Understanding artemisinin-resistant malaria: what a difference a year makes

Rick M. Fairhurst

Purpose of review
The emergence of artemisinin resistance in Southeast Asia (SEA), where artemisinin combination therapies (ACTs) are beginning to fail, threatens global endeavors to control and eliminate Plasmodium falciparum malaria. Future efforts to prevent the spread of this calamity to Africa will benefit from last year’s tremendous progress in understanding artemisinin resistance.

Recent findings
Multiple international collaborations have established that artemisinin resistance is associated with slow parasite clearance in patients, increased survival of early-ring-stage parasites in vitro, single-nucleotide polymorphisms (SNPs) in the parasite’s kelch protein gene (K13), parasite ‘founder’ populations sharing a genetic background of four additional SNPs, parasite transcriptional profiles reflecting an ‘unfolded protein response’ and decelerated parasite development, and elevated parasite phosphatidylinositol-3-kinase activity. In Western Cambodia, where the K13 C580Y mutation is approaching fixation, the frontline ACT is failing to cure nearly half of patients, likely due to partner drug resistance. In Africa, where dozens of K13 mutations have been detected at low frequency, there is no evidence yet of artemisinin resistance.

Summary
In SEA, clinical and epidemiological investigations are urgently needed to stop the further spread of artemisinin resistance, monitor the efficacy of ACTs where K13 mutations are prevalent, identify currently-available drug regimens that cure ACT failures, and rapidly advance new antimalarial compounds through preclinical studies and clinical trials.

Keywords
artemisinin, parasite kelch protein, malaria, Plasmodium falciparum, resistance

INTRODUCTION
For decades, Southeast Asia (SEA) has been ground zero for the evolution of drug-resistant Plasmodium falciparum malaria. After spawning generations of parasites resistant to chloroquine, sulfadoxine-pyrimethamine, quinine, and mefloquine, this region has now given rise to parasites resistant to artemisinins – the world’s frontline antimalarial drugs. These include artemisinin and its derivatives artesunate and artether, all of which are metabolized to the active compound dihydroartemisinin (DHA) in vivo. Parenteral artesunate has been highly efficacious in reducing malaria morbidity and mortality in SEA [1] and Africa [2]. Artemisinin combination therapies (ACTs) – oral coformulations of a potent, short-acting artemisinin and a less-potent, long-acting partner drug – have effectively reduced the world’s malaria burden, but now face the clear and present danger of artemisinin resistance [3]. This is because higher numbers of parasites that survive exposure to the artemisinin component are now exposed to the partner drug alone. This larger parasite biomass is, thus, more likely to develop partner-drug resistance, which is readily defined by a triad of findings following directly-observed treatment with a high-quality ACT: recrudescent parasitemia within 28–42 days (depending on the ACT), identified by expert microscopic examination of weekly blood smears; adequate partner drug exposure, confirmed by measurement of drug plasma concentrations on day 7; and decreased in-vitro susceptibility of recrudescent parasites to the
parasite clearance curve [6] using a ‘Parasite Clearance Estimator’ tool (http://www.wwarn.org/tools/resources/toolkit/analyse/parasite-clearance-estimator-or-pce) [7], and then calculating the parasite clearance half-life (in hours) from the slope of this line.

Artemisinin resistance was first reported from Pailin Province, Western Cambodia, as a slow parasite clearance rate in 2009 [8]. Since then, this clinical phenotype has been documented elsewhere in Cambodia [9,10], Vietnam [10,11,12], Thailand [10,13], Myanmar [10,14], and China [15]. Here, I review a recent series of clinical, epidemiological, genomics, and in-vitro studies that has rapidly transformed our understanding of artemisinin resistance in the human and parasite populations of these Southeast Asian countries.

WHAT IS THE DEFINITION OF ARTEMISININ RESISTANCE?

