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Ring stage dormancy of *Plasmodium falciparum* tolerant to artemisinin and its analogues – A genetically regulated "Sleeping Beauty"

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ABSTRACT

The appearance in 2008 in western Cambodia of *Plasmodium falciparum* tolerant to artemisinin, defined by longer parasite clearance time following drug administration and *in vitro* by a slightly higher survival rate of the ring stage after a 3-h treatment with 700 nM artemisinin (or analogues, collectively termed ART), has raised concerns of the possible loss of this frontline antimalarial [used in the form of an artemisinin combination therapy (ACT)], with its low IC_{50} value against the ring stage and pleiotropic pro-drug/poison property. The key genetic marker of ART tolerance phenotype is a number of non-synonymous mutations in *Pfkelch13* propeller domain. This results in defective assembly at the ring stage of a cytostome structure located at cytoplasmic side of the parasite membrane required for invagination of a double-membrane endosome carrying host cytosol haemoglobin to the digestive vacuole. The consequential deprivation of amino acids initiates ring stage parasites bearing the causal mutations in *Pf*K13 (or other key cytostome components) entry into a dormant state ("Sleeping Beauty"), which, after a duration longer than that the short-lived ART, "Sleeping Beauty" ring parasite resumes its normal, but accelerated, development to maintain the 48-h intra-erythrocytic life-cycle. We posit that when ART-tolerant *P. falciparum* has acquired under ART stress the causative *Pf*K13 mutation (not obligatory if mutations occur in other critical cytostome components), together with other necessary mutations to adjust to the new normalcy and to provide survival competitiveness, ART-tolerant parasite has now evolved into a genetically programmed "Sleeping Beauty". The onus of preventing the spread of ART-tolerant *P. falciparum* lies with the efficacy of ACT partner drug, hence the recommendation of a triple ACT (TACT). Nevertheless, attention should also be focussed on understanding the mechanisms of dormancy, such as induction, maintenance and recovery, to enable discovery and development of novel antimalarials targeting this unique parasite stage.

Since the discovery by Yuyu Tu and her colleagues in 1972 of the novel antimalarial artemisinin (qinghaosu), a sesquiterpene trioxane lactone whose endoperoxide bridge is essential for antimalarial activity (Tu, 2011), artemisinin and its analogues (collectively named ART) in combination with another antimalarial [with longer drug clearance time than that of ART (~5 h)] (ACT) has been the first-line chemotherapy against malaria in Southeast Asia, where *Plasmodium falciparum* resistance had developed to nearly all other antimalarials used as monotherapy including the antifolate combination sulfadoxine/pyrimethamine (Fansidar) (Blasco et al., 2017). However, the first report in 2008 of ACT-treated *falciparum* malaria cases in western Cambodia having longer parasite clearance times raised concern that *P. falciparum* has become "resistant" to ART (Noedl et al., 2008).

The early debate whether this phenomenon is due to "resistance" or "recrudescence" (Ferreira et al., 2013; Wellems et al., 2020) has largely been resolved with the realisation that in the field there exists a large preponderance of *P. falciparum* sensitive to ART, some of which have recrudescence properties but with a smaller proportion that have "resistance" phenotype. This small "ART-resistant" population will become predominant in regions where there is resistance to the ACT partner drug. This ART "resistant" *P. falciparum* is now characterized phenotypically as having in a treated patient a median clearance half-time of >5.5 h (WWARN K13 Genotype-Phenotype Study Group, 2019) and in culture a significantly higher survival rate of the ring stage after a short treatment (0–3 h) with 700 nM ART compared to control parasites [known as a ring survival assay ($RSA^{0-3\ h}$)] (Witkowski et al., 2013). In this article, the term "ART tolerance" will be used since the

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term "ART resistance" is a misnomer given ART IC_{50} values of the tolerant ring stage parasites are (for the present) only <10 folds higher than wild-type parasites.

