Global **Malaria** Programme

Report on antimalarial drug efficacy, resistance and response

years of surveillance (2010–2019)

World Health Organization

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Report on antimalarial drug efficacy, resistance and response: 10 years of surveillance (2010–2019)

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ABBREVIATIONS AND ACRONYMS

ACPR	adequate clinical and parasitological response
ACT	artemisinin-based combination therapy
AL	artemether-lumefantrine
AQ	amodiaquine
AS	artesunate
AUC	area under the plasma drug concentration curve
CQ	chloroquine
DHA	dihydroartemisinin
ETF	early treatment failure
G6PD	glucose-6-phosphate dehydrogenase
GMP	Global Malaria Programme
GMS	Greater Mekong subregion
GPARC	Global Plan for Artemisinin Resistance Containment
HIV	human immunodeficiency virus
iDES	integrated drug efficacy surveillance
IPTi	intermittent preventive treatment of infants
IPTp	intermittent preventive treatment of pregnant women
LCF	late clinical failure
LPF	late parasitological failure
MDA	mass drug administration
MQ	mefloquine
PCR	polymerase chain reaction
Pfcrt	*P. falciparum chloroquine resistance transporter*
Pfcytb	*P. falciparum cytochrome b*
Pfdhfr	*P. falciparum dihydrofolate reductase*
Pfdhps	*P. falciparum dihydropteroate synthase*
PfK13	*P. falciparum Kelch 13*
Pfmdr1	*P. falciparum multidrug resistance 1 protein*
Pfpm 2–3	*P. falciparum plasmepsin 2–3*
PPQ	piperaquine
PQ	primaquine
Pvcrt-o	*P. vivax chloroquine resistance transporter*
Pvdhfr	*P. vivax dihydrofolate reductase*
Pvdhps	*P. vivax dihydropteroate synthase*
Pvmdr1	*P. vivax multidrug resistance 1 protein*
PSA	piperaquine survival assay
PY	pyronaridine
RSA	ring-stage survival assay
SMC	seasonal malaria chemoprevention
SP	sulfadoxine-pyrimethamine
TES	therapeutic efficacy studies
WHO	World Health Organization

EXECUTIVE SUMMARY

Background

A relatively small number of drugs are currently being used to save the lives of millions of people infected each year by malaria. These drugs need to remain efficacious until new drugs and tools become available.

Monitoring antimalarial drug efficacy and resistance is important for the early detection of resistance which in turn enables timely action to prevent its spread and limit the impact on global health. Measurement of drug efficacy and resistance in malaria is complex. This report provides an overview of the tools currently used to monitor drug efficacy and resistance. The report also provides a summary of activities needed to minimize any public health impact of antimalarial drug resistance as well a review of the data collected from 2010–2019 in the World Health Organization (WHO) global database on antimalarial drug efficacy and resistance.

The last report reviewing the data available on antimalarial drug efficacy and resistance was published in 2010, less than two years after the first report from Cambodia of *Plasmodium falciparum* parasite with delayed clearance following treatment with artemisinins.[1] Artemisinins are the core component of artemisinin-based combination therapies (ACTs). This delayed clearance has been termed artemisinin partial resistance. Over the past 10 years much more data have become available on artemisinin partial resistance as well as on the impact of resistance to ACT partner drugs.

Mutations in the *P. falciprum Kelch 13* (*PfK13*) BTB/POZ and propeller domain have been shown to be associated with artemisinin partial resistance. High rate of ACT failure with dihydroartemisinin-piperaquine (DHA-PPQ) has been documented in the Greater Mekong subregion (GMS) and mutations associated with resistance to the ACT partner drug piperaquine were identified. The identification of molecular markers makes surveillance of parasite genotypes an important supplement to monitoring of the parasite response to different treatments.

Responding to the threat of drug resistance

Imperfect coverage and quality of malaria interventions contribute to the emergence and spread of resistance. Correct diagnosis is not always provided, drugs are sometimes misused, some patients may not have access to quality treatments and the coverage of vector control may remain low for some key populations. These failures lead to increased exposure of the malaria parasites to drugs, increasing the risk of drug resistance.

Prolonging the efficaciousness of the currently used drugs will require addressing shortcomings in the quality and coverage of malaria interventions, and adding specific activities that could help to minimize the risk of drug resistance and limit the public health consequences when drug resistance emerges and spreads.

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[1] Artemisinin and its derivatives.

At present, the main challenge of artemisinin and ACT partner drug resistance centres on the need for systems that can quickly inform on the need for changes in treatment policy, and a health system that can implement rapid policy changes so as to provide patients with the specific treatment needed.

WHO global database on antimalarial drug efficacy and resistance

The WHO global database on antimalarial drug efficacy and resistance contains data from therapeutic efficacy studies (TES) conducted on *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*, as well as molecular marker studies of *P. falciparum* drug resistance. TES are mainly done using first- and second-line treatment as well as treatments considered for introduction into the treatment policy.

The main findings from the analysis of the WHO global database on antimalarial drug efficacy and resistance are:

- Overall, where tested, first- and second-line treatments are efficacious for *P. falciparum*. Where high treatments failures rates were reported, policy changes have been made or are ongoing.

- In four countries in the GMS – Cambodia, Lao People's Democratic Republic, Thailand and Viet Nam – high rates of treatment failures have been detected after treatment with some ACTs. However, there are still at least two and sometimes three ACTs available that can effectively treat *P. falciparum* in these countries.

- Outside the GMS, resistance to sulfadoxine-pyrimethamine has meant that some countries (Sudan, Somalia, north-east India) have had to abandon artesunate+sulfadoxine-pyrimethamine (AS+SP) as a treatment for *P. falciparum*. These countries have changed to an alternative, highly efficacious ACT.

- The efficacy of ACTs in Africa is being monitored in most malaria-endemic countries. Artemether-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) are the first-line treatment policies used in most African countries, with some countries adding DHA-PPQ. Between 2010 and 2019, the overall average efficacy rates of AL, AS-AQ and DHA-PPQ were 98.0%, 98.4%, and 99.4% respectively. Efficacy is consistently high with a >10% failure rate only being identified in studies of AL and only in four of the 300 AL studies conducted over the past 10 years. Treatment failures following treatment with AL have been reported in travellers coming back from Africa to Europe, but resistance to lumefantrine has not been confirmed in Africa.

- While *P. vivax* resistance to chloroquine has been reported from all WHO regions, chloroquine remains efficacious in most part of the world. *P. vivax* resistance to artemisinin has not be identified.

- Data on *PfK13* mutations are available from all regions. Of the samples collected 2010-2019, 83.4% were found to be *PfK13* wild type. However, sampling is undertaken more frequently where resistance is suspected, so the prevalence in the samples may differ from the overall prevalence in parasites. The validated marker for artemisinin partial resistance C580Y is the mutation

most frequently identified; it was found in 9.8% of samples. The highest prevalence of *PfK13* mutations is in countries in the GMS where the majority of the samples is found to carry *PfK13* mutations.

- Outside GMS, findings of *PfK13* mutations in two countries give cause for concern:
    - In Guyana, C580Y mutations were found in surveys in 2010 and 2017.
    - In Rwanda, R561H was found in 11.9% of all the samples collected in 2018 (n=219). R561H is a validated marker of artemisinin partial resistance. There is evidence suggesting that the R561H mutation may be affecting the clearance rate, although to date, the ACTs tested remain efficacious.

- High prevalence of markers of *P. falciparum* resistance to piperaquine has been identified in the four GMS countries Cambodia, Lao People's Democratic Republic, Thailand and Viet Nam where high failure rates after treatment with DHA-PPQ have been detected. In several African countries, studies and surveys have detected significant proportions of the samples carrying the marker of piperaquine resistance.

- After a change in treatment policy in Cambodia from DHA-PPQ to artesunate-mefloquine (AS-MQ), fewer parasites appear to carry both C580Y and the marker of piperaquine resistance.

Conclusions

Countries and partners need to continue to work to improve coverage and quality of malaria interventions. This will both ensure better patient care and decrease the risk and impact of drug resistance. Up-to-date, quality data are needed on the efficacy of the recommended treatments, to ensure that patients receive efficacious treatment. Conducting these studies can be challenging, but the investment of time and resources is small when compared with the funding spent on treatments and the millions of patients depending on the continued efficacy of these treatments. Molecular markers are an asset for confirming resistance, in the analysis of trends and as an early warning signal. The identification of additional markers of resistance will further strengthen the efforts of resistance monitoring.

While chloroquine resistance will continue to pose a challenge for *P. vivax*, the primary challenge of *P. vivax* chemotherapeutics is that of successful radical cure. More countries are likely to have to change to the more expensive ACT treatments if chloroquine resistance continues to spread. The use of 8-aminoquinolines is limited by its efficacy, safety, patient adherence and drug interactions.

Currently, there are ACTs available capable of treating all *P. falciparum* strains. In some countries of the GMS, most of the *P. falciparum* parasites now carry mutations associated with artemisinin partial resistance. Where resistance to the ACT partner drug has also been identified, high failure rates to treatments have been identified. However, even in the GMS there are highly efficacious ACTs available to treat patients.

There is evidence that R561H, a validated marker of artemisinin partial resistance, has emerged and is being selected for in Rwanda. The ACTs tested in Rwanda remain efficacious, meaning that any immediate impact for patients is unlikely. However,

it is of concern that parasites have emerged with partial resistance to the central component in the drugs used to treat millions across Africa. In the GMS, artemisinin partial resistance is likely to have been involved in the spread of resistance to partner drugs.

It is worth noting that China was able to eliminate malaria despite the presence of malaria parasites partially resistant to artemisinins, and that great progress is being made towards elimination in the GMS where resistance poses the greatest challenge.

1. INTRODUCTION

Resistance to antimalarial drugs challenges our ability to save lives threatened by malaria, and to eliminate the burden that malaria places on individuals and societies. This burden is substantial, with malaria having caused an estimated 228 million cases and 405 000 deaths in 2018 *(1)*. The *Global technical strategy for malaria 2016–2030*, adopted at the World Health Assembly in 2015, highlights the potential of antimalarial drug resistance to seriously weaken the effectiveness of malaria responses and erode the gains achieved *(2)*.

Monitoring antimalarial drug efficacy and resistance is important for the early detection of resistance, which in turn enables timely action to prevent its spread and limit the impact on global health. Measurement of drug efficacy and resistance in malaria is complex. Studies of clinical and parasitological outcomes are the main sources of information on which national malaria control programmes base treatment policy; however, other studies are needed to confirm drug resistance if suspected.

Chapter 2 of this report gives an overview of the currently recommended treatments as well as other recommended uses of antimalarial medicine.

Chapter 3 defines drug resistance and antimalarial drug efficacy. In addition, the chapter provides a summary of activities needed to minimize any public health impact of antimalarial drug resistance.

Chapter 4 describes the tools currently used to monitor drug efficacy and resistance. This includes an overview of methods used to evaluate the cause of treatment failure.

Chapter 5 provides a summary of the data in the World Health Organization (WHO) global database on antimalarial drug efficacy and resistance. This database contains data from therapeutic efficacy studies (TES) conducted on *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*, as well as molecular marker studies of *P. falciparum* drug resistance. The report summarizes these data by region. It will be updated as new information becomes available.

2. ANTIMALARIAL TREATMENT

2.1 WHO-recommended treatments for malaria

WHO recommends artemisinin-based combination therapies (ACTs) as first- and second-line treatment for uncomplicated malaria caused by *P. falciparum* (Table 1). ACTs combine an artemisinin derivative with a partner drug. The role of the artemisinin compound is to reduce the number of parasites during the first 3 days of treatment (i.e. reduce parasite biomass), while the role of the partner drug is to eliminate the remaining parasites (i.e. cure the infection). For the treatment of blood-stage parasites in patients with uncomplicated *P. vivax*, WHO recommends either chloroquine (CQ) or an ACT for areas with CQ-resistant *P. vivax*. WHO currently recommends six ACTs: artemether-lumefantrine (AL), artesunate-amodiaquine (AS-AQ), artesunate-mefloquine (AS-MQ), artesunate-pyronaridine (AS-PY), artesunate+sulfadoxine-pyrimethamine (AS+SP) and dihydroartemisinin-piperaquine (DHA-PPQ). CQ is still being used as treatment for locally acquired cases of *P. falciparum* in some Mesoamerican countries and Hispaniola.

TABLE 1
WHO-recommended malaria treatment for uncomplicated malaria *(3)*

P. falciparum	
Uncomplicated	ACT
Pregnant women	
First trimester	Quinine+clindamycin
Second and third trimester	ACT
Other species *(P. vivax, P. ovale, P. malariae, P. knowlesi)*	
Blood-stage infection	
Uncomplicated	Chloroquine or ACT
Pregnant women	
First trimester	Chloroquine or quinine
Second and third trimester	Chloroquine or ACT
Liver-stage infection *(P. vivax, P. ovale)*	Primaquine*

ACT: artemisinin-based combination therapy; *P. Plasmodium*; WHO: World Health Organization.

* Not recommended for infants aged <6 months, pregnant women, women breastfeeding infants aged <6 months, or those with glucose-6-phosphate dehydrogenase (G6PD) deficiency.

The recommended treatment for severe malaria is injectable AS (intramuscular or intravenous) for at least 24 hours, followed by a complete 3-day course of an ACT once the patient can tolerate oral medicines. If parenteral AS is not available, the use of artemether is recommended in preference to quinine. Where injectable treatment cannot be given, children aged under 6 years with severe malaria should receive a single pre-referral treatment with rectal AS before being referred immediately to a health care facility where the full level of care can be provided *(3)*.

2.2 Other recommended use of antimalarial medicine

Antimalarial medicines are used not only to treat confirmed malaria cases but also to prevent malaria. For example, antimalarial medicines are used for chemoprophylaxis, chemoprevention through intermittent presumptive treatment of certain risk groups in highly endemic areas, and mass drug administration (MDA) in certain settings.

Drugs recommended for chemoprophylaxis for travellers are doxycycline or atovaquone–proguanil. In addition, MQ chemoprophylaxis can be used in areas with no *P. falciparum* resistance against MQ, and CQ can be used in areas with no risk of *P. falciparum* infection *(4)*. Primaquine (PQ) has been recommended for certain circumstances by the United States Centers for Disease Control and Prevention.

P. falciparum infection is frequently asymptomatic in older children and adults living in high transmission settings, where levels of acquired immunity tend to be high. To prevent the adverse consequences of malaria during pregnancy, WHO recommends intermittent preventive treatment of pregnant women (IPTp) with SP in areas with moderate to high malaria transmission in Africa. WHO recommends that IPTp be given to all pregnant women during antenatal care visits, starting as early as possible in the second trimester. Each IPTp-SP dose should be given at least 1 month apart, with a total of at least three doses during each pregnancy. In several countries in Africa, some *P. falciparum* parasites carry quintuple mutations linked to SP resistance.[2] However,

[2] Quintuple mutations are *Pfdhps* (A437G and G540E) and *Pfdhfr* (N51I, C59R and S108N).

IPTp-SP appears to remain effective in preventing the adverse consequences of malaria on maternal and fetal outcomes even in areas where quintuple mutations linked to SP resistance are prevalent in *P. falciparum*. Consequently, IPTp-SP is still recommended in all areas, irrespective of SP resistance status *(5)*. This recommendation may be reviewed and revised if necessary, when newer data become available.

WHO recommends a similar intervention for infants: intermittent preventive treatment of infants (IPTi). This is a full therapeutic course of antimalarial medicine that is given three times during the first year of life through routine immunization services, regardless of whether the child is infected with malaria. IPTi reduces clinical malaria, anaemia and severe malaria in the first year of life. WHO recommends IPTi with SP in areas with moderate to high malaria transmission in sub-Saharan Africa that have less than 50% prevalence of the mutation *Pfdhps* 540 *(3)*. As for IPTp-SP, this recommendation will be reviewed if necessary, when new data become available.

Seasonal malaria chemoprevention (SMC) is the intermittent administration of full presumptive treatment courses of an antimalarial medicine to children during the malaria season in areas of highly seasonal transmission. WHO recommends SMC with AQ+SP in areas with highly seasonal malaria transmission in the Sahel region of sub-Saharan Africa, where *P. falciparum* is sensitive to both these antimalarial medicines *(3)*.

MDA is the administration of a full therapeutic course of antimalarial medicine to a defined population living in a geographical area at about the same time, often repeated at intervals. It is a time-limited intervention with specific targets and objectives: to interrupt transmission of *P. falciparum* malaria in areas approaching elimination; to achieve a rapid reduction of morbidity and mortality during malaria epidemics, complex emergencies or situations where health systems may be overwhelmed; or as part of the response in the Greater Mekong subregion (GMS), to reduce the risk of spread of multidrug resistance. Medicines used must be of proven efficacy and preferably should have a long half-life. WHO recommends that MDA be conducted with a medicine different from that used for first-line treatment *(6)*. Under certain circumstances, such as complex emergencies, the first-line treatment may be considered for MDA.

3. ANTIMALARIAL DRUG RESISTANCE

3.1 Defining antimalarial drug resistance

As shown in Box 1, antimalarial drug resistance is defined as *the ability of a parasite strain to survive or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended, but within the tolerance of the subject (7)*. This definition can be expanded to specify that *the form of the drug active against the parasite must be able to gain access to the parasite or the infected erythrocyte for the duration of the time necessary for its normal action (8)*.

Drug resistance arises as a result of randomly occurring genetic mutations in the parasite population. Thus, patients with hyperparasitaemia are thought to be an important source of de novo resistance *(9)*. If a genetic trait gives a parasite a survival advantage when exposed to a drug, that genetic trait may be selected for under drug pressure. For some drugs, a single genetic event may be all that is required; in other cases, multiple independent genetic events may be necessary. Selection of a genetic

trait providing a survival advantage is more likely when the parasite population is exposed to sub-therapeutic levels of an antimalarial drug *(10)*. Host immunity augments the killing of parasites by chemotherapeutics, even those parasites carrying resistant traits. Therefore, resistance is more likely to arise and spread rapidly in a non-immune population *(9)*.

BOX 1
Drug resistance definitions

Antimalarial drug resistance is the ability of a parasite strain to survive and/or multiply despite administration and absorption of a drug given in doses equal to or higher than those usually recommended, but within tolerance of the subject.

Antimalarial multidrug resistance is resistance to more than two antimalarial compounds of different chemical classes. This term usually refers to *Plasmodium falciparum* resistance to chloroquine, sulfadoxine-pyrimethamine and a third antimalarial compound.

Artemisinin partial resistance is delayed parasite clearance following treatment with artemisinin-based monotherapy or with an artemisinin-based combination therapy (ACT).

3.2 Artemisinin partial resistance

In general, artemisinin and its derivatives rapidly reduce the number of parasites in the blood. In most malaria endemic areas, few patients treated with artemisinin-based monotherapy or with an ACT are identified as having parasites in the blood on day 3 following the initiation of therapy (day 0). In the GMS, a shift has been seen where parasite clearance is delayed; the result is that more patients still have parasites in the blood on day 3 after a treatment with artemisinin-based monotherapy or with an ACT. This delayed clearance of *P. falciparum* malaria parasites has been termed "artemisinin partial resistance". However, other factors (e.g. high initial parasitaemia or splenectomy) can lead to delayed clearance. Therefore, failure to clear parasites from the blood within the first three days does not necessarily mean that a patient is infected with parasites with artemisinin partial resistance. In areas where parasites are still fully sensitive to artemisinin, analysis of data reported to WHO suggests that 3.0–5.0% of patients may have parasitaemia persisting to day 3.

In TES, having more than 10% of patients with asexual parasites in the blood on day 3 after the start of treatment is used as an indicator of suspected artemisinin partial resistance in an area. Where frequent measurement of parasitaemia is available until parasite clearance, the rate at which parasitaemia declines after treatment can be used to assess the initial clearance due to treatment. The slope half-life (i.e. the time needed for parasitaemia to be halved during the log-linear phase of parasite clearance) is used to define delayed clearance. Unlike the measurement of day 3 parasitaemia, the slope half-life is not affected by the initial parasitaemia; however, it is affected by factors such as immunity, drug absorption, and the quality and procedures of the slide reading. Estimations of the slope half-life also ignore what is identified as the initial lag phase and tail part of the parasite clearance curve, where clearance is typically much slower *(11)*.

P. falciparum's vulnerability to artemisinin varies over the life cycle, being highest in the ring stage and lowest in the trophozoite stage. The mechanism of artemisinin partial resistance has been linked to an altered life cycle, where parasites exhibit an

extended ring stage (also called dormancy) and an abbreviated trophozoite stage; effectively, this increases the proportion of parasites likely to survive a short exposure to artemisinins and thereby increases parasite clearance time *(12)*. The changes in clearance time are associated with several genetic mutations in *P. falciparum Kelch 13 (PfK13) (13)*. Even in Cambodia, where up to 50% of the patients can be found to have parasites on day 3, WHO data analysis indicates that the day 3 parasitaemia is on average less than 1% of the pretreatment parasitaemia.

The recommended ACTs are given as 3-day treatments only. Owing to artemisinin's short half-life (1–2 hours), delayed clearance can result in more patients with parasites in the blood during a period when only the ACT partner drug remains in the blood at therapeutic levels. Given the very low parasitaemia by day 3, artemisinin partial resistance is unlikely to have caused emergence of partner drug resistance. However, artemisinin partial resistance and the higher proportion of patients with parasites on day 3 can make the selection and spread of partner drug resistance more likely. Resistance to the ACT partner drugs PPQ and MQ appears to have emerged independently of artemisinin partial resistance, although artemisinin partial resistance may have played a role in the spread of such resistance *(14)*. Increased production of gametocytes by parasites partially resistant to artemisinin (as assessed by the Tracking Resistance to Artemisinin Collaboration [TRAC I] and WHO data analysis) may have played a role in the spread of resistance *(15)*. Understanding of the implications and risks associated with the emergence of artemisinin partial resistance is still evolving. For example, it is not known whether slow clearance will eventually develop into resistance affecting all the parasite stages, or whether delayed parasite clearance will eventually lead to the loss of artemisinin as an effective treatment for severe malaria. Therefore, monitoring and tracking the emergence of artemisinin resistance remain essential.

3.3 Global public health implications of antimalarial drug resistance

Drug resistance poses a continuous threat to our capacity to successfully prevent and treat malaria. For the individual, antimalarial drug resistance can mean increased transmission and thus a higher risk of contracting malaria.

A 2003 study observed an association between the spread of CQ resistance in Africa during the 1980s and increased mortality in East and Southern Africa. Increasing mortality was exacerbated by deteriorating health systems, which could not effectively manage patients with treatment failures. The authors emphasized the importance of effective case management for preventing efficacy-related malaria deaths *(16)*. In addition, several studies highlighted the correlation between treatment failure and increased severe anaemia and mortality, and the correlation (at least in the early years) between the need for transfusion and the risk of transfusion-transmitted HIV.

Over the past 20 years, there have been significant improvements in health systems, vector control and case management; hence, the public health consequences of resistance to antimalarial medicines are likely to be less severe today than they were in the 1980s and 1990s. In the 1980s and 1990s, there was a lack of available alternatives to CQ and SP, and most patients suspected of having malaria were not provided with a parasitological diagnosis. In 2001, WHO first recommended the use of ACTs in countries where *P. falciparum* parasites were resistant to CQ, SP and AQ. In 2010, WHO recommended parasitological testing of all suspected malaria cases. Additional interventions such as IPTp, IPTi and SMC have been introduced, helping to protect the most vulnerable against malaria. The surveillance of efficacy and resistance had been weak, and only resistance at very high levels (>25% treatment failures on day 14) triggered changes in treatment

policy. With the introduction of more effective combination therapies, WHO has, since 2006, recommended policy change if the efficacy falls below 90% (on day 28 or 42) *(17)*.