In Southeast Asian patients with uncomplicated *P. falciparum* malaria and a starting parasite count at least 10000 parasites per μl of blood, artemisinin resistance is defined as a parasite clearance half-life at least 5 h following treatment with artemesunate monotherapy or an ACT (http://www.who.int/malaria/publications/atoz/status_rep_artemisinin_resistance_sep2014.pdf?ua=1). This 5-h cutoff value reflects the upper limit of parasite clearance half-lives in areas without artemisinin-resistant parasites [10]. Importantly, parasite clearance half-lives in SEA are not significantly modified by age [13]; hemoglobin E, a polymorphism carried by 50% of Cambodians [9]; starting parasite density [9,13]; or relatively lower drug exposures, that is, parasite clearance half-lives were similar in patients randomized to receive 2 or 4 mg/kg artemesunate [10]. Although immunity likely plays a role in parasite clearance in SEA, this has not yet been adequately studied, largely because age is a poor surrogate of adaptive immunity and no in-vitro correlate of parasite-clearing immunity has been established in this region.

To investigate whether adaptive immunity accelerates parasite clearance, two recent studies were conducted in a Malian village where artemisinin resistance is absent and age-dependent reductions in both malaria incidence and parasite density were clearly demonstrated [16]. In the first study [17], parasite clearance half-lives decreased significantly as age increased, suggesting that age-dependent immunity is involved in clearing ring-infected RBCs within hours of artemesunate exposure. In the second study [18], younger children cleared their parasites mostly by pitting, suggesting they lacked immune responses that rapidly clear ring-infected RBCs, whereas older children cleared their parasites mostly...
by a nonpitting, artemisinin-independent mechanism, suggesting they possessed such immune responses. As parasite clearance half-lives are likely influenced by immunity in many areas of Africa, site-specific, age-stratified data are needed to define baseline cutoff values for suspected artemisinin resistance in the future. In areas of Africa where malaria is being eliminated, the progressive loss of immunity may cause a lengthening of parasite clearance half-lives over time, which would not necessarily herald emerging artemisinin resistance.

Artemisinin resistance in *P. falciparum* has also been defined as a parasite survival rate at least 1% in the ring-stage survival assay (RSA\(^{0–3h}\)) *in vitro* [19]. In this assay, clinical parasite isolates are adapted to culture, synchronized at the early-ring stage (0–3 h postinvasion of RBCs), exposed to a pharmacologically-relevant dose of DHA for 6 h, and then cultured for an additional 66 h. The percentage of parasites surviving DHA exposure is then calculated as the ratio of parasites surviving exposure to DHA versus those surviving exposure to dimethyl sulfoxide (the DHA solvent). This assay discriminates two groups of parasites, one with less than 1% survival and another with at least 1% survival, which are generally defined as artemisinin sensitive and artemisinin resistant, respectively [19,20,21,22]. Importantly, this assay is unable to discriminate these two groups of parasites at the mid-ring and late-ring stages [19], suggesting that artemisinin resistance is an early-ring-stage phenotype. This finding may account for some discrepancies between parasite clearance half-lives and parasite survival rates in the RSA\(^{0–3h}\) [19]. For example, parasite isolates that are artemisinin resistant in the RSA\(^{0–3h}\) may clear rapidly in patients if they are circulating as mid-to-late ring stages during the time that parasite clearance is measured.