An early genome-wide association study of ART-tolerant *P. falciparum* from Cambodia revealed an association with single non-synonymous nucleotide variants in *Pfkelch13* (*Pf3D7_1343700*), with *Pf*kelch13 (*Pf*K13)C580Y variant being predominant followed by Y493H and R539T, remarkably all located in the "blade" sequences of the C-terminal propeller (kelch) domain (Ariey et al., 2014). A meta-analysis of individual patients globally revealed almost 200 different variant *Pf*K13 proteins, the majority being present at low prevalence and have unknown function, but in Asia some 20 different *Pf*K13 propeller variants associated with ART-tolerant phenotype have been confirmed, with *Pf*K13Y580 being the most prevalent (WWARN K13 Genotype-Phenotype Study Group, 2019; Kagoro et al., 2022).

Although there was speculation that *Pf*K13 mutations protect ring-stage parasites from ART-related oxidative stress similar to transcriptional responses regulated by the mammalian ortholog Keap1 (Straimer et al., 2015), in a seminal study Birnbaum et al. (2020) demonstrated localisation of other K13-colocalizing factors to the cytostome, overall endocytic pathway activity, and the K13 interactome. This led raised awareness that defective formation of the cytostome in ring stage ART-tolerant *P. falciparum* carrying the causative *Pf*K13 mutation produces reduced formation of the double-membrane endocytic vesicles bearing red cell cytosolic haemoglobin to the parasite digestive food vacuole. The association of *Pf*K13 with other parasite proteins to form the cytostome and the disturbance to this structure wrought by *Pf*K13 variants in the propeller domain has recently been reviewed (Behrens et al., 2021). It is worth noting that *Pf*K13 is apparently involved only in parasite ring stage cytostome formation (Sutherland et al., 2021), emphasizing its key importance in the evolution of ART-tolerant parasites, but other pathways can be anticipated (Wilairat et al., 2016). Ring stage parasites are the most sensitive to ART compared to the more mature forms (trophozoites and schizonts) (Kümpornsin et al., 2021; Auparakkitanon et al., 2022), and so it is not surprising that ART tolerance phenotype would have evolved at this parasite stage and involving factors associated with the formation of ring-stage-specific cytostome, such as *Pf*K13 and other co-acting proteins in the cytostome collar complex (Sutherland et al., 2021); mutations in components also involved in cytostome formation of the mature stages would be self-defeating.

As with other higher eukaryotes, response to stress such as food deprivation initially results in activation of initiation factor eIF2α kinase (which in turn requires phosphorylation by specific stress-responsive eIF2α kinase or through autophosphorylation), resulting in inhibition of GDP-eIF2α complex exchange with GTP (eIF2β-bound), thereby preventing further protein biosynthesis (Cao et al., 2019). Prevention of non-ribosome-bound mRNAs from degradation by RNases is achieved by accumulating these untranslated transcripts into so-called stress granules. At the same time, gene expression enters into a stress-induced transcriptional attenuation, involving inhibition of transcription of a multitude of genes and up-regulation of a few stress-response genes to ameliorate damages and also in preparation for return to normalcy once the crisis has passed (Sawarkar, 2022). This is reminiscent of the dormancy state observed with ring-stage ART-tolerant parasites in face of ART exposure *in vitro*, in which ring stage parasites of several *Plasmodium falciparum* lines were exposed to different doses of dihydroartemisinin (DHA), producing an abrupt arrest in parasite development after a single exposure to DHA, followed by recovery over a period of several days (Teuscher et al., 2010). Direct evidence of ART-induced dormancy was demonstrated by observation in culture of dormant parasites that were obtained from blood samples of *P. falciparum* 3D7-or K13-infected participants 48–72 h after single-dose artesunate (AS) treatment (Peatey et al., 2021). The molecular signature of dormancy, an up-regulation of acetyl CoA carboxylase, is detected in 3D7 and K13 samples post-AS but not pre-AS treatment (Chen et al., 2014; Peatey et al., 2021).