Emergence and spread of resistance to antimalarial drugs also carry economic costs: patients with treatment failure require repeated consultations leading to lost work days, increased school absences and greater health care costs; new drugs must be constantly discovered and developed to replace those that are lost to resistance; and changing and implementing new national treatment policies require additional training of health staff.

A 2014 review of the potential human and economic costs of widespread resistance to ACTs evaluated a hypothetical scenario in which all malaria endemic areas experience a 30% treatment failure rate with ACTs, and all severe malaria cases require treatment with quinine instead of AS *(18)*. Compared with a scenario where the treatment failure rates are stable at 5% and where severe malaria is effectively treated with AS, a widespread resistance scenario was found to have a yearly excess of 22 million treatment failures, 116 000 deaths, and costs including an estimated US$ 130 million to change treatment policy. This is clearly a hypothetical scenario that associates artemisinin partial resistance with treatment failures and has all endemic countries simultaneously experiencing 30% treatment failure rates for all treatments. Nevertheless, it gives an idea of the human and economic costs that would emerge if all ACTs should lose their efficacy in all malaria endemic areas.

Artemisinin partial resistance alone has not yet resulted in any documented increases in morbidity or mortality in the GMS, despite the delay in parasite clearance. Parenteral AS is still effective for the treatment of severe malaria, but resistance to ACT partner drugs has resulted in treatment failures, with recurrence of symptomatic malaria. At present, the main challenge of artemisinin and ACT partner drug resistance outside the GMS centres on the need for systems that can quickly inform on the need for changes in treatment policy, and a health system that can implement rapid policy changes so as to provide patients with the specific treatment needed. The progress made towards elimination of *P. falciparum* malaria in the GMS will help to mitigate the risk of spread of drug resistant parasites. The work in the GMS needs to be complemented by continued efforts to limit the risk of independent emergence of resistance outside this area, and strengthened surveillance of resistance and efficacy in all endemic countries to reduce the impact should resistance emerge.

3.4 Responding to the global threat of resistance

A relatively small number of drugs are currently being used to save the lives of millions of people infected each year by malaria. These drugs need to remain efficacious until new drugs and tools become available. Imperfect coverage and quality of malaria interventions contribute to the emergence and spread of resistance. Correct diagnosis is not always provided, drugs are sometimes misused, some patients may not have access to quality treatments and the coverage of vector control may remain low for some key populations. These failures lead to increased exposure of the malaria parasites to drugs, increasing the risk of drug resistance.

Past WHO guidance on antimalarial drug resistance has mainly focused on the threat of *P. falciparum* resistance to the antimalarial compound artemisinin. This includes guidance given in the *Global Plan for Artemisinin Resistance Containment* (GPARC) *(19)*, released in 2011, and the *Emergency Response to Artemisinin Resistance in the Greater Mekong subregion, Regional Framework for Action 2013–2015* (ERAR) *(20)*, released in 2013. Since

the development of GPARC, our understanding of artemisinin partial resistance has improved and the growing impact of resistance to ACT partner drugs has been recognized.

The primary challenge of *P. vivax* chemotherapeutics is that of successful radical cure. Chemotherapeutic management of latency in *P. vivax* and *P. ovale* remains a complex clinical and public health problem *(21)*. The challenge has many aspects:

- variable efficacy of 8-aminoquinoline antirelapse therapy with CYP2D6 polymorphisms;
- patient adherence to a 14-day regimen of primaquine, or access to single-dose tafenoquine; and
- providers coping with the universal therapeutic dilemma of 8-aminoquinoline haemolytic toxicity (i.e. give therapy and risk a life-threatening drug reaction in a minority of patients, or withhold therapy from all and invite repeated preventable malaria attacks).

A further challenge is that almost complete *P. vivax* resistance to CQ occurs in South-East Asia at high frequencies and has been reported from several other endemic areas.

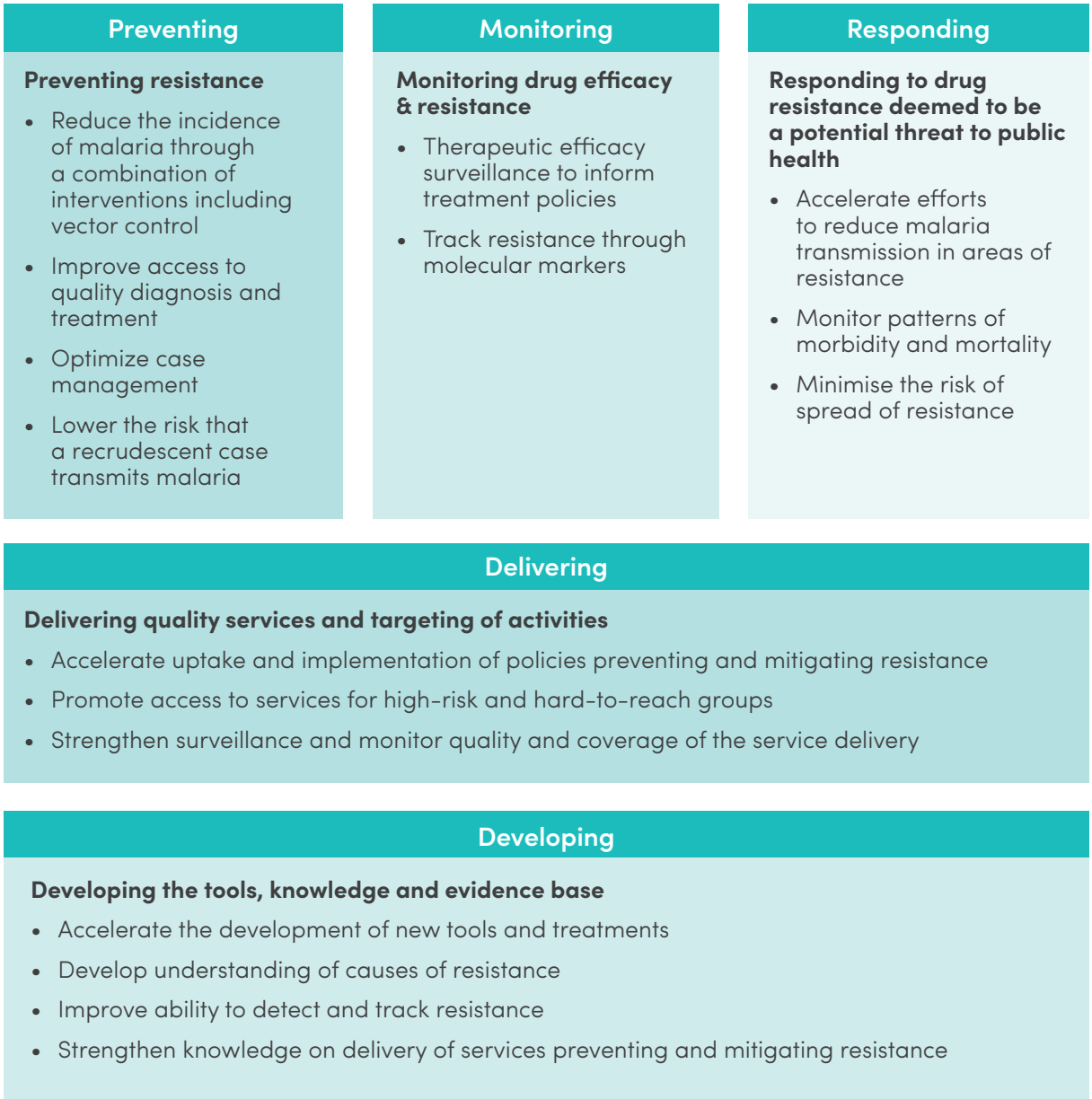
Prolonging the efficaciousness of the currently used drugs will require addressing shortcomings in the quality and coverage of malaria interventions, and adding specific activities that could help to minimize the risk of drug resistance and limit the public health consequences when drug resistance emerges and spreads. Fig. 1 gives an overview of the activities needed:

- **Preventing resistance:** Resistance develops and spreads when genetic mutations provide parasites with an advantage when they are exposed to a given drug. For the de novo resistant parasites to spread, they need to survive the given treatment so that they can develop gametocytes and be transmitted. Consequently, prevention of resistance can be pursued through activities that reduce the drug pressure on parasites, and reduce the risk of onward transmission of malaria from a recrudescent case.
- **Monitoring drug efficacy and resistance:** Surveillance of therapeutic efficacy and antimalarial drug resistance is required to detect changing patterns of parasite susceptibility, and make timely revisions to national and global policies. TES remain the gold standard used to inform treatment policies. Information from TES can be supplemented with information on molecular markers of drug resistance.
- **Responding to resistant strains deemed to be a potential threat to public health:** When resistance develops to a drug where alternative drugs are limited or unavailable, the aim of the response is twofold: to minimize the public health impact in the area where resistance has developed, and to contain the resistance within the affected areas. In the areas where resistance has emerged, a reduction of transmission and (where possible) the achievement of elimination will serve both these purposes.
- **Delivering quality services and targeting of activities:** The ability to prevent, monitor and respond to antimalarial drug resistance depends on the ability to deliver quality interventions and implement any changes needed. Delays in the uptake and implementation of policies can lead to worsening or spreading

resistance. Ensuring that all those at risk of malaria have access to prevention, early diagnosis and quality treatment can save lives and help prevent resistance. This can be achieved by using and expanding the available service delivery channels, including community health workers.

- **Developing the tools, knowledge and evidence base:** Although it may be possible to prolong the use of the currently available drugs, to achieve malaria eradication, new drugs, tools and strategies will be needed. Therefore, research will continue to be important both in the development of new drugs and tools, and to improve the understanding of resistance and the ability to manage it *(22)*.

FIG. 1
Activities to minimize risk of emergence and spread of resistance



4. TOOLS FOR MONITORING ANTIMALARIAL DRUG EFFICACY AND DRUG RESISTANCE

In both high and low endemic areas, TES are used to monitor how the malaria parasite strains in an area respond to antimalarial treatment. In very low endemic areas where cases are routinely provided with supervised treatment and are followed up to confirm cure, integrated drug efficacy surveillance (iDES) can provide information on efficacy (see Section 4.2).

TES, and to some extent iDES, can provide the information on efficacy needed to inform treatment policies. However, the efficacy depends on a range of factors other than resistance, including how well drugs are absorbed by the patients. In areas where lower than expected drug efficacy has been identified, additional studies are needed to confirm and characterize drug resistance as the cause; these may include in vitro studies of the parasite phenotype, and studies of genetic mutations known to be associated with drug resistance in the parasite. Increased access to genetic analyses and identification of molecular markers of resistance for both artemisinin partial resistance and some ACT partner drugs have resulted in an increasingly important role for genotypic surveillance that can be done independently of any phenotypic monitoring.

4.1 Therapeutic efficacy studies

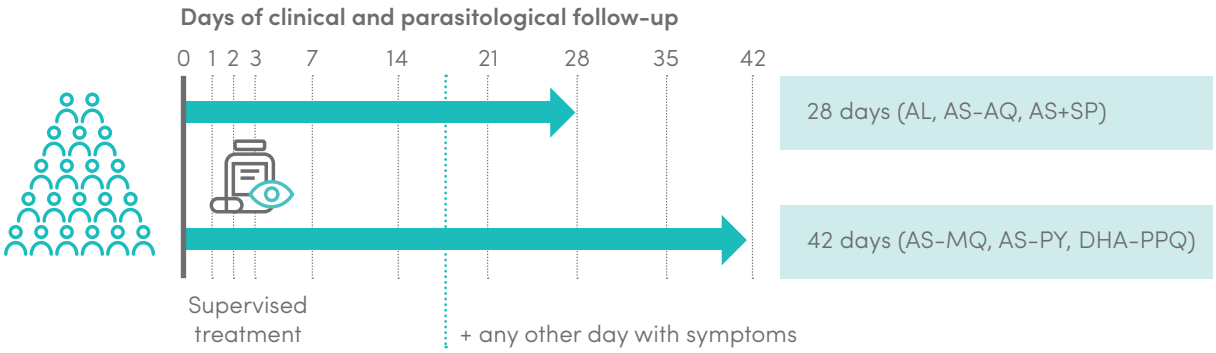
TES are prospective evaluations of patients' clinical and parasitological responses to treatment for uncomplicated malaria. They are conducted with diagnosis validated by microscopy, and using a quality assured treatment and supervised drug administration. Patients who are lost to follow-up or excluded from the study (e.g. due to self-medication or refusal to continue participation) are excluded or censored from the analysis.

TES remain the gold standard and they are being used to inform national treatment policies. WHO has developed a standard protocol for TES *(23)*. A limited number of sentinel sites, representing all the epidemiological strata in a country, are adequate to collect consistent longitudinal data and to document trends. The recommendation is to test the efficacy of the first- and second-line treatment at all sentinel sites at least every 2 years. In areas where multiple *Plasmodium* species are prevalent (typically *P. falciparum* and *P. vivax*), sentinel sites can be used to monitor therapeutic efficacy against these species simultaneously.

4.1.1 TES for *P. falciparum*

Clinical and parasitological responses to treatment are evaluated on days 0, 1, 2, 3, 7, 14, 21 and 28 (and on days 35 and 42 for some partner drugs). Therapeutic outcomes are assessed on the final day of the study (i.e. on day 28 or day 42). It is recommended that ACTs with a partner drug with a relative short elimination half-life should be followed up for at least 28 days, and ACTs with partner drugs with longer elimination half-lives should be followed up for at least 42 days (see Fig. 2).

FIG. 2
TES for *P. falciparum*



AL: artemether-lumefantrine; AS-AQ: artesunate-amodiaquine; AS-MQ: artesunate-mefloquine; AS-PY: artesunate-pyronaridine; AS+SP: artesunate+sulfadoxine-pyrimethamine; DHA-PPQ: dihydroartemisinin-piperaquine; *P. Plasmodium*; TES: therapeutic efficacy studies.

A minimum period of follow-up is mandatory to detect enough of the overall treatment failures. The objective of follow-up is not to detect every single recrudescence (for definition of recrudescence see Box 2), but to obtain a robust estimate of failure rates within a target time period. A longer follow-up period may capture a few additional recrudescences, but in higher transmissions settings it can also mean several reinfections that need to correctly be classified as such.

Modelling shows that more than 95% of the failures would be detected for DHA-PPQ and AS-MQ by 42 days of follow-up, and by 28 days for AL *(24)*. The current recommendation is to follow up AS-AQ and AS+SP and for a minimum of 28 days, and AS-PY for a minimum of 42 days.

BOX 2
Terms commonly used in therapeutic efficacy monitoring *(25)*

Recurrent parasitaemia is reappearance of asexual parasitaemia after treatment, due to recrudescence, relapse (in *P. vivax* and *P. ovale* infections only) or a new infection.

Recrudescence is recurrence of asexual parasitaemia of the same genotype(s) that caused the original illness, due to incomplete clearance of asexual parasites after antimalarial treatment.

Reinfection is a new infection that follows a primary infection; can be distinguished from recrudescence by the parasite genotype, which is often (but not always) different from that which caused the initial infection.

Relapse is recurrence of asexual parasitaemia in *P. vivax* or *P. ovale* infections arising from hypnozoites.

Note: Relapse occurs when the blood-stage infection has been eliminated but hypnozoites persist in the liver and mature to form hepatic schizonts. After an interval (generally between 3 weeks and 1 year), the hepatic schizonts rupture and liberate merozoites into the bloodstream.

Treatment outcomes are classified as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), or adequate clinical and parasitological response (ACPR) (see Box 3). Including monitoring of ETF in the protocol is more for the

safety of the patient rather than a means to detect very resistant parasites because it can take some time for the drug to be effective against the parasites and very resistant parasites most often appear late in the evolution of resistance. ETF occurs when the patient develops signs of severe malaria or does not have a rapid resolve of clinical symptoms and quick decline in the number of asexual parasites.

The patient is closely monitored during the first 3 days to ensure that appropriate rescue treatment is given if signs of severe malaria develop. If severe malaria develops within the first 24 hours, it may be that the inclusion criteria were not respected; whatever the cause, the patient should be withdrawn from the study and admitted to hospital for appropriate care unrelated to TES objectives. In some areas of artemisinin partial resistance, up to 50% of the patients may have a few remaining parasites in the blood on day 3. Some of these patients are likely to have low grade fever, sometimes for reasons other than malaria, and become classified as ETF. When these patients are followed up, almost all are found to be aparasitaemic on day 4, and remain so to the end of follow-up. Consequently, any analysis of efficacy studies with unexpectedly high treatment failure rates needs to include examination of the type of failures reported (in particular, the criteria on which ETF were classified).

The efficacy of drugs for *P. knowlesi* can be tested using the same protocol as for *P. falciparum*.

BOX 3
WHO classification of responses to treatment for TES *(23)*

Early treatment failure (ETF)

- danger signs or severe malaria on day 1, 2 or 3, in the presence of asexual parasitaemia; or
- asexual parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature; or
- asexual parasitaemia on day 3 with axillary temperature ≥37.5 °C; or
- asexual parasitaemia on day 3 ≥25% of count on day 0.

Late clinical failure (LCF)

- danger signs or severe malaria in the presence of asexual parasitaemia on any day between day 4 and day 28 (or day 42) in patients who did not previously meet any of the criteria of ETF; or
- presence of asexual parasitaemia on any day between day 4 and day 28 (or day 42) with axillary temperature ≥37.5 °C in patients who did not previously meet any of the criteria of ETF.

Late parasitological failure (LPF)

- presence of asexual parasitaemia on any day between day 7 and day 28 (or day 42) with axillary temperature <37.5 °C in patients who did not previously meet any of the criteria of ETF or LCF.

Adequate clinical and parasitological response (ACPR)

- absence of asexual parasitaemia at the end of follow-up (on day 28 or 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of ETF, LCF or LPF.

4.1.2 Classification of recurrent *P. falciparum* infections

In patients in whom recurrent parasitaemia appears during follow-up, molecular genotyping is performed to distinguish new *P. falciparum* infections from recrudescences *(23, 24, 26)*. The distinction is significant, because recrudescence indicates failure of the treatment, whereas a new infection does not. The method currently recommended by WHO uses the highly polymorphic markers of *P. falciparum msp1, msp2* and *glurp*. Based on these markers, reinfection and recrudescence are defined as follows:

- A "new infection" is reappearance of parasitaemia after initial parasite clearance in which all the alleles in parasites from the post-treatment sample are different from those in the admission (day 0) sample, for one or more loci tested. Consequently, a single marker (i.e. *msp1*, *msp2* or *glurp*) in which all alleles are different between pre- and post-treatment samples is sufficient to conclude a new infection.

- In a "recrudescence", at least one allele at each locus is common to both paired samples.

Other methods used to classify recurrent parasitaemia as a reinfection or a recrudescence include microsatellites (i.e. simple sequence repeats) or an algorithm with the microsatellites *(27, 28)*. Different algorithms based on *msp1, msp2* and *glurp* (e.g. looking at *msp1* and *msp2* only, or requiring a common allele in only 2 out of 3 markers) have been suggested, but could lead to higher estimates of failure rates, particularly in high transmission settings. Given that the true failure rates are unknown, it is not currently possible to compare the accuracy of these different methods.

The classification of recurrent parasitaemia is complicated by the occurrence of polyclonal infections, which are especially frequent in high transmission settings. If a minority clone is present on day 0 but is not identified, a recrudescence of this strain would incorrectly be classified as a reinfection. In very low transmission settings with low genetic diversity of the parasite population, any reinfections are likely to be due to parasites with a genotype close to the initial infection, making it more difficult to reliably distinguish reinfection from recrudescence by genotyping. The use of deep sequencing strategies could potentially improve the discriminatory power of these molecular tools, but this approach is not currently feasible in many settings *(24)*.

Changing the WHO-recommended method without a gold standard and knowledge of the accuracy of the methods could mean overestimating failure rates. The current thresholds (a minimum efficacy of 90% for existing first-line treatments and of 95% for newly introduced first-line treatments) would also have to be changed if a proposed alternative method means that no drugs are identified that can meet the criteria.

Until methods providing improved discriminatory power are widely available, WHO will maintain the current recommendation regarding the use of *msp1*, *msp2* and *glurp* to classify recurrent *P. falciparum*. Using the same methods across studies has the benefit of allowing for comparison. WHO will continue to review the situation and work with partners on potential revisions in this recommendation if and when they become feasible. No matter which method is used, the possible classification errors need to be considered in the review of the data and in the policy decisions.

4.1.3 *P. falciparum* TES in different settings

Studies conducted according to the WHO TES protocol, at the same sites and at regular intervals, allow study results to be compared within and across regions over time, and ultimately serve to detect the first signs of changes in treatment efficacy. In

high transmission settings, only febrile children with parasitaemia ranging between 2000 and 200 000 asexual parasites/µL are included. In lower transmission settings where fewer infections occur, older patients and patients with lower parasitaemia are also included (see Table 2). TES do not enrol patients who are pregnant; have known comorbidities, such as HIV or tuberculosis; or are asymptomatic without fever or without recent history of fever (24 h before consultation) .

TABLE 2
Adapting the *P. falciparum* TES protocol inclusion criteria depending on transmission level

Transmission level[a]	Inclusion criteria
High transmission	Patients with fever, aged 6–59 months, with an asexual parasitaemia ranging between 2000 and 200 000 parasites/µL.
Moderate transmission	Modified inclusion criteria to also include older children with an history of fever 24 h before consultation and an asexual parasitaemia ranging between 1000 and 100 000 parasites/µL.
Low transmission	Modified to also include adults and patients with an asexual parasitaemia of more than 500 parasites/µL (250 parasites/µL in South America).
Very low transmission	To achieve the required sample size, data from different sites can be combined (country aggregated data). The studies are conducted less frequently and only where possible. The use of molecular markers of resistance as an early warning system and an additional source of data can be of particular importance in these settings.

P: Plasmodium; TES: therapeutic efficiency studies.

[a] The following definitions are frequently used: *high transmission* – annual parasite incidence per 1000 population (API) ≥450 cases and a *P. falciparum* malaria prevalence of ≥35%; *moderate transmission* – API of 250–450 cases and a *P. falciparum/P. vivax* prevalence of 10–35%; *low transmission* – API of 100–250 cases and a *P. falciparum/P. vivax* prevalence of 1–10%; *very low transmission* – API of <100 cases and a *P. falciparum/P. vivax* prevalence of 0–1% *(29)*.

4.1.4 TES for *P. vivax* and *P. ovale*

Radical cure of patients having acute *P. vivax* or *P. ovale* infections requires effective treatment of both the asexual parasite stages in the blood that are responsible for patency, and the dormant liver stages (hypnozoites) that are responsible for latent infection and subsequent clinical attacks in the weeks and months that follow. Routine TES for *P. vivax* aim to estimate the efficacy of the treatment of the blood-stage parasites only.

A recurrent blood-stage infection following treatment of *P. vivax* or *P. ovale* can be a relapse (due to activation of hypnozoite), a recrudescence (due to blood-stage treatment failure) or a reinfection. It is not possible to reliably distinguish between these different causes of recurrent blood-stage infection. Genetically similar parasites across primary and recurrent infections can be caused by recrudescence or relapse. Genetically unrelated *P. vivax* parasites across primary and recurrent infections can be caused by reinfection or relapse *(30)*.

