

Fighting drug-resistant *Plasmodium falciparum*: the challenge of artemisinin resistance

C. Wongsrichanalai¹ and C. H. Sibley²

1) Bangkok, Thailand and 2) Department of Genome Sciences, University of Washington School of Medicine, WorldWide Antimalarial Resistance Network, Seattle, WA, USA

Abstract

Following a decade-long scale up of malaria control through vector control interventions, the introduction of rapid diagnostic tests and highly efficacious Artemisinin-based Combination Therapy (ACT) along with other measures, global malaria incidence declined significantly. The recent development of artemisinin resistance on the Cambodia-Thailand border, however, is of great concern. This review encompasses the background of artemisinin resistance in *Plasmodium falciparum*, its situation, especially in the Greater Mekong Sub-region (GMS), and the responses taken to overcome this resistance. The difficulties in defining resistance are presented, particularly the necessity of measuring the clinical response to artemisinins using the slow parasite-clearance phenotype. Efforts to understand the molecular basis of artemisinin resistance and the search for molecular markers are reviewed. The markers, once identified, can be applied as an efficient tool for resistance surveillance. Despite the limitation of current surveillance methods, it is important to continue vigilance for artemisinin resistance. The therapeutic efficacy “in vivo study” network for monitoring antimalarial resistance in the GMS has been strengthened. GMS countries are working together in response to artemisinin resistance and aim to eliminate all *P. falciparum* parasites. These efforts are crucial since a resurgence of malaria due to drug and/or insecticide resistance, program cuts, lack of political support and donor fatigue could set back malaria control success in the sub-region and threaten malaria control and elimination if resistance spreads to other regions.

Keywords: ACTs, artemisinin resistance, day 3 parasitaemia, Mekong, molecular markers, *Plasmodium falciparum*

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Corresponding author: C. Wongsrichanalai, 130 Sub Street, Bangkok 10500, Thailand
E-mail: dr.chansuda@gmail.com

Introduction

Along with AIDS and tuberculosis, malaria remains one of the major killers, despite a decline in its incidence in recent years. It was estimated that, in 2010, there were 219 million malaria cases, and 660 000 deaths from malaria; *Plasmodium falciparum* accounted for 91% of the overall cases [1]. *P. falciparum* is the main cause of severe clinical manifestations and deaths. Chloroquine and sulphadoxine–pyrimethamine were the mainstays of antimalarial therapy for decades, but, by the 1990s, resistance to both drugs had spread to almost all *P. falciparum*-endemic areas worldwide, resulting in rising malaria-related morbidity and mortality, particularly in Africa. As a result, the treatment of *falciparum* malaria has become complicated,

and combination therapy is required, as it has been for tuberculosis and AIDS. Since 2001, the WHO has recommended artemisinin-based combination therapy (ACT), a combined regimen of artemisinin and a longer-acting partner drug, as the treatment of choice for *falciparum* malaria [2].

The recent emergence of *P. falciparum* strains with reduced susceptibility to artemisinins in Pailin, western Cambodia, near the south-eastern border of Thailand, raised the possibility that these valuable drugs might already be losing their usefulness. Even worse is the potential spread of resistance beyond the Greater Mekong Sub-region (GMS, consisting of Yunnan Province of China, Myanmar, Thailand, Laos, Cambodia, and Vietnam), or *de novo* development of such resistance elsewhere.

Historical Patterns of Evolution and Spread of Drug Resistance

P. falciparum resistance to chloroquine and sulphadoxine–pyrimethamine first developed on the Thailand–Cambodia border in the late 1950s and 1960s, respectively. The spread of resistant parasite strains elsewhere, including Africa, has been well documented retrospectively with molecular markers of the resistance to each drug [3–5]. After chloroquine and sulphadoxine–pyrimethamine failures, Thailand introduced mefloquine as the first-line drug for uncomplicated falciparum malaria. To protect the lifespan of mefloquine, Thailand imposed strict controls on its use. Nonetheless, mefloquine-resistant falciparum malaria outbreaks occurred during the late 1980s and early 1990s in association with the influx of migrants for gem mining in Pailin. In 1995, Thailand replaced mefloquine with artesunate–mefloquine. The same combination was the first-line therapy in Cambodia from 2000 to 2012.

