resistance [3]. However, there is a lack of evidence linking more recent use of MDA with the evolution of anti-malarial resistance in *P. falciparum*. This review is focused on dihydroartemisinin-piperaquine (DHA-PPQ); an increasingly used artemisinin-combination therapy (ACT) in MDA for malaria. The first section of this review details current evidence on the molecular

<sup>†</sup>Susana Campino, Taane G. Clark and Anna Last are joint authors

\*Correspondence: [sophie.moss@lshtm.ac.uk](mailto:sophie.moss@lshtm.ac.uk); [anna.last@lshtm.ac.uk](mailto:anna.last@lshtm.ac.uk)

<sup>1</sup> Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

Full list of author information is available at the end of the article



© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

mechanisms behind resistance of *P. falciparum* to DHA-PPQ. This section also includes a comprehensive overview of the prevalence of molecular markers associated with DHA-PPQ resistance globally, using Whole Genome Sequence data from the MalariaGEN Pf3k project. The second section of this review systematically evaluates the impact of MDA with DHA-PPQ on the evolution of anti-malarial resistance. The authors systematically reviewed the evidence from the available literature reporting molecular markers of anti-malarial resistance following MDA with DHA-PPQ.

**Section I: the evolution of drug resistance**

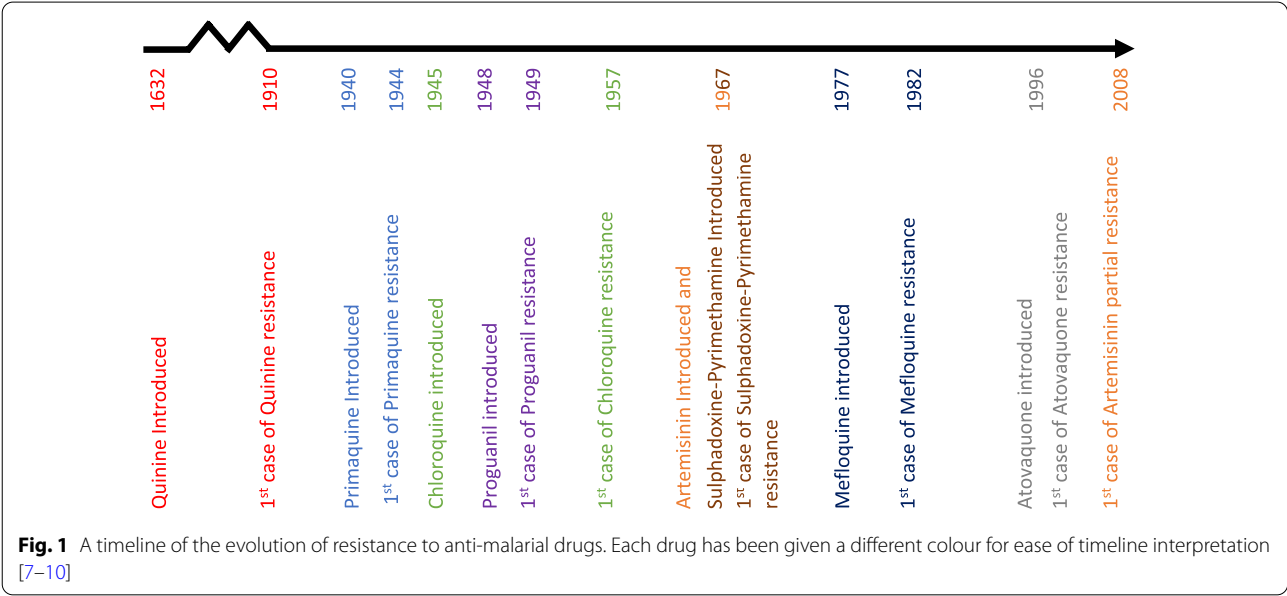
Anti-malarial drugs can be grouped into broad classes (Table 1). Widespread resistance to monotherapies led the World Health Organization (WHO) to recommend artemisinin-based combination therapy (ACT) as

first-line treatment in all malaria endemic countries for uncomplicated malaria, with artesunate recommended to treat severe cases [4]. ACT includes a combination therapy of an artemisinin derivative (artesunate, artemether or dihydroartemisinin) with a partner drug (either lumefantrine, amodiaquine, piperaquine, mefloquine or sulfadoxine-pyrimethamine) [4]. The most common artemisinin-based combinations used in Africa are artemether-lumefantrine (AL), artesunate-amodiaquine (AS-AQ) and dihydroartemisinin-piperaquine DHA-PPQ [4]. All currently have a high clinical efficacy in Africa, achieving 98%, 98.4% and 99.4%, respectively [4].

Resistance to many anti-malarial drugs has now evolved, but the speed at which resistance has emerged has differed depending on the drug (Fig. 1). Understanding the molecular mechanisms behind the evolution of resistance in these different drugs is crucial in understanding why resistance has evolved at different rates. Furthermore, the methods used for detecting and classifying anti-malarial resistance have changed over time [5]. In addition to in vivo, in vitro and ex vivo methods, molecular methods for detecting resistance have now been developed. This includes identifying genomic polymorphisms in the malaria parasite genome which are associated with resistance to anti-malarial drugs [5]. Historically, anti-malarial drug resistance has often spread from Southeast Asia to Africa, so monitoring molecular markers of resistance in different continents may enable the scientific community to pre-empt the spread of drug resistant malaria in Africa [6], depending on the mechanisms of resistance.

**Table 1** Anti-malarial drugs can be broadly grouped into different classes [27]

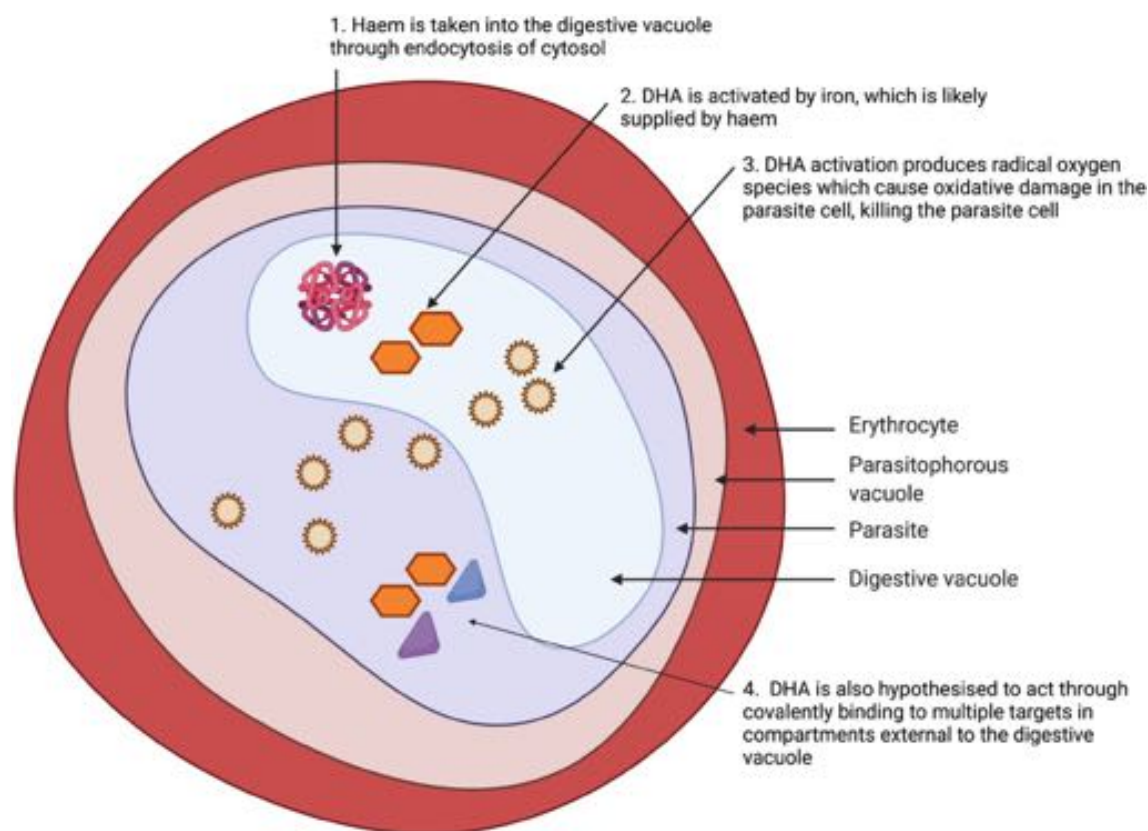
| Class             | Antimalarial drugs                                                                                 |
|-------------------|----------------------------------------------------------------------------------------------------|
| 4-Aminoquinolines | Chloroquine, amodiaquine, piperaquine                                                              |
| 8-Aminoquinolines | Primaquine, tafenoquine                                                                            |
| Antifolates       | Pyrimethamine, sulphadoxine, proguanil                                                             |
| Amino-alcohols    | Lumefantrine, mefloquine, quinine                                                                  |
| Mannich base      | Pyronaridine, naphthoquinone, atovaquone                                                           |
| Sesquiterpenes    | Artemisinin and its derivatives; dihydroartemisinin, artemether, artesunate, arteether, artemisone |
| Antibiotics       | Doxycycline, clindamycin                                                                           |



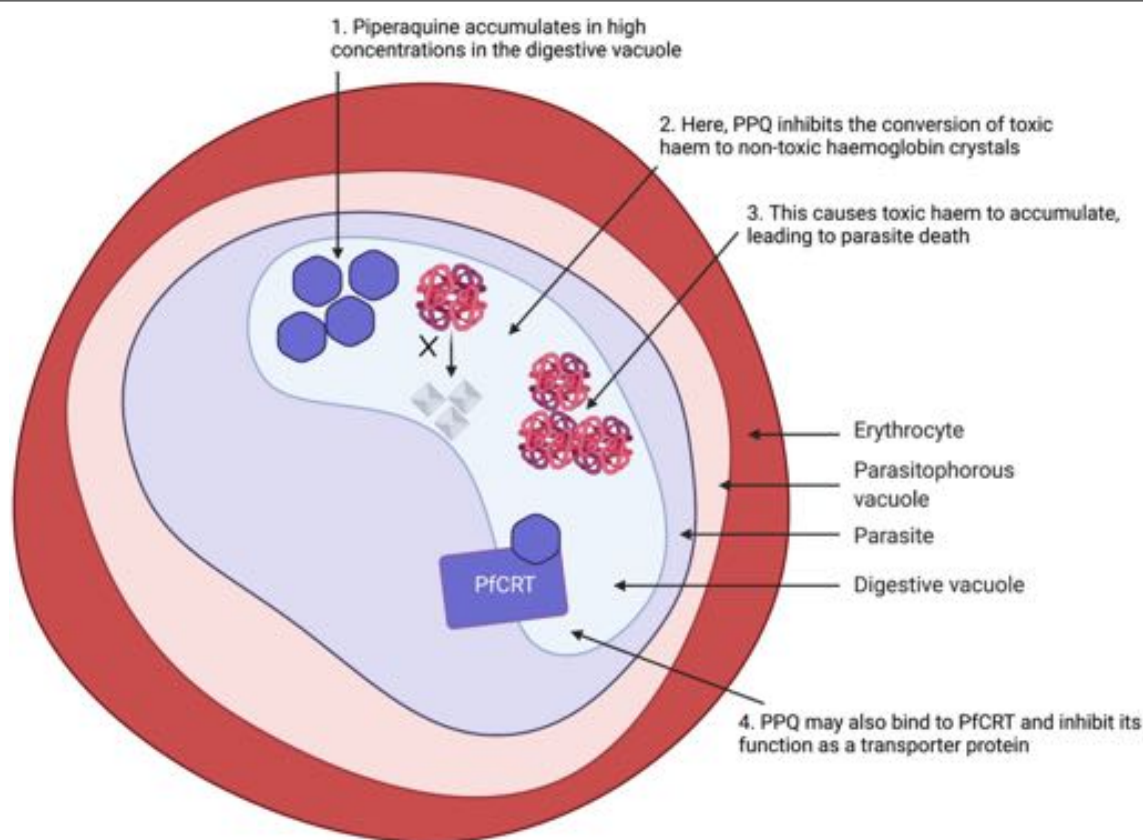
### Dihydroartemisinin-piperaquine: how does it work?

Dihydroartemisinin-piperaquine (DHA-PPQ) is an artemisinin-based combination composed of fast acting dihydroartemisinin, and slow acting piperaquine. Dihydroartemisinin (DHA) is a synthetic derivative of artemisinin, which is a sesquiterpene lactone first extracted from the plant *Artemisia annua* in 1972 [11, 12]. DHA is activated by iron, which is likely supplied by haem [13] which is taken into the parasite digestive vacuole (DV) through endocytosis of cytosol [14]. The most popular hypothesis for DHA's mechanism of action is that, once activated by haem, DHA produces radical oxygen species which cause oxidative damage in the parasite cell, killing the parasite [13, 15]. DHA is also hypothesized to act through the formation of covalent bonds with multiple targets in compartments external to the DV [16]. These mechanisms of action are illustrated in Fig. 2. DHA acts quickly and has a short half-life of approximately 0.85–2 h in adults [17–19]. To clear any residual parasites following the rapid action of DHA, it is paired in combination therapy with the long-acting partner drug, piperaquine [20].