**WHAT ARE THE GENETIC DETERMINANTS OF ARTEMISININ RESISTANCE?**

Initial genome-wide association studies of parasite clearance half-life implicated two regions of parasite chromosome 13 in artemisinin resistance [23,24], but the specific genetic determinant(s) remained elusive. In a parallel investigation, Ariey *et al.* [20] successfully induced artemisinin resistance in a Tanzanian parasite line by exposing it to increasing doses of artemisinin *in vitro*. By comparing the whole-genome sequences of drug-selected and unselected parasite lines, they identified a single-nucleotide polymorphism (SNP) in the *PF3D7_1343700* gene on chromosome 13, which encodes a M476I substitution in the propeller domain of a kelch protein. When compared with a known mammalian ortholog Keap1, the parasite kelch protein (‘K13’) consists of *Plasmodium* specific sequences, a bric-à-brac, tramtrack, broad-complex/poxvirus zinc fingers (BTB–POZ) domain, and a six-blade propeller domain (Fig. 1). Validation of K13-propeller polymorphism as a molecular marker of artemisinin resistance in Cambodia was achieved by showing that 17 different K13 mutations were present in parasites from this country (with each parasite clone carrying only one mutation); that the predominant CS80Y mutation had rapidly increased in prevalence in areas of Western Cambodia where artemisinin resistance had become common; and that the CS80Y, Y493H, and R539T mutations were associated with long parasite clearance half-lives and elevated RSA\(^{0–3h}\) survival rates.

Multiple groups have since made rapid progress in demonstrating K13-propeller polymorphism as a marker of artemisinin resistance elsewhere in SEA, including Vietnam, Thailand, Myanmar, and China by associating the same and additional K13-propeller mutations with slow parasite clearance [10,15,25,26]. Molecular surveillance studies have greatly expanded the map of K13-propeller polymorphism to include additional areas of Cambodia [27], Thailand [28], Myanmar [29,30], China [30,31], and Bangladesh [32]; some of these mutations have been previously associated with slow parasite clearance at other sites, but most have not and require validation. Currently, CS80Y predetermines in Cambodia [20,26,27,33,34] and along the Thailand–Myanmar border [25,26,29], whereas F446I predominates along the Myanmar–China border [15,29,31]. At present, it is unclear how CS80Y is approaching fixation in Western Cambodia given that this mutation does not seem to confer higher RSA\(^{0–3h}\) survival rates than other prevalent mutations (e.g., R539T and R543T) [20,21,22]. Multiple studies in Africa have detected dozens of K13-propeller mutations – many of which have not yet been observed in SEA – at very low frequency in 17 countries [35,36–40] (http://biorxiv.org/content/early/2015/05/22/019737). Whether these K13-propeller mutations cause artemisinin resistance in patients and *in vitro* also awaits further investigation. Table 1 [10,15,20,21,22,25,26,27,28,29,30–32,34,35,36–40] (http://biorxiv.org/content/early/2015/05/22/019737) lists all K13-propeller mutations discovered to date, according to their geographic location and association with artemisinin resistance.

In population genetics studies of artemisinin resistance, several surprising and unprecedented findings were made [41,42]. First, multiple parasite ‘founder’ populations were identified in Cambodia and Vietnam. These groups of highly-differentiated, clonal subpopulations are as different from each other as each of them is to African parasites, suggesting they have undergone extreme recent bottlenecking and subsequent expansion. Second, seven of the 11
founders were found to be artemisinin resistant in patients [41,42], and three of them were additionally confirmed to be artemisinin resistant in the RSA 0–3 h [21], suggesting that most of them were naturally selected by artemisinins. Third, each founder was tagged by a single K13-propeller mutation, with the C580Y mutation independently emerging on three different founders in Cambodia. Fourth, all seven artemisinin-resistant founders share a common genetic background comprised of four SNPs in genes encoding apicoplast ribosomal protein s10 (arp10 V127 M), ferredoxin (filD193Y), multidrug resistance 2 transporter (mdr2 T484I), and chloroquine resistance transporter (crt N326S) [42]. The roles of these mutations in the natural selection of these founders are unknown, but are likely to include increases in fitness. Some possibilities are that they compensate for putative deleterious effects of K13-propeller mutations; potentiate resistance to artemisinins; mediate resistance to previously used drugs (i.e., chloroquine, sulfadoxine-pyrimethamine, quinine, and doxycycline) or currently used ACT partner drugs (i.e., mefloquine, piperaquine, and lumefantrine); or increase parasite transmission to Anopheles mosquito vectors.