Current Therapy for *P. falciparum* Infection

Several ACT regimens are pre-qualified by the WHO and are commercially available (<http://apps.who.int/prequal/query/ProductRegistry.aspx>). By 2010, >80 endemic countries had adopted ACT as the first-line therapy [1]. The antimalarials currently used by national control programmes in the GMS are listed in Table 1.

The Early Indications of Diminishing Efficacy of ACTs in the GMS

It was not possible to strictly control antimalarial drug use in Cambodia as had been done in Thailand, partly because of the less developed public health system and the sparse infrastructure in rural areas. The initial plan was also to make ACTs readily accessible in remote endemic areas, to rapidly reduce the morbidity and mortality caused by malaria. As a result, in Cambodia, artesunate, mefloquine and other ACTs were available in the private sector without prescription or parasitological diagnosis. Clinical diagnosis was commonly used in both public and private sectors. This situation has improved only in the past 4–5 years; in the decade prior to that, these were excellent conditions for the selection of artemisinin-resistant parasites.

The first hints that the efficacy of artemisinins might be compromised emerged from studies of patients treated with

TABLE 1. First line therapies against uncomplicated falciparum malaria adopted by the six GMS countries to replace earlier drugs (chloroquine, SP and mefloquine monotherapy)

Country	Regimen(s)	Year implemented
Cambodia	Dihydroartemisinin-Piperaquine, ^a country-wide except Pailin	2012–present
	Atovaquone-Proguanil, ^a in Pailin only	2012–present
China	Artesunate plus Mefloquine ^a	2000–2012
	Dihydroartemisinin-Piperaquine	
	Artesunate plus Amodiaquine	
Laos, PDR	Artesunate plus Naphthoquine ^b	
	Artesunate plus Piperaquine	
	Artemether-Lumefantrine	2005–present
Myanmar	Artemether-Lumefantrine	2008–present
	Artesunate plus Mefloquine	(with revision to add primaquine 45 mg ^c in 2011)
	Dihydroartemisinin-Piperaquine	1995–present
Thailand	Artesunate plus Mefloquine (plus primaquine 30 mg)	
	Atovaquone-Proguanil (plus primaquine 30 mg) on the southeastern border with Cambodia (Trat and Chanthaburi provinces) only.	2009–2013
	Artesunate monotherapy	
Vietnam	Dihydroartemisinin-Piperaquine (plus primaquine 0.5 mg base/kg) ^c	>20 years until 2009
		2009–present

^aWith primaquine 45 mg according to policy, but not yet implemented due to safety concern.

^bNot part of WHO guidelines on malaria case management.

^cIn practice, primaquine is not regularly prescribed.

artesunate–mefloquine in western Cambodia and south-eastern Thailand in the 2000s [6–8], but it was not clear which of the two drug components was responsible.

Artemisinin Resistance

Definition of resistance

The standard definition of antimalarial resistance has been based on an assessment of the fraction of patients in a clinical trial who fail treatment during a follow-up period of 28 days or more. There are good reasons for this: for the 'old' drugs, chloroquine and sulphadoxine–pyrimethamine, there were few other tools, so resistance was acknowledged only after treatment failures rose to levels high enough to dispel any doubts.

Determination of artemisinin resistance in an ACT trial involves both an artemisinin and its partner drug, and so distinguishing the effects of artemisinins from those of the partner drug is hampered by our dependence on a definitive clinical therapeutic outcome. Drugs in the artemisinin class are unique: they have a very short elimination half-life and the ability to clear parasites extremely quickly [9]. The only indicator of artemisinin action is the very rapid decline in parasite burden observed after treatment, so a delay in parasite clearance during the first few days after ACT treatment is considered to be suggestive of artemisinin resistance.