Piperaquine (PPQ) was first introduced as a monotherapy in the 1960s, and later as the partner drug in DHA-PPQ combination therapy [20]. Piperaquine is thought to act by accumulating in high concentrations in the parasite's digestive vacuole. Here, it inhibits the conversion of toxic haem to non-toxic haemozoin crystals during parasite haemoglobin digestion, which is an essential metabolic process for the parasite. Inhibiting the conversion of haem to haemozoin results in high concentrations of toxic haem accumulating in the digestive vacuole, leading to parasite death [21, 22]. Furthermore, in vitro studies have demonstrated that *P. falciparum* exposed to PPQ accumulate more undigested haemoglobin, suggesting that PPQ decreases the rate of haemoglobin digestion, possibly killing the parasite through 'starvation' [23]. There is also evidence suggesting that PPQ binds directly to the *P. falciparum* chloroquine resistance transporter, PfCRT [24], where it may inhibit PfCRT's usual function as a transporter protein. These mechanisms of action are illustrated in Fig. 3.



**Fig. 2** A diagram illustrating how DHA is predicted to attack the parasite cell. Created with BioRender.com



**Fig. 3** A diagram illustrating how PPQ is predicted to attack the parasite cell. Created in Biorender.com

### Dihydroartemisinin-piperaquine: what are the resistance mechanisms?

Resistance to DHA-PPQ has emerged in Southeast Asia [25, 26] but the molecular mechanism of resistance is not fully understood. Molecular markers for partial resistance to DHA include single nucleotide polymorphisms (SNPs) on the *pfk13* *Plasmodium* gene, including the mutations F446I, N458Y, M476I, Y493H, R539T, Y543T, P553L, R561H, P574L and C580Y, which have been validated by the WHO [27]. Resistance to the partner drug PPQ is less well understood. Resistance to PPQ is associated with gene duplication of the plasmepsins *pfpm2* and *pfpm3* [28–32], and inactivation of either of these genes increases sensitivity to PPQ [33]. Plasmepsins II and III are proteases which work in a complex with other proteases in the digestive vacuole (DV) to digest haemoglobin and produce essential amino acids for the parasite [34]. Plasmepsin duplications may facilitate resistance to PPQ by increasing the rate of haemoglobin digestion. This may counteract the inhibitory effects of PPQ on haemoglobin digestion. Of note, resistance to PPQ has also been shown without duplication of *pfpm2* [35–37] and there is some evidence that increased expression of

*pfpm2* and *pfpm3* does not alter PPQ susceptibility [38]. Therefore, although PPQ resistance is correlated with *pfpm2* and *pfpm3* duplication, this is unlikely to be the sole mechanism of PPQ resistance. Considering this, plasmepsin copy number should not be used as the only indicator for surveying PPQ resistance. As with other anti-malarial drugs, such as mefloquine [39], all cases of resistance cannot usually be explained completely by one specific genetic polymorphism.

Resistance to DHA-PPQ has been associated with other genetic polymorphisms, including on the *pfexo* and *pfert* genes. *Plasmodium falciparum* Exonuclease (*pfexo*) is a putative exonuclease encoding gene. The E415G polymorphism on *pfexo* is strongly linked to increased copy number of *pfpm2* and *pfpm3* and has been correlated with treatment failure of DHA-PPQ in Cambodia [29, 40]. However, the functional role of this protein is uncertain.

Multiple polymorphisms in *pfert* are correlated with PPQ resistant phenotypes. The mutations T93S, H97Y, F145I and I218P have been associated with DHA-PPQ resistance in Cambodian isolates. Furthermore, in vitro studies have found that H97Y, F145I, M343L, G353V

[21] and C101F [22] cause the Dd2 (“Indo-China”) laboratory strain to be PPQ resistant and CQ sensitive. In vitro data has shown that F145I and C350R can mediate efflux of PPQ from the DV in the 7G8 (“Brazil”) laboratory strain at the same time as reducing CQ transport, suggesting that PPQ resistance may arise through efflux of PPQ from the digestive vacuole via PfCRT, in a similar mechanism to CQ resistance [24]. The mutations T93S and I218P have been shown to confer resistance to PPQ without the presence of *pfpm2* duplications [35]. Furthermore, T93S, H97Y, F145I and I128F each conferred resistance to PPQ on a background of *pfexo* E145G, but again without *pfpm2* duplication [36]. These data further show that plasmepsin duplications are not required for PPQ resistance. Many of these *pfCRT* mutations also resulted in a swollen digestive vacuole [21, 22, 36, 41]. This vacuole swelling indicates that the PfCRT protein has a role in maintaining vacuole morphology, and that mutations in *pfCRT* disrupt this function [41]. The structure of PfCRT has recently been elucidated [24], showing that *pfCRT* mutations associated with PPQ resistance are at moderately conserved sites in selected helices of the protein, including T93S, H97Y and C101F. This work has highlighted a number of other amino acid sites with similar properties, which may be under similar selection pressures from PPQ and may be useful for future PPQ resistance surveillance [24].

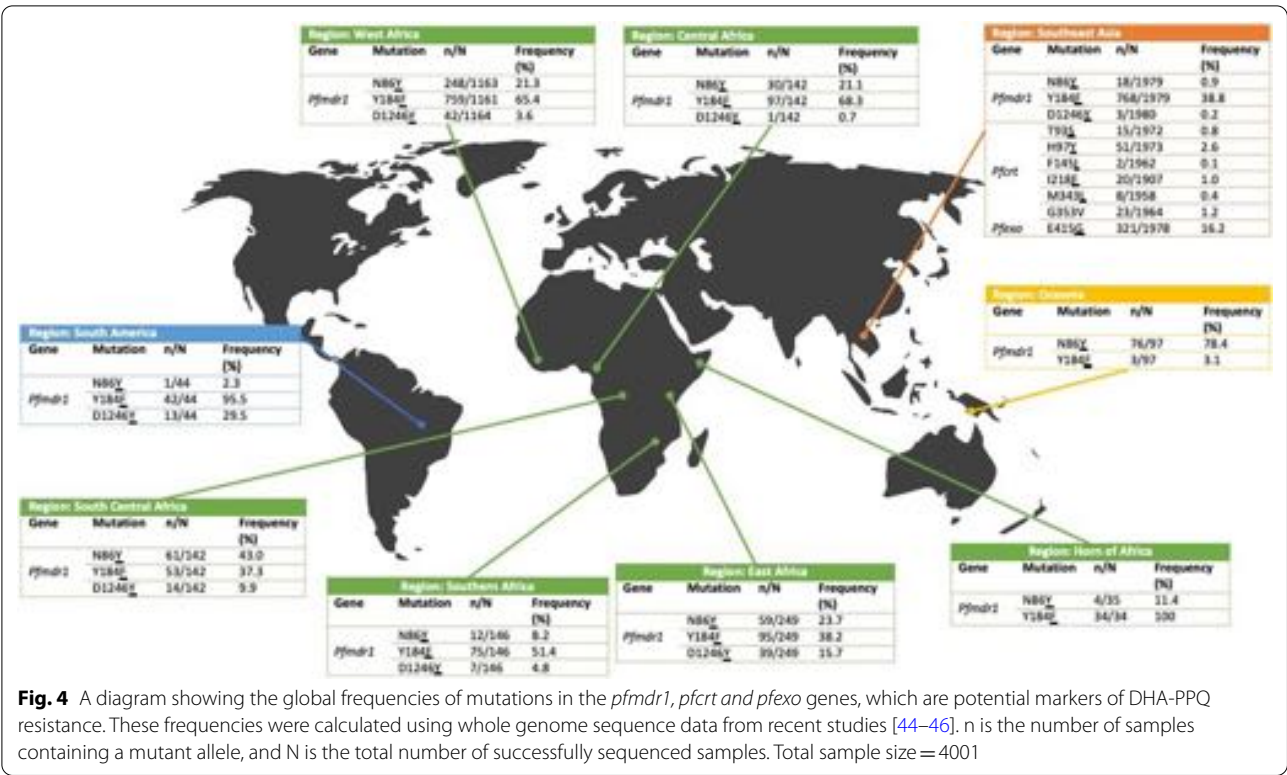
One hypothesis for the mechanism of PPQ resistance is that *pfCRT* mutations enable PfCRT to transport PPQ out of the digestive vacuole, away from its putative site of action, similarly to CQ resistance. However, some of these *pfCRT* mutations conveyed PPQ resistance without changing the rate of PPQ transport out of the digestive vacuole [21]. A competing hypothesis is that PPQ binds to PfCRT as part of its mode of action, disrupting its role as a transporter and DV morphology regulator, causing parasite death. These *pfCRT* mutations may inhibit PPQ from binding, causing PPQ resistance, whilst simultaneously removing the transporter’s ability to transport CQ, leading to CQ susceptibility [22].

Polymorphisms in *pfmdr1* have been associated with PPQ sensitivity. Conrad et al. found that DHA-PPQ treatment selected for the *pfmdr1* haplotype 86Y/Y184/Y1246. Interestingly, treatment with Artemether-Lumefantrine (AL) selected for opposite alleles; N86/184F/D1246 [42]. These opposing selection pressures suggest that DHA-PPQ may be a good choice of partner drug in areas where AL was previously used. Furthermore, increased *pfmdr1* copy number has been associated with enhanced sensitivity to piperazine [29, 43]. Veiga et al. hypothesized that increased *pfmdr1* copy number is associated with enhanced accumulation of PPQ in the DV, leading to increased sensitisation to PPQ [43].

In summary, PPQ is likely to kill parasites by disrupting haemoglobin digestion and may also act by binding to PfCRT, disrupting its role as a transporter. *Pfpm2* and *pfpm3* duplications correlate with PPQ resistance, but duplication is not essential for resistance. Therefore, plasmepsin copy number should not be used as a sole indicator of PPQ resistance. Additionally, some polymorphisms in *pfCRT* can confer resistance to PPQ in Dd2 parasites and the E415G *pfexo* mutation has been correlated with DHA-PPQ resistance in Cambodian isolates. Finally, increased *pfmdr1* copy number has been associated with enhanced sensitivity to PPQ.

### How prevalent are these putative PPQ-resistance conferring mutations?

As part of this review, whole genome sequence data was used to analyse the prevalence of the above-mentioned SNPs in *P. falciparum* samples from studies worldwide [44–46] (n = 4001) (Fig. 4). For frequency calculations, the authors considered isolates with monoclonal infections based on the Fws metric. The *pfmdr1* N86Y mutation was found at a prevalence of between 21.1% and 23.7% in samples from Central, West and East Africa, and a lower prevalence of 11.4% in the Horn of Africa and 8.2% in Southern Africa. A higher prevalence was found in Southern Central Africa, at 43%. The prevalence of N86Y was also higher in Oceania, at 78.4%. Whereas, the prevalence was lower in samples from South America, at 2.3% and Southeast Asia, at 0.9%. The *pfmdr1* Y184F mutation was found at a prevalence between 37.3% and 51.4% in samples from South Central Africa, East Africa, Southeast Asia and Southern Africa. The prevalence was 65.4% in West Africa and 68.3% in Central Africa. This mutation was found at a much higher prevalence of 95.5% in South America and in the Horn of Africa, where it was found to be 100%. In comparison, the prevalence of Y184F was very low in Oceania, at a prevalence of 3.1%. The D1246Y mutation was not found in samples from Oceania or the Horn of Africa, and ranged from a very low prevalence of 0.2% in Southeast Asia, to 29.5% in South America. *PfCRT* mutations of interest, including T93S, H97Y, F145I, I218F, M343I and G353V, were only present in samples from Southeast Asia, with a mutation prevalence of 0% in the other global regions. The prevalence of these mutations was low, with T93S, F145I, M343I in under 1% of the samples analysed. I218F had a prevalence of 1% and G353V had a prevalence of 1.2%, while H97Y had a prevalence of 2.6%. The *pfexo* mutation E415G was only found in samples from Southeast Asia, at a prevalence of 16.2%.



**Fig. 4** A diagram showing the global frequencies of mutations in the *pfmdr1*, *pfprt* and *pfexo* genes, which are potential markers of DHA-PPQ resistance. These frequencies were calculated using whole genome sequence data from recent studies [44–46]. n is the number of samples containing a mutant allele, and N is the total number of successfully sequenced samples. Total sample size = 4001

**Section II: how has mass drug administration with DHA-PPQ impacted molecular markers of resistance?**

Monitoring the prevalence of molecular markers associated with DHA-PPQ resistance enables widespread surveillance of *P. falciparum* markers in populations undergoing mass drug administration (MDA). This molecular surveillance can then be used to inform treatment policy specific to different populations. This review has investigated the impact of MDA with DHA-PPQ on the evolution of molecular markers associated with anti-malarial resistance.

