International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 07 Issue: 07 | July 2020 www.irjet.net p-ISSN: 2395-0072

Future Implications of K13 and Artimisnine on Malaria Research

Sultan Ahmad*[1], Aqeel Ahmad[2], Alkama Aziz[3]

[1]Senior Research Fellow, Department of Microbiology, Shri Guru Ram Rai University, Dehradun, UK, India
[2]Senior Research Fellow, Department of Biotechnology, Jamia MilliaIslamia, New Delhi, India
[3]Junior Research Fellow, Department of Botany, Ch. Charan Singh University, Meerut, UP, India

---

Abstract: According to the WHO world malaria report 2018, there were an estimated 219 million cases and 435 000 malaria related deaths in 2017 worldwide. In absence of a successful vaccine that would offer protection against malaria, we need to rely on antimalarial medication to treat as well as reduce the chances of getting the disease.Artemisinin in combination with other slow acting drugs is recommended for the treatment of P. falciparum malaria but to our dismay first reports of Artemisinin resistance started surfacing around 2008. Thus it is the need of the hour to identify and characterize the markers of drug resistance as well as track the resistant genotypes to follow the path and spread of these resistant parasites.

Keywords: Malaria, Plasmodium falciparum, drug resistance, artemisinin, k13

Introduction

The only approved vaccine for malaria as of 2015 is RTS,S, that has a relatively low efficacy. Since malaria causes high mortality worldwide, it is essential to identify and validate new candidates for vaccine as well as find novel strategies that would help in developing a better vaccine. In this direction the identified a thrombospondin related protein (PfTRAMP) that is involved in invasion of the red blood cells by the malaria parasite and antibodies against this protein inhibit this invasion process. But blood-stage malaria vaccines that target single antigens involved in erythrocyte invasion have not induced optimal protection in field trials due to antigenic polymorphisms and molecular redundancy. Vaccines that target multiple invasion-related parasite proteins may inhibit erythrocyte invasion more efficiently. Therefore the importance of developing a receptor-blocking blood-stage vaccine approach against *P. falciparum* that targets the erythrocyte binding domains of multiple parasite proteins, blocking their interaction with their receptors and thus inhibiting erythrocyte invasion.Malaria has been the widespread and deadly parasitic infection caused by anopheline mosquitoes. It has become a global issue with 214 million recent cases and 438,000 deaths in 2015, mainly in the sub-Saharan African regions[1].Over the last decade, malaria endemic regions have determined the dropping rate of malaria and shifting the focus from reducing to eliminating this life-threatening disease. It has been shown in the past that the decrease in malarial spread is achievable but very hard to sustain. The worldwide campaign to eradicate malaria has seriously failed due to the development of parasitic resistance to efficient antimalarial drugs and resistance of mosquito to insecticides. Now the global concern remains to introduce efficient drugs as a replacement of old and failed drugs [2, 3].

Humans are affected by mainly four types of parasitic Plasmodium, but the *falciparum* species is notable for the majority of fatalities worldwide. Most of the studies are focused on the falciparum malaria, but more efforts should be directed towards the interpretation of other malaria species that would help us grasp the severity of malaria infections and design better intervention strategies [4, 5].At the same time, it is need of the hour to look for new vaccine candidates and design vaccines using novel strategies for malaria elimination[6-8]. It has been noted as the species, rendering dreadful malarial syndromes, has developed resistance to every antimalarial compound available. The use of antimalarial drugs like chloroquine, sulfadioxine and pyrimethamine has been extensively implemented in the past, and therefore, been misused. The parasites have developed resistance under selective pressure due to the preparation of antimalarial drugs on a broad scale[9, 10]. When the parasites developed resistance to these antimalarial drugs in Southeast Asia, the P. falciparum endemic regions, mefloquine replaced other drugs, but soon resistance developed for this compound [11].

Artemisinin-based Combination Therapy (ACT)

Except one notable drug – artemisinin (ART), resistance has been developed to all known antimalarial drugs. For centuries, Artemisinins have been used as traditional Chinese herbal medicine, derived from the plant, *Artemisia annua* and proved its efficacy against the life-threatening disease [12, 13]. To prevent the emergence of resistance, artemisinins in combination with partner drugs, where other antimalarial drugs like chloroquine, sulfadioxine-pyrimethamine etc. are used as partners, are

widely used. Due to the high potency of ART with slow-acting and less potent partner drugs, ACTs are known for higher parasite killing rate, lack of side effects and absence of resistance[14-16].