Parasite clearance as a measure of artemisinin efficacy

Delayed parasite clearance is the basis for the current WHO working definition of artemisinin resistance [10]. The proportion of patients who are still parasite-positive on day 3 after ACT treatment began in a trial with directly observed therapy and standardized, quantitative microscopy is a parameter currently used as an early signal of poor therapeutic response to an artemisinin. According to the definition, if $\geq 10\%$ of cases still have detectable *P. falciparum* parasites 72 h after initiation of ACT ('day 3 parasitaemia'-positive), artemisinin resistance is suspected. This requires verification by standardized measurement of the parasite clearance with oral artesunate in curative monotherapy to confirm/refute the presence of artemisinin resistance in that endemic area [10].

On the basis of this definition, the WHO has confirmed two foci of artemisinin resistance so far: the Cambodia–Thailand border area, and the northern part of the Thailand–Myanmar border [11–13].

Since 2010, the day 3 parasitaemia-'positive' or slow-clearance phenotype in association with different ACT regimens has been observed at additional sites: south-western Myanmar,

southern Vietnam, and the Thailand–Myanmar border [14,15] (Fig. 1). There has not been confirmation of artemisinin resistance at these sites. However, subsequent declines in artesunate–mefloquine efficacies are being observed in the Thailand–Myanmar border areas [14].

Additional observations in Suriname showed a worrying increase in the proportion of patients treated with artemether–lumefantrine who were day 3 parasitaemia-positive. An investigation is ongoing for clarification at this northern Amazon site, the first site reported outside of the GMS (Jitan *et al.*, 61st Annual Meeting of the American Society of Tropical Medicine and Hygiene, 2012, Abstract 1326).

The difficulties in measuring the response to artemisinins with the parasite-clearance phenotype are manifold. The actual clearance of parasites in each patient depends on the initial parasitaemia, the synchrony and stage of the majority of the parasites, and many host factors [16]. 'Parasite-clearance rate' is currently the primary tool used to standardize measurement of the resistance phenotype [17,18]. For research purposes, a precise assessment requires careful quantitative measurement of the parasitaemia every 6–8 h over a period that can be as



FIG. 1. Map of the Greater Mekong Sub-region showing *Plasmodium falciparum*-endemic sites with suspected (black dots) and confirmed (black stars) artemisinin resistance as of March 2013 (modified from Reference 14).

long as 3 or 4 days [19]. In any individual patient, a standardized approach has been proposed: the linear portion of the curve that defines the half-life of the parasite numbers as they decline [20]. This common parameter allows comparison of the clearance rate from different regions or over a span of time.

***In vitro* artemisinin susceptibility**

There is not yet a validated *in vitro* assay method that correlates parasite susceptibility of *P. falciparum* to artemisinins with ACT treatment outcomes or parasite-clearance time, although experimental selection of resistance by drug pressure in a rodent model has been attempted [21]. Scientists are exploring modified assays that target the artemisinin treatment to the very early ring stages of the development cycle. These may allow the design of accurate *in vitro* methods that can identify parasites with the slow-clearance phenotype [22,23]. Validation of these methods is currently in progress.

Molecular basis of resistance

As a basis for the molecular study of artemisinin resistance, the rate of parasite clearance following artemisinin therapy is the only defined phenotype that we can currently use. Despite the complexity of that trait, scientists have shown that, in both western Cambodia and western Thailand, the genes of the parasites exercise strong control over their response to artemisinins [13,24,25]. This insight made it worthwhile to try to identify the specific gene or genes that control the clearance phenotype.

The most straightforward way to define a marker of drug resistance is to demonstrate that a parasite is much less susceptible to the drug than expected in a laboratory-based test. One can then investigate the genetic changes that are found in those resistant parasites but are absent in closely related parasites that are still sensitive to the drug at the usual low level [26]. Unfortunately, a laboratory-based test that distinguishes a slow-clearing from a rapidly clearing parasite is at an early stage of development [22], so this approach is not yet an option.