**WHAT IS THE MOLECULAR MECHANISM OF ARTEMISININ RESISTANCE?**

As mammalian kelch proteins can detect oxidants and other stressors, K13-propeller mutations were reasonably implicated in mediating resistance to artemisinin [20], a prooxidant drug. In one hypothetical model of artemisinin sensitivity and resistance (Fig. 2a), wildtype K13 binds a putative transcription factor and delivers it to ubiquitin ligase, which targets it for proteosomal degradation. When wildtype K13 senses oxidants like artemisinins, it undergoes a conformational change to liberate the transcription factor, which then upregulates the expression of genes involved in counteracting oxidative damage. In this model, the response of wildtype parasites is believed to be too little too late, such that the action of artemisinins is too potent and too rapid for parasites to successfully overcome. In artemisinin-resistant parasites, on the contrary, K13-propeller mutations destabilize the K13-transcription factor interaction, leading to constitutive activation of transcriptional changes that ‘prime’ the parasite to withstand oxidative damage caused by artemisinins.

Given the logical assumption that K13-propeller mutations mediate artemisinin resistance, Straimer et al. [22] tested this hypothesis directly by using zing-finger nuclease technology to edit the K13 locus in contemporary Cambodian parasite isolates. When three different K13-propeller mutations (CS80Y, RS39T, and IS43T) were edited to the wildtype sequence, the artemisinin-resistance phenotype – as measured in the RSA 0–3 h – was completely lost. They also showed that the introduction of five
different K13-propeller mutations confer increasing levels of resistance to the Indochinese Dd2 parasite line (Y493H<C580Y<M476I<R539T<I543T), and that introduction of the C580Y mutation confers higher levels of resistance to contemporary parasite isolates from Cambodia than to older parasite lines from Indochina. These data provide compelling evidence that different K13-propeller mutations mediate different levels of artemisinin resistance, and that the level of resistance can be influenced by parasite genetic background. Evidence that C580Y confers artemisinin resistance to the African NF54 parasite line has also been reported [43].

Although these studies established that K13 mutations confer artemisinin resistance to a variety of parasite clinical isolates and laboratory lines, additional studies were needed to further define the molecular mechanism. In a large population

---

Table 1. K13-propeller mutations, according to propeller blade number, geographic location, and artemisinin-resistance association