Concomitant treatment with PQ against liver-stage parasites can increase the efficacy of treatment (e.g. with CQ against blood-stage parasites). Therefore, in a TES the initiation of treatment with an 8-aminoquinoline (PQ or tafenoquine) should be delayed until day 28 if locally acceptable. Before 8-aminoquinoline administration on day 28,

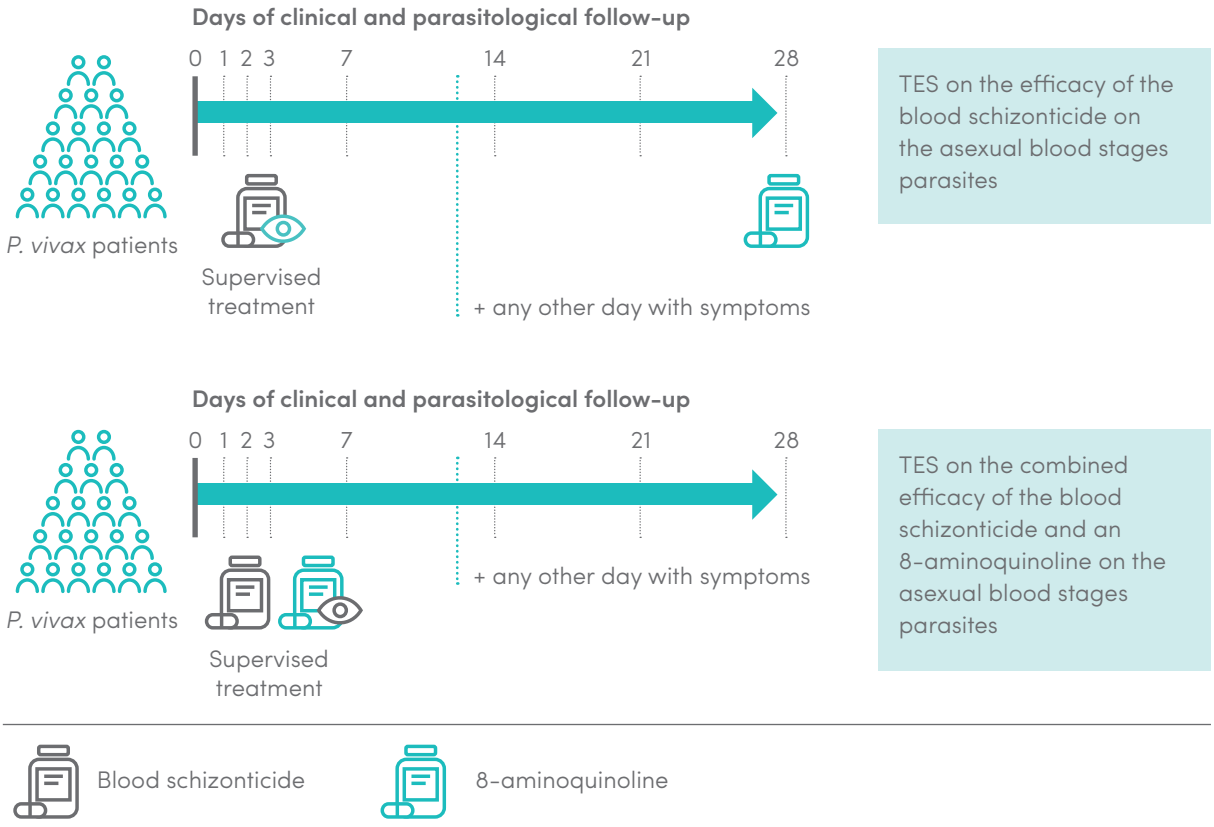
the patient's risk for relapse is low because of the prophylactic effect of CQ or the ACTs; however, if a relapse occurs, careful follow-up and treatment will prevent unnecessary morbidity. The delay in an 8-aminoquinoline therapy to day 28 allows for much more sensitive detection of asexual parasite resistance to the blood schizonticide.

The efficacy of radical cure with blood schizonticides and an 8-aminoquinoline can be monitored by providing supervised treatment with an 8-aminoquinoline and adding an additional period of monitoring to the routine TES.

Monitoring efficacy against asexual stages

The *P. falciparum* protocol can be used to monitor the efficacy of treatment for asexual stages of *P. vivax* and *P. ovale* with minor adaptations. The adaptations needed relate to the general lower level of parasitaemia and the risk of relapse. Consequently, inclusion criteria generally allow for parasitaemia as low as 250 asexual parasites/µL, and afebrile patients with a history of fever within 48 hours. The studies are prospective evaluations of clinical and parasitological responses on days 0, 1, 2, 3, 7, 14, 21 and 28. An additional consideration for *P. vivax* or *P. ovale* is that recurrent parasitaemia can be caused not only by reinfection or recrudescence but also by relapse due to activation of hypnozoites. Therefore, the follow-up is stopped at day 28. Classification of responses to treatment used for *P. falciparum* can also be used for *P. vivax* and *P. ovale* (see Box 3).

FIG. 3
TES on the efficacy against *P. vivax* and *P. ovale* asexual blood stages



P: Plasmodium; TES: therapeutic efficiency studies.

In TES against *P. vivax* asexual blood stages, the 8-aminoquinoline can be given at the end of follow-up (on day 28) or together with the blood schizonticide. Because 8-aminoquinoline has an effect on asexual blood-stage parasites, TES giving an 8-aminoquinoline from the start of the study will result in an estimate of the combined efficacy of the two treatments (see Fig. 3). Primaquine is often not prescribed with acute *P. vivax* due to fear of acute haemolytic anaemia in patients deficient in glucose-6-phosphate dehydrogenase (G6PD). Often, screening for that common inherited abnormality is not available. Thus, treatment failure rates in routine practice may be much higher where primaquine cannot always be safely prescribed and consumed.

Monitoring efficacy of hypnozoitocidal therapy

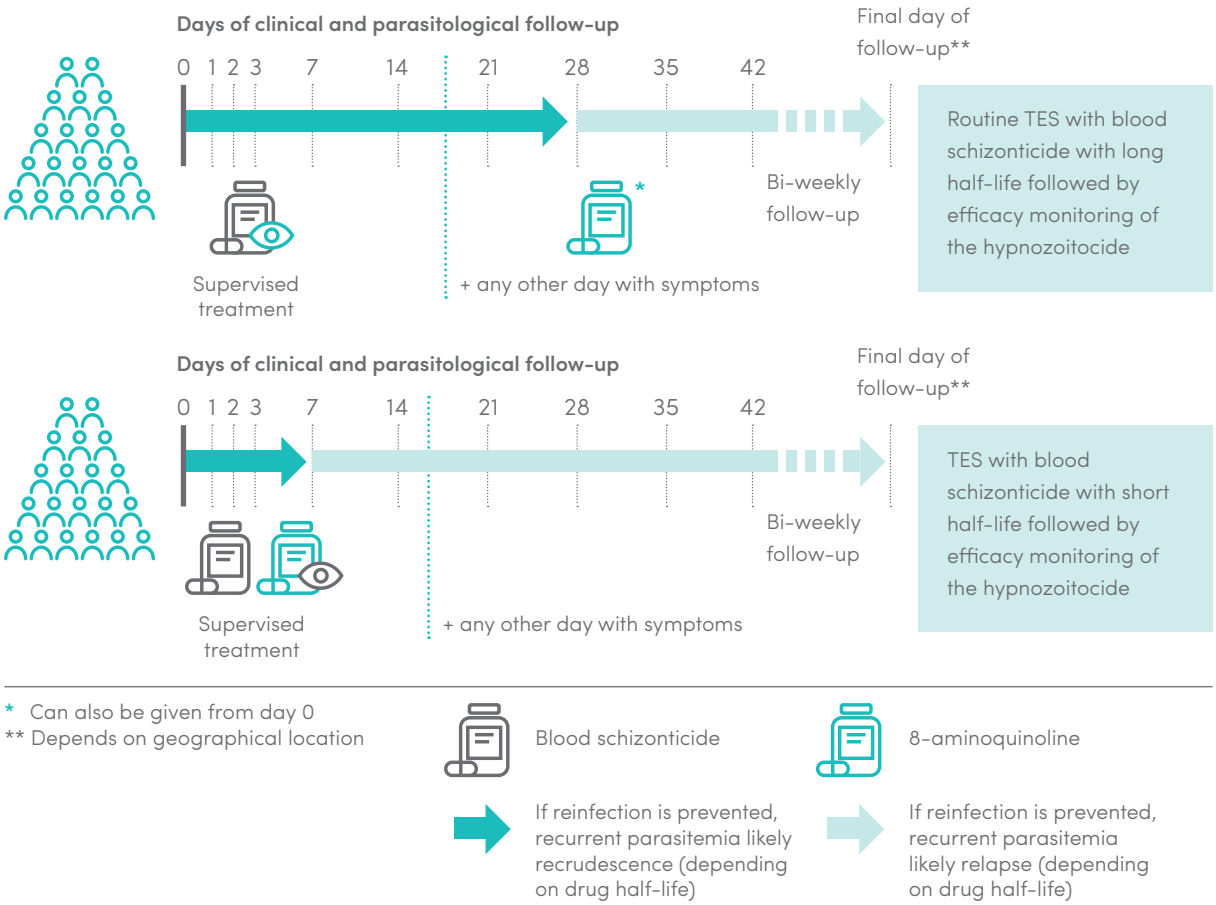
P. vivax and *P. ovale* are treated with hypnozoitocidal therapy against latency in order to prevent one or more relapses. Primaquine is given at 0.25 or 0.5 mg base/kg body weight daily over 14 days, or at 0.75 mg base/kg body weight once a week for 8 weeks *(3)*. Tafenoquine is a single 300 mg dose approved only for administration with CQ in non-pregnant, G6PD-normal adult patients. Patients should be assessed for G6PD deficiency using a qualitative or quantitative test before administration of primaquine, or should be closely monitored for onset of acute haemolytic anaemia. In cases of acute haemolytic anaemia, dosing should cease and the patient should be immediately referred to hospital for assessment. In the instance of tafenoquine, no patient should be dosed without first ascertaining more than 70% of normal G6PD activity. This can be done by a quantitative or semi-quantitative test.

Studies of the efficacy of 8-aminoquinolines in preventing relapse can be combined with the routine TES examining the efficacy of the blood schizonticide (treatment with both for radical cure). There are significant geographical variations in the frequency and timing of relapses. This variation needs to be taken into account when developing protocols for monitoring efficacy of radical cure *(31)*. Relapses may occur as early as 16 days and (rarely) as late as 3 years after the start of the initial treatment *(23)*. However, recurrent parasitaemia would not be expected before day 28 if the blood schizonticide given includes a drug with a long half-life, with normal absorption and in a setting where the parasites are fully sensitive to the drug. Drugs expected to prevent relapses before day 28 include CQ and the WHO-recommended ACTs other than AL, because lumefantrine has a short half-life *(32)*. In clinical trials undertaken primarily to monitor the efficacy of the radical cure, a treatment with a short half-life drug (e.g. 7-day quinine or AS) is useful to rule out the suppression of early relapses *(33)*.

The period of follow-up should be adapted to the regional relapse characteristics of the parasite. The follow-up phase varies in the literature from 3 to 12 months. The ideal follow-up period for all areas is 12 months. Based on the best available information on relapse patterns *(31, 33)*, the minimum recommended follow-up period is 8 months for Northeast Asia, South Asia and Central America, and 3 months for all other areas.

When the blood schizonticide given has a relatively long half-life, its efficacy is monitored with patient follow-up until day 28 according to the standard WHO protocol for surveillance of antimalarial drug efficacy *(23)*. To subsequently monitor the efficacy of primaquine for radical cure, blood should be examined and assessed for malaria weekly until day 42 after the start of treatment with a blood schizonticide, and then on a bi-weekly basis until the end of follow-up. TES using a drug with a short half-life should follow the same schedule of follow-up (see Fig. 4).

FIG. 4
TES of efficacy of blood schizonticide and hypnozoitocide



TES: therapeutic efficiency studies.

The usage and efficacy of primaquine is affected by genetic polymorphism. When given at hypnozoitocidal doses, primaquine induces dose-dependent acute haemolytic anaemia in patients with genetic G6PD – a widely prevalent genetic disorder with a prevalence of 3–35% in tropical areas. More than 180 different genetic variants of G6PD deficiency are known, with a varying effect on activity of the enzyme *(34)*. Additionally, primaquine is a prodrug that requires metabolism into an active metabolite to have an effect against hypnozoites. Studies have shown that cytochrome P450 CYP2D6 isoenzyme is important in the metabolic activation of primaquine; thus, mutations in the highly polymorphic CYP2D6 gene in patients may affect primaquine efficacy. In patients with the "null metabolizer" genotype, primaquine may have no efficacy at any dose; similarly, patients with the "impaired activity" genotype (especially common in Asian populations) may be at higher risk of treatment failure. Among 21 confirmed primaquine treatment failures in Indonesia, 20 had impaired CYP2D6 metabolism genotypes and phenotypes *(35)*. Hence, CYP2D6 polymorphisms represent the most probable cause of primaquine treatment failures.

Ideally, the therapeutic efficacy of primaquine should be studied in an environment where there is no risk of reinfection, or at least where the risk is low. Ensuring that reinfection is prevented is only possible where patients have left the areas with ongoing transmission. However, information on primaquine efficacy is also needed for areas where reinfection is a risk, and in groups of patients in whom reinfection is a risk. In such settings, it is not possible to make conclusions regarding the cause of recurrent *P. vivax* infection in individual patients; nevertheless, data collected from many patients may offer insights about the efficacy of prescribed primaquine treatment

when compared with the general relapse rate in an area or in a control group that does not receive primaquine.

Confirmed recurrent *P. vivax* after full adherence to primaquine therapy and in the absence of a risk of reinfection may confidently be classified as a therapeutic failure. Primaquine failure is defined as a confirmed positive blood smear for *P. vivax* during the follow-up phase after treatment with an effective blood schizonticide and primaquine therapy, in a patient for whom reinfection has been prevented. Confirmation of infection as resistant to primaquine should come with demonstration of a normal CYP2D6 genotype-predicted activity score above 1.0 *(35)*. Where there is a risk of reinfection, the results need to be analysed based on the local patterns of relapse and the risk of reinfection during the follow-up period, ideally compared with groups of patients not receiving hypnozoitocidal therapy.

4.1.5 Frequent deviations from the WHO TES standard protocol

Studies on antimalarial drug efficacy sometimes deviate from the WHO standard protocol in ways that have implications for study outcomes or analysis of results. In the reporting of the data, crucial information (e.g. dates of the study) is often missing. The minimum follow-up time for each medicine should be respected to allow comparison; where longer follow-up periods are used, the data with the standard follow-up should be reported.

The Technical Expert Group on malaria chemotherapy recommended that TES be conducted as single-arm studies rather than as comparative studies. Conducting TES as comparative studies has led to delays or failure to finish the studies, in part due to the need for large sample sizes. Sample size calculation can also differ for other reasons. Final sample sizes are often influenced by recruitment capacity; therefore, it is important to analyse the local malaria epidemiology before choosing a sentinel site.

The standard protocol allows some modifications regarding age and initial parasitaemia; however, some protocols exclude children or include adults without clear rationale. Similarly, some studies have included asymptomatic individuals and patients with parasitaemia either below or above the suggested limits, making interpretation of results difficult. Mixed infections are sometimes included, despite the recommendations against this. Including mixed infections can affect efficacy; it also complicates slide reading and data analysis.

Previous administration of antimalarial drugs within the past 4 weeks is not a criterion for exclusion under the WHO protocol but nonetheless has been used as such in some studies. Pregnant women and patients with known comorbidities (e.g. HIV or tuberculosis) are excluded in routine TES; however, having data from these patients is essential, and such data should be collected in research trials where feasible. When pregnant patients or patients with comorbidities are included in studies, this needs to be noted and taken into account in the data analysis.

Parasite density – expressed as the number of asexual parasites per microlitre of blood – can be calculated by dividing the number of asexual parasites counted against the number of white blood cells counted, and then multiplying by an assumed white blood cell density. Typically, the analysis uses an assumed white blood cell density of 6000 or 8000/µL. The WHO protocol considers a blood slide to be negative when examination of microscopic fields that includes 1000 white blood cells reveals no asexual parasites. Protocol variations in these examinations and counting methods make certain comparisons difficult, such as the day 3 positivity rate over time and between sites. Quality and quality control of slide reading remains the main challenge in many of the TES.

TES require supervised treatment of all doses given. Some studies do not supervise the evening AL dose. Consequently, AL studies that show high failure rates often need to be repeated with supervision of all treatment doses ensured, or with AL blood levels measured. Both of these corrective measures are costly and can lead to unfounded questions on the efficacy of AL or, in case of actual resistance, can risk delaying any needed changes in treatment policy.

Classification of treatment outcomes is only rarely modified by TES investigators. One major issue involves patients classified as ETF on day 1 due to development of severe malaria within the first 24 hours. This is rarely, if ever, due to resistance, but rather is due to the inclusion criteria being disregarded (in such cases, these patients should be excluded from the TES analysis). Another issue is that patients must have a haemoglobin level of more than 8 g/dL at enrolment. A malaria patient with a haemoglobin level of less than 5 g/dL is defined as having severe malaria. Therefore, a patient with too low a haemoglobin level at enrolment is at high risk of being classified as ETF. Finally, ETF is not synonymous with artemisinin partial resistance (delayed clearance) and vice versa.

The analysis of the data allows the use of per protocol and Kaplan-Meier methodologies. In some cases, TES analyses employ an intention-to-treat approach, which is not useful in single-arm efficacy studies. Some analyses classify patients with reinfections with the same or another species, or patients missing polymerase chain reaction (PCR) analysis, as ACPR rather than properly excluding them from the analysis. It is not recommended to merge data (except in very low transmission areas) in a multicentre trial, even if the protocol was designed as such. Even in cases of low number of enrolled patients, data from all sites should be described separately, because merged data from sites could obscure the appearance of emerging resistance.

The PCR analysis used in the classification of *P. falciparum* patients as having recrudescence or reinfection sometimes differs from the methodology recommended in the WHO protocol. Some studies do not use PCR correction, whereas others use the *msp2* marker alone, or use *msp1* and *msp2* without *glurp*. Sometimes, microsatellites or barcodes are used rather than *msp1*, *msp2* and *glurp*, and in some analyses, different algorithms than the standard WHO protocol are used to classify recurrent *P. falciparum* (see Section 4.1.2). Thus, it is critical to clearly describe the molecular methods and algorithms used for these analyses, to assist with interpreting comparability among studies.

4.2 Integrated drug efficacy surveillance

In areas pursuing malaria elimination, the systems for case management and surveillance have been strengthened to enable identification, tracking and classification of all malaria cases. The aim is to provide rapid, complete treatment for all malaria cases and to ensure cure. Where this is done, drug efficacy monitoring can be integrated into the routine surveillance system, and is referred to as iDES. In iDES, information is collected from all cases, including those with asymptomatic infections, detected through the public or private system. The minimum information needed is quality assured diagnosis on day 0 to confirm malaria, supervised treatment to confirm complete adherence, and quality assured diagnosis on the last day of follow-up to confirm cure. Additional information can be collected, depending on the needs and the systems in place. As with TES, the follow-up for *P. falciparum* is 28 or 42 days, depending on the ACT partner drug. For *P. vivax*, the follow-up for the efficacy monitoring of the treatment of the blood-stage parasites is 28 days. The follow-up for the efficacy monitoring of radical cure will vary between geographical regions owing to differences in relapse frequency and timing (see Section 4.1.4).

4.3 Evaluating the cause of treatment failure

Drug resistance is only one of the potential causes of treatment failure. Other potential causes include incorrect dosage, poor treatment adherence, poor drug quality, incomplete drug metabolism or drug interactions. Consequently, it is important to consider other causes before concluding that a treatment failure occurred because of drug resistance. In TES, or in the context of a strong malaria elimination programme, the risk of failure due to some of these factors can be reduced; for example, supervised treatment prevents treatment failures that might otherwise occur due to patient noncompliance or incorrect dosing. Similarly, when medicines are confirmed to be of high quality, the risk of failure due to poor drug quality is minimized.

4.3.1 Studies of drug blood levels

Even after supervised administration of a full regimen of an antimalarial medicine, various factors can cause treatment failure in the absence of drug resistance; these factors include poor absorption, rapid elimination (e.g. diarrhoea or vomiting) and poor biotransformation of prodrugs. Measurement of blood drug concentrations is needed to help distinguish clinical treatment failure due to inadequate drug concentration from failure due to drug resistant parasites. TES sometimes include measurement of concentrations of longer acting antimalarial drugs on day 7. A normal day 7 concentration suffices to rule out poor drug quality, dosing or absorption as a cause of therapeutic failure. The drug concentration on day 7 is a surrogate of the area under the plasma drug concentration curve (AUC);[3] it reflects the actual exposure of parasites to a drug and is predictive of the treatment outcome because it reflects the concentrations to which the small numbers of residual parasites are exposed *(36)*. It is more common to include measurement of drug concentrations in studies that aim to confirm resistance in areas where previous TES have shown a decline in efficacy.

For some antimalarial drugs, no therapeutic blood or plasma concentration ranges have been defined. Also, the bioavailability of certain antimalarial drugs varies widely between population groups. When the dosage has been recommended in the absence of information on blood concentrations in important patient subgroups (e.g. children and pregnant women), the recommended dosages for these groups can be too low, potentially causing treatment failures *(36)*.

There is good evidence that CQ-sensitive *P. vivax* will be eliminated or suppressed (in the instance of post-treatment relapses) by a whole blood concentration of about 100 ng/mL of CQ and its primary metabolite (desethylchloroquine, measured separately). In most patients, that threshold is not crossed until about day 35 post-treatment. Any parasitaemia recurring up to day 28 may thus be presumed to be resistant, whether that recurrent parasiteamia is from recrudescence, reinfection or relapse. *P. vivax* CQ resistance may be confirmed using blood samples collected on day 7, the day of failure or on day 28. Recurrent *P. vivax* parasitaemia at a whole blood CQ and desethylchloroquine concentrations exceeding 100 ng/mL would confirm CQ resistance *(23, 37)*.

4.3.2 In vitro and ex vivo tests

In vitro assays test the sensitivity of culture-adapted parasites to antimalarial drugs by exposing them to a precise concentration of a drug (typically for 48 or 72 hours) in culture plates and observing the effect on parasite growth. An advantage of in vitro assays is that the results are not confounded by host factors such as drug absorption or immunity.

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[3] AUC estimation requires repeated measures and sophisticated mathematics.

Various in vitro and ex vivo tests have been used in the past, with the key difference being the methods used to quantify parasite growth. Methods include using microscopy to count parasites, incorporating radioisotope precursors into the parasites, using fluorescent-based techniques employing SYBR® green I, or using enzyme-linked immunosorbent assays (ELISAs) to provide measures of parasite growth by quantifying biomolecules produced during parasite development such as histidine-rich protein 2 (HRP2) or plasmodial lactate dehydrogenase (pLDH) *(38-40)*. Results are often expressed as the mean drug concentration that inhibits 50% of the parasite's growth (50% inhibitory concentration, IC_{50}), mean concentration that inhibits 90% of the parasite's growth (90% inhibitory concentration, IC_{90}) or the minimum inhibitory concentration (MIC).