The following discussion is focused only on *P. falciparum* carrying *Pf*K13 mutations causally linked to ART tolerance phenotype, albeit some normal parasite lines upon ART stress can exhibit a number of the described properties, e.g., prolonged ring stage duration. Thus, it could be argued that ring stage dormancy does not require mutations in genes linked to *P. falciparum* ART tolerance, but this ART-induced ring dormancy cannot ensure continuing persistence and robustness of ART-tolerant parasites in the field (see discussion below).

P. falciparum contains three *Pf*eIF2α kinases, namely, eIK1, eIK2 and PK4, among which active PK4 phosphorylates *Pf*eIF2α that is critical for ART-tolerant ring-stage dormancy and importantly, PK4 inhibitor GSK2606414 restores ART sensitivity of *P. falciparum* Dd2C580Y; activation of PK4 is by means of autophosphorylation (Zhang et al., 2017). A study employing liquid chromatography-mass spectrometry-based proteomics, peptidomics and metabolomics revealed ART-tolerant *P. falciparum* carrying causal *Pf*K13 mutations [Cam3.IIR539T, Cam3.IIC580Y (isogenic derivative of Cam3.IIR539T) and PL7R539T (field isolate from western Cambodia)] contained lower abundance of mutant *Pf*K13 and several endogenous peptides derived from haemoglobin, as well as accumulation of glutathione and its precursor γ-glutamylcysteine compared to wild type parasites (Siddiqui et al., 2017). A more detailed study examining differences in intra-erythrocytic development, transcriptomics, proteomics, and metabolomics of *Pfk13*-edited isogenic parasites on two different genetic backgrounds (Dd2 and Cam3.II) between untreated rings and those pulsed 3–6 h with nM DHA revealed a delay in DHA-treated ring-stage development apparent in both parasite lines carrying *Pf*K13C580Y and R539T mutations, but subsequent development is accelerated in both mutant parasites to maintain the normal 48-h cycle, indicating a role of additional survival (repair) mechanisms (Mok et al., 2021). Of 667 genes examined, 400 genes are differentially expressed across the 48-h cycle in Dd2R539T and Dd2C580Y mutant parasites compared to isogenic Dd2WT. Notably, differentially expressed gene changes during the first 6 h of DHA treatment are qualitatively more similar between Dd2WT and Dd2Y580 than Dd2T539. Presence of *Pf*K13 mutants affects unfolded protein response, protein degradation, vesicular trafficking, and mitochondrial metabolism. Interestingly, mutant *Pf*K13-mediated tolerance to DHA in Cam3.II line is abrogated by treatment with atovaquone, a mitochondrial electron transport chain inhibitor (but this phenomenon may only apply to this particular parasite line) (Mok et al., 2021).

A more recent transcriptome-wide association analysis conducted on 459 *P. falciparum* isolates collected in the Greater Mekong Subregion between 2016 and 2018 at 0 and 6 h post-ACT treatment of patients, shows a high prevalence of *Pf*K13 variants causally linked to ART tolerance phenotype with a specific ART-tolerant associated transcriptional profile that involves a broad but discrete set of biological functions related to proteotoxic stress, host cytoplasm remodelling and REDOX metabolism (Zhu et al., 2022). The authors concluded the ART tolerance associated transcriptional profile presumably evolves from initial transcriptional responses of susceptible parasites to artemisinin, but the genetic basis for this adapted response is likely to be complex. It is worth noting a report of changes in mitochondrial morphology and of reduced mito-nuclear distances and metabolic consequences both in parasites carrying *Pf*K13WT as well as C580Y mutations that survived a short nM DHA exposure *in vitro* at the ring stage ("persisters") (Connelly et al., 2021). Further evolution of such *P. falciparum* K13Y580 (and similar variants) might favour trajectories ultimately resulting in ART-tolerant parasites.