A relatively complete reference genome has been available for *P. falciparum* for >10 years [27], and comparing the complete genomic sequences of a diverse group of parasites allows the definition of important tools for analysis [28–31]. Using these tools, one can identify regions of the genome that show signs of having been selected very recently in populations of parasites subjected to heavy use of artemisinin-based drugs [32]. Comparing such genomes with those of parasites that have not been under that selective pressure should identify candidate regions of the genome that contain genes responsible for the phenotype. When applied to parasites from the Thailand–Myanmar border, this approach identified a region on chromosome 13 that fits this definition [25].

An alternative approach involves comparison of the genomes of parasites that show the slow-clearing phenotype with those of closely related parasites that do not, to identify regions of the genome that are associated with the phenotype: a genome-wide association study. In contrast to the first approach, one needs to correlate the phenotype with the genotype in individual parasites. Using this method, Takala-Harrison *et al.* have recently identified three regions on chromosomes 10, 13 and 14 that show strong correlations with slow clearance in populations from the GMS [33]. Although chromosome 13 was identified in both types of genomic screen, these specific changes (single-nucleotide polymorphisms (SNPs)) on chromosome 13 do not overlap with the region described by Cheeseman *et al.* Even more recently, a study that focused on the population structure of four parasite groups that are all found in the area of Pailin, Cambodia revealed that the ‘artemisinin-resistant’ parasites constitute an unusual and genetically distinct subpopulation, another indication that genetic factors strongly influence the artemisinin resistance trait [34].

The identification of specific changes in genes correlated with the slow-clearing phenotype could greatly simplify widespread surveillance for artemisinin-resistant parasites. When these SNPs have been identified, they can be used to develop an assay performed simply by collecting a small spot of blood from an infected person on filter paper, and using basic molecular methods to determine whether or not the parasites in the sample carry the diagnostic SNP. For example, an early method for detecting chloroquine-resistant parasites was fundamental to understanding the changes in response to chloroquine use and their extent [5]. Simple genetic markers correlated with resistance to chloroquine and sulphadoxine–pyrimethamine also allowed low-cost surveillance of the geographical extent [35] and changes in the prevalence of resistance [36], and identification of the mechanisms that underlie the resistance phenotype [37].

The slow-clearance phenotype could arise in parasites elsewhere as a result of a different set of genetic changes, so the correlation of these SNPs is currently being tested in other parasite populations [33]. If the candidate SNP markers suggested by Takala-Harrison *et al.* or newly identified by others are validated, this could be a crucial step in tracking artemisinin resistance in Southeast Asia, or elsewhere, when there is suspicion of its emergence. Much earlier, *in vitro* studies in French Guiana reported that SNPs in the ATPase6 gene were associated with lower susceptibility to artemether *in vitro* [38], but those ATPase6 mutations could neither be confirmed in parasites from other regions [39] nor found to be associated with the slow-clearing parasites from nearby Suriname [40].

There have been suggestions that some 'traditional' resistance marker genes, *pfmdr1* and *pfmrpl*, may be involved in resistance to artemisinin, at least in a supportive role [41]. As the artemisinin is only one component of ACTs, it is important not to lose track of markers that may allow tracking of resistance to the partner drugs. There are indications that *pfmdr1* copy number and particular alleles of *pfmdr1* and *pfcr* are associated with reduced susceptibility to lumefantrine and amodiaquine, two of the partner drugs commonly deployed [42].

Resistance to Partner Drugs

The basis of the ACT design is the pairing of a short-lived artemisinin with another antimalarial drug having a different mode of action, and a much longer duration in the patient's body, the 'partner drug'. Therefore, ACT efficacy also depends on the partner drug. Continuing use of an ACT in an endemic community when there is significant resistance to the partner drug can be equivalent to exposing the parasites to artemisinin monotherapy. Routine monitoring of ACT therapeutic efficacies cannot distinguish whether diminishing efficacy is attributable to the artemisinin or partner drug component, or both.