In vitro testing has played a relatively limited role in the monitoring of drug resistance, because the findings have not been able to accurately predict the treatment outcome for patients. However, the in vitro methodology has recently been adapted to better simulate the in vivo exposure of parasites to specific antimalarial drugs, increasing the correlation between the findings of in vivo and in vitro studies. Artemisinins have a short half-life, and artemisinin partial resistance has been shown to mostly affect the early ring stage of parasite development. To mimic the in vivo response of parasites to artemisinins and increase the sensitivity, a ring-stage survival assay (RSA^{0-3h}) has been developed, to test the response of tightly synchronized early ring-stage parasites (0–3 hours post-invasion) to pharmacologically relevant levels of DHA[4] for 6 hours. The results are evaluated by assessing the proportion of viable parasites that developed into second-generation rings or trophozoites with normal morphology at 66 hours compared with untreated controls *(41)*. For PPQ, a PPQ survival assay (PSA) has been developed, exposing rings appearing 0–3 hours post-invasion to PPQ for 48 hours, thus potentially exposing all parasite stages to PPQ *(42)*. Recently, an amodiaquine survival assay (AQSA) has been developed; it is based on the PSA and involves 0–3-hour post-invasion rings exposed to AQ for 48 hours *(43)*.

In vitro assays have been used in tracking the trends of the in vitro response of parasites over time, following the introduction of a drug *(36)*. RSA^{0-3h} and PSA have been used in the identification of molecular markers for artemisinin partial resistance and PPQ resistance *(13, 44)*.

Ex vivo assays use parasites taken directly from patients before they receive treatment. Such assays are simpler and there is no potential confounding effect from metabolic changes in the parasites (resulting from culture adaptation) or elimination of some of the parasite populations during long-term cultivation. However, ex vivo assays are not reproducible. Because parasites obtained ex vivo are not synchronized, this will affect the drug resistance results, particularly when comparing results from assays using synchronized parasites (e.g. RSA^{0-3h}).

Use of in vitro tests in species other than *P. falciparum* is limited. Maintaining a continuous culture of *P. vivax* is still difficult; also, the use of in vitro assays for *P. vivax* is hampered by the generally lower parasitaemia in *P. vivax* infections, the frequent presence of several developmental stages of both asexual and sexual erythrocytic phases in the peripheral circulation, and the deleterious effect of synchronization with sorbitol on in vitro parasite growth. Only a few studies have used ex vivo assays for *P. vivax (45)*.

4 DHA is the active metabolite of AS and artemether.

4.3.3 Molecular markers

Molecular markers are genetic changes identified as being associated with a change in parasite susceptibility to antimalarial drugs. As noted in Section 4.3, treatment outcomes are affected by factors other than intrinsic parasite susceptibility; such factors include patient acquired immunity, initial parasite biomass, treatment adherence, dosing, drug quality and pharmacokinetics. Therefore, even markers that correlate almost perfectly with in vitro resistance are of limited use in predicting the treatment outcome in the individual patient. However, molecular markers can be used to help confirm resistance as a cause of treatment failure, and a changing prevalence of a molecular marker for resistance in a geographical area can serve as an early warning signal. When, and to what degree, a change in the prevalence of a molecular marker will be reflected in a decrease in the efficacy of a drug will differ among population groups and areas, with a stronger correlation expected among non-immune populations.

In the past, returning travellers with malaria have been an important supplementary source of information on the spread of resistance. Recently, there have been reports of detection of molecular markers of resistance in infections acquired by travellers returning from areas where these markers were not previously reported. However, based on sampling from one or a few travellers, it is not possible to determine whether a mutation was present in parasites circulating in the area of acquisition at a prevalence indicating selection of this mutation. Therefore, identification of these parasite mutations in infected returning travellers needs to be followed up by studies or surveys seeking to clarify whether selection is taking place, and whether the mutations identified affect the efficacy of the first- or second-line treatment in the area of interest. One challenge is that it may be difficult to identify precisely the area where the parasite with a given mutation was transmitted.

Many molecular markers of *P. falciparum* resistance to antimalarial drugs have been identified (see Table 3). Currently, the markers most closely monitored are those of artemisinin partial resistance and resistance to ACT partner drugs, although molecular markers have not yet been identified for some of the ACT partner drugs.

Molecular markers for resistance in *P. falciparum*

TABLE 3
Molecular markers of resistance to antimalarial drugs for *P. falciparum*

Drug	Molecular markers		References
	Gene	Mutation	
4-aminoquinolines			
Chloroquine	*Pfcrt*	K76T + different sets of mutations at other codons (including C72S, M74I, N75E, A220S, Q271E, N326S, I356T and R371I)	*(46-49)*
	Pfmdr1 (in combination with *Pfcrt* mutations only)	N86Y, Y184F, S1034C, N1042D and D1246Y	*(50-52)*
Amodiaquine (mannich base)	Yet to be validated	Studies show that amodiaquine selects for *Pfmdr1* mutations (86Y)	*(53-57)*
Piperaquine	*Pfpm2–3*	*Pfpm2–3* increased copy number	*(44)*
	Pfcrt	Detected in vivo: T93S, H97Y, F145I, I218F and C350R	*(58-62)*
		Detected in vitro: T93S, H97Y, F145I, I218F, M343L and G353V	
Antifolates			
Pyrimethamine	*Pfdhfr*	N51I, C59R, S108N and I164L	*(63, 64)*
Sulfadoxine	*Pfdhps*	S436A/F, A437G, K540E, A581G and A613T/S	*(64)*
Proguanil	*Pfdhfr*	A16V, N51I, C59R, S108N and I164L	*(65)*
Amino-alcohols			
Lumefantrine	Yet to be validated	Studies show that lumefantrine selects for *Pfmdr1* mutations (N86)	*(54, 55, 57, 66)*
Mefloquine	*Pfmdr1*	*Pfmdr1* increased copy number	*(51)*
Quinine	Yet to be validated		
Mannich base			
Pyronaridine	Yet to be validated		
Naphthoquinone			
Atovaquone	*Pfcytb*	Y268N/S/C	*(65, 67)*
Sesquiterpene lactones			
Artemisinin and its derivatives	*PfK13*	List of candidate and validated markers developed (see Table 4)	*(13, 68)*
Antibiotics			
Doxycycline	Resistance not documented		
Clindamycin	Resistance not documented		
8-aminoquinolines			
Primaquine	Resistance not documented		
Tafenoquine	Resistance not documented		

P: Plasmodium; Pfcrt: P. falciparum chloroquine resistance transporter; Pfcytb: P. falciparum cytochrome b; Pfdhfr: P. falciparum dihydrofolate reductase; Pfdhps: P. falciparum dihydropteroate synthase; PfK13: P. falciparum Kelch 13; Pfmdr1: P. falciparum multidrug resistance 1 protein; Pfpm2–3: P. falciparum plasmepsin 2–3; TES: therapeutic efficacy studies.

Molecular markers of artemisinin partial resistance

Both in vitro and in vivo studies have shown that mutations in the *PfK13* BTB/POZ and propeller domain are associated with delayed parasite clearance. To date, more than 260 non-synonymous mutations in the *PfK13* gene have been reported. However, not all the non-synonymous *PfK13* mutants reported are associated with artemisinin partial resistance; mutants can also represent genotypes arising de novo but not being selected for, and thus not detected in any later studies or surveys. Different *PfK13* mutations have varying effects on the clearance phenotype. WHO has established a list of candidate or associated and validated markers of artemisinin partial resistance. The criteria for classification of *PfK13* markers of artemisinin partial resistance can be seen in Box 4.

BOX 4

Criteria for classification of *PfK13* markers of artemisinin partial resistance *(69)*

Candidate or associated *PfK13* markers of artemisinin partial resistance

1. A statistically significant association ($p < 0.05$) between a *PfK13* mutation and clearance half-life >5 hours or day 3 parasitaemia via a chi-squared test or appropriate multivariable regression model on a sample of at least 20 clinical cases.

OR

2. Survival of >1% using the RSA^{0-3h} in at least five individual isolates with a given mutation or a statistically significant difference ($p < 0.05$) in the RSA^{0-3h} assay between culture-adapted recombinant isogenic parasite lines, produced using transfection and gene editing techniques, which express a variant allele of *PfK13* as compared with the wild-type allele.

Validated *PfK13* markers of artemisinin partial resistance

Both requirements 1 and 2 are met.

The current list of validated and candidate *PfK13* mutations is provided in Table 4; all are located in the *PfK13* BTB/POZ and propeller domain. Outside these domains, two mutations were reported frequently in clinical studies: K189T and E252Q. E252Q has been associated with delayed clearance, but in vitro this association appears to be dependent on other mutations. The mutation A578S has been identified in several studies in Asia and Africa, but has not been associated with clinical or in vitro resistance to artemisinin.

TABLE 4
Current list of validated and candidate or associated *PfK13* mutations

Validated		Candidate or associated	
F446I	**P553L**	P441L	N537I/D
N458Y	**R561H**	G449A	G538V
M476I	**P574L**	C469F/Y	V568G
Y493H	**C580Y**	A481V	R622I
R539T		R515K	A675V
I543T		P527H	

Less frequent variants have allegedly been associated with delayed parasite clearance; however, sample sizes were too small to determine statistical significance. These variants include K479I, G533A, R575K, M579I, D584V, P667T, F673I and H719N.

Molecular markers for resistance in *P. vivax*

Understanding of the mechanisms of resistance in *P. vivax* is still limited. Decreased *P. vivax* sensitivity to CQ has been linked with overexpression of *Pvcrt-o* and mutations in *Pvmdr1 (70-73)*, while MQ resistance has been linked to amplification of *Pvmdr1 (71, 74)*. As with *P. falciparum*, exposing *P. vivax* to antifolate leads to sequential acquisition of mutations in *Pvdhfr* and *Pvdhps (75-78)*.

4.4 Informing policy change

Data on efficacy – and thus on the proportion of patients with treatment failure – are used to inform policy. WHO recommends that the national treatment guideline be changed if the treatment failure rate exceeds 10% in a study that complies with the WHO protocol. Where quality data show a sharp increase in the rate of treatment failures, a change in policy can be considered even if the rate is below 10%. The antimalarial medicines adopted should have a parasitological cure rate greater than 95%.

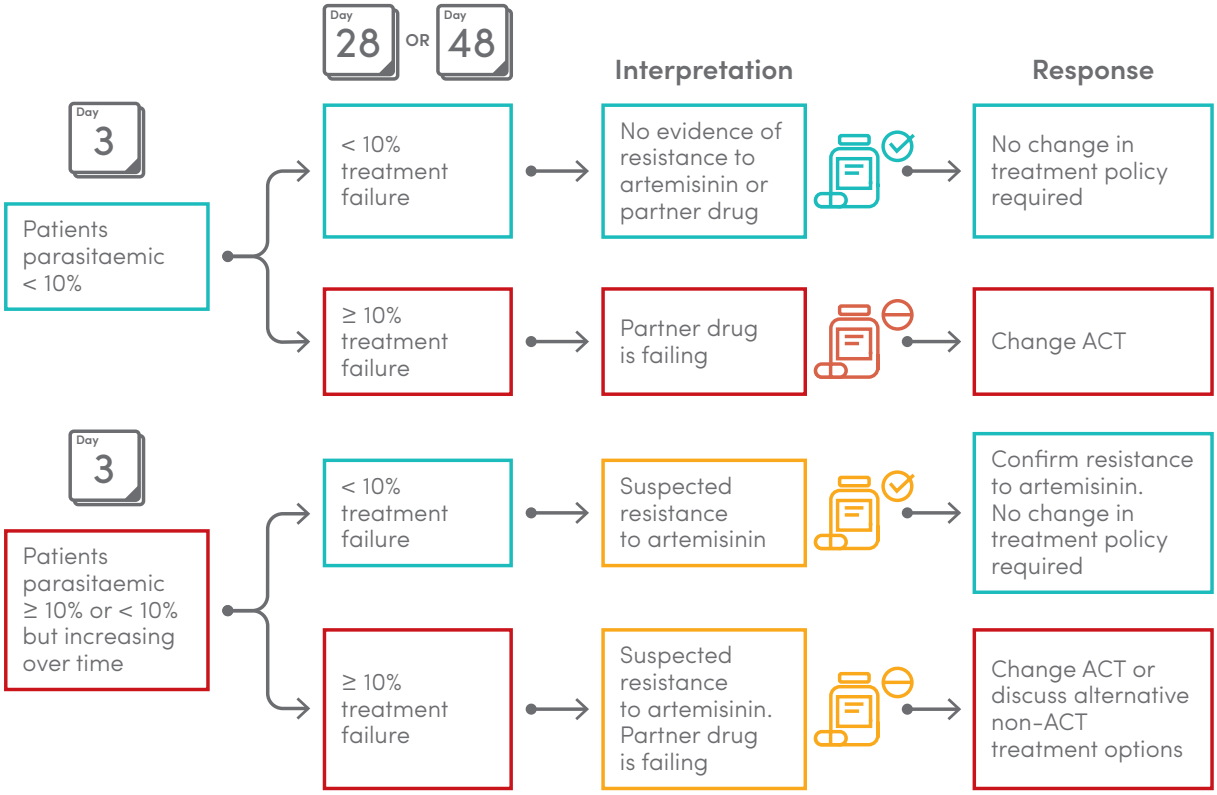
To facilitate a rapid change of treatment policy when needed, updated information on the efficacy of alternative first-line treatments should be available; any treatments considered as future potential first-line treatments must be registered or authorized for use in the country. The recommended second-line treatment for all malaria species should be an ACT *(3)*. Given that the efficacy of the second-line treatment should be known, and that the treatment should already be registered and available in the country, one option when the first-line treatment fails is to use the established second-line treatment as first-line treatment while finding the efficacy of alternative second-line treatments.

Information on the prevalence of molecular markers of drug resistance can supplement the information on efficacy. If there is a marked increase in the prevalence of the molecular marker for resistance to the partner drug in the first-line ACT, countries should consider changing their policy, or should at least prepare for a change in policy, before a 10% failure rate is reached.

Even where artemisinin partial resistance is widespread, ACTs remain the most efficacious and safe treatment for *P. falciparum* malaria, provided that the partner drug is highly efficacious. On its own, detection of high rates of day 3 positivity (>10%) or validated markers of artemisinin partial resistance does not necessitate a change

in the treatment policy. However, where there are high rates of day 3 positivity or validated markers of artemisinin partial resistance, ensuring that a highly efficacious ACT is in use is important to delay the spread of artemisinin partial resistance. Fig. 5 provides an overview of the interpretation of and response to data gathered from TES testing of an ACT for the treatment of uncomplicated *P. falciparum* malaria.

FIG. 5
Interpretation of and response to data from TES testing of an ACT for *P. falciparum*



ACT: artemisinin-based combination therapy; *P: Plasmodium*; TES: therapeutic efficacy studies.

5. DATA ON ANTIMALARIAL DRUG EFFICACY AND DRUG RESISTANCE (2010–2019)

5.1 Summary

The WHO global database on antimalarial drug efficacy and resistance contains data from TES conducted on *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*, as well as molecular marker studies of *P. falciparum* drug resistance (*PfK13*, *Pfpm2–3* and *Pfmdr1*, and *Pfcrt* in Central America). The data come from both published and unpublished studies, and when collating these data, rates are re-estimated in accordance with the WHO protocol where possible and necessary.

For *P. falciparum*, the global database contains data from 1046 TES conducted between 2010 and 2019, with data from 65 749 patients; most (53.8%) of these studies were undertaken in the WHO African Region. Overall, the efficacies of the tested drugs against *P. falciparum* remain high. In four countries in the GMS – Cambodia, Lao People's Democratic Republic, Thailand and Viet Nam – high rates of treatment

failures have been detected after treatment with some ACTs. There are still regimens available that can effectively treat *P. falciparum* in these countries, but the situation requires close monitoring. Outside the GMS, resistance to SP has meant that some countries have had to abandon AS+SP as a treatment for *P. falciparum*. Treatment failures following treatment with AL have been reported in travellers coming back from Africa to Europe. Only in 4 of the 300 AL studies conducted in the WHO African region found more than >10% treatment failures. When repeated, further studies did not find the same high failure rates. Lumefantrine resistance has not been confirmed in Africa.

The global database contains data from 198 TES undertaken between 2010 and 2019 for *P. vivax*, with information from 12 372 patients. The region with most studies is the WHO South-East Asia Region (41.9% of studies). Although CQ is still an efficacious treatment for *P. vivax* in many countries, CQ resistance has been identified in all WHO regions. Annex 1 lists the countries where CQ failure or resistance has been detected for *P. vivax* patients.

The availability of data from recent studies differs between countries and regions, with studies being more frequent where resistance has recently posed a challenge. Up-to-date, quality data are needed on the efficacy of the recommended treatments, to ensure that patients receive efficacious treatment. Conducting these studies can be challenging, but the investment of time and resources is small when compared with the funding spent on treatments and the millions of patients depending on the continued efficacy of these treatments. Too often, study findings are reported years after a study is concluded. Swift reporting and sharing of data with relevant partners in countries and with WHO is needed to enable actions (e.g. a change in the recommended treatment, or studies to confirm the basis for a high failure rate being reported) to be taken when necessary. Overall, where tested, first- and second-line treatment are efficacious for *P. falciparum* and *P. vivax* in all endemic areas. Where high treatments failures rates were reported, policy changes have been made or are ongoing.

Data from samples tested for *PfK13* mutations are available from 1044 studies and surveys undertaken between 2010 and 2019. More than half (52.2%) were samples from the WHO African Region. Of the samples collected, 83.4% were found to be *PfK13* wild type. However, sampling is undertaken more frequently where resistance is suspected, so the prevalence in the samples may differ from the overall prevalence in parasites. C580Y is the mutation most frequently identified; it was found in 9.8% of samples. The highest prevalence of *PfK13* mutations is found in the GMS. Outside South-East Asia, the findings in two countries gives cause for concern. In Guyana, C580Y mutations were found in surveys in 2010 and 2017, and in Rwanda, R561H was found in 11.9% of all the samples collected in 2018 (n=219). There is evidence suggesting that the R561H mutation may be affecting the clearance rate, although to date, the ACTs tested remain efficacious, meaning that any immediate impact for patients is unlikely. Nevertheless, it is of concern that parasites have emerged with partial resistance to the central component in the drugs used to treat millions across Africa. In the GMS, artemisinin partial resistance is likely to have been involved in the spread of resistance to partner drugs. Antimalarial drug efficacy combined with known molecular markers needs to be continually monitored, to ensure that treatment policy can change rapidly in response to signs of emerging ACT partner drug resistance. It is worth noting that China was able to eliminate malaria despite the presence of malaria parasites partially resistant to artemisinins. The change in treatment policy in Cambodia from DHA-PPQ to AS-MQ resulted in selection against strains carrying both C580Y and PPQ resistance.

Although artemisinin partial resistance is a concern, resistance to ACT partner drugs is the cause of the high failure rates detected after treatment with ACTs. Data on *Pfpm2–3* copy numbers are available from 194 studies, and data on *Pfmdr1* copy numbers from 251 studies. Overall, 79.4% of the sampled parasites were found to

have *Pfpm2–3* single copies and 90.6% of the sampled parasites were found to have *Pfmdr1* single copy; these findings indicate that most parasites tested did not carry mutations associated with PPQ or MQ resistance. Studies have identified samples with both *PfPm2–3* and *Pfmdr1* amplifications; this has been reported in Cambodia where a study in 2016 found that 21.3% of samples (n=75) carried both *PfPm2–3* and *Pfmdr1* amplifications. Samples with both *PfPm2–3* and *Pfmdr1* amplifications were also found in Lao People's Democratic Republic and Viet Nam, as well as in 16 studies in the following African countries: Burkina Faso, Burundi, Comoros, Congo, the Democratic Republic of the Congo, Equatorial Guinea, Gabon and Uganda.

The latest available information and references can be found online in the Malaria Threats Map, which provides a geographical representation of drug efficacy and resistance data.[5] The data from the most recent TES are also summarized in reports available online and in Annex 2.[6] Information on the markers *Pfdhfr* and *Pfdhps* in Africa can be found on the IPTi Consortium website.[7]

The following sections outline the status of antimalarial drug efficacy for the treatment of uncomplicated malaria, and the prevalence of selected molecular markers. The information is presented by WHO region, based on data collected during the period 2010–2019 from TES; note that such studies should not be done with drugs for which high-level resistance has been reported and high failure rates are expected.

5.2 WHO African Region

P. falciparum

Most of the global burden of *P. falciparum* and most of the *P. falciparum* endemic countries are in the WHO African Region. The data on efficacy of treatment for *P. falciparum* and selected molecular markers of resistance are presented below for three African subregions, as defined by the countries being supported by the three WHO inter-country support teams: Central, Eastern and Southern, and West Africa (Annex 3 lists countries by region).

5.2.1 WHO African Region: Central Africa

Central Africa includes 10 countries, of which two recommend AL as first-line treatment for uncomplicated *P. falciparum*, seven recommend AS-AQ, and one recommends either AL or AS-AQ.

Data on the efficacy of WHO-recommended ACTs for *P. falciparum* are available from 94 studies undertaken in 10 countries, with 91 of those studies enrolling at least 20 patients[8] (see Fig. 6). High failure rates were found in two AL studies undertaken in northern Angola in 2013 (13.6%, n=81)[9] and in 2015 (11.7%, n=69). These failure rates may have been due to non-adherence to WHO standard protocol, given that an AL study in the same location in 2017 found a low failure rate (4.5%, n=91). AS-AQ has been tested

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5 See https://www.who.int/teams/global-malaria-programme/surveillance/malaria-threats-maps

6 See https://www.who.int/teams/global-malaria-programme/case-management/drug-efficacy-and-resistance/antimalarial-drug-efficacy-database

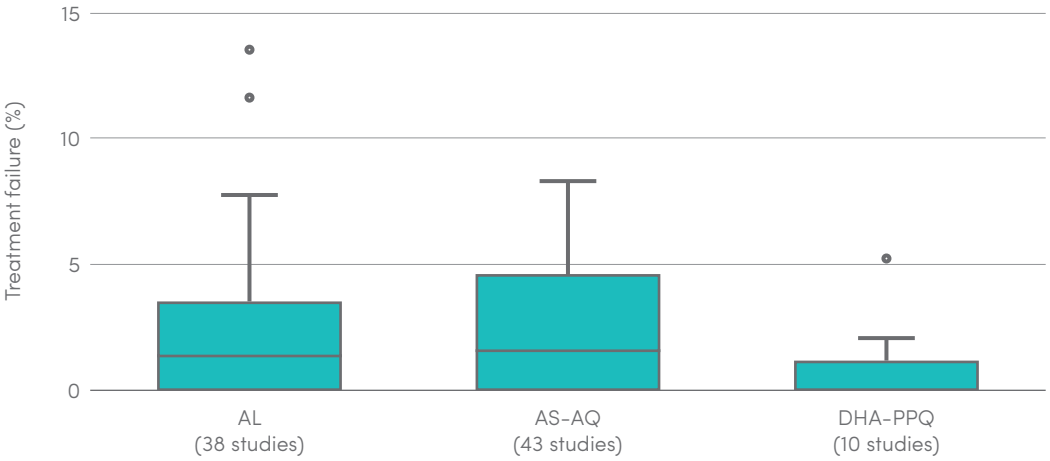
7 See https://drugresistancemaps.org/ipti/

8 Figures and sections focus on studies with at least 20 patients. However, the WHO TES protocol recommends that in the case of a medicine with an expected failure rate of 5%, a confidence level of 95% and a precision level of 5%, a minimum of 73 patients should be enrolled. It is also possible to include 50 patients with 10% precision. Where studies with <20 patients are deemed to provide potentially important additional information, these are mentioned in the text.