Taken altogether, we posit that once sufficient mutations have evolved under ART stress to produce ART-tolerant *P. falciparum* with its concomitant ring-stage dormancy and competitive fitness, these field ART-tolerant parasites will continue to retain this ring dormancy phenotype in the absence of ART treatment, and after a quiescent period, these ART-tolerant parasites will return to normal but accelerated development to compensate for the delay, i.e., a genetically

programmed "Sleeping Beauty". Consistent with this proposition is the recent report by Yu et al. (2022) of the construction of *P. falciparum* 3D7 strain containing *Pf*K13Y493H or C580Y mutation having reduced sensitivity to DHA (using RSA method) and delayed progression of ring stages by ~6 h compared to 3D7WT, and with a subsequent shortened trophozoite-stage duration to achieve the standard 48 h growth cycle in normal culture medium *without DHA pulsing*. This type of dormancy is in contrast to mosquito's salivary gland dormant sporozoites (entry into dormancy requiring active eIK2), which are "awaken" upon injection into host blood capillary (Turque et al., 2016). In the case of dormant ring the return to intraerythrocytic development cycle in the field appears to be a stochastic process, occurring after a period of hours, days or even weeks. This might be due to temporal variability of appropriate microenvironmental cues, which are required to activate binding of a set of transcription ApiAP2 factors, master regulators of intraerythrocytic stage switching, to initiate requisite genetic and epigenetic changes (Serrano-Durán et al., 2022).

Thus, the spread of ART-tolerant *P. falciparum* is also dependent on parasite co-resistance to the ACT partner drug. Such genetically regulated "Sleeping Beauty" parasites, especially those having acquired genes enabling survival competitiveness and resistance to other antimalarials, can expand into a region whenever conditions are favourable, as reported in a recent detailed genomic epidemiology study of a *P. falciparum* outbreak in Attapeu Province, PDR Lao, during the 2020–2021 malaria season, which revealed the aetiology as a rapid clonal expansion of a hitherto minor population of circulating *Pf*K13Thr 539 variant carrying multidrug-resistant gene (LAA1); interestingly LAA1 ancestry was traced to a western Cambodian strain (KH3) isolated in 2008 (Wasakul et al., 2022). For the immediate future, in order not to abandon ART's unique properties, i.e., high sensitivity against ring-stage parasites and pleiotropic pro-drug/poison mode of action, a third antimalarial has been suggested be included in ACT (TACT) in regions where both partner drugs are still effective (van der Pluijm et al., 2021). The two chosen partner drugs should exhibit no antagonistic effects and have comparable pharmacokinetics (so that should one drug fail, there is no antimalarial void allowing survival and spread of emerging ART-tolerant parasites). Prior to launching a region-wide administration inclusion of endpoint markers of both TACT partner drugs' efficacy should also be taken into consideration (phenotyping is direct and preferable indicator than genotyping of resistance emergence given the plasticity of evolutionary trajectories) (Wilairat et al., 2016; Bassat et al., 2022; Chotsiri et al., 2022). In order to prevent transmission, an additional low safe dose of gametocidal primaquine should also be taken into consideration (Stepniewska et al., 2022). Suggestions of alternatives to TACT include a 6-day ACT regimen using two different drug partners in tandem (but with risk of poor compliance) (van Schalkwyk and Sutherland, 2015; Schallig et al., 2017) or a two-drug combination in which ART is replaced with a novel endoperoxide with longer pharmacokinetics, e.g., arteflene or arterolane (but these ART-mimics are still under clinical trials) (Woodley et al., 2021).

Nonetheless, a better understanding of the mechanisms of ART-tolerant *P. falciparum* ring stage dormancy would lead to development of drugs that could prevent the occurrence or inhibit essential biochemical properties of the dormancy state, for instance, targeting PK4 (Zhang et al., 2017), protein biosynthesis (Laleu et al., 2022; Xie et al., 2022) or proteasome function (Xie et al., 2021). A mechanism-based view of dormancy in ART-tolerant *P. falciparum* will enable us to see the forest for the trees.

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Declaration of competing interest

The authors declare no competing interest.