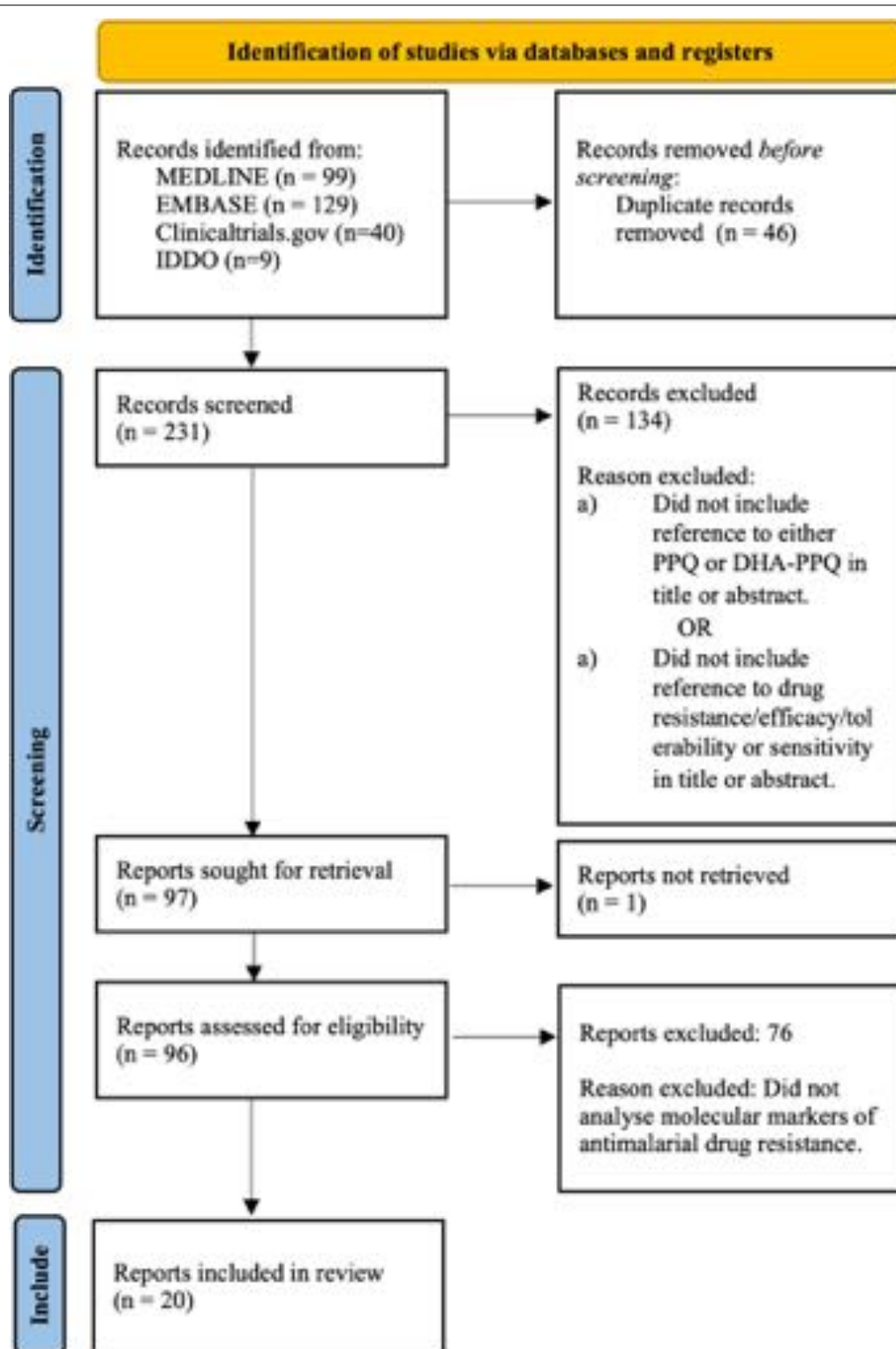
**Methodology**

This review included relevant studies from clinicaltrials.gov, EMBASE, MEDLINE and the Infectious Diseases Data Observatory (IDDO). Searches were dated back to 2011, when DHA-PPQ was first approved as the ACT Eurartesim®, by the European Medicines Agency. A detailed search strategy and methodology can be found in Appendix 1, which follows PRISMA guidelines. In brief, MDA studies were included from [www.clinicaltrials.gov](http://www.clinicaltrials.gov) which were completed, with results, which used DHA-PPQ or PPQ as an intervention. Associated publications with reference to molecular markers of resistance were included in this review. Two further publications were

included from IDDO, following search terms ‘malaria’ and ‘piperaquine’. Search terms for malaria, mass drug administration, anti-malarial resistance and PPQ or DHA-PPQ were used to extract publications from EMBASE and MEDLINE. These were then filtered for publications which included analysis of molecular markers of resistance. The methodology flowchart can be seen in Fig. 5.

**Results**

A total of 20 studies passed the screening criteria and were included for analysis in this systematic review. Each study was analysed to understand the reported impact of treatment with DHA-PPQ or PPQ on molecular markers associated with resistance to DHA-PPQ. Molecular marker data extracted from these studies included *pfpm2* copy number, *pfexo* E415G, *pfprt* mutations, *pfmdr1* mutations and copy number variations, and artemisinin-resistance associated mutations. Of the 20 studies reviewed, 7 included analysis of *pfpm2* copy number, 2 included analysis of *pfexo* E415G, 9 included analysis of mutations in *pfprt*, 13 included analysis of mutations and/or copy number variation in *pfmdr1* and 11 included analysis of mutations associated with reduced artemisinin-sensitivity. 16 of the studies were associated with clinical trials which included the use of DHA-PPQ. 2 used piperaquine phosphate and 1 used artemisinin-piperaquine. 14



**Fig. 5** A flowchart demonstrating how studies were identified for this review following PRISMA guidelines

studies were associated with MDA trials, 7 of which were associated with IPTp, which is a form of targeted MDA. 5 studies were associated with clinical trials for treatment of confirmed malaria, but were retained in this review to provide the breadth of genomic data available related to DHA-PPQ use. A summary of the relevant molecular markers reported in each study can be seen in Table 2, sorted by molecular marker of interest. One report analysed molecular markers in the *pf dhfr* and *pf dhps* genes associated with SP resistance, but did not analyse polymorphisms associated with resistance to DHA-PPQ in *pfpm2*, *pfmdr1*, *pf crt* or *pf kelch13* [49]. Therefore, this study is not included in Table 2.

## Discussion

Advances in sequencing technology have resulted in an explosion in the generation, availability, and analysis of sequencing data. This includes genomic data from the deadliest malaria parasite, *P. falciparum*. Genomic surveillance has consequently gained an increasingly important role in monitoring anti-malarial drug resistance, through the surveillance of molecular markers in the *P. falciparum* genome. The surveillance of molecular markers associated with drug resistance is recognized as a surveillance tool by the WHO [47]. Genomic surveillance is particularly important in the case of mass drug administration programmes, where drug treatment is given to members of a population whether or not they are symptomatic for malaria. MDA is endorsed by the WHO in certain settings, such as endemic island communities, where there is limited risk of importation of infection, good access to treatment and implementation of vector control and surveillance [2]. Furthermore, randomized controlled trials with MDA using DHA-PPQ have been shown to be safe and to significantly lower the burden of malaria in pre-elimination settings [48]. Therefore, with continued use of MDA, surveillance of molecular markers of resistance is crucial.

## What impact did these studies have on molecular markers of drug resistance?

### *Pfpm2* copy number

There was no evidence for selection for increased *pfpm2* copy number following MDA with DHA-PPQ in Kayin state, Myanmar [51] or after MDA for 2 months over 2 consecutive years in Mozambique [52] or after MDA taken for 3 days for 3 months in Myanmar [55]. Conrad et al. found modest increases in *pfpm2* copy number in 1 of 18 samples from patients receiving DHA-PPQ IPTp, where participants received DHA-PPQ every 8 weeks or every 4 weeks during pregnancy [53]. Taken together, this suggests that short term MDA treatments are unlikely to select for amplification in *pfpm2* copy number. However,

Imwong et al. have found *pfpm2* amplification in their longitudinal observational studies in the eastern Greater Mekong subregion [51, 57]. This is an area where DHA-PPQ has been used extensively for many years, and may suggest that longer periods of DHA-PPQ use can select for increased *pfpm2* copy number.

### *Pfexo* E415G

Two studies included in this review measured the frequency of the *pfexo* E415G mutation. Conrad et al. sequenced this locus and did not detect the *pfexo* E415G mutation in the samples that they analysed [53]. Son et al. identified the *pfexo* E415G mutation in their study population prior to MDA, but found no statistically significant increase in the prevalence of this mutation following MDA with forest rangers in Vietnam [58]. This evidence does not demonstrate a correlation between MDA with DHA-PPQ and increased prevalence of the *pfexo* E415G mutation. However, only 2 of the studies analysed this marker. Of note, some of the 20 studies analysed in this review were published before the association between the *pfexo* mutation and PPQ resistance was reported. This includes Ochong et al., Conrad et al., Somé et al., Zongo et al., Tumwebaze et al., Taylor et al. and Madanitsa et al. [42, 49, 61–65].

### *Pf crt* mutations

Nayebare et al. found that *pf crt* K76T prevalence was higher in samples collected from women in Uganda during IPTp with DHA-PPQ than in parasites collected prior to the start of IPTp, or while women received IPTp with SP [59]. Similarly, Conrad et al. found that the prevalence of the K76T mutation was higher in samples collected from the DHA-PPQ arm of IPTp in Uganda, than in the SP arm or in samples collected prior to the start of IPTp [53]. This increase in K76T prevalence was also correlated with increased PPQ exposure.

In contrast, Somé et al. found no significant selection for *pf crt* K76T following Seasonal Malaria Chemoprevention (SMC) with DHA-PPQ for 3 months in Burkina Faso [62]. Imwong et al. analysed *pf crt* mutations F145I, I218E, N326S, M343I/L and G353V and found no evidence of selection of *pf crt* mutations associated with PPQ resistance following MDA in Kayin State, Myanmar [51]. In support of this, Gupta et al. found no statistically significant difference in the prevalence of *pf crt* polymorphisms after MDA with DHA-PPQ for 2 months, for 2 years, in Mozambique [52]. Tumwebaze et al. found that monthly MDA with DHA-PPQ in Uganda was not associated with changes in the prevalence of *pf crt* polymorphisms [63]. Finally, Zongo et al. found no significant difference in the prevalence of the

**Table 2** A summary of the papers which were analysed in this systematic literature review, detailing which molecular markers were detected following treatment, and any changes in the frequency of molecular markers investigated

| Molecular marker associated with<br>DHA-PPQ resistance | Associated trial(s)                                                                                                                                                                                                                                              | Intervention                                                                                                                                                                                                                                                                           | Publication                                                                                                                                                                                                                                                                                                                                                                                                 | Impact of intervention on molecular<br>marker                                                                                                                                                                                                                                                                                                                                                                                                                  |
|--------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Pfpm2</i> copy number                               |                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                        | Samples from multiple trials and epidemiological studies conducted between 1st Jan 2007 and 31st December 2018 were analysed by Imwong et al. (2020). Therapeutic efficacy studies of artesunate [50] and DHA-PPQ in Yunnan Province of China, from 2009 to 2012 | Multiple trials and epidemiological studies, including MDA with DHA-PPQ in Kayin State, Myanmar                                                                                                                                                                                        | Imwong et al. (2020) [51] Molecular epidemiology of resistance to anti-malarial drugs in the Greater Mekong subregion: an observational study                                                                                                                                                                                                                                                               | This study found that between Jan 1 2007 and Dec 31 2018, <i>pfp</i> m2 amplification was more frequent in the eastern Grater Mekong subregion than in Myanmar. Regarding mass drug administration, there was no evidence of selection for increased <i>pfp</i> m2 copy number following MDA with DHA-PPQ in Myanmar and Cambodia                                                                                                                              |
|                                                        | NCT02914145<br>Mass Drug Administration of Monthly DHA-PPQ to Accelerate Towards Malaria Elimination in Magde District, Southern Mozambique                                                                                                                      | Mass Drug Administration with DHA-PPQ. Two monthly rounds of MDA with DHA-PPQ for two consecutive years in Magde, Southern Mozambique                                                                                                                                                  | Gupta et al. (2020) [52] Effect of mass dihydroartemisinin-piperaquine administration in southern Mozambique on the carriage of molecular markers of antimalarial resistance                                                                                                                                                                                                                                | There was no statistically significant difference in the proportion of isolates with multicopy <i>pfp</i> m2 when comparing samples collected pre and post MDA                                                                                                                                                                                                                                                                                                 |
|                                                        | NCT02282293<br>Reducing the Burden of Malaria in HIV-Infected Pregnant Women and Their HIV-Exposed Children (PRO-MOTE Birth Cohort 2)                                                                                                                            | Randomized controlled trial among 300 pregnant women in Uganda. Participants were randomized to receive either: (i) SP (500 mg Sulphadoxine and 25 mg pyrimethamine) every 8 weeks (ii) DHA-PPQ (40 mg dihydroartemisinin and 320 mg PQ) every 8 weeks or (iii) DHA-PPQ every 4 weeks  | Conrad et al. (2017) [53] Impact of Intermittent Preventive Treatment During Pregnancy on <i>Plasmodium falciparum</i> Drug Resistance-Mediating Polymorphisms in Uganda                                                                                                                                                                                                                                    | This study found a modest increase in <i>pfp</i> m2 copy number in 1 of 18 samples from patients receiving DHA-PPQ IPTp                                                                                                                                                                                                                                                                                                                                        |
|                                                        | NCT01872702<br>Targeted Chemo-elimination (TCE) to Eradicate Malaria in Areas of Suspected or Proven Artemisinin Resistance in Southeast Asia and South Asia                                                                                                     | Active comparator: Three monthly rounds of DHA-PPQ and low dose primaquine<br>Placebo comparator: No intervention used<br>Two villages randomly allocated to intervention at each of 4 sites, and two villages randomly allocated to control (no intervention). 500 people per village | von Seidlein et al. (2019) [54] The impact of targeted malaria elimination with mass drug administrations on <i>falciparum</i> malaria in Southeast Asia: A cluster randomized trial<br><br>Landier et al. (2017) [55] Safety and effectiveness of mass drug administration to accelerate elimination of artemisinin-resistant <i>falciparum</i> malaria: a pilot trial in four villages of Eastern Myanmar | This study analysed blood specimens from before MDA initiation. They found that 4% of the individuals sampled (10 of 269) had <i>pkelch13</i> C580Y and multiple copies of <i>pfp</i> m2/3. This study did not measure the difference in prevalence of markers pre and post MDA<br><br>This study found no amplification of <i>pfp</i> m2 copy number in the 69 samples that they analysed, which were 53 collected before the MDA and 16 collected afterwards |