9 For TES, n indicates the number of patients followed up until treatment failure or ACPR at the last day of follow-up.

in 44 studies in 10 countries, with 43 of those studies enrolling at least 20 patients; none of those studies found a failure rate of more than 10%. However, of five AS-AQ studies in Burundi, four found failure rates in the range 6.8–7.7%, and three of these four studies were undertaken in 2019. DHA-PPQ has been tested in 10 studies in two countries; all 10 studies found a failure rate of 5.2% or less.

FIG. 6
Treatment failure rates in TES studies in Central Africa with *P. falciparum*[a]



AL: artemether-lumefantrine; AS-AQ: artesunate-amodiaquine; DHA-PPQ: dihydroartemisinin-piperaquine; *P: Plasmodium*; TES: therapeutic efficacy studies.

[a] The box-and-whisker plots show the distribution of values for each drug, with the boxes extending from the 25th to the 75th percentile and the middle line indicating the median. The whiskers denote adjacent values extending from the top of the box to the largest data element, which is ≤1.5 times the interquartile range (IQR; that is, the distance from the 25th to the 75th percentile), and down from the bottom of the box to the smallest data element, which is ≥1.5 times the IQR. The dots denote observations outside the range of adjacent values.

In the Central African countries, data on *PfK13* mutations are available from 141 studies that collected a total of 9652 samples from 10 countries. Most of the samples were *PfK13* wild type (98.6%). A total of 61 different mutations have been identified, 45 of which have only been found in one sample. Five of the mutations detected are validated molecular markers, and one is a candidate molecular marker of artemisinin partial resistance. M476I and P574L (validated markers) and C469F (candidate marker) were detected in Chinese travellers returning from Equatorial Guinea, and P553L and R539T (validated markers) were detected in Chinese travellers returning from Angola. Additionally, in 2012, the validated marker R561H was identified in a TES in the Democratic Republic of the Congo in one sample (0.6%, n=179). In Equatorial Guinea, none of the 405 patients enrolled in TES in 2017 and 2018 were found to be positive on day 3. Furthermore, none of the markers identified in the travellers returning to China were identified in the 474 samples collected in Equatorial Guinea in 2017 and 2018, and tested for *PfK13* mutations; this includes the mutation M579I, which had been detected in one traveller and had been reported by some researchers as evidence of the emergence of artemisinin partial resistance in Africa. In Angola, four TES have been conducted since the identification of the markers in Chinese travellers. None of the markers identified in the returning travellers have been found in the 507 samples collected in Angola, and none of the cases enrolled in TES were found to be positive on day 3. Of note, the mutation Q613E has been detected in one sample in 11 different studies and surveys (5 in Angola and 6 in the Democratic Republic of the Congo). To date, there is no information available to suggest that this mutation is associated with clinical or in vitro resistance to artemisinin.

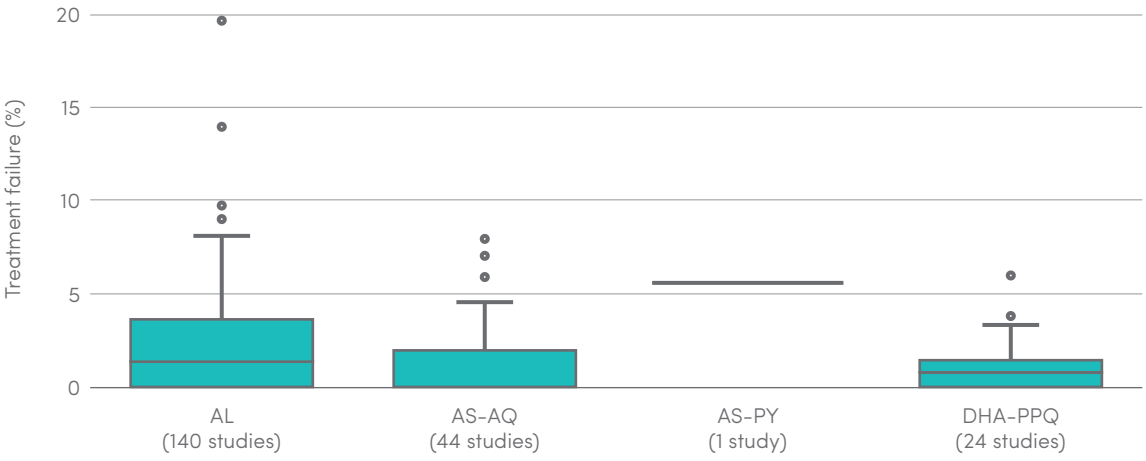
Data on *Pfpm2–3* amplifications are available for 822 samples in seven countries in 15 studies or surveys, with *Pfpm2–3* amplifications being found in 19.3% of the samples. The prevalence of *Pfpm2–3* amplifications varied between and within countries, with amplification being found in 0.0–50.0% of samples. The highest prevalence was found in Burundi in 2019, with half of the samples carrying *Pfpm2–3* amplifications (n=78).

5.2.2 WHO African Region: Eastern and Southern Africa

Eastern and Southern Africa covers 20 countries, of which 14 recommend AL as first-line treatment for uncomplicated *P. falciparum*, three recommend AS-AQ, and one recommends either AL or AS-AQ.

A total of 149 studies on AL were undertaken in 2010–2019 in 12 countries, with 140 of those studies enrolling at least 20 patients (see Fig. 7). Two AL studies found more than 10% treatment failures. In Malawi, one study in 2010 found a failure rate of 19.5% (n=41); a study at the same site in 2012 found a failure rate of 4.3% (n=46). In Uganda, a study in 2015 found a failure rate of 13.9% and 5.1% (n=36) using per-protocol and Kaplan-Meier analysis, respectively. Some AL studies have found failure rates close to 10%; the highest rates were in Zimbabwe in 2010, with a treatment failure rate of 9.1% (n=77), and in Kenya in 2016, with a treatment failure rate of 9.0% (n=130). AS-AQ was tested in 48 studies in seven countries, with 44 of those studies enrolling at least 20 patients. None of these studies found a failure rate higher than 10%. The highest rates were in Eritrea, with a failure rate of 7.9% (n=63) in 2010 and of 7.3% (n=41) in 2012. Since then, studies done at the same sites have found lower failure rates (≤4.5%). AS-PY has only been tested once, in Kenya in 2015, where the failure rate was 5.6% (n=71). The efficacy of DHA-PPQ has been tested in 26 studies in eight countries, with 24 of those studies enrolling at least 20 patients. The highest failure rate, 6.0% (n=140), was identified in Kenya in 2016.

FIG. 7
Treatment failure rates in TES studies in Eastern and Southern Africa with *P. falciparum*



AL: artemether-lumefantrine; AS-AQ: artesunate-amodiaquine; AS-PY: artesunate-pyronaridine; DHA-PPQ: dihydroartemisinin-piperaquine; *P: Plasmodium*; TES: therapeutic efficacy studies.

In the Eastern and Southern African countries, data on *PfK13* mutations are available from 214 studies collecting 11 271 samples from 16 countries. Most samples were found to be *PfK13* wild type (96.5%). A total of 129 different mutations have been identified, more than in any other region, with 86 of these mutations being found in only one sample. Two validated markers of artemisinin partial resistance have been detected in the Eastern and Southern African countries: P574L and R561H. The marker P574L has been detected in three studies; two in Rwanda (in 2013 and 2015) and one in Uganda

(in 2012). In all three studies, P574L was found in one sample only (0.4–1.5%). The marker R561H has been detected in five studies in Rwanda in 2012–2018; in 2018, 11.9% of the 219 samples collected carried R561H. Despite high positivity rate at day 3 detected in 2018, the efficacy of the ACTs tested between 2012-2018 (AL and DHA-PIP) remained high. Additionally, R561H was detected in two samples from travellers returning to China from Rwanda. Previously, R561H had been detected mainly in the western GMS, in Myanmar and in western Thailand bordering Myanmar. It had also been detected in older samples from Cambodia (collected before 2005) and in one sample from each of the following countries: China (Yunnan province), the Democratic Republic of the Congo, India, Sudan, United Republic of Tanzania and Viet Nam.

R622I (a candidate marker for partial artemisinin resistance) was detected in Eritrea, in Ethiopia, in a traveller returning to China from Mozambique, and in one sample in a survey in Zambia. In Ethiopia in two small studies in 2013, R622I was detected in three samples but has not been detected since then. In Eritrea, the highest prevalence of R622I was found in a study from 2016 (16.7%, n=24); overall, R622I was found in 8.7% of 275 samples collected in Eritrea in 2016 and in 7.7% of 207 samples in 2017. Importantly, the ACTs tested remained highly efficacious. In 2016, four studies on AS-AQ were conducted in Eritrea; the highest failure rate found was 4.5% (n=67). Of the five AL studies in 2017 enrolling 200 patients, none found any failures, but one study found a day 3 positivity of 6.3% (n=64). Among other *PfK13* mutations not yet classified as candidate or validated markers, those most frequently identified were C469F, A675V and N585K. The C469F marker has been detected in five studies in Uganda and in three studies in Rwanda, but at a prevalence of less than 5%. Similarly, the A675V marker has been detected in six studies in Uganda and in one study in Rwanda, at a prevalence close to 10% in one study in Uganda (9.3%, n=43). The N585K marker has been detected in six studies in Kenya in 2013, with a highest prevalence of 10.6% (n=47). This mutation has only been detected in Kenya, and has not been detected there or anywhere else since 2013.

In summary, the situation in Rwanda is of particular note. There is considerable evidence of selection of R561H, and this appears to be associated with delayed clearance of parasites after treatment with an ACT. Although this is a cause for concern, at present, the ACTs tested (AL and DHA-PPQ) remain efficacious. The situation in Eritrea also warrants close monitoring.

Data on *Pfpm2–3* amplifications are available for 1572 samples in six countries from 32 studies or surveys. *Pfpm2–3* amplifications were found in 8.3% of the samples. The prevalence of *Pfpm2–3* amplifications varied considerably, with a prevalence range of 0.0–33.9% of samples.

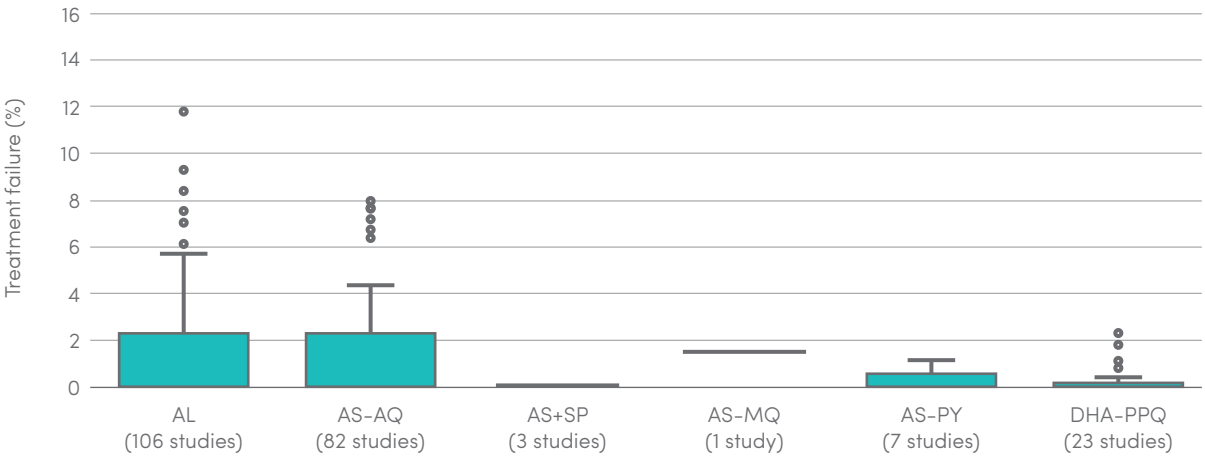
5.2.3 WHO African Region: West Africa

West Africa includes 17 countries, of which five recommend AL as first-line treatment for uncomplicated *P. falciparum*, three recommend AS-AQ, seven recommend either AL or AS-AQ, and one recommends three different first-line treatments: AL, AS-AQ and DHA-PPQ.

A total of 113 studies on AL were undertaken in 2010–2019 in 14 countries in this region, with 106 of those studies enrolling at least 20 patients (see Fig. 8). One study in Gambia in 2010 found a failure rate of 11.9% (n=42). In the 12 AL studies performed in Gambia since that 2010 study, all had low failure rates (≤2.7%). Studies in Ghana have found failures rates close to 10%, with the highest (from 2010) being 9.4% (n=53). Two studies in the same sites in Ghana in 2013 and 2015 found low failure rates (≤1.9%). AS-AQ has been tested in 91 studies in 12 countries, with 82 of those studies enrolling at least

20 patients. None of those studies found a failure rate higher than 10%. In Liberia, two AS-AQ studies in 2017 identified failure rates of 7.3% (n=82) and 8.0% (n=49). The efficacy of AS+SP was tested in three studies in Mali in 2010–2013; none of those studies found any treatment failures. AS-MQ was tested in one study in Senegal in 2010, which found a low failure rate (1.5%, n=70). In 2011, seven studies looked at the efficacy of AS-PY; those studies were done in Burkina Faso, Guinea and Mali, and all found low failure rates (≤1.2%). All 23 DHA-PPQ studies also found low failure rates (≤2.4%).

FIG. 8.
Treatment failure rates in TES studies in West Africa with *P. falciparum*



AL: artemether-lumefantrine; AS-AQ: artesunate-amodiaquine; AS-PY: artesunate-pyronaridine; DHA-PPQ: dihydroartemisinin-piperaquine; *P: Plasmodium*; TES: therapeutic efficacy studies.

In the West African countries, data on *PfK13* mutations are available from 150 studies that collected a total of 9218 samples from 10 countries. Most of the samples were *PfK13* wild type (98.1%). A total of 91 different mutations were identified, 64 of which were found in only one sample. Three validated markers have been detected in West African countries: M476I, R539T and C580Y. M476I was detected in one sample in Nigeria in 2018 (2.0%, n=51). In samples from 113 Chinese travellers returning from Ghana in 2013, 0.9% carried R539T and 2.7% carried C580Y. Neither R539T nor C580Y have been detected in the 958 samples from Ghana analysed since 2013.

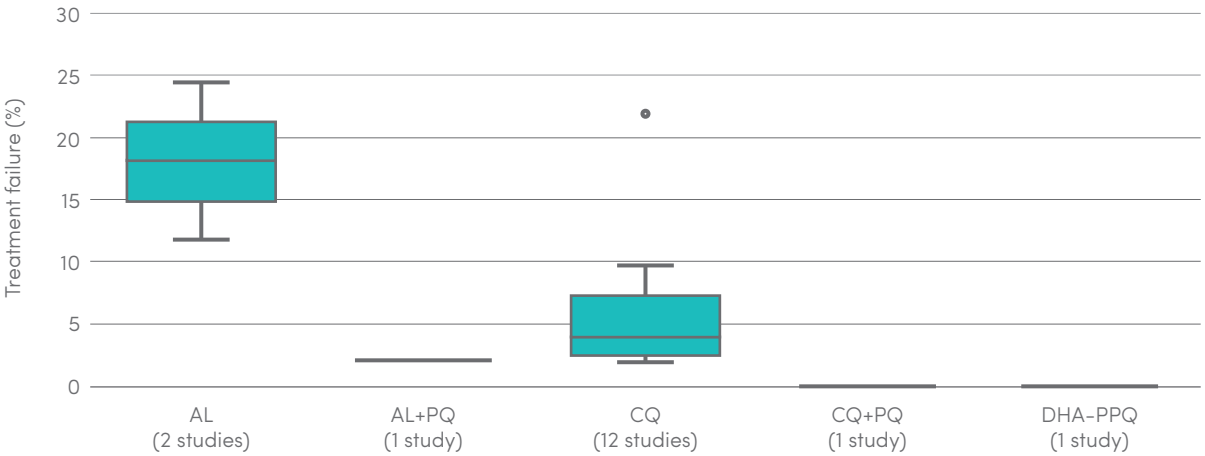
Data on *Pfpm2–3* amplifications are available for 1789 samples from six countries from 20 studies or surveys. *Pfpm2–3* amplifications were found in 12.4% of the samples. The prevalence of *Pfpm2–3* amplifications was 0.0–49.3%. Studies in Liberia, Senegal and Sierra Leone did not identify any amplifications, but all four studies in Burkina Faso found a prevalence of amplifications of at least 21.5%.

P. vivax

P. vivax is rarely reported from the WHO African Region, and only three countries – Ethiopia, Madagascar and Mauritania – have undertaken TES for *P. vivax*. In Madagascar, two small studies in 2012 and 2013 with AS-AQ found no failures (n=13 in both). In two studies testing CQ in Mauritania in 2013, neither study found any failures (n=57, n=62). Ethiopia has undertaken 18 studies, with 17 of those studies enrolling at least 20 patients. Two AL studies found failures rates of 11.9% (n=92) and 24.5% (n=114), probably due to lumefantrine's short half-life resulting in early relapses. The only study done at the same site with AL+PQ found a 2.3% failure rate (n=86). The first-line treatment for *P. vivax* in Ethiopia is CQ. Among the 13 CQ

studies in Ethiopia, three from 2010 found high failure rates: 22.0% (n=50), 9.8% (n=82) and 9.3% (n=108). Studies after 2010 found lower failure rates (<6.6%). One study with CQ+PQ in 2012 found no failures (n=91). Two studies with DHA-PPQ in 2017 (one enrolling only eight patients) found no failures (see Fig. 9).

FIG. 9
Treatment failure rates in TES studies in the WHO African Region with *P. vivax*



AL: artemether-lumefantrine; AL+PQ: artemether-lumefantrine+primaquine; CQ: chloroquine; CQ+PQ: chloroquine+primaquine; DHA-PPQ: dihydroartemisinin-piperaquine; *P: Plasmodium*; TES: therapeutic efficacy studies; WHO: World Health Organization.

5.3 WHO Region of the Americas

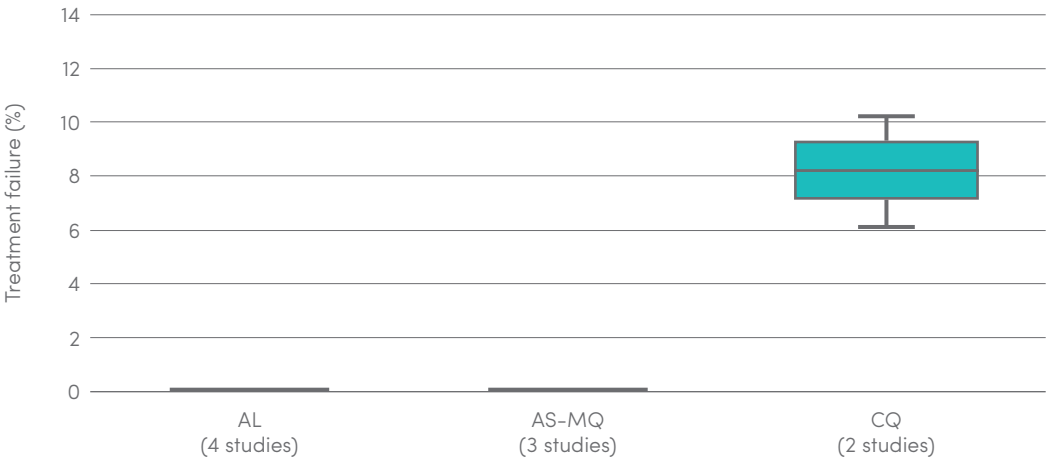
P. falciparum

In the WHO Region of the Americas, three different treatments are recommended as first-line treatments for uncomplicated *P. falciparum*. AL is the recommended first-line treatment in most South American countries (including Bolivia, Colombia, Ecuador, French Guiana, Guyana, Suriname and Venezuela), Brazil recommends both AL and AS-MQ and Peru recommends AS-MQ. Mesoamerican countries where *P. falciparum* cases are imported (Belize, Costa Rica, El Salvador and Mexico) also recommend AS-MQ. In the rest of the Central American countries and in Hispaniola, countries still recommend CQ for the treatment of uncomplicated *P. falciparum*; this includes the Dominican Republic, Guatemala, Haiti, Honduras, Nicaragua and Panama, where most *P. falciparum* cases are mainly locally acquired.

Data on the efficacy of WHO-recommended ACTs for *P. falciparum* are available from 14 studies in four countries, with seven of those studies enrolling at least 20 patients (see Fig. 10). The only study using AL reported from Brazil was undertaken in 2015 and found no failures (n=74). AL was tested in five studies in Colombia in 2011–2018. No treatment failures were identified among the 220 patients enrolled across the studies. AS-MQ was tested in six studies in Brazil in 2010–2012 and no treatment failures were identified in 284 patients. Efficacy data on CQ are available from only two studies in Haiti, both of which enrolled more than 20 patients. A study in 2011 found a failure rate of 10.3% (n=68), and a study in 2013 found a failure rate of 15.3% (n=39). The studies in Haiti were not PCR corrected; thus, some of the failures may be reinfections. Information on the molecular marker of resistance to CQ, *Pfcrt*, could have helped in determining whether these failures were caused by resistance, but such information was not available for these studies.

Data on *Pfcrt* are available for 22 different surveys in Haiti in 2010–2017, with samples from 1158 patients. Only 0.3% of these samples carried the *Pfcrt* mutations linked with CQ resistance. The highest prevalence was found in a survey in 2010 among malaria patients returning to France from Haiti (10.5%, n=19). *Pfcrt* information is also available from Guatemala (16 samples), Honduras (152 samples) and Nicaragua (123 samples). *Pfcrt* mutations have not been identified in Guatemala, but were identified in two (1.3%) of the samples tested in Honduras, and in one (0.8%) of the samples tested in Nicaragua.

FIG. 10
Treatment failure rates in TES in the WHO Region of the Americas with *P. falciparum*



AL: artemether-lumefantrine; AS-MQ: artesunate-mefloquine; CQ: chloroquine; *P: Plasmodium*; TES: therapeutic efficacy studies; WHO: World Health Organization.