References

Ariey, F., Witkowski, B., Amaratunga, C., Beghain, J., Langlois, A.C., Khim, N., Kim, S., Duru, V., Bouchier, C., Ma, L., Lim, P., Leang, R., Duong, S., Sreng, S., Suon, S., Chuor, C.M., Bout, D.M., Ménard, S., Rogers, W.O., Genton, B., Fandeur, T., Miotto, O., Ringwald, P., Le Bras, J., Berry, A., Barale, J.-C., Fairhurst, R.M., Benoit-Vical, F., Mercereau-Puijalon, O., Ménard, D., 2014. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature 505, 50–55.

Auparakkitanon, S., Wilairat, P., Wilairat, P., 2022. Will the in situ activator(s) of artemisinin please stand up? Mol. Biochem. Parasitol. 248, 111461.

Bassat, Q., Maïga-Ascofaré, O., May, J., Clain, J., Mombo-Ngoma, G., Groger, M., Adegnika, A.A., Agobé, J.-C.D., Djimde, A., Mischlinger, J., Ramharter, M., ASAAP, Consortia, Multimal, 2022. Challenges in the clinical development pathway for triple and multiple drug combinations in the treatment of uncomplicated falciparum malaria. Malar. J. 21, 61.

Behrens, H.M., Schmidt, S., Spielmann, T., 2021. The newly discovered role of endocytosis in artemisinin resistance. Med. Res. Rev. 41, 2998–3022.

Birnbaum, J., Scharf, S., Schmidt, S., Jonscher, E., Hoeijmakers, W.A.M., Flemming, S., Toenhake, C.G., Schmitt, M., Sabitzki, R., Bergmann, B., Fröhlke, U., Mesén-Ramírez, P., Soares, A.B., Herrmann, H., Bártfai, R., Spielmann, T., 2020. A Kelch13-defined endocytosis pathway mediates artemisinin resistance in malaria parasites. Science 367, 51–59.

Blasco, B., Leroy, D., Fidock, D.A., 2017. Antimalarial Drug Resistance: Linking *Plasmodium Falciparum* Parasite Biology to the Clinic, vol. 23, pp. 917–928.

Cao, Y., Liu, S., Liu, K., Abbasi, I.H.R., Cai, C., Yao, J., 2019. Molecular mechanisms relating to amino acid regulation of protein synthesis. Nutr. Res. Rev. 32, 183–191.

Chen, N., LaCrue, A.N., Teuscher, F., Waters, N.C., Gatton, M.L., Kyle, D.E., Cheng, Q., 2014. Fatty acid synthesis and pyruvate metabolism pathways remain active in dihydroartemisinin-induced dormant ring stages of *Plasmodium falciparum*. Antimicrob. Agents Chemother. 58, 4773–4781.

Chotsiri, P., White, N.J., Tarning, J., 2022. Pharmacokinetic considerations in seasonal malaria chemoprevention. Trends Parasitol. 38, 673–682.

Connelly, S.V., Manzella-Lapeira, J., Levine, Z.C., Brzostowski, J., Krymskaya, L., Rahman, R.S., Ellis, A.C., Amin, S.N., Sá, J.M., Wellems, T.E., 2021. Restructured mitochondrial-nuclear interaction in *Plasmodium falciparum* dormancy and persister survival after artemisinin exposure. mBio 12, e0075321.

Ferreira, P.E., Culleton, R., Gil, J.P., Meshnick, S.R., 2013. Artemisinin resistance in *Plasmodium falciparum*: what is it really? Trends Parasitol. 29, 318–320.

Kagoro, F.M., Barnes, K.I., Marsh, K., Ekapirat, N., Mercado, C.E.G., Sinha, I., Humphreys, G., Dhorda, M., Guerin, P.J., Maude, R.J., 2022. Mapping genetic markers of artemisinin resistance in *Plasmodium falciparum* malaria in Asia: a systematic review and spatiotemporal analysis. Lancet Microbe 3, e184–e192.