<table>
<thead>
<tr>
<th>Propeller Blade</th>
<th>Mutation</th>
<th>Location</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P441L</td>
<td>F442</td>
<td>P443S</td>
</tr>
<tr>
<td></td>
<td>G449D</td>
<td>G450</td>
<td>F451</td>
</tr>
<tr>
<td></td>
<td>N458I</td>
<td>S459L</td>
<td>M460</td>
</tr>
<tr>
<td></td>
<td>Q468</td>
<td>C469Y</td>
<td>C469F</td>
</tr>
<tr>
<td>2</td>
<td>P475</td>
<td>M476I</td>
<td>S477</td>
</tr>
<tr>
<td></td>
<td>S485N</td>
<td>A486</td>
<td>V487I</td>
</tr>
<tr>
<td></td>
<td>F495L</td>
<td>G496F</td>
<td>G497</td>
</tr>
<tr>
<td></td>
<td>L505</td>
<td>F506</td>
<td>E507</td>
</tr>
<tr>
<td></td>
<td>R515T</td>
<td>D516Y</td>
<td>V517</td>
</tr>
<tr>
<td></td>
<td>L524</td>
<td>N525D</td>
<td>S526</td>
</tr>
<tr>
<td></td>
<td>T535</td>
<td>S536</td>
<td>N537I</td>
</tr>
<tr>
<td></td>
<td>G545E</td>
<td>Y546</td>
<td>S547</td>
</tr>
<tr>
<td></td>
<td>V555</td>
<td>E556D</td>
<td>A557S</td>
</tr>
<tr>
<td></td>
<td>A564</td>
<td>W565</td>
<td>V566I</td>
</tr>
<tr>
<td></td>
<td>R574L</td>
<td>R575K</td>
<td>S575G</td>
</tr>
<tr>
<td></td>
<td>F583</td>
<td>T583I</td>
<td>N584</td>
</tr>
<tr>
<td></td>
<td>G592</td>
<td>T593S</td>
<td>N594</td>
</tr>
<tr>
<td></td>
<td>E602</td>
<td>V603</td>
<td>Y604</td>
</tr>
<tr>
<td></td>
<td>W666</td>
<td>Q667A</td>
<td>P667L</td>
</tr>
<tr>
<td></td>
<td>A675Y</td>
<td>A676S</td>
<td>A676D</td>
</tr>
<tr>
<td></td>
<td>I684</td>
<td>T685</td>
<td>G686</td>
</tr>
<tr>
<td></td>
<td>N694</td>
<td>S695</td>
<td>C696</td>
</tr>
<tr>
<td></td>
<td>N704</td>
<td>E705</td>
<td>W706</td>
</tr>
<tr>
<td></td>
<td>V714</td>
<td>P715</td>
<td>R716</td>
</tr>
<tr>
<td></td>
<td>A724</td>
<td>N725</td>
<td>I726</td>
</tr>
</tbody>
</table>
A transcriptomics study of *P. falciparum* isolates obtained directly from Southeast Asian patients with malaria [44], Mok et al. first identified a subset of parasites that was collected at the early-ring stage of development, that is, when the artemisinin-resistance phenotype is expressed. In analyzing the transcriptional profiles of these isolates against a wide range of corresponding half-lives, these investigators found that artemisinin resistance is associated with increased expression of an ‘unfolded protein response’ pathway involving two major chaperone complexes: *Plasmodium* reactive oxidative stress complex (PROSC) and TCP-1 ring complex (TRiC). Artemisinin-resistant clinical isolates, on the contrary, mutant K13 fails to bind PI3K, leading to increased PI3K activity and phosphatidylinositol-3-phosphate (PI3P) levels. In this ‘protected’ state, high PI3P levels are presumably able to promote the survival of parasites exposed to artemisinins, for example, my mediating membrane fusion events involved in parasite growth. Adapted from [47**].

**FIGURE 2.** Two proposed mechanisms of artemisinin sensitivity and resistance in *Plasmodium falciparum*. (a) In artemisinin-sensitive parasites, wildtype *Plasmodium falciparum* kelch13 protein (K13) (green) binds a putative transcription factor and targets it for degradation. In artemisinin-resistant parasites, on the contrary, mutant K13 (red) fails to bind this putative transcription factor, which is then free to upregulate genes involved in the antioxidant response. In this ‘protected’ state, parasites are better prepared to handle the oxidative stress imposed by activated artemisinins, for example, by refolding oxidatively-damaged proteins. (b) In artemisinin-sensitive parasites, wildtype K13 binds phosphatidylinositol-3-kinase (PI3K) and targets it for degradation. In artemisinin-resistant parasites, the contrary, mutant K13 fails to bind PI3K, leading to increased PI3K activity and phosphatidylinositol-3-phosphate (PI3P) levels. In this ‘protected’ state, high PI3P levels are presumably able to promote the survival of parasites exposed to artemisinins, for example, my mediating membrane fusion events involved in parasite growth. Adapted from [47**].

Evidence that artemisinins target the sole *P. falciparum* phosphatidylinositol-3-kinase (PI3K), and that PI3K is the putative binding partner of K13. In their model of artemisinin sensitivity (Fig. 2b), wildtype K13 binds PI3K and delivers it to ubiquitin ligase, which polyubiquitinates K13 and marks it for proteosomal degradation. As these parasites have low basal levels of phosphatidylinositol-3-phosphate (PI3P), the product of PI3K activity, they are highly sensitive to artemisinins, which inhibit PI3K and, thus, prevent the increase in PI3P levels that is presumably needed for parasite growth (PI3P levels normally increase as parasites develop from rings to schizonts). In their corresponding model of artemisinin resistance, mutant K13 fails to bind PI3K. PI3K, thus, avoids being degraded, resulting in high basal levels of PI3K and its product PI3P. As resistant parasites already have high basal levels of PI3P when exposed to artemisinin, they can better withstand the PI3K-inhibiting effects of this drug and, thus, continue their PI3P-dependent growth. How elevated levels of PI3P might mediate artemisinin resistance is not known, but one possibility is that PI3P is involved in membrane biogenesis and fusion events required for parasite growth. Future work is needed to integrate these findings with those of the aforementioned population transcriptomics study [44**], which found no association between PI3K transcript levels and either parasite.
clearance half-lives or K13-propeller mutations, and to reconcile two very disparate artemisinin modes of action: namely, nonspecific oxidation of multiple parasite proteins versus specific inhibition of PI3K.