**Table 2** (continued)

| Molecular marker associated with DHA-PPQ resistance | Associated trial(s)                                                                                                                                                                                                                                                | Intervention                                                                                                                                                                                                                                                                            | Publication                                                                                                                                                                                           | Impact of intervention on molecular marker                                                                                                                                                                                                                                                                                                                                                                             |
|-----------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Pfexo</i> E415G mutation                         | NCT02083380<br>Randomized Phase IIb Study of Efficacy, Safety, Tolerability & Pharmacokinetics of a Single Dose Regimen of Artefenomel (OZ439) in Loose Combination With Piperazine in Adults and Children With Uncomplicated <i>Plasmodium falciparum</i> Malaria | Experimental: A) Artefenomel 800 mg and Piperazine Phosphate 640 mg<br>Experimental: B) Artefenomel 800 mg and Piperazine Phosphate 960 mg<br>Experimental: C) Artefenomel 800 mg and Piperazine Phosphate 1440 mg                                                                      | Leroy et al. (2019) [56] African isolates show a high proportion of multiple copies of the <i>Plasmodium falciparum</i> plasmepsin-2 gene, a piperazine resistance marker                             | This study analysed <i>pfpm2</i> copy number in samples collected from patients before treatment. They found a higher proportion of multicopy parasites in African samples compared to Asian samples. Parasites with multicopy <i>pfpm2</i> and single copy <i>pfmdr1</i> (hypothetical to favour PPQ resistance) were found at similar prevalence in Asian and African samples                                        |
|                                                     | Samples from multiple trials and epidemiological studies were analysed by Imwong et al. (2017). Those that were trials that included PPQ or DHA-PPQ were NCT02453308                                                                                               | Longitudinal observational study between 1st January 2008 and 31st December 2015                                                                                                                                                                                                        | Imwong et al. (2017) [57] The spread of artemisinin-resistant <i>Plasmodium falciparum</i> in the Greater Mekong subregion: a molecular epidemiology observational study                              | This study found <i>pfpm2</i> amplification in isolates collected between 1 <sup>st</sup> January and 31 <sup>st</sup> December 2008, in western Cambodia and north-eastern Thailand. <i>Pfpm2</i> amplification was only observed in parasites with the C580Y 'long haplotype'                                                                                                                                        |
|                                                     | NCT02282293<br>Reducing the Burden of Malaria in HIV-Infected Pregnant Women and Their HIV-Exposed Children (PRO-MOTE Birth Cohort 2)                                                                                                                              | Randomized controlled trial among 300 pregnant women in Uganda. Participants were randomized to receive either: (i) SP (500 mg Sulphadoxine and 25 mg pyrimethamine) every 8 weeks, (ii) DHA-PPQ (40 mg dihydroartemisinin and 320 mg PQ) every 8 weeks, or (iii) DHA-PPQ every 4 weeks | Conrad et al. (2017) [53] Impact of Intermittent Preventive Treatment During Pregnancy on <i>Plasmodium falciparum</i> Drug Resistance—Mediating Polymorphisms in Uganda                              | Conrad et al. sequenced a 395 bp amplicon surrounding the E415G locus but did not find the E415G mutation in the samples that they analysed. They detected a different non-synonymous mutation in <i>pfexo</i> , R346I, in 1 of 19 samples collected from patients receiving DHA-PPQ treatment                                                                                                                         |
| <i>Pfcr</i> mutations                               | NCT02788864<br>A Randomized, Placebo-controlled, Double-blind Trial Using Dihydroartemisinin and Piperazine (DHA-PPQ) to Protect Forest Workers From Malaria in Bu Gia Map National Park                                                                           | Active comparator: DHA-PPQ for 3 days prior to forest visit<br>Placebo comparator: Placebo for 3 days prior to forest visit<br>150 participants                                                                                                                                         | Son et al. (2017) [58] The prevalence, incidence and prevention of <i>Plasmodium falciparum</i> infections in forest rangers in Bu Gia Map National Park, Binh Phuoc province, Vietnam: a pilot study | This study found that before MDA, 11 out of 30 samples had the E415G mutation. Following MDA and returning from working in the forest, two study participants were infected with <i>P. falciparum</i> . One in the DHA-PPQ arm and one in the placebo arm. The participant in the placebo arm was infected with wild-type <i>P. falciparum</i> when they entered the forest, but the E415G mutation when they returned |
|                                                     | NCT02793622<br>Prevention of Malaria in HIV-uninfected Pregnant Women and Infants                                                                                                                                                                                  | IPtP during pregnancy:<br>Active comparator arm: Monthly SP during pregnancy<br>Active comparator arm: Monthly DHA-PPQ during pregnancy<br>782 participants                                                                                                                             | Nayebare et al. (2020) [59] Associations between Malaria-Preventive Regimens and <i>Plasmodium falciparum</i> Drug Resistance-Mediating Polymorphisms in Ugandan Pregnant Women                       | This study found that <i>pfcr</i> K76T was more prevalent in parasites collected while women received IPtP with DHA-PPQ, than in parasites collected before the start of IPtP, or while women received IPtP with SP                                                                                                                                                                                                    |

**Table 2** (continued)

| Molecular marker associated with DHA-PPQ resistance                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Associated trial(s)                                                                                                                                                                                                                                                                     | Intervention                                                                                                                                                                                              | Publication                                                                                                                                                                                                                                                                                                                                                      | Impact of intervention on molecular marker |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|
| Samples from multiple trials and epidemiological studies conducted between 1st Jan 2007 and 31st December 2018 were analysed by Imwong et al. (2020).<br>Therapeutic efficacy studies of artesunate [50] and DHA-PPQ in Yunnan Province of China, from 2009 to 2012<br>NCT02914145<br>Mass Drug Administration of Monthly DHA-PQP to Accelerate Towards Malaria Elimination in Magude District, Southern Mozambique<br>NCT02282293<br>Reducing the Burden of Malaria in HIV-Infected Pregnant Women and Their HIV-Exposed Children (PRO-MOTE Birth Cohort 2) | Multiple trials and epidemiological studies, including MDA with DHA-PPQ in Kayin State, Myanmar                                                                                                                                                                                         | Imwong et al. (2020) [51] Molecular epidemiology of resistance to anti-malarial drugs in the Greater Mekong subregion: an observational study                                                             | This study found no evidence for selection of <i>pfcr</i> mutations associated with piperazine resistance following MDA with DHA-PPQ in Kayin State, Myanmar, or in Cambodia                                                                                                                                                                                     |                                            |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Mass Drug Administration with DHA-PPQ. Two monthly rounds of MDA with DHA-PPQ for two consecutive years in Magude, Southern Mozambique                                                                                                                                                  | Gupta et al. (2020) [52] Effect of mass dihydroartemisinin-piperazine administration in southern Mozambique on the carriage of molecular markers of antimalarial resistance                               | This study found no statistically significant difference between the frequency of <i>pfcr</i> polymorphisms when comparing samples collected before and after MDA                                                                                                                                                                                                |                                            |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Randomized controlled trial among 300 pregnant women in Uganda. Participants were randomized to receive either: (i) SP (500 mg Sulphadoxine and 25 mg pyrimethamine) every 8 weeks, (ii) DHA-PPQ (40 mg dihydroartemisinin and 320 mg PQ) every 8 weeks, or (iii) DHA-PPQ every 4 weeks | Wallender et al. (2019) [60] Modelling Prevention of Malaria and Selection of Drug Resistance with Different Dosing Schedules Dihydroartemisinin-Piperazine Preventive Therapy during Pregnancy in Uganda | This study used non-linear mixed effects modelling to describe the relationship between PPQ concentration and the probability of finding the mutation N86Y in <i>pfmdr1</i> and K76T in <i>pfcr</i> . The models predicted that higher median PPQ concentrations would be required to prevent infections with mutant haplotypes compared to wild-type haplotypes |                                            |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                         | Conrad et al. (2017) [53] Impact of Intermittent Preventive Treatment During Pregnancy on <i>Plasmodium falciparum</i> Drug Resistance—Mediating Polymorphisms in Uganda                                  | Treatment with DHA-PPQ was associated with increased prevalence of <i>pfcr</i> K76T compared with samples collected before treatment, or treatment with SP. Selection increased with increasing PPQ exposure. Receipt of DHA-PPQ selected for the <i>pfcr</i> 76T- <i>mdr1</i> N86-184F-1246D haplotype                                                          |                                            |

**Table 2** (continued)

| Molecular marker associated with DHA-PPQ resistance | Associated trial(s)                                                                                                                                                                        | Intervention                                                                                                                                                                                                                                                                                                                                                                                                           | Publication                                                                                                                                                                                                                                                                                                                                | Impact of intervention on molecular marker                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|-----------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                     | NCT00527800<br>Interactions Between HIV and Malaria in African Children<br>5 year longitudinal trial from 2007 to 2012 in Tororo, Uganda                                                   | Experimental arm 1: Treatment for episodes of uncomplicated malaria with DHA-PPQ, once daily for 3 days<br>Comparator arm 2: Treatment for uncomplicated malaria with artemether-lumefantrine, twice daily for 3 days<br>Experimental A: Prevention of malaria in HIV uninfected, exposed children, with trimethoprim-sulfamethoxazole<br>No intervention B: Prevention of malaria in HIV uninfected, exposed children | Conrad et al. (2014) [42] Comparative Impacts Over 5 Years of Artemisinin-Based Combination Therapies on <i>Plasmodium falciparum</i> Polymorphisms That Modulate Drug Sensitivity in Ugandan Children                                                                                                                                     | Treatment over time with DHA-PPQ or AL was associated with higher prevalences of wildtype K76. The extent of selection was lower than that with recent treatment with AL                                                                                                                                                                                                                                                                                                                                                                                    |
|                                                     | NCT00941785<br>Randomized Trial of the Efficacy, Safety, Tolerability and Pharmacokinetics of Dihydroartemisinin-piperaquine for Seasonal IPT to Prevent Malaria in Children Under 5 Years | Experimental: Three monthly rounds of DHA-PPQ in August, September and October<br>Active comparator: Three monthly rounds of Sulphadoxine-Pyrimethamine plus Amodiaquine<br>1500 participants                                                                                                                                                                                                                          | Zongo et al. (2015) [61] Randomized Noninferiority Trial of Dihydroartemisinin-Piperaquine Compared with Sulphadoxine-Pyrimethamine plus Amodiaquine for Seasonal Malaria Chemoprevention in Burkina Faso                                                                                                                                  | This study found no significant difference in prevalence of the <i>pfcr</i> mutation K76T following SMC with DHA-PPQ                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                                     | NCT00948896 and NCT00978068                                                                                                                                                                | Experimental arm 1: TS; TMP/SMX given daily<br>Experimental arm 2: SP given monthly as a single dose<br>Experimental arm 3: DHA-PPQ given monthly, once a day for 3 consecutive days<br>Arm 4: no intervention                                                                                                                                                                                                         | Somé et al. (2014) [62] Selection of Drug Resistance-Mediating <i>Plasmodium falciparum</i> Genetic Polymorphisms by Seasonal Malaria Chemoprevention in Burkina Faso<br><br>Tumwebaze et al. (2015) [63] Impact of antimalarial treatment and chemoprevention on the drug sensitivity of malaria parasites isolated from Ugandan children | This study measured the prevalence of mutations in parasites collected before the initiation of the intervention, a month after the completion of three-monthly treatments, and from a control group of children not subject to the intervention. They found no significant selection of <i>pfcr</i> K76T following treatment with DHA-PPQ<br><br>This study found minor differences in the prevalence of SNPs associated with drug resistance between different trial arms. Monthly DHA-PPQ treatment was not associated with polymorphisms in <i>pfcr</i> |