Kümpornsin, K., Loesbanluechai, D., de Cozar, C., Kotanan, N., Chotivanich, K., White, N. J., Wilairat, P., Gomez-Lorenzo, M.G., Gamo, F.J., Sanz, L.M., Lee, M.S.C., Chookajorn, T., 2021. Lumefantrine attenuates *Plasmodium falciparum* artemisinin resistance during the early ring stage. Int. J. Parasitol. Drugs Drug Resist. 17, 186–190.

Laleu, B., Rubiano, K., Yeo, T., Hallyburton, I., Anderson, M., Crespo-Fernandez, B., Gamo, F.-J., Antonova-Koch, Y., Orjuela-Sanchez, P., Wittlin, S., Jana, G.P., Maity, B. C., Chenu, E., Duffy, J., Sjö, P., Waterson, D., Winzeler, E., Guantai, E., Fidock, D.A., Hansson, T.G., 2023. Exploring a tetrahydroquinoline antimalarial hit from the Medicines for Malaria Pathogen Box and identification of its mode of resistance as PfeEF2. ChemMedChem (in press).

Mok, S., Stokes, B.H., Gnädig, N.F., Ross, L.S., Yeo, T., Amaratunga, C., Allman, E., Solyakov, L., Bottrill, A.R., Tripathi, J., Fairhurst, R.M., Llinás, M., Bozdech, Z., Tobin, A.B., Fidock, D.A., 2021. Artemisinin-resistant K13 mutations rewire *Plasmodium falciparum*'s intra-erythrocytic metabolic program to enhance survival. Nat. Commun. 12, 530.

Noedl, H., Se, Y., Schaecher, K., Smith, B.L., Socheat, D., Fukuda, M.M., Artemisinin Resistance in Cambodia 1 (ARC1) Study Consortium, 2008. Evidence of artemisinin-resistant malaria in western Cambodia. N. Engl. J. Med. 359, 2619–2620.

Peatey, C., Chen, N., Gresty, K., Anderson, K., Pickering, P., Watts, R., Gatton, M.L., McCarthy, J., Cheng, Q., 2021. Dormant *Plasmodium falciparum* parasites in human infections following artesunate therapy. J. Infect. Dis. 223, 1631–1638.

Sawarkar, R., 2022. Transcriptional lockdown during acute proteotoxic stress. Trends Biochem. Sci. 47, 660–672.

Schallig, H., Tinto, H., Sawa, P., Kaur, H., Duparc, S., Ishengoma, D.S., Magnussen, P., Alifrangis, M., Sutherland, C.J., 2017. Randomised controlled trial of two sequential artemisinin-based combination therapy regimens to treat uncomplicated falciparum malaria in African children: a protocol to investigate safety, efficacy, and adherence. B.M.J. Global Health. 2, e000371.

Serrano-Durán, R., López-Farfán, D., Gómez-Díaz, E., 2022. Epigenetic and epitranscriptomic gene regulation in *Plasmodium falciparum* and how we can use it against malaria. Genes 13, 1734.

Siddiqui, G., Srivastava, A., Russell, A.S., Creek, D.J., 2017. Multi-omics based identification of specific biochemical changes associated with PfKelch13-mutant artemisinin-resistant *Plasmodium falciparum*. J. Infect. Dis. 215, 1435–1444.

Stepniewska, K., Allen, E.N., Humphreys, G.S., Poirot, E., Craig, E., Kennon, K., Yilma, D., Bousema, T., Guerin, P.J., White, N.J., Price, R.N., Raman, J., Martensson, A., Mwaiswelo, R.O., Bancone, G., Bastiaens, G.J.H., Bjorkman, A.,

Brown, J.M., D'Alessandro, U., Dicko, A.A., El-Sayed, B., Elzaki, S.-E., Eziefula, A.C., Gonçalves, B.P., Hamid, M.M.A., Kaneko, A., Kariuki, S., Khan, W., Kwambai, T.K., Ley, B., Ngasala, B.E., Nosten, F., Okebe, J., Samuels, A.M., Smit, M.R., Stone, W.J. R., Sutanto, I., Kuile, F.T., Tine, R.C., Tiono, A.B., Drakeley, C.J., Gosling, R., Stergachis, A., Barnes, K.I., Chen, I., 2022. Safety of single-dose primaquine as a *Plasmodium falciparum* gametocytocide: a systematic review and meta-analysis of individual patient data. BMC Med. 20, 350.