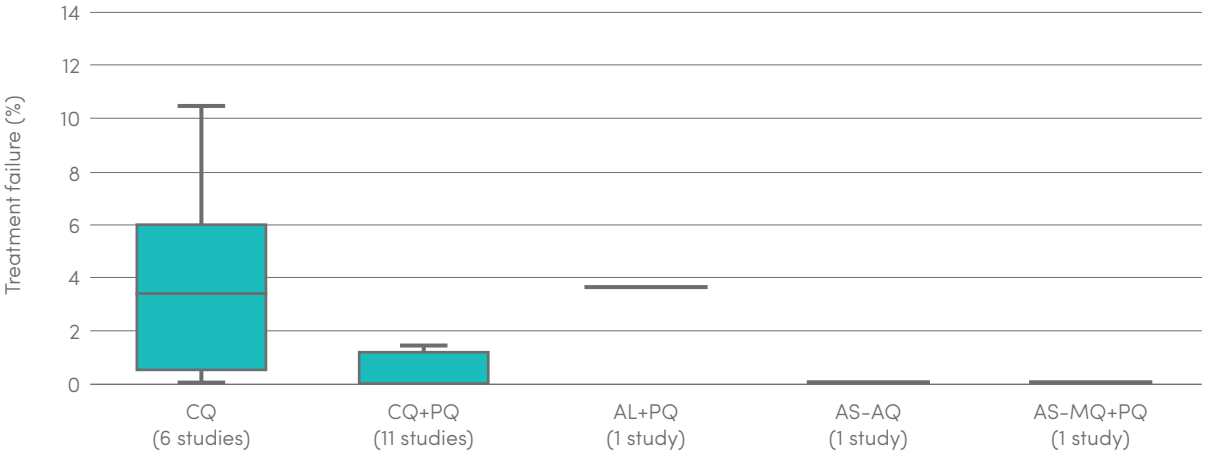
In the WHO Region of the Americas, data on *PfK13* mutations are available from 68 studies collecting 5338 samples from eight countries. Most samples were found to be *PfK13* wild type (99.5%). Only six different mutations have been identified in this region – significantly fewer than in any other region. The mutation C580Y, which has been validated as a marker of artemisinin partial resistance, was detected in 19 samples in Guyana but not in any other country in the region. The candidate marker A481V was found in one sample in two different surveys in Manaus, Brazil, in 2012 and in 2014, but was not found in a smaller survey in the same area in 2015. With the possible exception of C580Y, there is no evidence of selection of mutations associated with artemisinin partial resistance.

Data on the molecular marker for MQ resistance, *Pfmdr1* amplifications, are available for 660 samples collected in Brazil and Colombia in seven studies or surveys. *Pfmdr1* amplifications were found in 8.3% of the samples. The highest prevalence of *Pfmdr1* amplifications was found in a study in Colombia (32.1%, n=81). No data are available for *Pfpm2–3* amplifications in this region.

P. vivax

All the countries in the WHO Region of the Americas recommend CQ as the first-line treatment for *P. vivax*. In the period 2010–2019, six studies were performed with CQ and 11 with CQ+PQ, with all studies enrolling more than 20 patients (see Fig. 11). A high failure rate was reported in one CQ study in northern Bolivia in 2011 (10.4%, n=96). In Brazil, the efficacy of three ACTs (AL, AS-AQ and AS-MQ) was tested in 2012. The efficacy of all three ACTs was found to be high, and only the AL study identified treatment failures (3.6%, n=84).

FIG. 11
Treatment failure rates in TES in the WHO Region of the Americas with *P. vivax*



AL+PQ: artemether-lumefantrine+primaquine; AS-AQ: artesunate-amodiaquine; AS-MQ+PQ: artesunate-mefloquine+primaquine; CQ: chloroquine; CQ+PQ: chloroquine+primaquine; *P: Plasmodium*; TES: therapeutic efficacy studies; WHO: World Health Organization.

5.4 WHO South-East Asia Region

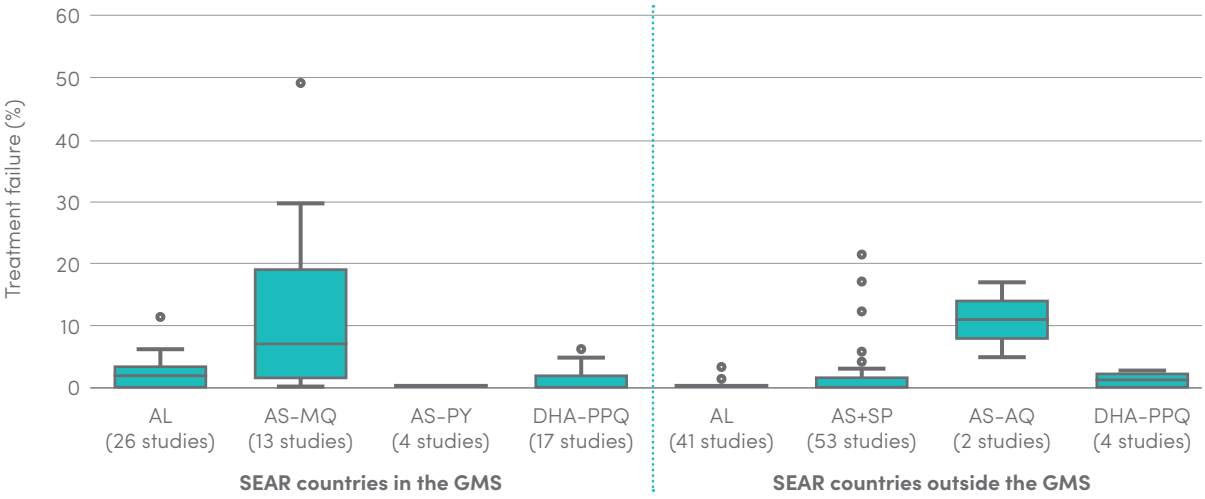
P. falciparum

The patterns of *P. falciparum* resistance and efficacy clearly differ between countries within and outside the GMS; thus, the efficacy data are presented stratified by this geographical factor (see Fig. 12). Two countries in the GMS are part of the WHO South-East Asia Region: Myanmar and Thailand. Myanmar has three recommended first-line treatments for uncomplicated *P. falciparum*: AL, AS-MQ and DHA-PPQ. AL is widely used by the public sector. Thailand recommends DHA-PPQ in most of the country and AS-PY in two provinces along the border with Cambodia. In Myanmar and Thailand, data are available from 28 AL studies, with 26 of these studies enrolling at least 20 patients (24 in Myanmar and two in Thailand). All AL studies in Myanmar showed low treatment failure rates (≤3.8%). An AL study in southern Thailand in 2012 had a high failure rate (11.3%, n=44). The AS-MQ studies undertaken in Myanmar found low failure rates. In contrast, in Thailand, five of the 10 AS-MQ studies enrolling more than 20 patients found failure rates greater than 10%, with the highest being in 2011 in western Thailand (49.1%, n=53). Initially, AS-MQ was the first-line treatment in Thailand; however, in 2015, in response to the high failure rates, this was changed to DHA-PPQ. Four AS-PY studies in Myanmar in 2017–2018, enrolling a total of 189 patients, found no treatment failures. Among the 14 DHA-PPQ studies in Myanmar and the one DHA-PPQ study in Thailand enrolling more than 20 patients, none found a failure rate of more than 10%. However, one study conducted in 2015–2018 (n=15) in Thailand's Sisaket province bordering Cambodia found a Kaplan-Meier estimated failure rate of 86.7% through day 42. DHA-PPQ has not been recommended as first-line treatment for *P. falciparum* for this part of Thailand since 2018; it has been replaced by AS-PY.

Outside the GMS, AL is the recommended first-line treatment for uncomplicated *P. falciparum* in Bangladesh, Bhutan, north-eastern India and Nepal. The southern and western states of India still recommend AS+SP, whereas Indonesia recommends DHA-PPQ. Data from 60 AL TES are available, with 41 of those studies enrolling at least 20 patients; all showed low treatment failure rates (≤3.9%). Two small studies in Bangladesh, enrolling nine and seven patients, identified one treatment failure

each, but no inferences can be made owing to the low numbers of patients. Four small AL studies in 2010–2013 in Nepal enrolled a total of 40 patients and identified one treatment failure. Bhutan has only a few indigenous *P. falciparum* cases; four AL studies in Bhutan in 2010–2013 enrolled a total of 30 patients and found no treatment failures. In India, 56 studies were done for AS+SP in 2010–2019, with 53 of those studies enrolling more than 20 patients. High failure rates were noted in three studies in 2012 in the north-eastern states: Tripura (25.9%, n=58), Arunachal Pradesh (21.4%, n=28) and Mizoram (19.0%, n=58). In response, in 2013, the first-line treatment in the north-eastern states was changed to AL. A high failure rate was also reported in an AS+SP study in West Bengal state in in 2014–2016 (15.8%, n=226). This study did not follow the WHO protocol and definitions, and there were major issues in the analysis of both the clinical data and molecular markers (see below). The data also contrast with other available data on drug efficacy from this part of India, and thus should be interpreted with caution. In the rest of India, AS+SP failure rates were less than 10%. AS-AQ was tested in two studies in Indonesia in 2011 and 2012; the 2012 study (in Lampung province, Sumatra) had a high failure rate (16.7%, n=24). In Indonesia in 2010–2017, four efficacy studies for DHA-PPQ enrolling at least 20 patients found treatment failure rates of 2.3% or less.

FIG. 12
Treatment failure rates in TES in the WHO South-East Asia Region with *P. falciparum*



AL: artemether-lumefantrine; AS-AQ: artesunate-amodiaquine; AS-MQ: artesunate-mefloquine; AS-PY: artesunate-pyronaridine; AS+SP: artesunate+sulfadoxine-pyrimethamine; DHA-PPQ: dihydroartemisinin-piperaquine; GMS: Greater Mekong subregion; *P: Plasmodium*; SEAR: WHO South-East Asia Region; TES: therapeutic efficacy studies; WHO: World Health Organization.

In the period 2010–2019, 5893 samples were tested for *PfK13* mutations and reported on from the GMS countries in the WHO South-East Asia Region. The samples were collected in 115 studies and surveys, and 66 different genotypes were detected. Only 56.4% of the samples were found to be *PfK13* wild type. In Thailand, the most frequent mutation was C580Y (a validated marker of artemisinin partial resistance), which was found in 31.0% of samples. In Myanmar, the most frequent mutation was F446I, which was found in 12.7% of samples. Other frequent mutations in Myanmar that are validated markers include R561H (3.2%) and P574L (2.8%).

Outside the GMS, information on *PfK13* genotype was available from 3189 samples collected from 69 studies and surveys. In total, 32 different genotypes were detected. Of the samples collected, 97.8% were *PfK13* wild type genotype. C580Y

was identified in one sample (1.8%, n=55) in Bangladesh in 2018. Three other mutations identified in one patient only were identified in Bangladesh. No mutations were detected in Indonesia or Nepal. In India, 28 different mutations have been detected, including single samples carrying the validated markers R561H and P553L, and the candidate marker P441L. Only one study – a TES with AS+SP in West Bengal in 2014–2016 (n=226) – found more than two samples carrying any specific mutation. Overall, 21 (9.3%) samples were reported to carry G625R, seven samples (3.1%) carried R539T (considered to be imported from Cambodia, where the prevalence of this mutant was extremely low), four samples (1.8%) carried N672S and two samples (0.9%) carried F446I. The G625R mutation has not been reported from anywhere else in the region, including in a study by the Mahidol-Oxford Research Unit in West Bengal in 2015–2018 (n=89). In addition, this study found no patients with delayed clearance.

Data on the molecular marker for MQ resistance, *Pfmdr1* amplifications, are available for 2042 samples in the WHO South-East Asia Region (collected in Bangladesh, India, Myanmar and Thailand) from 41 studies or surveys. *Pfmdr1* amplifications were found in 15.8% of the samples. None of the samples from Bangladesh (n=280) or India (n=66) found any *Pfmdr1* amplifications. Of the 550 samples collected in Thailand, 40.9% carried *Pfmdr1* amplifications; the highest prevalence was in 2011 in Tak province in eastern Thailand (73.9%, n=23). In Myanmar, 8.6% of 1146 samples carried *Pfmdr1* amplifications. High prevalence has been found in studies in Myanmar; for example, a study in northern Myanmar in 2011 found *Pfmdr1* amplifications in 81.7% of the 60 samples collected. However, a large survey (n=437) in Myanmar in 2016, in Kayin state bordering Thailand, found *Pfmdr1* amplifications in only 2.0% of the samples. These findings indicate that MQ resistance is not being selected for in Myanmar and that MQ resistance has not spread outside the GMS.

Data on the molecular marker for PPQ resistance, *Pfpm2–3* amplifications, are available for 1391 samples in the WHO South-East Asia Region, representing 25 studies or surveys in Bangladesh, India, Indonesia, Myanmar and Thailand. DHA-PPQ has been used as first-line treatment for uncomplicated *P. falciparum* in Indonesia and Thailand, and as one of several recommended first-line treatments in Myanmar. Only in eastern Thailand has *Pfpm2–3* been detected at high prevalence; two studies conducted in Sisaket province in 2015–2018 (n=43) found 36 samples (83.7%) with *Pfpm 2–3*. In this province, DHA-PPQ was found to have a very low efficacy and the treatment policy was changed. *Pfpm2–3* amplifications were found in one other country, Indonesia, where a 2017 study found a 3.2% prevalence (n=95).

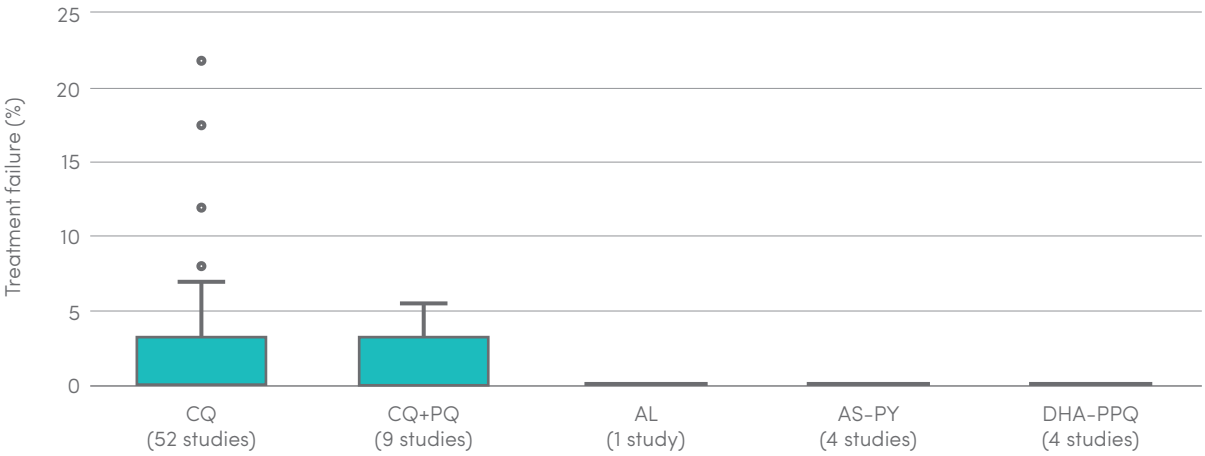
P. vivax

CQ is the first-line treatment for uncomplicated *P. vivax* in Bangladesh, Bhutan, the Democratic People's Republic of Korea, India, Myanmar, Nepal, Sri Lanka and Thailand. AL is first-line treatment for uncomplicated *P. vivax* in Timor-Leste. In Indonesia, DHA-PPQ is the first-line treatment for uncomplicated *P. vivax*.

Data are available from 61 CQ TES conducted in 2010–2017, with 52 of those studies enrolling at least 20 patients (see Fig. 13). Three studies found a failure rate of more than 10%: in Myanmar in 2010 (11.9%, n=67) and in 2012 (21.7%, n=60), and in Timor-Leste in 2011 (17.5%, n=80). Data are available from 14 studies where CQ and PQ were given from day 0; with nine of those studies enrolling at least 20 patients. The studies all found low failure rates (≤5.5%). Three studies with AL were undertaken in the Democratic People's Republic of Korea in 2015, with one of those studies enrolling more than 20 patients; no failures were identified. Four studies of AS-PY were

undertaken in Myanmar in 2017 and 2018; no treatment failures were identified among the 201 patients. Five DHA-PPQ studies were undertaken in Indonesia, with four of those studies enrolling more than 20 patients. None of the studies found any treatment failures.

FIG. 13
Treatment failure rates in TES in the WHO South-East Asia Region patients with *P. vivax*



AL: artemether-lumefantrine; AS-PY: artesunate-pyronaridine; CQ: chloroquine; CQ+PQ: chloroquine+primaquine; DHA-PPQ: dihydroartemisinin-piperaquine; *P: Plasmodium*; TES: therapeutic efficacy studies; WHO: World Health Organization.

5.5 WHO Eastern Mediterranean Region

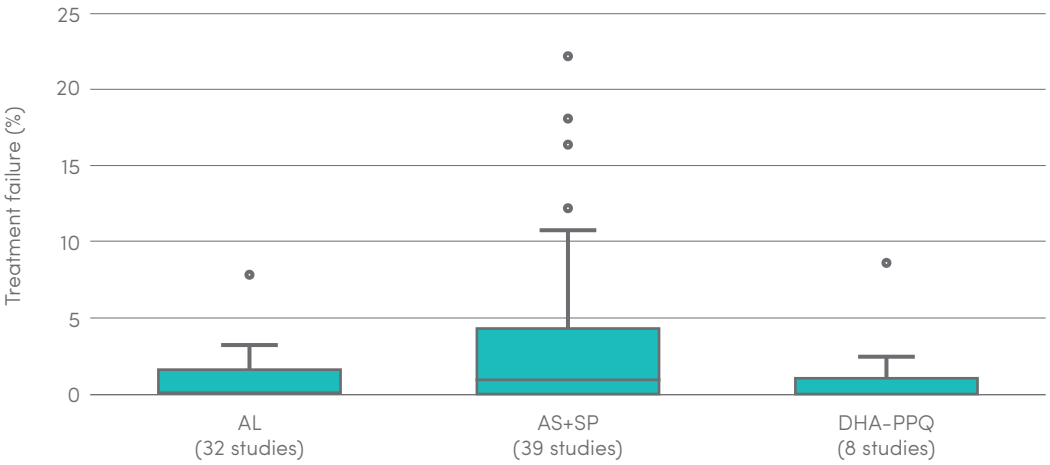
P. falciparum

Currently, only two different ACTs are recommended as first-line treatment for uncomplicated *P. falciparum* in the WHO Eastern Mediterranean Region: AL and AS+SP. AL is recommended in Afghanistan, Djibouti, Pakistan, Somalia and Sudan, whereas AS+SP is recommended in the Islamic Republic of Iran and Saudi Arabia. Yemen is in the process of changing from AS+SP to AL as the first-line treatment.

A total of 33 studies on AL were undertaken in 2010–2019 in five countries, with 32 of those studies enrolling at least 20 patients (see Fig. 14). All AL TES for *P. falciparum* in the region found failure rates of less than 10%; the highest failure rate, identified in Sudan in 2017, was 7.9% (n=38). AS+SP has been tested in 42 studies in six countries in this region, with 39 of those studies enrolling at least 20 patients. A treatment failure rate of more than 10% was identified in two countries: Somalia and Sudan. In Somalia, two studies found a high failure rate: one in Jamame in southern Somalia in 2011 (22.2%, n=81) and one in Bossaso in north-eastern Somalia in 2015 (12.3%, n=81). Three studies in south-eastern Sudan found a high failure rate: two in Gadaref state in 2014 (10.8%, n=37; 18.1%, n=44) and one in Blue Nile state in 2015 (16.4%, n=61). Based on these data, Somalia and Sudan changed their first-line treatment for uncomplicated *P. falciparum* from AS+SP to AL in 2016 and 2017, respectively. Efficacy data on DHA-PPQ are available from eight studies in three countries, and all showed low failure rates (≤2.5%).

FIG. 14

Treatment failure rates in TES in the WHO Eastern Mediterranean Region with *P. falciparum*



AL: artemether-lumefantrine; AS+SP: artesunate+sulfadoxine-pyrimethamine; DHA-PPQ: dihydroartemisinin-piperaquine; *P: Plasmodium*; TES: therapeutic efficacy studies; WHO: World Health Organization.

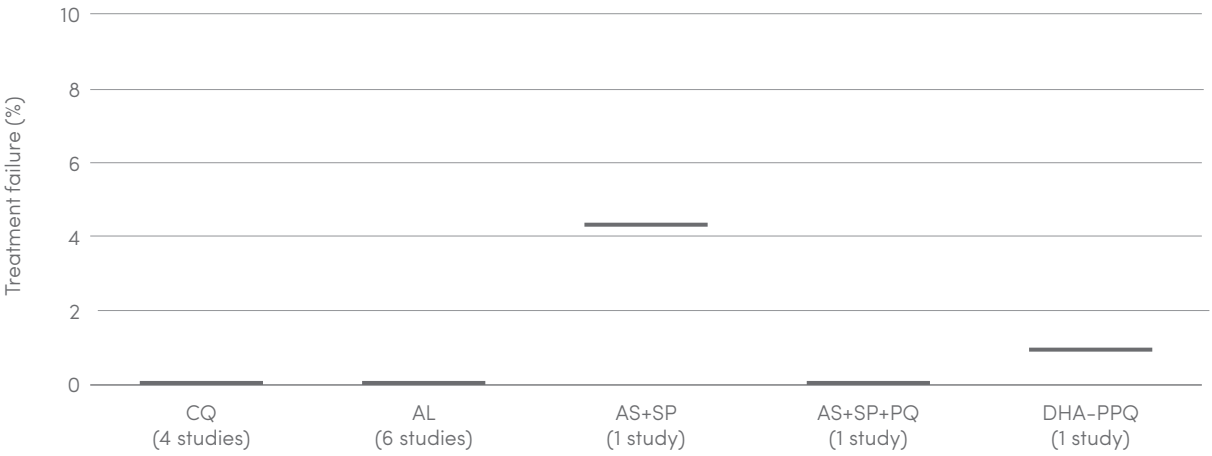
In the WHO Eastern Mediterranean Region, data on *PfK13* mutations are available from 59 studies representing 2442 samples from seven countries. Most samples were *PfK13* wild type (98.4%). A total of 30 different mutations have been identified, one of which – R622I (a candidate maker for artemisinin partial resistance) – has been identified in more than one sample. The R622I mutation was identified in Sudan and Somalia, with the highest prevalence being found in Sudan in 2016 (11.8%, n=34). Two validated molecular markers of artemisinin partial resistance have been identified in this region; M476I in Somalia in 2016 (1.0%, n=102) and R561H in Sudan in 2017 (1.3%, n=80). With the possible exception of R622I, there is no evidence of selection of mutations associated with artemisinin partial resistance. However, continued surveillance is warranted, and R622I needs to be validated as a marker for artemisinin partial resistance.

Data on *Pfpm2–3* amplifications are available for 1351 samples in the region collected in Afghanistan (114 samples), Pakistan (456 samples), Somalia (236 samples) and Sudan (545 samples) through 16 studies or surveys. Overall, *Pfpm2–3* amplifications were found in 0.3% of samples, with the highest prevalence being 1.3% in Sudan in 2017 (n=78).

P. vivax

In the WHO Eastern Mediterranean Region, six countries – Afghanistan, Djibouti, the Islamic Republic of Iran, Pakistan, Saudi Arabia and Yemen – recommend CQ as the first-line treatment for *P. vivax* malaria, whereas Somalia and Sudan recommend AL. CQ studies have been undertaken in Afghanistan (1 study), the Islamic Republic of Iran (2 studies) and Pakistan (1 study) (see Fig. 15). No failures were detected in the CQ studies. The efficacy of AL has been assessed in Afghanistan (4 studies), Somalia (1 study) and Sudan (1 study); none of the studies identified any treatment failures. The efficacy of AS+SP and AS+SP+PQ was studied in two sites in Sudan in 2015. No failures were found in the patients treated with AS+SP+PQ in the 28 days following treatment. In the patients treated with AS+SP only, three of 29 patients enrolled and followed up in two sites had treatment failure by day 28. One DHA-PPQ study in Pakistan in 2013 found a low failure rate (1.0%, n=103).

FIG. 15
Treatment failure rates in TES in the WHO Eastern Mediterranean Region with *P. vivax*



AL: artemether-lumefantrine; AS+SP: artesunate+sulfadoxine-pyrimethamine; AS+SP+PQ: artesunate+sulfadoxine-pyrimethamine+primaquine; CQ: chloroquine; DHA-PPQ: dihydroartemisinin-piperaquine; *P: Plasmodium*; TES: therapeutic efficacy studies; WHO: World Health Organization.