**Table 2** (continued)

| Molecular marker associated with<br>DHA-PPQ resistance | Associated trial(s)                                                                                                                                                                                                                                                                                                                                                                                                        | Intervention                                                                                                                                                                                                                                                                            | Publication                                                                                                                                                                                                                                                                                                                      | Impact of intervention on molecular<br>marker                                                                                                                                                                                                                                                                                                                                     |
|--------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Pfmdr1</i> polymorphisms                            | NCT02793622<br>Prevention of Malaria in HIV-uninfected Pregnant Women and Infants                                                                                                                                                                                                                                                                                                                                          | IPTp during pregnancy:<br>Active comparator arm: Monthly SP during pregnancy<br>DHA-PPQ during pregnancy<br>Active comparator arm: Monthly DHA-PPQ during pregnancy<br>782 participants                                                                                                 | Nayebare et al. (2020) [59] Associations between Malaria-Preventive Regimens and <i>Plasmodium falciparum</i> Drug Resistance-Mediating Polymorphisms in Ugandan Pregnant Women                                                                                                                                                  | This study found that <i>pfmdr1</i> N86Y and Y184F were more prevalent in parasites collected while women received IPTp with DHA-PPQ, than in parasites collected before the start of IPTp, or while women received IPTp with SP. The prevalence of D1246Y was similar in parasites collected before and after IPTp.                                                              |
|                                                        | NCT02914145<br>Mass Drug Administration of Monthly DHA-PQP to Accelerate Towards Malaria Elimination in Magde District, Southern Mozambique<br><br>Samples from multiple trials and epidemiological studies conducted between 1st Jan 2007 and 31st December 2018 were analysed by Imwong et al. (2020).<br><br>Therapeutic efficacy studies of artesunate [50] and DHA-PPQ in Yunnan Province of China, from 2009 to 2012 | Mass Drug Administration with DHA-PPQ. Two monthly rounds of MDA with DHA-PPQ for two consecutive years in Magde, Southern Mozambique<br><br>Multiple trials and epidemiological studies, including MDA with DHA-PPQ in Kayin State, Myanmar                                            | Gupta et al. (2020) [52] Effect of mass dihydroartemisinin-piperaquine administration in southern Mozambique on the carriage of molecular markers of antimalarial resistance<br><br>Imwong et al. (2020) [51] Molecular epidemiology of resistance to antimalarial drugs in the Greater Mekong subregion: an observational study | This study found no evidence of statistically significant differences in <i>pfmdr1</i> polymorphisms, including <i>pfmdr1</i> copy number, when comparing samples collected before and after MDA<br><br>This study found that <i>pfmdr1</i> amplification is at low prevalence across the Greater Mekong subregion                                                                |
|                                                        | NCT02083380<br>Randomized Phase IIb Study of Efficacy, Safety, Tolerability & Pharmacokinetics of a Single Dose Regimen of Artefenomel (OZ439) in Loose Combination With Piperazine in Adults and Children With Uncomplicated <i>Plasmodium falciparum</i> Malaria                                                                                                                                                         | Experimental: A) Artefenomel 800 mg and Piperazine Phosphate 640 mg<br>Experimental: B) Artefenomel 800 mg and Piperazine Phosphate 960 mg<br>Experimental: C) Artefenomel 800 mg and Piperazine Phosphate 1440 mg                                                                      | Leroy et al. (2019) [56] African isolates show a high proportion of multiple copies of the <i>Plasmodium falciparum</i> plasmepsin-2 gene, a piperazine resistance marker                                                                                                                                                        | This study analysed <i>pfmdr1</i> copy number in samples collected from patients before treatment. They found a threefold higher prevalence of multicopy <i>pfmdr1</i> in Africa than in Asia. Parasites with multicopy <i>plpm2</i> and single copy <i>pfmdr1</i> (which is hypothesized to favour PPQ resistance) were found at similar prevalence in Asian and African samples |
|                                                        | NCT0282293<br>Reducing the Burden of Malaria in HIV-Infected Pregnant Women and Their HIV-Exposed Children (PRO-MOTE Birth Cohort 2)                                                                                                                                                                                                                                                                                       | Randomized controlled trial among 300 pregnant women in Uganda. Participants were randomized to receive either: (i) SP (500 mg Sulphadoxine and 25 mg pyrimethamine) every 8 weeks, (ii) DHA-PPQ (40 mg dihydroartemisinin and 320 mg PQ) every 8 weeks, or (iii) DHA-PPQ every 4 weeks | Wallender et al. (2019) [60] Modelling Prevention of Malaria and Selection of Drug Resistance with Different Dosing Schedules Dihydroartemisinin-Piperaquine Preventive Therapy during Pregnancy in Uganda                                                                                                                       | This study used non-linear mixed effects modelling to describe the relationship between PPQ concentration and the probability of finding the mutation N86Y in <i>pfmdr1</i> and K76T in <i>pfcr1</i> . The models predicted that higher median PPQ concentrations would be required to prevent infections with mutant haplotypes compared to wild-type haplotypes                 |

**Table 2** (continued)

| Molecular marker associated with DHA-PPQ resistance | Associated trial(s)                                                                                                                                                                      | Intervention                                                                                                                                 | Publication                                                                                                                                                                                           | Impact of intervention on molecular marker                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                     |                                                                                                                                                                                          |                                                                                                                                              | Conrad et al. (2017) [53] Impact of Intermittent Preventive Treatment During Pregnancy on <i>Plasmodium falciparum</i> Drug Resistance—Mediating Polymorphisms in Uganda                              | Treatment with DHA-PPQ was associated with increased prevalence of <i>pfmdr1</i> N86Y and Y184F mutations compared with before treatment, or treatment with SP. Increased frequency of DHA-PPQ exposure was associated with increased prevalence of <i>pfmdr1</i> N86Y. Treatment with DHA-PPQ was associated with decreased prevalence of D1246Y compared with samples collected before treatment, or treatment with SP. DHA-PPQ treatment selected for the <i>pfmdr1</i> 86Y-184F, 86Y-D1246, N86-184F-1246D and the <i>pfprt</i> 76T- <i>mdr1</i> N86-184F-1246D haplotypes. Consistent with this, a generalized linear model of PPQ exposure demonstrated that increasing PPQ concentration was associated with increasing prevalence of N86Y. There was no evidence of increased <i>pfmdr1</i> copy number associated with DHA-PPQ treatment |
|                                                     | NCT0278864<br>A Randomized, Placebo-controlled, Double-blind Trial Using Dihydroartemisinin and Piperaquine (DHA-PPQ) to Protect Forest Workers From Malaria in Bu Gia Map National Park | Active comparator: DHA-PPQ for 3 days prior to forest visit<br>Placebo comparator: Placebo for 3 days prior to forest visit 150 participants | Son et al. (2017) [58] The prevalence, incidence and prevention of <i>Plasmodium falciparum</i> infections in forest rangers in Bu Gia Map National Park, Binh Phuoc province, Vietnam: a pilot study | This study found that before MDA, no <i>P. falciparum</i> isolates that were genotyped had multiple copies of <i>pfmdr1</i> . Following MDA and returning from working in the forest, two study participants were infected with <i>P. falciparum</i> . Neither had multicopy <i>pfmdr1</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

**Table 2** (continued)

| Molecular marker associated with DHA-PPQ resistance | Associated trial(s)                                                                                                                                                                        | Intervention                                                                                                                                                                                                                                                                                                                                                                                                               | Publication                                                                                                                                                                                                                                                                                                                                                                                                          | Impact of intervention on molecular marker                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-----------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                     | NCT00941785<br>Randomized Trial of the Efficacy, Safety, Tolerability and Pharmacokinetics of Dihydroartemisinin-piperaquine for Seasonal IPT to Prevent Malaria in Children Under 5 Years | Experimental: Three monthly rounds of DHA-PPQ in August, September and October<br>Active comparator: Three monthly rounds of Sulphadoxine-Pyrimethamine plus Amodiaquine<br>1500 participants                                                                                                                                                                                                                              | Zongo et al. (2015) [61] Randomized Noninferiority Trial of Dihydroartemisinin-Piperaquine Compared with Sulphadoxine-Pyrimethamine plus Amodiaquine for Seasonal Malaria Chemoprevention in Burkina Faso<br><br>Somé et al. (2014) [62] Selection of Drug Resistance-Mediating <i>Plasmodium falciparum</i> Genetic Polymorphisms by Seasonal Malaria Chemoprevention in Burkina Faso                               | This study found no significant difference in the prevalence of <i>pfmdr1</i> mutations N86Y, F184Y or D1246Y following SMC with DHA-PPQ<br><br>Measured the prevalence of mutations in parasites collected from children before the initiation of the intervention, from children a month after the completion of three-monthly treatments, and from a control group of children not subject to the intervention. They found borderline significant selection for <i>pfmdr1</i> D1246Y following treatment with DHA-PPQ                                                                                                                                                                                                                                                                  |
|                                                     | NCT00527800<br>Interactions Between HIV and Malaria in African Children<br>5 year longitudinal trial from 2007 to 2012 in Tororo, Uganda                                                   | Experimental arm 1: Treatment for episodes of uncomplicated malaria with DHA-PPQ, once daily for 3 days<br>Comparator arm 2: Treatment for uncomplicated malaria with artemether-lumefantrine, twice daily for 3 days<br><br>Experimental A: Prevention of malaria in HIV uninfected, exposed children, with trimethoprim-sulfamethoxazole<br>No intervention B: Prevention of malaria in HIV uninfected, exposed children | Taylor et al. (2016) [64] Artemether-lumefantrine and Dihydroartemisinin-Piperaquine Exert Inverse Selective Pressure on <i>Plasmodium falciparum</i> Drug Sensitivity-Associated Haplotypes in Uganda<br><br>Conrad et al. (2014) [42] Comparative Impacts Over 5 Years of Artemisinin-Based Combination Therapies on <i>Plasmodium falciparum</i> Polymorphisms That Modulate Drug Sensitivity in Ugandan Children | This study fit a haplotype frequency estimation model to study <i>pfmdr1</i> N86Y, Y184F and D1246Y alleles. They found that DHA-PPQ selected for 86Y, but only when this was combined with Y184 and 1246Y, selecting for the haplotype YYY, and against haplotypes NFD and NYY<br><br>The prevalences of N86, 184F and D1246 increased over the time of the study. When comparing the AL and DHA-PPQ treatment arms, the prevalences of these alleles was greater in the AL treatment arm. Recent treatment with DHA-PPQ was associated with increased prevalence of <i>pfmdr1</i> N86Y, D1246Y and a lower prevalence of Y184F. The extent of selection was lower than that with recent treatment with AL. There was no association between treatment arm and <i>pfmdr1</i> copy number |

**Table 2** (continued)

| Molecular marker associated with DHA-PPQ resistance | Associated trial(s)                                                                                                                                                                                                                                                 | Intervention                                                                                                                                                                                                   | Publication                                                                                                                                                                                                                                                                                                                      | Impact of intervention on molecular marker                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Artemisinin-resistance associated mutations         | NCT00948896<br>A Randomized Controlled Trial of Monthly Dihydroartemisinin-piperaquine Versus Monthly Sulfadoxine-pyrimethamine Versus Daily Trimethoprim-sulfamethoxazole Versus No Therapy for the Prevention of Malaria                                          | Experimental arm 1: TS; TMP/SMX given daily<br>Experimental arm 2: SP given monthly as a single dose<br>Experimental arm 3: DHA-PPQ given monthly, once a day for 3 consecutive days<br>Arm 4, no intervention | Tumwebaze et al. (2015) [63] Impact of antimalarial treatment and chemoprevention on the drug sensitivity of malaria parasites isolated from Ugandan children                                                                                                                                                                    | This study found minor differences in the prevalence of SNPs associated with drug resistance between different trial arms. Monthly DHA-PPQ treatment was not associated with polymorphisms in <i>pfmdr1</i> . However, when samples from the DHA-PPQ treatment arm were sorted based on circulating PPQ levels, parasites with higher PPQ exposure showed evidence for selection for <i>pfmdr1</i> 86Y and 1246Y mutations                                                                                                                                                                |
|                                                     | NCT02914145<br>Mass Drug Administration of Monthly DHA-PQP to Accelerate Towards Malaria Elimination in Magude District, Southern Mozambique                                                                                                                        | Mass Drug Administration with DHA-PPQ. Two monthly rounds of MDA with DHA-PPQ for two consecutive years in Magude, Southern Mozambique                                                                         | Ochong et al. (2013) [65] Fitness Consequences of <i>Plasmodium falciparum</i> <i>pfmdr1</i> Polymorphisms Inferred from Ex Vivo Culture of Ugandan Parasites                                                                                                                                                                    | This study did not measure changes in molecular markers of resistance in trial participants; this study cultured parasites from participants to evaluate fitness advantages of certain <i>pfmdr1</i> polymorphisms. Their results suggest fitness advantages for parasites with the <i>pfmdr1</i> N86Y mutation and wild-type D1246Y                                                                                                                                                                                                                                                      |
|                                                     | Samples from multiple trials and epidemiological studies conducted between 1st Jan 2007 and 31st December 2018 were analysed by Imwong et al. (2020).<br>Therapeutic efficacy studies of artesunate [50] and DHA-PPQ in Yunnan Province of China, from 2009 to 2012 | Multiple trials and epidemiological studies, including MDA with DHA-PPQ in Kayin State, Myanmar                                                                                                                | Gupta et al. (2020) [52] Effect of mass dihydroartemisinin-piperaquine administration in southern Mozambique on the carriage of molecular markers of antimalarial resistance<br><br>Imwong et al. (2020) [51] Molecular epidemiology of resistance to antimalarial drugs in the Greater Mekong subregion: an observational study | There was no statistically significant difference between the frequency of polymorphisms when comparing samples collected pre and post MDA. No <i>pfkelch13</i> polymorphisms associated with partial-resistance to artemisinin were found in the isolates analyzed<br><br>The longitudinal study found that two genotypes of <i>pfkelch</i> have come to dominate: the Cys580Tyr mutation in the eastern Greater Mekong subregion, and the Phe446Ile mutation in Myanmar. They found no evidence for further selection of <i>pfkelch</i> mutations following mass treatment with DHA-PPQ |