Straimer, J., Gnädig, N.F., Witkowski, B., Amaratunga, C., Duru, V., Ramadani, A.P., Dacheux, M., Khim, N., Zhang, L., Lam, S., Gregory, P.D., Urnov, F.D., Mercereau-Puijalon, O., Benoit-Vical, F., Fairhurst, R.M., Ménard, D., Fidock, D.A., 2015. Drug resistance. K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. Science 347, 428–431.

Sutherland, C.J., Henrici, R.C., Artavanis-Tsakonas, K., 2021. Artemisinin susceptibility in the malaria parasite *Plasmodium falciparum*: propellers, adaptor proteins and the need for cellular healing. FEMS Microbiol. Rev. 45, fuaa056.

Teuscher, F., Gatton, M.L., Chen, N., Peters, J., Kyle, D.E., Cheng, Q., 2010. Artemisinin-induced dormancy in *Plasmodium falciparum*: duration, recovery rates, and implications in treatment failure. J. Infect. Dis. 202, 1362–1368.

Tu, Y., 2011. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nat. Med. 17, 1217–1220.

Turque, O., Tsao, T., Li, T., Zhang, M., 2016. Translational repression in malaria sporozoites. Microb. Cell. 3, 227–229.

van der Pluijm, R.W., Amaratunga, C., Dhorda, M., Dondorp, A.M., 2021. Triple artemisinin-based combination therapies for malaria - a new paradigm? Trends Parasitol. 37, 15–24.

van Schalkwyk, D.A., Sutherland, C.J., 2015. Malaria resistance to non-artemisinin partner drugs: how to reACT. Lancet Infect. Dis. 15, 621–623.

Wasakul, V., Disratthakit, A., Mayxay, M., Chindavongsa, K., Sengsavath, V., Thuy-Nhien, N., Pearson, R.D., Phalivong, S., Xayvanghang, S., Maude, R.J., Gonçalves, S., Day, N.P., Newton, P.N., Ashley, E.A., Kwiatkowski, D.P., Dondorp, A.M., Miotto, O., 2022. Malaria outbreak in Laos driven by a selective sweep for *Plasmodium falciparum kelch13* R539T mutants: a genetic epidemiology analysis. Lancet Infect. Dis. S1473–3099 (22), 00697-1.

Wellems, T.E., Sá, J.M., Su, X.-Z., Connelly, S.V., Ellis, A.C., 2020. Artemisinin resistance': something new or old? Something of a misnomer? Trends Parasitol. 36, 735–744.

Wilairat, P., Kümpornsin, K., Chookajorn, T., 2016. *Plasmodium falciparum* malaria: convergent evolutionary trajectories towards delayed clearance following artemisinin treatment. Med. Hypotheses 90, 19–22.

Witkowski, B., Amaratunga, C., Khim, N., Sreng, S., Chim, P., Kim, S., Lim, P., Mao, S., Sopha, C., Sam, B., Anderson, J.M., Duong, S., Chuor, C.M., Taylor, W.R.J., Suon, S., Mercereau-Puijalon, O., Fairhurst, R.M., Menard, D., 2013. Novel phenotypic assays for the detection of artemisinin-resistant *Plasmodium falciparum* malaria in Cambodia: in-vitro and ex-vivo drug-response studies. Lancet Infect. Dis. 13, 1043–1049.