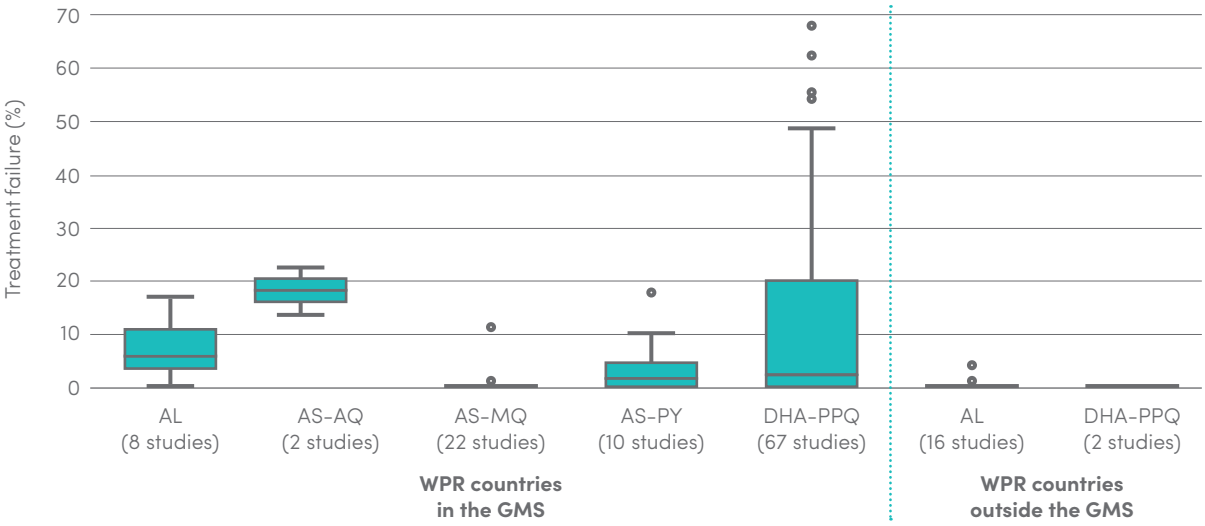
5.6 WHO Western Pacific Region

P. falciparum

In the WHO Western Pacific Region, similar to the South-East Asia Region, the patterns of *P. falciparum* resistance and efficacy differ clearly between countries within and outside the GMS (see Fig. 16). In the GMS, Cambodia recommends AS-MQ as first-line treatment for *P. falciparum*, Lao People's Democratic Republic recommends AL, and Viet Nam recommends DHA-PPQ or AS-PY. High failure rates have been detected for AL in three studies in Lao People's Democratic Republic. However, there are questions about protocol adherence in these studies, and about the relatively low number of patients enrolled; thus, a study is ongoing in 2019–2020 to enrol and follow up a sufficient number of patients to provide more robust information on treatment efficacy. Preliminary data from this ongoing study appear to show high efficacy. AS-AQ had been tested twice in 2016 in Cambodia but was discarded as a potential treatment for *P. falciparum* owing to high failure rates. A failure rate of more than 10% for AS-MQ was detected in Cambodia in a 2010 study enrolling more than 20 patients (11.1%, n=45); however, the 19 studies undertaken in 2014–2019 enrolling more than 20 patients showed low AS-MQ treatment failure rates (≤1.9%). A small AS-MQ study in Cambodia in 2019 (n=16) had two treatment failures (12.5%). Fifteen studies have been conducted with AS-PY in the WHO Western Pacific Region GMS countries, with 10 of these studies enrolling at least 20 patients. Two of these 10 AS-PY studies detected a failure rate of more than 10%, both of which were in Cambodia in 2014 (10.2%, n=59; 18.0%, n=50). The AS-PY studies in Cambodia since 2014 have detected low failure rates (≤3.3%). One small AS-PY study in Viet Nam in 2017 enrolling 19 patients found three treatment failures (15.8%), whereas the three studies in Viet Nam in 2017 enrolling more than 20 patients found low failure rates (≤5.1%). One AS-PY study in Lao People's Democratic Republic in 2018 detected no failures (n=29). For DHA-PPQ, high failure rates have been detected in Cambodia, Lao People's Democratic Republic and Viet Nam. Consequently, Cambodia discarded DHA-PPQ as first-line treatment, initially in 2014 in five provinces, and then in 2016 for the whole country. Viet Nam now recommends AS-PY in provinces where high DHA-PPQ failure rates have been detected.

All five WHO Western Pacific Region countries[10] outside the GMS that reported indigenous *P. falciparum* cases in 2019 recommend AL as first-line treatment against uncomplicated *P. falciparum* malaria. Between 2010 and 2019, data are available from 21 studies on AL, with 16 of those studies enrolling at least 20 patients. AL treatment failure rates have not exceeded 10%. Two studies with DHA-PPQ in Papua New Guinea in 2012 recorded no treatment failures.

FIG. 16
Treatment failure rates in TES in the WHO Western Pacific Region with *P. falciparum*



AL: artemether-lumefantrine; AS-AQ: artesunate-amodiaquine; AS-MQ: artesunate-mefloquine; AS-PY: artesunate-pyronaridine; DHA-PPQ: dihydroartemisinin-piperaquine; GMS: Greater Mekong subregion; *P: Plasmodium*; TES: therapeutic efficacy studies; WHO: World Health Organization; WPR: WHO Western Pacific Region.

In the period 2010–2019, 10 266 samples were tested for *PfK13* mutations and reported on from the WHO Western Pacific Region GMS countries. The samples represent 204 studies and surveys, and a total of 38 different genotypes were detected. Only 42.2% of the samples were found to be *PfK13* wild type. There are clear temporal and spatial patterns in the distribution of the *PfK13* mutations. The mutation C580Y was already highly prevalent in Cambodia in 2010, being identified in 35.5% of all samples that year. In contrast, in 2010, C580Y had not been identified in Lao People's Democratic Republic or China, and was present in only 4.3% of samples in Viet Nam. Since then, it has become the most prevalent genotype in Cambodia, Lao People's Democratic Republic and Viet Nam. This increase has probably been driven in part by the spread of a strain carrying both C580Y and PPQ resistance. More recently, the prevalence of C580Y appears to have been decreasing in Cambodia. Of the samples collected, 87.8% carried C580Y in 2016 (n=238), 58.2% in 2018 (n=225) and 57.3% in 2019 (n=110). This decrease in prevalence has been accompanied by an increase in the prevalence of *PfK13* wild type, from 11.3% in 2016 to 35.6% in 2018 and 25.5% in 2019. The validated marker Y493H also appears to be increasing in prevalence in Cambodia, from 0.0% in 2016 to 5.8% in 2018 and 17.3% in 2019. The changes in the *PfK13* genotypes found in Cambodia could have been driven by a change in treatment policy from DHA-PPQ to AS-MQ, resulting in selection against strains carrying both C580Y and PPQ resistance. In the WHO Western Pacific Region, the validated marker F446I has only been identified in China, among cases likely to have been imported. The validated markers I543T and P553L have been found mostly in Viet Nam, and Y493H mostly in Cambodia and Viet Nam.

[10] Papua New Guinea, the Philippines, Solomon Islands, Timor-Leste and Vanuatu. Malaysia last reported indigenous cases of *P. falciparum* in 2018.

Outside the GMS countries, information on *PfK13* genotype is available from 380 samples collected from 24 studies and surveys. Of the samples collected, 98.9% are *PfK13* wild type. Two *PfK13* mutations were identified. In Solomon Islands in 2012, one sample with G592R was identified that has not been reported elsewhere. A TES in Papua New Guinea in 2019 identified C580Y in 5.5% of the samples (n=55); C580Y has also been found in a traveller returning to Australia from Papua New Guinea. It has been confirmed that these are not strains imported to Papua New Guinea, and further studies are ongoing.

Data on the molecular marker for MQ resistance, *Pfmdr1* amplifications, are available for 5173 samples in the WHO Western Pacific Region, collected in 106 studies or surveys. Overall, amplifications were found in 10.5% of the samples. Cambodia is the only country where AS-MQ has been used as first-line treatment and is the only country in this region where *Pfmdr1* amplifications have been found at high prevalence. A variation in secular trends for this marker has been noted. For example, from 2010 to 2012, 20 of 28 studies found more than 10% of samples with *Pfmdr1* amplifications. In contrast, among the 11 studies undertaken from 2017 to 2018, no study found more than 10% of *Pfmdr1* amplifications. However, in 2019, studies again documented a high prevalence of *Pfmdr1* amplifications, with the highest prevalence being in Ratanakiri province (50.0%, n=52). The change to AS-MQ as first-line treatment in 2014 means this increase was expected, and underlines the need for continued close monitoring of efficacy. *Pfmdr1* amplifications have been found in Lao People's Democratic Republic and Viet Nam, but at low frequencies and typically close to the border to Cambodia. In Papua New Guinea, a study in 2019 identified one sample (4.6%) with *Pfmdr1* amplifications.

Data on the molecular marker for PPQ resistance, *Pfpm2–3* amplifications, are available for 3727 samples in the WHO Western Pacific Region, collected in 85 studies or surveys. DHA-PPQ has been used as first-line treatment in Cambodia and Viet Nam, and in both countries, high prevalence of *Pfpm2–3* amplifications has been found. All eight studies in Cambodia in 2015 found a prevalence of *Pfpm2–3* amplifications of more than 50%. In 2018 and 2019, none of the 10 studies reported a prevalence of *Pfpm2–3* amplifications of more than 50%, which probably reflects the shift in treatment policy from 2014, changing from DHA-PPQ to AS-MQ. In Viet Nam, two studies in 2019 found a high prevalence of *Pfpm2–3* amplifications: in Gia Lai province, amplifications were found in 69.2% (n=52) and in Dak Lak province in 75.0% (n=92). In Lao People's Democratic Republic, a 2016 study found that 25 (59.5%) of 42 samples carried *Pfpm2–3* amplifications, despite the fact that DHA-PPQ has never been used as first-line treatment. Outside the GMS, only Papua New Guinea has data on *Pfpm2–3* amplifications. Three studies in 2017–2019 found no samples with *Pfpm2–3* amplifications. Parasites carrying both *Pfpm2–3* and *Pfmdr1* increased copy numbers were reported in Cambodia (up to 21.3% in one study in 2016), Lao People's Democratic Republic and Viet Nam.

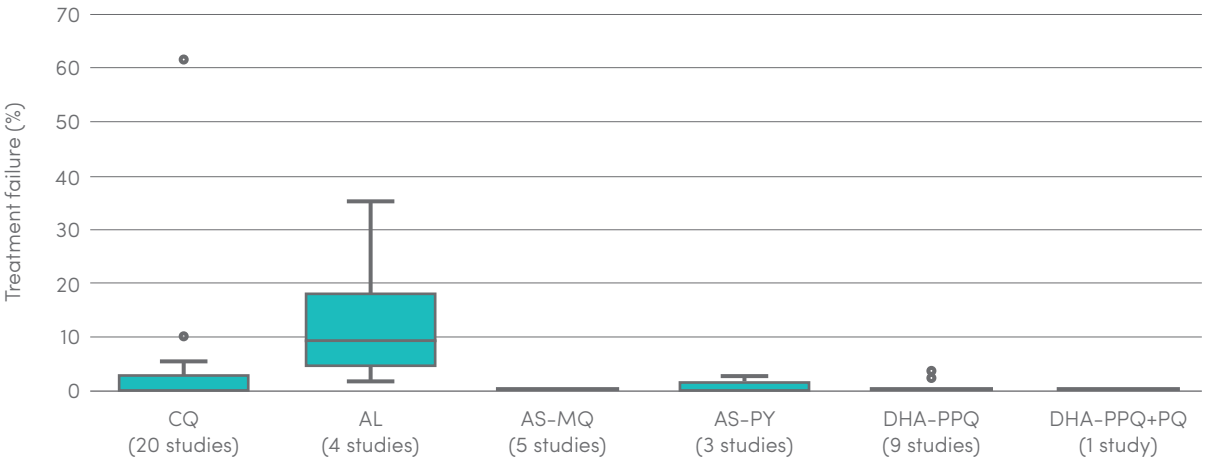
P. vivax

CQ is the first-line treatment for uncomplicated *P. vivax* in Viet Nam, the Philippines and the Republic of Korea. AL is first-line treatment for uncomplicated *P. vivax* in Lao People's Democratic Republic, Malaysia, Papua New Guinea, Solomon Islands and Vanuatu. In Cambodia, AS-MQ is the first-line treatment for uncomplicated *P. vivax*.

Data are available from 27 CQ TES conducted from 2010 to 2019, with 20 of those studies enrolling at least 20 patients (see Fig. 17). One of these studies, in Malaysia in 2012, reported a treatment failure rate of more than 10% (61.9%, n=42). Among

seven studies on AL, four enrolled at least 20 patients. Two of those studies reported treatment failures of more than 10%: one in Papua New Guinea in 2011 (35.0%, n=20) and another in Vanuatu in 2011 (12.1%, n=33). The high treatment failure with AL is probably due to primaquine only being administered on day 28, and the short half-life of lumefantrine leading to relapse prior to day 28. Five studies have been undertaken on AS-MQ in this region: in Cambodia (3 studies), Lao People's Democratic Republic (1 study) and Malaysia (1 study). None of the studies identified any treatment failures. AS-PY was evaluated in two studies in Cambodia in 2018 and one study in Lao People's Democratic Republic in 2019. Only one failure was identified, occurring in Cambodia (1.7%, n=60). DHA-PPQ for *P. vivax* has been studied in Cambodia (7 studies with DHA-PPQ and 1 with DHA-PPQ+PQ), Papua New Guinea (1 study) and Viet Nam (1 study). Treatment failures were only identified in two studies, with the highest failure rate being in Cambodia in 2013 (3.3%, n=60).

FIG. 17
Reatment failure rates in TES in the WHO Western Pacific Region with *P. vivax*



AL: artemether-lumefantrine; AS-MQ: artesunate-mefloquine; AS-PY: artesunate-pyronaridine; CQ: chloroquine; DHA-PPQ: dihydroartemisinin-piperaquine; DHA-PPQ+PQ: dihydroartemisinin-piperaquine+primaquine; *P: Plasmodium*; TES: therapeutic efficacy studies; WHO: World Health Organization.

5.7. Other species

P. ovale

Data on drug efficacy for the treatment of *P. ovale* from 2010 to 2019 are available from 144 patients in four countries. As with *P. malariae, P. ovale* is relatively rare, and all the 22 studies enrolled few patients. All four countries with data on drug efficacy for *P. ovale* are in the WHO African Region: Burkina Faso, Gabon, Guinea and Mali. The studies collected data on 78 patients treated with AL, 24 with AS-AQ, 36 with AS-PY and 28 with DHA-PPQ. The only study detecting a treatment failure was in Gabon with AL (3.7%, n=27).

P. knowlesi

P. knowlesi is a zoonotic malaria species that has been reported from Asia. Malaysia reported 4124 cases of *P. knowlesi* in 2018. Since 2012, Malaysia has collected information on drug efficacy from 4156 patients infected with *P. knowlesi*. Of these, 1225 patients were treated with CQ, 96 with AS-MQ and 2835 with AL. No treatment

failures have been reported, showing that the available treatments have high efficacy against this zoonotic malaria species in Malaysia.

P. malariae

Data on drug efficacy for the treatment of *P. malariae* from 2010 to 2019 are available from 496 patients in five countries. Because *P. malariae* is relatively rare, most of the 27 studies enrolled only few patients. Only one country outside the WHO African Region has undertaken efficacy studies for *P. malariae*. Malaysia has collected data from 120 patients treated with CQ and 28 patients treated with AL; no treatment failures were detected. The four countries in the WHO African Region that have undertaken studies on *P. malariae* are Burkina Faso, Gabon, Guinea and Mali. These studies collected data on 78 patients treated with AL, 76 with AS-AQ, 93 with AS-PY and 101 with DHA-PPQ. No treatment failures were detected.

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ANNEX 1: COUNTRIES WHERE *PLASMODIUM VIVAX* CHLOROQUINE FAILURE OR RESISTANCE WAS REPORTED

Plasmodium vivax chloroquine treatment failure on or before day 28 has been observed in Afghanistan, Bolivia (Plurinational State of), Brazil, Cambodia, China, Colombia, Democratic People's Republic of Korea, Ethiopia, French Guiana, Guyana, India, Indonesia, Madagascar, Malaysia, Myanmar, Nepal, Pakistan, Papua New Guinea, Peru, Republic of Korea, Solomon Islands, Sri Lanka, Thailand, Timor-Leste, Turkey, Vanuatu and Viet Nam.

Confirmation of true chloroquine resistance, however, requires additional studies of drug concentrations in blood. The spread of chloroquine-resistant *P. vivax* is therefore not entirely clear. At least one confirmed case of chloroquine-resistant vivax malaria was reported in the following countries: Bolivia (Plurinational State of), Brazil, Colombia, Ethiopia, French Guiana, Indonesia, Malaysia, Myanmar, Papua New Guinea, Peru, Solomon Islands and Thailand.

Artemisinin-based combination therapies (ACTs) are the recommended treatment for chloroquine-resistant *P. vivax*.

ANNEX 2: SUMMARY OF TREATMENT FAILURE RATES GROUPED BY SPECIES, TREATMENT, COUNTRY AND WHO REGION

Data from the most recent TES are summarized in the reports below. The reports provide a complete overview of treatment failure rates grouped by species, treatment and country. Treatment failure rates are calculated using the per-protocol method. This analysis includes only patients who complete the entire study follow-up and have a clear outcome of either treatment success or failure. Patients who do not complete follow-up, deviate from the study protocol, or withdraw are excluded from the analysis. Studies have a minimum follow-up period of 28 days, with polymerase chain reaction (PCR)-correction to distinguish between treatment failures caused by reinfection from those caused by recrudescence.

Summary of treatment failure rates among patients infected with *P. falciparum*, grouped by country and treatment, per WHO region (October 2020)*

						Percentile	
	Study years	Number of studies	Median	Min	Max	25	75
WHO African Region							
Angola							
Artemether-lumefantrine	2013–2017	6	4.1	2.6	13.6	2.7	12.7
Artesunate-amodiaquine	2015–2017	4	0.0	0.0	6.3	0.0	3.2
Dihydroartemisinin-piperaquine	2015–2017	4	0.0	0.0	1.4	0.0	0.7
Benin							
Artemether-lumefantrine	2011–2017	6	0.7	0.0	3.5	0.0	2.7
Burkina Faso							
Artemether-lumefantrine	2011–2016	2	1.8	1.5	2.1	1.5	2.1
Artesunate-amodiaquine	2011–2016	3	3.2	1.0	4.4	1.0	4.4
Artesunate-pyronaridine	2011–2016	2	0.9	0.6	1.2	0.6	1.2
Dihydroartemisinin-piperaquine	2011–2016	2	0.0	0.0	0.0	0.0	0.0
Burundi							
Artesunate-amodiaquine	2015–2019	5	7.3	2.8	7.7	4.8	7.7
Cameroon							
Amodiaquine+sulfadoxine-pyrimethamine	2014–2014	1	8.6	8.6	8.6	8.6	8.6
Artemether-lumefantrine	2010–2013	2	3.3	3.2	3.4	3.2	3.4
Artesunate-amodiaquine	2010–2014	5	4.1	1.9	8.2	1.9	7.4
Dihydroartemisinin-piperaquine	2010–2013	2	3.5	3.1	3.8	3.1	3.8
Central African Republic							
Amodiaquine+sulfadoxine-pyrimethamine	2010–2010	1	3.6	3.6	3.6	3.6	3.6
Artemether-lumefantrine	2010–2017	2	1.1	0.0	2.2	0.0	2.2
Artesunate-amodiaquine	2010–2010	1	0.0	0.0	0.0	0.0	0.0

continues...

* Studies of artesunate-mefloquine, artesunate-pyronaridine and dihydroartemisinin-piperaquine have a 42-day follow-up, unless otherwise indicated. All other studies have a 28-day follow-up, unless otherwise indicated.

continued...

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
Chad							
Artemether-lumefantrine	2019–2019	2	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2011–2019	4	0.0	0.0	1.8	0.0	0.9
Comoros							
Artemether-lumefantrine	2011–2017	13	0.0	0.0	2.5	0.0	0.0
Congo							
Artemether-lumefantrine	2010–2019	6	0.0	0.0	3.6	0.0	2.7
Artesunate-amodiaquine	2010–2017	4	2.4	0.0	4.0	1.1	3.4
Côte d'Ivoire							
Artemether-lumefantrine	2012–2019	23	0.0	0.0	5.8	0.0	1.9
Artesunate-amodiaquine	2012–2019	18	0.0	0.0	2.1	0.0	0.0
Democratic Republic of the Congo							
Artemether-lumefantrine	2011–2018	15	0.0	0.0	5.9	0.0	1.6
Artesunate-amodiaquine	2011–2017	12	1.1	0.0	4.8	0.0	2.7
Dihydroartemisinin-piperaquine	2011–2017	6	0.0	0.0	5.2	0.0	3.6
Equatorial Guinea							
Artemether-lumefantrine	2018–2018	3	4.9	0.0	7.6	0.0	7.6
Artesunate-amodiaquine	2010–2018	6	0.7	0.0	4.9	0.0	3.6
Eritrea							
Artemether-lumefantrine	2017–2017	5	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2010–2016	18	2.4	0.0	7.9	0.0	5.2
Ethiopia							
Artemether-lumefantrine	2010–2018	22	1.2	0.0	8.0	0.0	2.3
Dihydroartemisinin-piperaquine	2017–2018	2	0.0	0.0	0.0	0.0	0.0
Gabon							
Artemether-lumefantrine	2014-2017	4	2.8	2.0	4.1	2.4	3.5
Artesunate-amodiaquine	2014-2018	3	3.2	0.0	4.8	0.0	4.8
Gambia							
Artemether-lumefantrine	2010–2016	13	0.0	0.0	11.9	0.0	2.1
Dihydroartemisinin-piperaquine	2014–2018	2	0.0	0.0	0.0	0.0	0.0
Ghana							
Artemether-lumefantrine	2010–2018	17	1.9	0.0	9.4	0.0	6.7
Artesunate-amodiaquine	2010–2017	26	0.0	0.0	6.6	0.0	2.1
Guinea							
Artemether-lumefantrine	2015-2019	6	2.0	0.0	7.6	0.5	7.6
Artesunate-amodiaquine	2011-2019	8	1.5	0.0	7.7	0.5	3.9
Artesunate-pyronaridine	2011-2016	1	0.5	0.5	0.5	0.5	0.5
Dihydroartemisinin-piperaquine	2011-2016	1	0.4	0.4	0.4	0.4	0.4
Guinea-Bissau							
Artemether-lumefantrine	2012–2015	1	5.0	5.0	5.0	5.0	5.0
Dihydroartemisinin-piperaquine	2012–2015	1	0.0	0.0	0.0	0.0	0.0

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continued...