This difficulty can be partly solved by testing *in vitro* the susceptibility of the partner drug of the ACT. The major partner drugs being used in Thailand, Cambodia and neighbouring countries are lumefantrine, mefloquine, and piperazine; all can be monitored with an *in vitro* assay [43,44]. Unfortunately, *in vitro* methods are technically and logistically demanding, so they are not consistently used for monitoring by malaria control programmes in the GMS.

Changes in the familiar loci, *pfcr* and *pfmdr1*, are implicated in resistance to mefloquine and lumefantrine. In Southeast Asia, parasites with increased copy numbers of *pfmdr1* have been shown to be more resistant to mefloquine [45–47], and the resistance is coupled with a particular SNP (I84F) in Cambodia [48]. However, in Africa, where mefloquine has been little used, copy number variation in this gene is rarely seen [49]. There is increasing evidence that the SNP K76 in *pfcr* and several codons in *pfmdr1* are associated with lower responsiveness to lumefantrine. Alleles of *pfmdr1* that carry the combination of codons 86N, I84F and I246D are most often implicated in lower responsiveness, both in Southeast Asia and in the East African region [42,50–57]. There is currently no information on mutations associated with resistance to piperazine, although its chemical similarity to chloroquine and other 4-aminoquinolines such as amodiaquine suggests that parasites with *pfcr* alleles containing the 76T codon and alleles of *pfmdr1* that carry the 86Y, I84Y and

I246Y combination would be expected to be more resistant to that class of drug. So far, molecular surveillance has not yet been adopted for routine monitoring of antimalarial drug resistance in the GMS.

Responses to Artemisinin Resistance

All foci of confirmed and suspected artemisinin resistance are currently in the GMS, with the exception of suspected sites in the northern Amazon (Suriname and nearby areas). Intensified malaria control in the Mekong was launched in 2008 to limit spread, with the eventual goal of eliminating *P. falciparum* on the Cambodia–Thailand border [58]. Updates of artemisinin resistance evidence and response activities can be found on the WHO Global Malaria Programme website (http://www.who.int/malaria/areas/drug_resistance/updates/en/index.htm).

The WHO recommends routine antimalarial resistance surveillance, and Mekong countries have been active in the monitoring of ACT efficacy by therapeutic trials through an established sub-regional network [14]. However, low *P. falciparum* incidence has complicated the enrolment of sufficient study participants to reach the required sample size. Application of molecular surveillance would be more practical as a supplemental/alternative tool once a marker of artemisinin resistance is identified. Africa has also been alerted to the threat of artemisinin resistance, and improved monitoring is being called for [59].

A number of other intensified control activities are needed, such as prompt diagnosis, effective treatment and gametocyte clearance, and vector control intervention. The ban on artemisinin monotherapy, including with law enforcement, in the Mekong is an example of an effort to reduce drug pressure in order to prevent the selection of artemisinin resistant parasites. Strategies targeting migrants and hard-to-reach populations, such as to improve their accessibility to rational therapy with ACTs, are important because of the potential of these populations to serve as reservoirs and spreaders of resistant parasites. The therapeutic and elimination strategies deserve more detailed discussion.

Therapeutic strategy—use of schizontocidal drugs

Eurartesim (Sigma-Tau, Pomezia, Italy), a fixed-dose dihydroartemisinin–piperazine combination, received European Medicines Agency clearance in 2011. Dihydroartemisinin–piperazine was adopted as the first-line therapy against both falciparum and vivax malaria for Cambodia in 2012, except in Pailin, where Malarone is the first-line drug for *P. falciparum*. Poor efficacy of dihydroartemisinin–piperazine has been observed in Pailin and its neighbouring province of Pursat

since 2009 [14]. The uncontrolled use of this ACT and piperaquine alone in the private sector in Cambodia over a decade before has been implicated.

A fixed-dose combination (FDC) of pyronaridine and artesunate, Pyramax (Shin Poong, Seoul, South Korea), was given a positive scientific opinion by the European Medicines Agency under Article 58 in 2012, and is being considered for recommendation by the WHO. The combination has the advantages of efficacy against both *P. falciparum* and *Plasmodium vivax*. It is considered to be safe in both adults and children (≥ 20 kg), but liver function monitoring is recommended [60,61]. It has not yet been adopted as the first-line antimalarial by any country in the GMS.