Woodley, C.M., Amado, P.S.M., Cristiano, M.L.S., O'Neill, P.M., 2021. Artemisinin inspired synthetic endoperoxide drug candidates: design, synthesis, and mechanism of action studies. Med. Res. Rev. 41, 3062–3095.

WWARN K13 Genotype-Phenotype Study Group, 2019. Association of mutations in the *Plasmodium falciparum* Kelch13 gene (*Pf3D7_1343700*) with parasite clearance rates after artemisinin-based treatments - a WWARN individual patient data meta-analysis. BMC Med. 17, 1.

Xie, S.C., Metcalfe, R.D., Mizutani, H., Puhalovich, T., Hanssen, E., Morton, C.J., Du, Y., Dogovski, C., Huang, S.-C., Ciavarri, J., Hales, P., Griffin, R.J., Cohen, L.H., Chuang, B.-C., Wittlin, S., Deni, I., Yeo, T., Ward, K.E., Barry, D.C., Liu, B., Gillett, D. L., Fernandez, B.F., Ottilie, S., Mittal, N., Churchyard, A., Ferguson, D., Aguiar, A.C. C., Guido, R.V.C., Baum, J., Hanson, K.K., Winzeler, E.A., Gamo, F.-J., Fidock, D.A., Baud, D., Parker, M.W., Brand, S., Dick, L.R., Griffin, M.D.W., Gould, A.E., Tilley, L., 2021. Design of proteasome inhibitors with oral efficacy in vivo against *Plasmodium falciparum* and selectivity over the human proteasome. Proc. Natl. Acad. Sci. U.S.A. 118, e2107213118.

Xie, S.C., Metcalfe, R.D., Dunn, E., Morton, C.J., Huang, S.-C., Puhalovich, T., Du, Y., Wittlin, S., Nie, S., Luth, M.R., Ma, L., Kim, M.-S., Pasaje, C.F.A., Kumpornsin, K., Giannangelo, C., Houghton, F.J., Churchyard, A., Famodimu, M.T., Barry, D.C., Gillett, D.L., Dey, S., Kosasih, C.C., Newman, W., Niles, J.C., Lee, M.C.S., Baum, J., Ottilie, S., Winzeler, E.A., Creek, D.J., Williamson, N., Parker, M.W., Brand, S., Langston, S.P., Dick, L.R., Griffin, M.D.W., Gould, A.E., Tilley, L., 2022. Reaction hijacking of tyrosine tRNA synthetase as a new whole-of-life-cycle antimalarial strategy. Science 376, 1074–1079.

Yu, X., Wang, C., Zhao, Y., Tang, J., Zhu, M., Platon, L., Culleton, R., Zhu, G., Ménard, D., Zhang, Q., Cao, J., 2022. Ring-stage growth arrest: metabolic basis of artemisinin tolerance in *Plasmodium falciparum*. iScience 26, 105725.

Zhang, M., Gallego-Delgado, J., Fernandez-Arias, C., Waters, N.C., Rodriguez, A., Tsuji, M., Wek, R.C., Nussenzweig, V., Sullivan Jr., W.J., 2017. Inhibiting the *Plasmodium* eIF2α kinase PK4 prevents artemisinin-induced latency. Cell Host Microbe 22, 766–776.

Zhu, L., van der Pluijm, R.W., Kucharski, M., Nayak, S., Tripathi, J., White, N.J., Day, N. P.J., Faiz, A., Phyo, A.P., Amaratunga, C., Lek, D., Ashley, E.A., Nosten, F., Smithuis, F., Ginsburg, H., von Seidlein, L., Lin, K., Imwong, M., Chotivanich, K., Mayxay, M., Dhorda, M., Nguyen, H.C., Nguyen, T.N.T., Miotto, O., Newton, P.N., Jittamala, P., Tripura, R., Pukrittayakamee, S., Peto, T.J., Hien, T.T., Dondorp, A.M., Bozdech, Z., 2022. Artemisinin resistance in the malaria parasite, *Plasmodium falciparum*, originates from its initial transcriptional response. Commun. Biol. 5, 274.