**Table 2** (continued)

| Molecular marker associated with DHA-PPQ resistance                                                                                                          | Associated trial(s)                                                                                                                                                                                                                                                                    | Intervention                                                                                                                                                                                                    | Publication                                                                                                                                                                                                                                                                                                                                                                                      | Impact of intervention on molecular marker |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|
| NCT01872702<br>Targeted Chemo-elimination (TCE) to Eradicate Malaria in Areas of Suspected or Proven Artemisinin Resistance in Southeast Asia and South Asia | Active comparator: Three monthly rounds of DHA-PPQ and low dose primaquine<br>Placebo comparator: No intervention used<br>Two villages randomly allocated to intervention at each of 4 sites, and two villages randomly allocated to control (no intervention). 500 people per village | von Seidlein et al. (2019) [54] The impact of targeted malaria elimination with mass drug administrations on <i>falciparum</i> malaria in Southeast Asia: A cluster randomized trial                            | This study analysed blood specimens from before MDA initiation. They found that 4% of the individuals sampled (10 of 269) had <i>pfkelch13</i> C580Y and multiple copies of <i>pfpv2/3</i> . This study did not measure the difference in prevalence of markers pre and post MDA. 9/10 of the participants with the C580Y genotyped parasites cleared their parasitaemia after receiving the MDA |                                            |
|                                                                                                                                                              |                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                  |                                            |
|                                                                                                                                                              |                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                  |                                            |
| NCT number not available<br>Large-scale Artemisinin-Piperaquine Mass Drug Administration with or Without Primaquine, Anjouan Island, Union of Comoros        | Artemisinin-piperaquine (AP), with or without primaquine, was given in 3 monthly rounds as MDA                                                                                                                                                                                         | Tripura et al. (2018) [66] A Controlled Trial of Mass Drug Administration to Interrupt Transmission of Multidrug-Resistant <i>Falciparum</i> Malaria in Cambodian Villages                                      | This study found that all genotyped <i>Plasmodium</i> infections carried the <i>pfkelch13</i> C580Y mutation. Of isolates genotyped up to April 2016, 34 had the C580Y mutation and 1 had the F446I mutation                                                                                                                                                                                     |                                            |
|                                                                                                                                                              |                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                  |                                            |
|                                                                                                                                                              |                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                  |                                            |
| NCT02282293<br>Reducing the Burden of Malaria in HIV-Infected Pregnant Women and Their HIV-Exposed Children (PRO-MOTE Birth Cohort 2)                        | Randomized controlled trial among 300 pregnant women in Uganda. Participants were randomized to receive either (i) SP (500 mg Sulphadoxine and 25 mg pyrimethamine) every 8 weeks (ii) DHA-PPQ (40 mg dihydroartemisinin and 320 mg PQ) every 8 weeks or (iii) DHA-PPQ every 4 weeks   | Landier et al. (2017) [55] Safety and effectiveness of mass drug administration to accelerate elimination of artemisinin-resistant <i>falciparum</i> malaria: A pilot trial in four villages of Eastern Myanmar | This study measured the prevalence of <i>pfkelch13</i> mutations before and after MDA. They found that the prevalence was 85.6% before and 56.7% afterwards. The most frequent mutations were C580Y and G358V                                                                                                                                                                                    |                                            |
|                                                                                                                                                              |                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                  |                                            |
|                                                                                                                                                              |                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                  |                                            |
| NCT02282293<br>Reducing the Burden of Malaria in HIV-Infected Pregnant Women and Their HIV-Exposed Children (PRO-MOTE Birth Cohort 2)                        | Deng et al. (2018) [67] Large-scale Artemisinin-Piperaquine Mass Drug Administration With or Without Primaquine Dramatically Reduces Malaria in a Highly Endemic Region of Africa                                                                                                      | Conrad et al. (2017) [53] Impact of Intermittent Preventive Treatment During Pregnancy on <i>Plasmodium falciparum</i> Drug Resistance-Mediating Polymorphisms in Uganda                                        | This study found no evidence for the selection of <i>pfkelch13</i> mutations between treatment groups                                                                                                                                                                                                                                                                                            |                                            |
|                                                                                                                                                              |                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                  |                                            |
|                                                                                                                                                              |                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                  |                                            |

**Table 2** (continued)

| Molecular marker associated with DHA-PPQ resistance                                                                                                                                                                                                                | Associated trial(s)                                                                                                                                                                                                | Intervention                                                                                                                                                                                                                                                                                     | Publication                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Impact of intervention on molecular marker |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|
| NCT02083380<br>Randomized Phase IIb Study of Efficacy, Safety, Tolerability & Pharmacokinetics of a Single Dose Regimen of Artefenomel (OZ439) in Loose Combination With Piperazine in Adults and Children With Uncomplicated <i>Plasmodium falciparum</i> Malaria | Experimental: A) Artefenomel 800 mg and Piperazine Phosphate 640 mg<br>Experimental: B) Artefenomel 800 mg and Piperazine Phosphate 960 mg<br>Experimental: C) Artefenomel 800 mg and Piperazine Phosphate 1440 mg | Leroy et al. (2019) [56] African isolates show a high proportion of multiple copies of the <i>Plasmodium falciparum</i> plasmepsin-2 gene, a piperazine resistance marker                                                                                                                        | This study analysed samples collected from patients before treatment. They found that 67.6% of isolates genotyped from Vietnam had <i>pfkelch13</i> resistance mutations. In contrast, none of the 332 isolates successfully genotyped from African patients carried validated or candidate <i>pfkelch13</i> mutations                                                                                                                                       |                                            |
|                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                    | Macintyre et al. (2017) [68] A randomised, double-blind clinical phase II trial of the efficacy, safety, tolerability and pharmacokinetics of a single dose combination treatment with artefenomel and piperazine in adults and children with uncomplicated <i>Plasmodium falciparum</i> malaria | This study analysed <i>pfkelch13</i> mutations and found a high mutation frequency in Vietnam of 70.1%. Five mutations were detected, including C580Y, I543T, P553L and V568G, all associated with artemisinin partial resistance, and C469P, which is not associated with artemisinin partial resistance. The presence of these mutations was associated with parasite clearance half-life. The most common <i>pfkelch13</i> genotypes were C580Y and P553L |                                            |
| Samples from multiple trials and epidemiological studies were analysed by Imwong et al. (2017). Those that were trials that included PPQ or DHA-PPQ were NCT02453308                                                                                               | Longitudinal observational study between 1st January 2008 and 31st December 2015                                                                                                                                   | Imwong et al. (2017) [57] The spread of artemisinin-resistant <i>Plasmodium falciparum</i> in the Greater Mekong subregion: a molecular epidemiology observational study                                                                                                                         | This study found that over a large area of the Greater Mekong subregion, a single long haplotype <i>pfkelch13</i> C580Y mutant lineage has come to dominate                                                                                                                                                                                                                                                                                                  |                                            |
| NCT02788864<br>A Randomized, Placebo-controlled, Double-blind Trial Using Dihydroartemisinin and Piperazine (DHA-PPQ) to Protect Forest Workers From Malaria in Bu Gia Map National Park                                                                           | Active comparator: DHA-PPQ for 3 days prior to forest visit<br>Placebo comparator: Placebo for 3 days prior to forest visit<br>150 participants                                                                    | Son et al. (2017) [58] The prevalence, incidence and prevention of <i>Plasmodium falciparum</i> infections in forest rangers in Bu Gia Map National Park, Binh Phuoc province, Vietnam: a pilot study                                                                                            | This study found that before MDA, 11 out of 30 <i>P. falciparum</i> had the C580Y <i>kelch13</i> mutation. Following MDA and returning from working in the forest, two study participants were infected with <i>P. falciparum</i> . One in the DHA-PPQ arm and one in the placebo arm. The participant in the placebo arm was infected with wild-type <i>P. falciparum</i> when they entered the forest, but the C580Y mutation when they returned           |                                            |

K76T polymorphism following SMC with DHA-PPQ in Burkina Faso [61].

Overall, evidence for selection of *pfprt* K76T following MDA with DHA-PPQ is mixed. Few studies analysed *pfprt* markers other than K76T. Other key polymorphisms have been associated with PPQ resistance in vitro. *Pfprt* H97Y, F145I, M343L, G353V [21] and C101F [22] mutations have been shown to confer PPQ resistance and CQ sensitivity in vitro, and F145I and C350R have been shown to efflux PPQ from the DV at the same time as reducing CQ transport [24]. Furthermore, T93S and I218P have been shown to confer PPQ resistance [35] along with T93S, H97Y, F145I and I128F. Each of these mutations conferred resistance to PPQ on a background of *pfexo* E415G [36]. This evidence suggests that that there may be other, more relevant *pfprt* markers than K76T, and that future studies would benefit from monitoring this range of identified putative *pfprt* polymorphisms which have conferred DHA-PPQ resistance in vitro.

#### **Pfmdr1 mutations and copy number**

Following IPTp with DHA-PPQ in Uganda, both Naye-bare et al. and Conrad et al. found increased prevalence of *pfmdr1* N86Y and Y184F mutations in samples collected during treatment than in samples collected before treatment [53, 59]. Increased exposure to PPQ also correlated with increased prevalence of N86Y [53, 63] and D1246Y [63] in Uganda. Mixed results were found regarding the D1246Y polymorphism. Naye-bare et al. found that the prevalence of *pfmdr1* D1246Y was similar in samples collected before and after IPTp [59]. Whereas, Conrad et al. found that D1246Y prevalence decreased in samples collected during IPTp with DHA-PPQ, compared with samples collected before treatment or during treatment with SP [53]. Somé et al. found borderline selection for wild-type D1246Y following treatment with DHA-PPQ in Burkina Faso [62]. Taylor et al. and Conrad et al. monitored changes in polymorphisms and haplotypes in *pfmdr1* in Uganda between 2007 and 2012 after treatment with AL or DHA-PPQ [42, 64]. Conrad et al. found that treatment with DHA-PPQ was associated with increased prevalence of N86Y and D1246Y, and a lower prevalence of Y184F [42]. Taylor et al. used a haplotype frequency estimation model and found that treatment with DHA-PPQ only selected for N86Y when this allele was found with D1246Y and Y184F, in the haplotype YYY, and that it selected against haplotypes NFD and NYY [64].

In contrast, Gupta et al. found no change in *pfmdr1* polymorphisms following MDA with DHA-PPQ in Mozambique [52]. Furthermore, Zongo et al. found no

significant difference in the prevalence of *pmdfr1* mutations following SMC with DHA-PPQ in Burkina Faso [61]. Gupta et al., Son et al., and Conrad et al., found no association between MDA with DHA-PPQ and increased *pfmdr1* copy number [42, 52, 53, 58]. This small number of studies suggests that MDA with DHA-PPQ may select for mutations in *pfmdr1*, particularly N86Y.

#### **Artemisinin partial resistance associated mutations**

No evidence for increased selection of *pfkelch13* mutations was found after MDA with DHA-PPQ in Kayin State, Myanmar [51] in Mozambique [52], Uganda [53], or in Eastern Myanmar [55], or following treatment with artemisinin-piperaquine in the Comoros [67]. This suggests that although the prevalence of some *pfkelch13* polymorphisms have become widespread in the GMS [51, 57], MDA with DHA-PPQ has not increased the prevalence of *pfkelch13* mutations associated with reduced artemisinin sensitivity, as least in the studies which have monitored molecular markers of resistance following MDA.

#### **Conclusion**

Despite molecular markers of drug resistance being a recognized surveillance tool by the WHO [27], the level of reporting of molecular markers associated with DHA-PPQ resistance found in this study was low. Of the total 96 papers screened for eligibility in this review, only 20 analysed molecular markers of drug resistance. This highlights considerations for future studies with DHA-PPQ, where further analysis and reporting of molecular markers related to DHA-PPQ resistance would greatly assist understanding of how MDA impacts polymorphisms associated with resistance. Importantly, molecular markers associated with DHA-PPQ have different prevalences in different geographic regions. Molecular surveillance data from future studies in different geographies will further increase understanding of how treatment with DHA-PPQ is impacting the evolution of resistance in these different geographies.