WHAT IS THE CLINICAL IMPACT OF ARTEMISININ RESISTANCE?

It is important to emphasize that ACTs still cure patients with slow parasite clearance, provided that the partner drug remains effective. However, slow parasite clearance in ACT-treated patients causes more parasites to be exposed to partner drugs alone, increasing their chance of developing resistance to these drugs and causing ACT failures. As predicted, DHA-piperaquine is now failing to cure malaria in Western Cambodia, where artemisinin resistance is most entrenched. Compared with earlier studies that documented 98% DHA-piperaquine efficacy, three recent studies have reported reduced efficacy in this region. In the first study [48], efficacy in Pailin and Pursat Provinces was 75 and 89% in 2008–2010. In the second study [33], in which data were pooled by region, efficacy was 85% in four Western Cambodian provinces (where artemisinin resistance is common) compared with 98% in four eastern Cambodian provinces (where artemisinin resistance is uncommon) in 2011–2013. In this study, the most significant risk factor for treatment failure was the presence of a resistance-associated K13-propeller mutation. In the third study [34], efficacy in Oddar Meanchey Province was 46% in 2012–2014. In this study, a significant risk factor for treatment failure was the presence of the K13 C580Y mutation and two other SNPs on chromosomes 10 and 13 that were previously associated with slow parasite clearance [23]. Surprisingly, all three studies were unable to associate treatment failures with elevated piperaquine IC\textsubscript{50} values \textit{in vitro}. As high ACT failure rates in SEA have only been observed in areas where resistance to the partner drug exists, it is likely that piperaquine resistance has indeed emerged. Although this possibility is further suggested by increasing piperaquine IC\textsubscript{50} values within multiple study sites over time (unpublished data; [49]), more robust evidence of piperaquine resistance is needed to identify its genetic markers, elucidate its molecular mechanism, and discover new drugs that circumvent it. Meanwhile, artesunate-mefloquine may be an effective treatment for DHA-piperaquine failures, as suggested by a contemporaneous reduction in mefloquine IC\textsubscript{50} values and disappearance of the multicopy \textit{pfmdr1} genotype – a molecular marker of mefloquine resistance [33,49,50].

CONCLUSION

The aggressive global use of ACTs was expected to weaken malaria’s stranglehold on the health and economies of the world’s most impoverished communities. Unfortunately, the eventual spread of artemisinin resistance from SEA, where ACTs have begun to fail, to Africa, where the world’s greatest malaria transmission, morbidity, and mortality occur, seems likely. Multiple international collaborations have defined in-vivo and in-vitro correlates of artemisinin resistance, identified its causal genetic determinant, begun to elucidate its molecular mechanism, and assessed its clinical impact. These collaborative efforts should now be extended to monitor ACT efficacy in areas where K13-propeller mutations are prevalent, test whether currently available drugs cure ACT failures, and advance newly-developed antimalarial compounds into clinical trials.

Acknowledgements

I thank Socheat Duong, Arjen Dondorp, Nick White, Nick Day, Joel Tarning, Olivo Miotto, Dominic Kwiatkowski, Frédéric Ariey, Didier Menard, David Fidock, Zbynek Bozdech, Mahamadou Diakité, Pierre Buffet, and Michael Fay for many years of transparent, productive, and enjoyable collaborations; and Chanaki Amaratunga, Seila Suon, Sokunthea Sreng, Pharakth Lim, Tatiana Loperas-Mesa, Jennifer Anderson, Dick Sakai, Robert Gwadz, and Thomas Wellemes for their efforts in supporting our field studies in Cambodia and Mali.