The artesunate–mefloquine combination used in the GMS has taken the form of individual tablets of each drug administered together. An FDC is now available under the trade name Mefliam Plus (Cipla, Mumbai, India), and is pre-qualified by the WHO [62]. The use of this FDC, which offers better compliance, may increase in some parts of the Mekong, such as Myanmar.

Malarone is a fixed-dose combination of two antimalarial agents, atovaquone and proguanil hydrochloride. It is a non-ACT indicated for malaria prophylaxis and the treatment of uncomplicated falciparum malaria. From 2009 to early 2013, Malarone was used for the first time on a large scale in south-eastern Thailand adjacent to Pailin, with the intention of temporarily reducing artemisinin drug pressure. Similarly, Cambodia decided to adopt Malarone in Pailin in 2012 after the dual failures of artesunate–mefloquine and dihydroartemisinin–piperaquine. However, high-level resistance to atovaquone is well documented *in vivo* and *in vitro* [63]. A 1000-fold decrease in susceptibility is caused by a single point mutation in the mitochondrial cytochrome *b* gene (Y268S) [64]. Resistance to cycloguanil, the active metabolite of proguanil, is conferred by specific changes in the dihydrofolate reductase gene (*dhfr*) (VI 6A, SI 08T, or II 64L), but its synergy with atovaquone is not thought to involve its antifolate specificity [65].

The limited availability of new drugs in the pipeline is a challenge in dealing with artemisinin-resistant malaria. As artemisinins are natural products, there have been serious efforts to produce synthetic endoperoxides. The first clinical product of this sort was OZ277 (Rbx I I 60 or arterolane) [66], an FDC of arterolane and piperaquine (Synriam; Ranbaxy, Gurgaon, Haryana, India). OZ439 is another synthetic endoperoxide undergoing clinical trials [67]. The development of these and other new drugs has been reviewed elsewhere [68,69].

Malaria elimination

Elimination of the artemisinin-resistant *P. falciparum* parasites, and eventually of malaria, is the ultimate goal of the coordi-

nated efforts to respond to artemisinin resistance in the Mekong. The intensified malaria control over the past 4–5 years has led to a very low malaria prevalence on the Cambodia–Thailand border.

In Pailin, Cambodia, there were 1474 malaria cases (62% *P. falciparum*) in 2009 and only 494 cases (118 or 24% *P. falciparum*) in 2012 (source: Cambodian National Malaria Control Programme). Across the border in Trat, Thailand, the number of cases for the whole province dropped from 310 (35% *P. falciparum*) to 92 cases in 2012. Only 12 of these cases (13%) were *P. falciparum* cases. In the adjacent province of Chanthaburi, there were 646 cases in 2009 (11% *P. falciparum*) and 152 cases in 2012, only six of these being *P. falciparum* cases (4%) (Source: Thai National Malaria Control Program).

At present, a combination of tools with which to target individual malaria cases for elimination is being considered. In the absence of an effective vaccine, antimalarial therapies that interrupt transmission or attack the gametocyte stage and surveillance measures to detect asymptomatic, low-parasitaemia cases are among the major issues to consider.

Gametocytocidal drugs. Declining artemisinin efficacy means that more parasites survive ACT treatment, enlarging the pool of parasites that produce gametocytes, and accelerating the spread of resistance. Primaquine has long been known to kill malaria gametocytes, and supplementing ACT with primaquine could substantially reduce transmission. The WHO now recommends this strategy in low-transmission countries such as those in the Mekong [2]. However, individuals who carry alleles of the glucose-6-phosphate dehydrogenase (G6PD) gene with diminished activity may suffer serious haemolysis when treated with primaquine. For this reason, the safety of the 45-mg single dose of primaquine previously recommended has been questioned in patients whose G6PD deficiency status is unknown [70]. In practice, adding primaquine to ACT is not regularly done in the Mekong. Recently, it was agreed that a 15-mg single dose of primaquine to supplement ACT would be sufficiently effective and safe, and this is the basis for the more recent WHO recommendation [71].