The choice of markers analysed is not currently standardized, which may be partly because *pfexo* E415G and *pfpm2* copy number have only recently emerged as molecular markers associated with PPQ resistance. Future studies with DHA-PPQ should monitor a broader range of molecular markers which have been associated with resistance to DHA-PPQ. This includes *pfexo* E415G, *pfpm2* copy number and *pfmdr1* copy number and N86Y, Y184F and D1246Y mutations. In addition to these markers, putative polymorphisms in *pfprt* should also be monitored, including mutations T93S, H97Y, F145I, I218P, M343L, C350R, G353V. This would contribute to a more

comprehensive analysis of resistance polymorphisms following MDA implementation.

To really understand the impact that DHA-PPQ MDA has had on the evolution of drug resistance, there needs to be much greater focus and investment on genomic surveillance in trial and programmatic settings. This would enable the research community to build on the already growing field of genomic surveillance to better understand the impact of using anti-malarial drugs on a large scale. Phenomenal steps have already been made, including through the Pan-African Malaria Genetic Epidemiology Network (PAMGEN). However, the lack of published molecular surveillance data from trials highlights the need for increasing focus on genomic surveillance if MDA is used as a population-based strategy for malaria control.

## Appendix 1

### Detailed search methodology

Detailed search terms used in MEDLINE

Ovid MEDLINE(R) ALL < 1946 to May 24, 2021 >

1. Malaria/or malaria, cerebral/or malaria, falciparum/or blackwater fever/or malaria, vivax/67560
2. (Malaria\* or falciparum or plasmodium infection\* or remittent fever or blackwater fever).mp. 107016
3. 1 or 2 107016
4. Chemoprevention/or mass drug administration/6658
5. Mass screening/or anonymous testing/or multiphasic screening/108880
6. [Mass adj3 (screening\* or administration\* or test\* or treatment\*)].mp.121669
7. (MSAT or MTAT or FSAT or FEMSE or MPPT or TME or DOT or RACD or IPTp or SMC).mp.52228
8. Direct observed therap\*.mp.27
9. Mass piperazine prophylactic treat\*.mp.0
10. Seasonal malaria chemo\*.mp.115
11. (Focal screening and treat\*).mp.6
12. Intermittent preventive treatment in pregnancy.mp.148
13. Reactive case detection.mp.71
14. (Focal screening and treatment).mp.6
15. 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 181089
16. 3 and 15 2086
17. Exp Antimalarials/85757
18. (Anti-malarial\* or antimalarial\*).mp.35587
19. Drug resistance/or drug resistance, multiple/or drug tolerance/83191

20. [(Drug or multidrug) adj3 (resist\* or toleran\* or tolerate\* or sensit\*)].mp.368770
21. [(Drug or multidrug) adj3 (resist\* or toleran\* or tolerate\* or sensit\*)].mp.368770
22. 17 or 18 or 19 or 20 or 21 448688
23. 16 and 22 1195
24. (Piperazine or dihydroartemisinin or dihydroartemisinin-piperazine).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] 2129
25. 23 and 24 117

These studies were filtered to included publication dates of 2011 onwards, resulting in 99 papers in total. Detailed search terms used in EMBASE.

Embase <1974 to 2021 May 24>

1. Malaria falciparum/or *Plasmodium vivax* malaria/ or malaria/ or *Plasmodium knowlesi* malaria/ or *Plasmodium ovale* malaria/88321
2. (Malaria\* or falciparum or plasmodium infection\* or remittent fever or blackwater fever).mp.130248
3. 1 or 2 130248
4. Mass drug administration/or chemoprophylaxis/26856
5. Mass screening/56612
6. [Mass adj3 (screening\* or administration\* or test\* or treatment\*)].mp.76289
7. Direct observed therap\*.mp.65
8. Mass piperazine prophylactic treat\*.mp.0
9. Seasonal malaria chemo\*.mp.255
10. (Focal screening and treat\*).mp.16
11. Intermittent preventive treatment in pregnancy.mp.226
12. Reactive case detection.mp.157
13. (Focal screening and treatment).mp.15
14. 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 102462
15. 3 and 14 3684
16. Antimalarial agent/24465
17. (Anti-malarial\* or antimalarial\*).mp.41035
18. Antimalarial drug resistance/ or antimalarial drug susceptibility/ or drug resistance/ or multidrug resistance/143743
19. [(Drug or multidrug) adj3 (resist\* or toleran\* or tolerate\* or sensit\*)].mp.470284
20. 16 or 17 or 18 or 19 502467
21. 15 and 20 1461

22. (Dihydroartemisinin or dihydroartemisinin plus piperazine or piperazine).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] 4363
23. 21 and 22 192

These studies were filtered to included publication dates of 2011 onwards, resulting in 129 papers in total. Removing duplicates resulted in 184 papers. These 134 papers were excluded because they either did not include reference to PPQ or DHA-PPQ, or they did not include reference to drug resistance/efficacy/tolerability or sensitivity in the abstract or title. This resulted in 50 publications which were assessed for eligibility. 35 studies were excluded because they did not include measurement of molecular markers of resistance, leaving 15 studies from MEDLINE or EMBASE which were included in this review.

#### IDDO search

Search date: 7/7/2021.

In the IDDO database (<https://www.iddo.org>), the terms 'Malaria' and 'Piperazine' were used to identify publications. This resulted in 9 publications, 2 of which included analysis of molecular markers of resistance and were included in this review.

#### Clinicaltrials.gov search

Search date: 7/7/2021.

The following search (Disease: malaria, Terms: Piperazine Status: Has results) resulted in 13 clinical studies. Four studies were excluded as these were pharmacokinetic studies of interventions in healthy volunteers. Three studies were excluded as these were studies involving induced blood stage malaria in healthy participants, as opposed to vector transmitted malaria. This resulted in 1 Phase 2 clinical trial and 5 Phase 3 clinical trial remaining. There were 7 publications associated with these clinical trials which included analysis of molecular markers of resistance. Four of these were unique and included in this review.

#### Abbreviations

MDA: Mass drug administration; DHA-PPQ: Dihydroartemisinin-piperazine; WGS: Whole genome sequencing; SNPs: Single nucleotide polymorphisms; WHO: World Health Organization; ACT: Artemisinin-combination therapy; AL: Artemether-lumefantrine; AS-AQ: Artesunate-amodiaquine; ITN: Insecticide-treated nets; IPTp: Intermittent preventive treatment in pregnancy; LLIN: Long-lasting insecticidal nets; SP: Sulfadoxine-pyrimethamine; AS: Artesunate; QN: Quinine; IVM: Ivermectin; DHA: Dihydroartemisinin; DV: Digestive vacuole; PPQ: Piperazine; CQ: Chloroquine; CNV: Copy number variant; PCR: Polymerase chain reaction; DNA: Deoxyribose nucleic acid.

#### Acknowledgements

Thank you to colleagues at LSHTM and SGUL for our many interesting and insightful discussions.

#### Author contributions

SM conceived, designed and conducted the study. EM, SC and TGC provided data and assisted with analysis. SM wrote the first draft of the manuscript. All authors commented on and edited the manuscript. All authors read and approved the final manuscript.

#### Funding

SM is funded by Medical Research Council UK (Grant No. MR/N013638/1). TGC is funded by Medical Research Council UK (Grant no. MR/M01360X/1, MR/N010469/1, MR/R025576/1, and MR/R020973/1) Grants. SC is funded by BloomsburySET and Medical Research Council UK Grants (MR/M01360X/1, MR/R025576/1, and MR/R020973/1). AL is funded by Joint Global Health Trials Scheme (MRC, Wellcome Trust, UKRI, NIHR, Grant no. MR/S005013/1).

#### Availability of data and materials

The processed datasets are available from the corresponding author. All data is available from the individual studies cited, including ENA accession numbers for raw sequences.

#### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

#### Author details

<sup>1</sup>Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK. <sup>2</sup>Institute of Infection and Immunity, St George's University of London, London, UK. <sup>3</sup>Faculty of Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, London, UK.

Received: 17 January 2022 Accepted: 10 May 2022

Published online: 11 June 2022

#### References

1. WHO. World malaria report 2021. Geneva: World Health Organization; 2021.
2. WHO. Mass drug administration, mass screening and treatment and focal screening and treatment for malaria. Malaria policy advisory committee meeting. Geneva: World Health Organization; 2015.
3. Payne D. Did medicated salt hasten the spread of chloroquine resistance in *Plasmodium falciparum*? *Parasitol Today*. 1988;4:112–5.
4. WHO. World malaria report 2020. Geneva: World Health Organization; 2020.
5. Slater L, Betson M, Ashraf S, Sargison N, Chaudhry U. Current methods for the detection of antimalarial drug resistance in *Plasmodium* parasites infecting humans. *Acta Trop*. 2021;216: 105828.
6. Takala-Harrison S, Laufer MK. Antimalarial drug resistance in Africa: key lessons for the future. *Ann N Y Acad Sci*. 2015;1342:62–7.
7. Looareesuwan S, Viravan C, Webster HK, Kyle DE, Hutchinson DB, Canfield CJ. Clinical studies of atovaquone, alone or in combination with other antimalarial drugs, for treatment of acute uncomplicated malaria in Thailand. *Am J Trop Med Hyg*. 1996;54:62–6.
8. Edgcomb JH, Arnold J, Yount Eh Jr, Alving AS, Eichelberger L, Jeffery GM, et al. Primaquine, SN 13272, a new curative agent in vivax malaria; a preliminary report. *J Natl Malar Soc*. 1950;9:285–92.
9. Noedl H, Se Y, Schaefer K, Smith BL, Socheat D, Fukuda MM, Artemisinin Resistance in Cambodia 1 (ARC1) Study Consortium. Evidence of artemisinin-resistant malaria in western Cambodia. *N Engl J Med*. 2008;359:2619–20.
10. Institute of Medicine (US) Committee on the Economics of Antimalarial Drugs. Saving lives, buying time: economics of malaria drugs in an age