Financial support and sponsorship

I am funded by the Intramural Research Program of the NIAID, NIH.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

Tropical and travel-associated diseases


This study showed that some K13-propeller mutations (e.g., C580Y) have independently emerged in different parasite populations in different regions of SEA, and that parasites do not seem to be spreading from Myanmar to these findings. Importantly, these results have important implications for the containment of artemisinin resistance in SEA, in that they increase the focus on preventing the local emergence of resistant parasites.

This study showed that some K13-propeller mutations (e.g., C580Y) have independently emerged in different parasite populations in different regions of SEA, and that parasites do not seem to be spreading from Myanmar to these findings. Importantly, these results have important implications for the containment of artemisinin resistance in SEA, in that they increase the focus on preventing the local emergence of resistant parasites.
42. Miotto O, Amato R, Ashley EA, et al. Genetic architecture of artemisinin-resistant Plasmodium falciparum. Nat Genet 2015; 47:226–234. This study associates artemisinin-resistant founder populations with the presence of K13-propeller polymorphism and a particular genetic background comprised of four SNPs in genes encoding apicoplast ribosomal protein s10 (arpS10 V127M), ferredoxin (df D193Y), multidrug resistance 2 transporter (mdr2 T484I), and chloroquine resistance transporter (crt N326S). This finding, which was observed both in the Greater Mekong Subregion and along the Thailand–Myanmar border, strongly suggests that K13-propeller mutations by themselves are not naturally selected in the human and mosquito populations of SEA. Instead, it seems that additional SNPs are needed to increase fitness in these geographical settings. The finding that this genetic background is not seen in Africa suggests that the naturally-occurring K13-propeller mutations observed on this continent cannot yet give rise to artemisinin-resistant subpopulations. The genetic background of four SNPs may, therefore, be considered a molecular marker for identifying areas most at risk of artemisinin resistance in the future.


44. Mok S, Ashley EA, Ferreira PE, et al. Drug resistance. Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science 2015; 347:431–435. This study reports the largest ex-vivo transcriptomics study of any infectious disease. By analyzing the transcriptional profiles of more than 1000 P. falciparum clinical isolates, this study identified parasites at the early-ring stage of parasite development that expressed a transcriptional profile associated with long parasite clearance half-lives and K13-propeller mutations. This profile indicates that parasites, before they have encountered artemisinin in the patient, have already decelerated their growth and upregulated their ‘unfolded protein response’ pathway. Together, these findings suggest that parasites have evolved to anticipate artemisinin exposure, and are, thus, better able to first repair oxidative protein damage before progressing through their lifecycle. The identification of two upregulated chaperone complexes (PROSC and TRiC), with multiple molecular components, has opened up myriad new avenues for future investigation.


47. Mbelengu A, Bhattacharjee S, Pandharkar T, et al. A molecular mechanism of artemisinin resistance in Plasmodium falciparum malaria. Nature 2015; 520:683–687. This study reports a novel candidate mechanism of artemisinin action and resistance. Artemisinins are proposed to target P. falciparum PI3K, leading to decreased PI3K activity, decreased PI3P levels, and inhibition of parasite growth. To overcome this effect, artemisinin-resistant parasites have evolved mutant K13-propeller proteins that fail to bind PI3K and to mark it for degradation. The resultant increases in PI3K activity and PI3P levels enable artemisinin-exposed parasites to continue their intraerythrocytic development until drug levels decrease and PI3K activity is restored. Future studies are needed to reconcile these findings with those of a recent population transcriptomics study showing that the PI3K transcript levels of artemisinin-sensitive and artemisinin-resistant parasites are not significantly different, and the widely-held belief that artemisinins act through an oxidation mechanism.