Nonetheless, a test for G6PD deficiency should be performed when possible to guide primaquine prescription. Rapid tests are available for point-of-care adoption, but the one product that has been cleared by the US Food and Drug Administration is not feasible for use under field conditions [72]. A product with a user-friendly design for field use, the CareStart G6PD deficiency screening test, still needs improvement in its accuracy, and is being subjected to further field clinical trials [73].

Currently, tafenoquine is the only other transmission-blocking drug that is still undergoing clinical trials. In terms of safety,

it is a primaquine analogue, and can therefore similarly induce haemolysis in G6PD-deficient individuals. Its advantage is its longer half-life, offering the possibility of administration as a single-dose therapy [68].

Highly sensitive diagnostic tools. Asymptomatic parasite carriers do not come to medical attention, and potentially form a reservoir for new epidemics. New diagnostic methods that can detect very low levels of parasitaemia are needed as a surveillance tool as countries move towards elimination. Designing PCR-based assays that are feasible for use in the field is one possibility [74,75].

Mass screening and treatment (MSAT). MSAT has been used for outbreak investigation and elimination. A smaller-scale, more manageable version of MSAT, focused screening and treatment with PCR support, was conducted in western Cambodia in 2010 [76]. It was found to be a potentially useful tool for identifying asymptomatic carriers of *P. falciparum* and providing valuable epidemiological information, although with a high price tag and logistical difficulties.

Mass drug administration (MDA). MDA is yet another strategy whereby everyone in a defined geographical area receives treatment, irrespective of the presence of symptoms and without malaria diagnosis. Currently, the WHO does not encourage the use of MDA, because of its short-lived impact on transmission and the likelihood that it will lead to selection of drug-resistant genotypes. However, given the urgent need for responses to artemisinin resistance, MDA was revisited as a potential measure to eliminate *P. falciparum* on the Cambodia–Thailand border [77]. Malarone–primaquine was considered as one of the drug choices for this purpose. However, the safety profile of Malarone and primaquine given in combination has not been studied. Treating a population with primaquine without screening for G6PD deficiency status also raises ethical concerns. Among the long list of other concerns are the unknown boundary of the existing distribution of the artemisinin resistant parasites and lack of a valid evaluation method with which to measure the success of MDA operations [77].

Conclusion

Over the past decade, the world has witnessed significant declines in the numbers of malaria cases and associated deaths. Improvements in case management, particularly with the introduction of rapid diagnostics tests and highly efficacious ACTs, the scale-up of vector control interventions and other

preventive strategies are among key factors that have contributed to this success. The recent emergence of artemisinin resistance and its potential spread, however, have threatened to reverse the accumulated gains.

Countries in the GMS are continuing their vigilance tracking of ACT therapeutic efficacy, and, for large areas in the GMS, the goal is to eliminate *P. falciparum*. The GMS therapeutic efficacy surveillance has been strengthened, and case management has been improved, along with other measures of malaria control. However, there are several limitations of the existing tools and strategies. *In vivo* therapeutic efficacy monitoring is becoming increasingly challenging, in part because of the success of malaria control, which has led to difficulties in enrolling an adequate number of study subjects. The potential application of molecular surveillance is being explored, and we have begun to understand artemisinin resistance mechanisms, but much more work is required to identify and validate molecular markers to serve as a surveillance tool.

As large areas in the Mekong are undergoing the transition from standard malaria control to preparation for elimination, control programmes need new, improved elimination tools and strategies. Research to develop such tools, including drugs, diagnostics, and surveillance strategies, is crucial. Globally, new categories of drugs as potent as artemisinins would be ideal as alternatives, now that artemisinin pressure is on the rise, especially in Africa.

Strong political support, cross-border collaboration, improved healthcare infrastructure, local economic development and a long-term funding commitment are among the important factors required to win our war against artemisinin resistance.

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Transparency Declaration

The authors declare that they have no conflict of interest.

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