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
Kenya							
Artemether-lumefantrine	2010–2017	7	2.8	0.0	9.0	1.3	3.8
Artesunate-pyronaridine	2015–2016	1	5.6	5.6	5.6	5.6	5.6
Dihydroartemisinin-piperaquine	2010–2017	4	2.5	0.5	6.0	0.9	4.9
Liberia							
Artemether-lumefantrine	2017–2018	2	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2010–2018	3	7.3	0.0	8.0	0.0	8.0
Madagascar							
Artemether-lumefantrine	2018–2018	3	1.8	0.0	4.0	0.0	4.0
Artesunate-amodiaquine	2012–2018	10	0.0	0.0	2.0	0.0	1.1
Malawi							
Artemether-lumefantrine	2010–2017	11	2.4	0.0	19.5	0.0	4.6
Artesunate-amodiaquine	2012–2017	3	1.0	1.0	2.0	1.0	2.0
Dihydroartemisinin-piperaquine	2013–2015	1	0.8	0.8	0.8	0.8	0.8
Mali							
Artemether-lumefantrine	2010–2016	11	0.9	0.0	2.6	0.0	2.2
Artesunate	2010–2011	1	0.0	0.0	0.0	0.0	0.0
Artesunate+sulfadoxine-pyrimethamine	2010–2014	3	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2011–2016	4	0.0	0.0	0.0	0.0	0.0
Artesunate-pyronaridine	2011–2016	4	0.0	0.0	0.6	0.0	0.3
Dihydroartemisinin-piperaquine	2011–2016	5	0.0	0.0	1.2	0.0	0.6
Mauritania							
Artesunate-amodiaquine	2012–2012	2	1.8	1.8	1.8	1.8	1.8
Mozambique							
Artemether-lumefantrine	2011–2018	13	1.5	0.0	5.8	0.0	3.9
Artesunate-amodiaquine	2011–2018	6	0.0	0.0	1.4	0.0	1.3
Dihydroartemisinin-piperaquine	2013–2015	1	0.0	0.0	0.0	0.0	0.0
Niger							
Artemether-lumefantrine	2011–2015	3	1.1	0.0	5.3	0.0	5.3
Artesunate-amodiaquine	2011–2014	2	1.0	0.5	1.4	0.5	1.4
Dihydroartemisinin-piperaquine	2013–2014	1	1.9	1.9	1.9	1.9	1.9
Nigeria							
Artemether-lumefantrine	2010–2015	10	0.0	0.0	7.8	0.0	1.7
Artesunate-amodiaquine	2010–2015	11	0.0	0.0	6.9	0.0	3.6
Dihydroartemisinin-piperaquine	2014–2015	9	0.0	0.0	1.9	0.0	0.0
Rwanda							
Artemether-lumefantrine	2012–2018	9	3.2	0.8	5.8	2.5	4.9
Dihydroartemisinin-piperaquine	2013–2015	2	2.1	0.8	3.3	0.8	3.3
Sao Tome and Principe							
Artemether-lumefantrine	2017–2018	2	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2017–2017	2	0.0	0.0	0.0	0.0	0.0

continues...

continued...

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
Senegal							
Artemether-lumefantrine	2010–2019	12	0.0	0.0	1.6	0.0	0.3
Artesunate-amodiaquine	2010–2019	7	0.0	0.0	1.7	0.0	0.5
Artesunate-mefloquine	2010–2010	1	1.5	1.5	1.5	1.5	1.5
Dihydroartemisinin-piperaquine	2010–2018	4	0.5	0.0	2.4	0.0	1.7
Sierra Leone							
Artemether-lumefantrine	2011–2016	4	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2011–2016	4	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2016–2016	2	0.0	0.0	0.0	0.0	0.0
Togo							
Artemether-lumefantrine	2012–2013	3	2.7	0.0	3.0	0.0	3.0
Artesunate-amodiaquine	2012–2013	3	0.0	0.0	3.8	0.0	3.8
Uganda							
Artemether-lumefantrine	2011–2019	12	1.8	0.0	13.9	0.0	3.3
Artesunate-amodiaquine	2013–2014	3	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2015–2019	6	1.3	0.0	1.6	0.0	1.5
United Republic of Tanzania (mainland)							
Artemether-lumefantrine	2010–2018	27	1.4	0.0	9.7	0.0	4.3
Artesunate-amodiaquine	2011–2017	5	0.0	0.0	2.0	0.0	1.0
Dihydroartemisinin-piperaquine	2014–2017	5	0.0	0.0	3.0	0.0	2.0
Zambia							
Artemether-lumefantrine	2012–2016	7	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2016–2016	3	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2016–2016	3	0.0	0.0	0.0	0.0	0.0
Zimbabwe							
Artemether-lumefantrine	2010–2017	20	1.5	0.0	9.1	0.0	3.9

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
WHO Region of the Americas							
Brazil							
Artemether-lumefantrine	2015–2016	1	0.0	0.0	0.0	0.0	0.0
Artesunate-mefloquine	2010–2017	6	0.0	0.0	0.0	0.0	0.0
Colombia							
Artemether-lumefantrine	2011–2019	5	0.0	0.0	0.0	0.0	0.0
Guyana							
Artesunate	2014–2014	1	0.0	0.0	0.0	0.0	0.0
Suriname							
Artemether-lumefantrine	2011–2011	1	0.0	0.0	0.0	0.0	0.0
Artesunate-mefloquine [28 days]	2013–2014	1	0.0	0.0	0.0	0.0	0.0

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
WHO South-East Asia Region							
Bangladesh							
Artemether-lumefantrine	2010–2019	19	0.0	0.0	14.3	0.0	0.0
Bhutan							
Artemether-lumefantrine	2010–2013	4	0.0	0.0	0.0	0.0	0.0
India							
Artemether-lumefantrine	2010–2019	31	0.0	0.0	4.0	0.0	1.1
Artesunate+sulfadoxine-pyrimethamine	2010–2017	56	0.0	0.0	25.9	0.0	1.5
Indonesia							
Artesunate-amodiaquine	2011–2013	2	10.8	4.8	16.7	4.8	16.7
Dihydroartemisinin-piperaquine	2010–2017	6	0.0	0.0	2.3	0.0	2.2
Myanmar							
Artemether-lumefantrine	2010–2018	26	1.9	0.0	6.0	0.0	2.7
Artesunate	2011–2011	1	0.0	0.0	0.0	0.0	0.0
Artesunate-mefloquine	2011–2013	5	0.0	0.0	2.2	0.0	1.8
Artesunate-pyronaridine	2017–2018	4	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2011–2018	22	0.0	0.0	4.8	0.0	0.0
Nepal							
Artemether-lumefantrine	2010–2014	4	0.0	0.0	6.3	0.0	3.2
Thailand							
Artemether-lumefantrine	2012–2012	2	8.5	5.6	11.3	5.6	11.3
Artesunate-mefloquine	2010–2016	18	7.4	0.0	49.1	0.0	19.5
Dihydroartemisinin-piperaquine	2014–2018	5	5.9	0.0	100.0	0.0	93.4
Timor-Leste							
Artemether-lumefantrine	2012–2019	2	0.0	0.0	0.0	0.0	0.0

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
WHO Eastern Mediterranean Region							
Afghanistan							
Artemether-lumefantrine	2011–2015	5	0.0	0.0	0.0	0.0	0.0
Artesunatesulfadoxine-pyrimethamine	2010–2016	4	0.0	0.0	1.0	0.0	0.5
Iran (Islamic Republic of)							
Artesunate+sulfadoxine-pyrimethamine	2010–2015	7	0.0	0.0	1.0	0.0	0.0
Pakistan							
Artemether-lumefantrine	2012–2017	4	0.0	0.0	1.2	0.0	0.6
Artesunate+sulfadoxine-pyrimethamine	2011–2017	6	0.0	0.0	1.5	0.0	1.3
Dihydroartemisinin-piperaquine	2015–2015	2	0.0	0.0	0.0	0.0	0.0

continues...

continued...

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
Somalia							
Artemether-lumefantrine	2013–2018	5	0.0	0.0	2.4	0.0	1.7
Artesunate+sulfadoxine-pyrimethamine	2011–2015	4	8.4	1.0	22.2	2.7	17.3
Dihydroartemisinin-piperaquine	2016–2016	2	1.8	1.0	2.5	1.0	2.5
Sudan							
Artemether-lumefantrine	2010–2018	16	1.4	0.0	7.9	0.0	2.4
Artesunate+sulfadoxine-pyrimethamine	2010–2016	16	4.0	0.0	18.1	1.3	7.9
Dihydroartemisinin-piperaquine	2015–2017	4	0.0	0.0	1.8	0.0	0.9
Yemen							
Artemether-lumefantrine	2010–2019	3	0.0	0.0	0.0	0.0	0.0
Artesunate+sulfadoxine-pyrimethamine	2010–2014	5	0.0	0.0	3.6	0.0	3.3

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
WHO Western Pacific Region							
Cambodia							
Artemether-lumefantrine	2010–2011	1	5.0	5.0	5.0	5.0	5.0
Artesunate+atovaquone-proguanil	2014–2015	2	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2016–2017	2	18.2	13.8	22.6	13.8	22.6
Artesunate-mefloquine	2010–2019	28	0.0	0.0	12.5	0.0	0.0
Artesunate-pyronaridine	2014–2019	9	1.7	0.0	18.0	0.0	6.8
Atovaquone-proguanil	2014–2015	2	0.8	0.0	1.5	0.0	1.5
Dihydroartemisinin-piperaquine	2010–2018	30	14.6	0.0	85.7	3.5	35.6
China							
Artesunate	2011–2011	1	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2012–2015	6	0.0	0.0	6.0	0.0	3.0
Lao People's Democratic Republic							
Artemether-lumefantrine	2010–2018	10	7.6	0.0	17.2	1.2	15.5
Artesunate-mefloquine	2018–2019	1	8.3	8.3	8.3	8.3	8.3
Artesunate-pyronaridine	2018–2019	1	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2016–2017	1	32.4	32.4	32.4	32.4	32.4
Malaysia							
Artemether-lumefantrine	2014–2018	5	0.0	0.0	0.0	0.0	0.0
Artesunate-mefloquine [28 days]	2012–2016	1	0.0	0.0	0.0	0.0	0.0
Papua New Guinea							
Artemether-lumefantrine	2011–2019	6	0.0	0.0	1.1	0.0	0.6
Dihydroartemisinin-piperaquine	2012–2014	2	0.0	0.0	0.0	0.0	0.0

continues...

continued...

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
Philippines							
Artemether-lumefantrine	2013–2018	7	0.0	0.0	9.1	0.0	4.3
Solomon Islands							
Artemether-lumefantrine	2011–2017	3	5.3	0.0	6.3	0.0	6.3
Viet Nam							
Artemether-lumefantrine	2015–2016	1	0.0	0.0	0.0	0.0	0.0
Artesunate	2010–2012	3	3.1	2.6	8.2	2.6	8.2
Artesunate-mefloquine	2019–2019	3	0.0	0.0	0.0	0.0	0.0
Artesunate-pyronaridine	2017–2018	5	1.6	0.0	15.8	0.0	10.5
Dihydroartemisinin-piperaquine	2010–2019	45	0.0	0.0	68.1	0.0	4.0

Summary of treatment failure rates among patients infected with *P. vivax*, grouped by country and treatment, per WHO region (October 2020)*

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
WHO African Region							
Ethiopia							
Artemether-lumefantrine	2012–2014	1	11.9	11.9	11.9	11.9	11.9
Artemether-lumefantrine+ primaquine	2012–2014	1	2.3	2.3	2.3	2.3	2.3
Chloroquine	2010–2018	11	4.2	2.3	22.0	2.5	6.6
Chloroquine+primaquine	2012–2014	1	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2017–2018	2	0.0	0.0	0.0	0.0	0.0
Madagascar							
Artesunate-amodiaquine	2012–2013	2	0.0	0.0	0.0	0.0	0.0
Mauritania							
Chloroquine	2012–2012	2	0.0	0.0	0.0	0.0	0.0

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
WHO Region of the Americas							
Bolivia (Plurinational State of)							
Chloroquine	2011–2011	1	10.4	10.4	10.4	10.4	10.4
Brazil							
Artemether-lumefantrine+ primaquine	2012–2015	1	3.6	3.6	3.6	3.6	3.6
Artesunate-amodiaquine	2012–2013	1	0.0	0.0	0.0	0.0	0.0

continues...

* All studies have a 28-day follow-up.

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	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
Artesunate-mefloquine+ primaquine	2012–2015	1	0.0	0.0	0.0	0.0	0.0
Chloroquine	2011–2015	3	2.2	0.0	6.4	0.0	6.4
Chloroquine+primaquine	2011–2018	8	0.0	0.0	1.2	0.0	1.1
Colombia							
Chloroquine	2011–2011	1	0.0	0.0	0.0	0.0	0.0
Chloroquine+primaquine	2012–2013	2	0.7	0.0	1.4	0.0	1.4
French Guiana							
Chloroquine	2010–2015	1	4.6	4.6	4.6	4.6	4.6
Peru							
Chloroquine	2011–2013	1	0.0	0.0	0.0	0.0	0.0
Chloroquine+primaquine	2011–2013	1	0.0	0.0	0.0	0.0	0.0
Venezuela (Bolivarian Republic of)							
Chloroquine+primaquine	2013–2013	1	0.0	0.0	0.0	0.0	0.0

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
WHO South-East Asia Region							
Bangladesh							
Chloroquine+primaquine	2014–2015	1	0.0	0.0	0.0	0.0	0.0
Bhutan							
Chloroquine	2013–2015	1	0.0	0.0	0.0	0.0	0.0
Chloroquine+primaquine	2010–2013	4	0.0	0.0	0.0	0.0	0.0
Democratic People's Republic of Korea							
Artemether-lumefantrine	2015–2015	2	0.0	0.0	0.0	0.0	0.0
Chloroquine	2012–2017	12	2.2	0.0	4.8	0.0	3.2
Chloroquine+primaquine	2012–2012	2	4.4	3.2	5.5	3.2	5.5
India							
Chloroquine	2010–2017	10	0.0	0.0	0.9	0.0	0.0
Chloroquine+primaquine	2012–2015	1	0.0	0.0	0.0	0.0	0.0
Indonesia							
Dihydroartemisinin-piperaquine	2015–2018	5	0.0	0.0	1.2	0.0	0.6
Myanmar							
Artesunate-pyronaridine	2017–2018	4	0.0	0.0	0.0	0.0	0.0
Chloroquine	2010–2016	31	0.0	0.0	21.7	0.0	4.0
Chloroquine+primaquine	2014–2017	6	0.0	0.0	5.6	0.0	4.1
Nepal							
Chloroquine	2010–2017	6	0.0	0.0	1.0	0.0	0.5
Chloroquine+primaquine	2015–2017	1	0.0	0.0	0.0	0.0	0.0
Timor-Leste							
Artemether-lumefantrine	2017–2019	1	0.0	0.0	0.0	0.0	0.0
Chloroquine	2011–2013	1	17.5	17.5	17.5	17.5	17.5

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
WHO Eastern Mediterranean Region							
Afghanistan							
Artemether-lumefantrine	2014–2014	4	0.0	0.0	0.0	0.0	0.0
Chloroquine	2010–2016	2	1.1	0.0	2.1	0.0	2.1
Chloroquine+primaquine	2010–2014	1	0.0	0.0	0.0	0.0	0.0
Iran (Islamic Republic of)							
Chloroquine	2010–2015	2	0.0	0.0	0.0	0.0	0.0
Pakistan							
Chloroquine	2013–2013	1	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2013–2013	1	1.0	1.0	1.0	1.0	1.0
Somalia							
Artemether-lumefantrine	2018–2019	1	0.0	0.0	0.0	0.0	0.0
Sudan							
Artemether-lumefantrine	2011–2011	1	0.0	0.0	0.0	0.0	0.0
Artesunate+sulfadoxine-pyrimethamine	2015–2016	2	18.8	4.3	33.3	4.3	33.3
Artesunate+sulfadoxine-pyrimethamine+primaquine	2015–2016	2	0.0	0.0	0.0	0.0	0.0

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
WHO Western Pacific Region							
Cambodia							
Artesunate-mefloquine	2018–2018	3	0.0	0.0	0.0	0.0	0.0
Artesunate-pyronaridine	2018–2018	2	0.9	0.0	1.7	0.0	1.7
Chloroquine	2012–2014	2	0.6	0.0	1.2	0.0	1.2
Dihydroartemisinin-piperaquine	2010–2016	8	0.0	0.0	3.3	0.0	0.0
Dihydroartemisinin-piperaquine + primaquine	2010–2010	1	0.0	0.0	0.0	0.0	0.0
China							
Chloroquine	2010–2015	8	0.4	0.0	2.4	0.0	1.6
Lao People's Democratic Republic							
Artesunate-mefloquine	2018–2019	1	0.0	0.0	0.0	0.0	0.0
Artesunate-pyronaridine	2018–2019	1	0.0	0.0	0.0	0.0	0.0
Malaysia							
Artesunate-mefloquine	2012–2014	1	0.0	0.0	0.0	0.0	0.0
Chloroquine	2012–2014	1	61.9	61.9	61.9	61.9	61.9
Papua New Guinea							
Artemether-lumefantrine	2011–2014	3	7.1	0.0	35.0	0.0	35.0
Dihydroartemisinin-piperaquine	2012–2014	2	0.0	0.0	0.0	0.0	0.0
Philippines							
Chloroquine	2010–2016	9	0.0	0.0	9.1	0.0	2.7
Solomon Islands							
Artemether-lumefantrine	2011–2013	2	18.4	5.1	31.6	5.1	31.6

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	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
Vanuatu							
Artemether-lumefantrine	2011–2013	2	6.7	1.2	12.1	1.2	12.1
Viet Nam							
Chloroquine	2010–2016	7	0.0	0.0	13.3	0.0	9.8
Dihydroartemisinin-piperaquine	2013–2014	1	1.7	1.7	1.7	1.7	1.7

Summary of treatment failure rates among patients infected with *P. ovale*, grouped by country and treatment, per WHO region (October 2020)*

	Study years	Number of studies	Median	Min	Max	Percentile 25	Percentile 75
WHO African Region							
Burkina Faso							
Artemether-lumefantrine	2011–2016	1	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2011–2016	1	0.0	0.0	0.0	0.0	0.0
Artesunate-pyronaridine	2011–2016	2	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2011–2016	2	0.0	0.0	0.0	0.0	0.0
Gabon							
Artemether-lumefantrine	2014–2016	1	3.7	3.7	3.7	3.7	3.7
Guinea							
Artesunate-amodiaquine	2011–2016	1	0.0	0.0	0.0	0.0	0.0
Artesunate-pyronaridine	2011–2016	1	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2011–2016	1	0.0	0.0	0.0	0.0	0.0
Mali							
Artemether-lumefantrine	2011–2016	3	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2011–2016	2	0.0	0.0	0.0	0.0	0.0
Artesunate-pyronaridine	2011–2016	4	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2011–2016	3	0.0	0.0	0.0	0.0	0.0

* All studies have a 28-day follow-up.

Summary of treatment failure rates among patients infected with *P. malariae*, grouped by country and treatment, per WHO region (October 2020)*

						Percentile	
	Study years	Number of studies	Median	Min	Max	25	75
WHO African Region							
Burkina Faso							
Artemether-lumefantrine	2011–2016	1	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2011–2016	1	0.0	0.0	0.0	0.0	0.0
Artesunate-pyronaridine	2011–2016	2	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2011–2016	2	0.0	0.0	0.0	0.0	0.0
Gabon							
Artemether-lumefantrine	2014–2016	1	0.0	0.0	0.0	0.0	0.0
Guinea							
Artesunate-amodiaquine	2011–2016	1	0.0	0.0	0.0	0.0	0.0
Artesunate-pyronaridine	2011–2016	1	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2011–2016	1	0.0	0.0	0.0	0.0	0.0
Mali							
Artemether-lumefantrine	2011–2016	3	0.0	0.0	0.0	0.0	0.0
Artesunate-amodiaquine	2011–2016	3	0.0	0.0	0.0	0.0	0.0
Artesunate-pyronaridine	2011–2016	4	0.0	0.0	0.0	0.0	0.0
Dihydroartemisinin-piperaquine	2011–2016	2	0.0	0.0	0.0	0.0	0.0

						Percentile	
	Study years	Number of studies	Median	Min	Max	25	75
WHO Western Pacific Region							
Malaysia							
Artemether-lumefantrine	2017–2018	2	0.0	0.0	0.0	0.0	0.0
Chloroquine	2014–2016	3	0.0	0.0	0.0	0.0	0.0

Summary of treatment failure rates among patients infected with *P. knowlesi*, grouped by country and treatment, per WHO region (October 2020)*

						Percentile	
	Study years	Number of studies	Median	Min	Max	25	75
WHO Western Pacific Region							
Malaysia							
Artemether-lumefantrine	2014–2018	3	0.0	0.0	0.0	0.0	0.0
Artesunate-mefloquine	2012–2014	1	0.0	0.0	0.0	0.0	0.0
Chloroquine	2012–2016	5	0.0	0.0	0.0	0.0	0.0

* All studies have a 28-day follow-up.

ANNEX 3: COUNTRIES BY WHO REGIONS AND INTER-COUNTRY SUPPORT TEAMS

WHO African Region

Inter-Country Support Team for Central Africa: Angola, Burundi, Cameroon, Central African Republic, Chad, Congo, Democratic Republic of the Congo, Equatorial Guinea, Gabon, Sao Tome and Principe.

Inter-Country Support Team for Eastern and Southern Africa: Botswana, Comoros, Eritrea, Eswatini, Ethiopia, Kenya, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, Namibia, Rwanda, Seychelles, South Africa, South Sudan, Uganda, United Republic of Tanzania, Zambia, Zimbabwe.

Inter-Country Support Team for West Africa: Algeria, Benin, Burkina Faso, Cape Verde, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone, Togo.

WHO Region of the Americas

Antigua and Barbuda, Argentina, Bahamas, Barbados, Belize, Bolivia (Plurinational State of), Brazil, Canada, Chile, Colombia, Costa Rica, Cuba, Dominica, Dominican Republic, Ecuador, El Salvador, Grenada, Guatemala, Guyana, Haiti, Honduras, Jamaica, Mexico, Nicaragua, Panama, Paraguay, Peru, Saint Kitts and Nevis, Saint Lucia, Saint Vincent and the Grenadines, Suriname, Trinidad and Tobago, United States of America, Uruguay, Venezuela (Bolivarian Republic of).

WHO South-East Asia Region

Bangladesh, Bhutan, Democratic People's Republic of Korea, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand, Timor-Leste.

WHO European Region

Albania, Andorra, Armenia, Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Malta, Monaco, Montenegro, Netherlands, Norway, Poland, Portugal, Republic of Moldova, Romania, Russian Federation, San Marino, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tajikistan, The former Yugoslav Republic of Macedonia, Turkey, Turkmenistan, Ukraine, United Kingdom of Great Britain and Northern Ireland, Uzbekistan.

WHO Eastern Mediterranean Region

Afghanistan, Bahrain, Djibouti, Egypt, Iran (Islamic Republic of), Iraq, Jordan, Kuwait, Lebanon, Libya, Morocco, Oman, Pakistan, Qatar, Saudi Arabia, Somalia, Sudan, Syrian Arab Republic, Tunisia, United Arab Emirates, Yemen.

WHO Western Pacific Region

Australia, Brunei Darussalam, Cambodia, China, Cook Islands, Fiji, Japan, Kiribati, Lao People's Democratic Republic, Malaysia, Marshall Islands, Micronesia (Federated States of), Mongolia, Nauru, New Zealand, Niue, Palau, Papua New Guinea, Philippines, Republic of Korea, Samoa, Singapore, Solomon Islands, Tonga, Tuvalu, Vanuatu, Viet Nam.

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