- of resistance. Washington (DC): National Academies Press (US); 2004. p. 1125–7.
11. van Aghtmael MA, Eggelte TA, van Boxel CJ. Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication. *Trends Pharmacol Sci.* 1999;20:199–205.
  12. You-you T, Mu-yun N, Yurong Z, Lan-na L, Shu-lian G, Mu-qun Z, et al. Studies on the constituents of *Artemisia annua*. *J Med Plant Res.* 1982;44:143–5.
  13. Wang J, Zhang CJ, Chia WN, Loh CC, Li Z, Lee YM, He Y, Yuan LX, Lim TK, et al. Haem-activated promiscuous targeting of artemisinin in *Plasmodium falciparum*. *Nat Commun.* 2015;6:10111.
  14. Spielmann T, Gras S, Sabitzki R, Meissner M. Endocytosis in *Plasmodium* and toxoplasma parasites. *Trends Parasitol.* 2020;36:520–32.
  15. Heller LE, Roepe PD. Artemisinin-based antimalarial drug therapy: molecular pharmacology and evolving resistance. *Trop Med Infect Dis.* 2019;4:89.
  16. Eckstein-Ludwig U, Webb RJ, Van Goethem ID, East JM, Lee AG, Kimura M, et al. Artemisinins target the SERCA of *Plasmodium falciparum*. *Nature.* 2003;424:957–61.
  17. Le Thi DT, Le NH, Nguyen CH, Phan Thi D, Na-Bangchang K. Pharmacokinetics of a five-day oral dihydroartemisinin monotherapy regimen in patients with uncomplicated falciparum malaria. *Drug Metab Pharmacokinet.* 2008;23:158–64.
  18. Nguyen DV, Nguyen QP, Nguyen ND, Le TT, Nguyen TD, Dinh DN, Nguyen TX, et al. Pharmacokinetics and ex vivo pharmacodynamic antimalarial activity of dihydroartemisinin-piperaquine in patients with uncomplicated falciparum malaria in Vietnam. *Antimicrob Agents Chemother.* 2009;53:3534–7.
  19. Newton PN, van Vugt M, Teja-Isavadharm P, Siriyanonda D, Rasameesoraj M, et al. Comparison of oral artesunate and dihydroartemisinin antimalarial bioavailabilities in acute falciparum malaria. *Antimicrob Agents Chemother.* 2002;46:1125–7.
  20. Wirth DF, Alonso PL. Malaria biology in the era of eradication. *Cold Spring Harbor Perspect Med.* 2017. <https://doi.org/10.1101/cshperspect.a025510>.
  21. Ross LS, Dhingra SK, Mok S, Yeo T, Wicht KJ, Kümpornsin K, et al. Emerging Southeast Asian PfCRT mutations confer *Plasmodium falciparum* resistance to the first-line antimalarial piperaquine. *Nat Commun.* 2018;9:25–8.
  22. Dhingra SK, Redhi D, Combrinck JM, Yeo T, Okombo J, Henrich PP, et al. A variant PfCRT isoform can contribute to *Plasmodium falciparum* resistance to the first-line partner drug piperaquine. *mBio.* 2017;8:3.
  23. Sachanonta N, Chotivanich K, Chairsri U, Turner GD, Ferguson DJ, Day NP, et al. Ultrastructural and real-time microscopic changes in *P. falciparum*-infected red blood cells following treatment with antimalarial drugs. *Ultrastruct Pathol.* 2011;35:214–25.
  24. Kim J, Tan YZ, Wicht KJ, Erramilli SK, Dhingra SK, Okombo J, Vendome J, et al. Structure and drug resistance of the *Plasmodium falciparum* transporter PfCRT. *Nature.* 2019;576:315–20.
  25. Saunders DL, Vanachayangkul P, Lon C, U.S. Army Military Malaria Research Program, National Center for Parasitology, Entomology, and Malaria Control (CNM), Royal Cambodian Armed Forces. Dihydroartemisinin-piperaquine failure in Cambodia. *N Engl J Med.* 2014;371:484–5.
  26. van der Pluijm RW, Imwong M, Chau NH, Hoa NT, Thuy-Nhien NT, Thanh NV, et al. Determinants of dihydroartemisinin-piperaquine treatment failure in *Plasmodium falciparum* malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. *Lancet Infect Dis.* 2019;19:952–61.
  27. WHO. Report on antimalarial drug efficacy, resistance and response: 10 years of surveillance (2010–2019). Geneva: World Health Organization; 2020.
  28. Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, et al. A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: a phenotype–genotype association study. *Lancet Infect Dis.* 2017;17:174–83.
  29. Amato R, Lim P, Miotto O, Amaratunga C, Dek D, Pearson RD, et al. Genetic markers associated with dihydroartemisinin–piperaquine failure in *Plasmodium falciparum* malaria in Cambodia: a genotype–phenotype association study. *Lancet Infect Dis.* 2017;17:164–73.
  30. Bopp S, Magistrado P, Wong W, Schaffner SF, Mukherjee A, Lim P, Dhorda M, et al. Plasmeppsin II–III copy number accounts for bimodal piperaquine resistance among Cambodian *Plasmodium falciparum*. *Nat Commun.* 2018;9:1769.
  31. Agrawal S, Moser KA, Morton L, Cummings MP, Parihar A, Dwivedi A, et al. Association of a novel mutation in the *Plasmodium falciparum* chloroquine resistance transporter with decreased piperaquine sensitivity. *J Infect Dis.* 2017;216:468–76.
  32. Ansbro MR, Jacob CG, Amato R, Kekre M, Amaratunga C, Sreng S, Suon S, et al. Development of copy number assays for detection and surveillance of piperaquine resistance associated plasmeppsin 2/3 copy number variation in *Plasmodium falciparum*. *Malar J.* 2020;19:181.
  33. Mukherjee A, Gagnon D, Wirth DF, Richard D. Inactivation of plasmeppsins 2 and 3 sensitizes *Plasmodium falciparum* to the antimalarial drug piperaquine. *Antimicrob Agents Chemother.* 2018;62:e02309–e2317.
  34. Nasamu AS, Polino AJ, Istvan ES, Goldberg DE. Malaria parasite plasmeppsins: more than just plain old degradative pepsins. *J Biol Chem.* 2020;295:8425–41.
  35. Dhingra SK, Small-Saunders JL, Ménard D, Fidock DA. *Plasmodium falciparum* resistance to piperaquine driven by PfCRT. *Lancet Infect Dis.* 2019;19:1168–9.
  36. Boonyalai N, Vesely BA, Thamnurak C, Praditpol C, Fagnark W, Kirativanich K, et al. Piperaquine resistant Cambodian *Plasmodium falciparum* clinical isolates: in vitro genotypic and phenotypic characterization. *Malar J.* 2020;19:269.
  37. Chebore W, Zhou Z, Westercamp N, Otieno K, Shi YP, Sergeant SB, et al. Assessment of molecular markers of anti-malarial drug resistance among children participating in a therapeutic efficacy study in western Kenya. *Malar J.* 2020;19:291.
  38. Loesbanluechai D, Kotanan N, de Cozar C, Kochakarn T, Ansbro MR, et al. Overexpression of plasmeppsin II and plasmeppsin III does not directly cause reduction in *Plasmodium falciparum* sensitivity to artesunate, chloroquine and piperaquine. *Int J Parasitol Drugs Drug Resist.* 2019;9:16–22.
  39. Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, et al. Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number. *Lancet.* 2004;364:438–47.
  40. Parobek CM, Parr JB, Brazeau NF, Lon C, Chaorattanakawee S, Gosi P, et al. Partner-drug resistance and population substructuring of artemisinin-resistant *Plasmodium falciparum* in Cambodia. *Genome Biol Evol.* 2017;9:1673–86.
  41. Pulcini S, Staines HM, Lee AH, Shafik SH, Bouyer G, Moore CM, Daley DA, et al. Mutations in the *Plasmodium falciparum* chloroquine resistance transporter, PfCRT, enlarge the parasite's food vacuole and alter drug sensitivities. *Sci Rep.* 2015;5:14552.
  42. Conrad MD, LeClair N, Arinaitwe E, Wanzira H, Kakuru A, Bigira V, Muhindo M, et al. Comparative impacts over 5 years of artemisinin-based combination therapies on *Plasmodium falciparum* polymorphisms that modulate drug sensitivity in Ugandan children. *J Infect Dis.* 2014;210:344–53.
  43. Veiga MI, Ferreira PE, Malmberg M, Jörnham L, Björkman A, Nosten F, et al. pfmdr1 amplification is related to increased *Plasmodium falciparum* in vitro sensitivity to the bisquinoline piperaquine. *Antimicrob Agents Chemother.* 2012;56:3615–9.
  44. Osborne A, Manko E, Takeda M, Kaneko A, Kagaya W, Chan C, Ngara M, et al. Characterizing the genomic variation and population dynamics of *Plasmodium falciparum* malaria parasites in and around Lake Victoria. *Kenya Sci Rep.* 2021;11:19809.
  45. Turkiewicz A, Manko E, Sutherland CJ, Diez Benavente E, Campino S, Clark TG. Genetic diversity of the *Plasmodium falciparum* GTP-cyclohydrolase 1, dihydrofolate reductase and dihydropteroate synthetase genes reveals new insights into sulfadoxine-pyrimethamine antimalarial drug resistance. *PLoS Genet.* 2020;16: e1009268.
  46. MalariaGEN, Ahouidi A, Ali M, Almagro-Garcia J, Amambua-Ngwa A, et al. An open dataset of *Plasmodium falciparum* genome variation in 7,000 worldwide samples. *Wellcome Open Res.* 2021;6:42.
  47. WHO and Technical Expert Group. Technical expert group (TEG) on drug efficacy and response. Geneva: World Health Organization; 2015.
  48. Eisele TP. Mass drug administration can be a valuable addition to the malaria elimination toolbox. *Malar J.* 2019;18:281.
  49. Madanitsa M, Kalilani L, Mwapa V, van Eijk AM, Khairallah C, Ali D, et al. Scheduled intermittent screening with rapid diagnostic tests and treatment with dihydroartemisinin-piperaquine versus intermittent preventive therapy with sulfadoxine-pyrimethamine for malaria in pregnancy

- in Malawi: an open-label randomized controlled trial. *PLoS Med.* 2016;13:e1002124.
50. Huang F, Takala-Harrison S, Jacob CG, Liu H, Sun X, Yang H, Nyunt MM, et al. A Single mutation in K13 predominates in southern China and is associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment. *J Infect Dis.* 2015;212:1629–35.
  51. Imwong M, Dhorda M, Myo Tun K, Thu AM, Phyo AP, Proux S, Suwannasin K, et al. Molecular epidemiology of resistance to antimalarial drugs in the Greater Mekong subregion: an observational study. *Lancet Infect Dis.* 2020;20:1470–80.
  52. Gupta H, Galatas B, Chidimatembue A, Huijben S, Cisteró P, Matambisso G, et al. Effect of mass dihydroartemisinin-piperaquine administration in southern Mozambique on the carriage of molecular markers of antimalarial resistance. *PLoS ONE.* 2020;15:e0240174.
  53. Conrad MD, Mota D, Foster M, Tukwasibwe S, Legac J, Tumwebaze P, et al. Impact of intermittent preventive treatment during pregnancy on *Plasmodium falciparum* drug resistance-mediating polymorphisms in Uganda. *J Infect Dis.* 2017;216:1008–17.
  54. von Seidlein L, Peto TJ, Landier J, Nguyen TN, Tripura R, Phommasone K, et al. The impact of targeted malaria elimination with mass drug administrations on *falciparum* malaria in Southeast Asia: a cluster randomised trial. *PLoS Med.* 2019;16:e1002745.
  55. Landier J, Kajeechiwa L, Thwin MM, Parker DM, Chaumeau V, Wiladphaingern J, et al. Safety and effectiveness of mass drug administration to accelerate elimination of artemisinin-resistant *falciparum* malaria: a pilot trial in four villages of eastern Myanmar. *Wellcome Open Res.* 2017;2:81.
  56. Leroy D, Macintyre F, Adoke Y, Ouoba S, Barry A, Mombongo G, et al. African isolates show a high proportion of multiple copies of the *Plasmodium falciparum* plasmepsin-2 gene, a piperaquine resistance marker. *Malar J.* 2019;18:126.
  57. Imwong M, Suwannasin K, Kunasol C, Sutawong K, Mayxay M, Rekol H, et al. The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study. *Lancet Infect Dis.* 2017;17:491–7.
  58. Son DH, Thuy-Nhien N, von Seidlein L, Le Phuc-Nhi T, Phu NT, Tuyen NTK, et al. The prevalence, incidence and prevention of *Plasmodium falciparum* infections in forest rangers in Bu Gia Map National Park, Binh Phuoc province, Vietnam: a pilot study. *Malar J.* 2017;16:444.
  59. Nayebara P, Asua V, Conrad MD, Kajubi R, Kakuru A, Nankabirwa JI, et al. Associations between malaria-preventive regimens and *Plasmodium falciparum* drug resistance-mediating polymorphisms in Ugandan pregnant women. *Antimicrob Agents Chemother.* 2020;64:e01047–e1120.
  60. Wallender E, Zhang N, Conrad M, Kakuru A, Muhindo M, Tumwebaze P, et al. Modeling prevention of malaria and selection of drug resistance with different dosing schedules of dihydroartemisinin-piperaquine preventive therapy during pregnancy in Uganda. *Antimicrob Agents Chemother.* 2019;63:e01393–e1418.
  61. Zongo I, Milligan P, Compaore YD, Some AF, Greenwood B, Tarning J, et al. Randomized noninferiority trial of dihydroartemisinin-piperaquine compared with sulfadoxine-pyrimethamine plus amodiaquine for seasonal malaria chemoprevention in Burkina Faso. *Antimicrob Agents Chemother.* 2015;59:4387–96.
  62. Somé AF, Zongo I, Compaoré YD, Sakandé S, Nosten F, Ouédraogo JB, Rosenthal PJ. Selection of drug resistance-mediating *Plasmodium falciparum* genetic polymorphisms by seasonal malaria chemoprevention in Burkina Faso. *Antimicrob Agents Chemother.* 2014;58:3660–5.
  63. Tumwebaze P, Jagannathan P, Legac J, Mota D, Tukwasibwe S, Whalen M, et al. Impact of antimalarial treatment and chemoprevention on the drug sensitivity of malaria parasites isolated from Ugandan Children. *Antimicrob Agents Chemother.* 2015;59:3018–30.
  64. Taylor AR, Flegg JA, Holmes CC, Guérin PJ, Sibley CH, Conrad MD, et al. Artemether-lumefantrine and dihydroartemisinin-piperaquine exert inverse selective pressure on *Plasmodium falciparum* drug sensitivity-associated haplotypes in Uganda. *Open Forum Infect Dis.* 2016;4:ofw229.
  65. Ochong E, Tumwebaze PK, Byaruhanga O, Greenhouse B, Rosenthal PJ. Fitness consequences of *Plasmodium falciparum* pfm1 polymorphisms inferred from ex vivo culture of ugandan parasites. *Antimicrob Agents Chemother.* 2013;57:4245–51.
  66. Tripura R, Peto TJ, Chea N, Chan D, Mukaka M, Sirithiranont P, et al. A controlled trial of mass drug administration to interrupt transmission of multidrug-resistant *falciparum* malaria in Cambodian villages. *Clin Infect Dis.* 2018;67:817–26.
  67. Deng C, Huang B, Wang Q, Wu W, Zheng S, Zhang H, et al. Large-scale artemisinin-piperaquine mass drug administration with or without primaquine dramatically reduces malaria in a highly endemic region of Africa. *Clin Infect Dis.* 2018;67:1670–6.
  68. Macintyre F, Adoke Y, Tiono AB, Duong TT, Mombongo G, Bouyou-Akotet M, et al. A randomised, double-blind clinical phase II trial of the efficacy, safety, tolerability and pharmacokinetics of a single dose combination treatment with artefenomel and piperaquine in adults and children with uncomplicated *Plasmodium falciparum* malaria. *BMC Med.* 2017;15:181.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.