The ARTs being highly active against the asexual cycle of *P. falciparum* are capable of reducing the biomass of the malarial parasite along with short half-life (<1h) of ARTs in plasma,which necessitates the use of long-lasting partner drugs [17]. However, in spite of the significant usage, clinical ART resistance has not yet been demonstrated. Interestingly, the mode of action and inhibition of parasite growth regarding Artemisinin remains a curious case and a mystery to-date [18, 19].

Derivatives of artemisinin that include dihydroartemisinin, artemether, arteether and artesunate and many others are called the first generation derivatives of ART and thus synthesized and used in treating malaria [20]. These derivatives are sesquiterpene lactones known for their high activity and rapid elimination of malarial parasites almost at all stages of development[16]. The suggested mechanism for ART activation is a Fe-heam mediated process cleaving the endoperoxide moiety of the ARTs and forming the reactive oxygen species (ROS), which targets the nucleophilic groups in parasitic proteins and lipids. The artemisinin is known to covalently bind to 124 parasitic proteins, most of which are involved in biological metabolism essential for survival.

As recommended by WHO in 2001, artemisinin-based combination therapies (ACTs) are widely used as first-line multidrug-treatment resistant to *P. falciparum*[21, 22]. In Southeastern Asia, artesunate-mefloquine has been effectively used for uncomplicated malaria caused by *P. falciparum*. The ACT being widely used and recommended is the dihydroartemisininpiperquine in the Southeastern countries due to its promising efficacy [15]. White and others suggested that ART derivatives used along with antimalarial partner drugs could rapidly decrease the parasite density to a minimum, whereas keeping the optimum levels of the by longer activation of the drug components[23]. High efficacy of ACTs has been shown in the past in treating uncomplicated malaria in Asia and Africa; local data is not available in spite of the clear determination of antimalarial inefficiency during recent years[24-28].

The efficacy of ACTs was demonstrated in Afghanistan by one of the recent studies while conducting clinical trials utilizing a combination therapy of an ART derivative - artesunate (AS) and sulfadoxine-pyrimethamine (SP). This study indicated the presence of drug resistant alleles which did not develop resistance against ACT treatment and hence, proved efficient for the intervention of malaria caused by *P. falciparum*[29].

ART Resistance Phenotypes

Since a few years, ART resistance has emerged as a rising concern. The first report of ART resistance dates back to early 2000s near the Thai-Cambodian border for which the results are still ambiguous.The signs of inefficacy of ACTs and artesunate monotherapy were clearly indicated in western Cambodian artesunate-resistant parasitic isolates [30]. According to WHO, the emergence of piperaquine resistance in association to ART resistance and its aid in the selection of piperaquine-resistant parasites are contemporarily unclear. It is, however, suggested that the piperaquine resistance may have independently emerged due to the long life of piperaquine and its prior use as monotherapy[31].

Various recent clinical, *in vitro*, transcriptomics and genomic studies in Southeast Asia have outlined the *in vivo* and *in vitro* ART-resistant phenotypes, determined its genetic basis, and have studied its clinical impact. Partial resistance is offered by the slow parasite clearance rates expressed only in the early-ring stages of the parasite[16].A productive insight was provided by Duru and others in demonstrating the failure of ACTs, particularly dihydroartemisinin-piperquine in Cambodian isolates. All of the parasites in this study indicated the selection of parasite that were already resistant to artemisinin [32].

Clinical ART Resistance

Clinical ART resistance can be defined as heightened half-life clearance of the parasite or the presence of detectable parasites on the 3rd day of ACT intervention. The parasitic half-life is highly associated with the *in vitro* and *ex vivo* ring-stage assays (RSAs), which evaluate the endurance of the initial ring-stage parasites exposed to the 700nM dosage for 6 hours of the active metabolite of ART – the DHA (dihydroartemisinin) [17, 33]. Nonetheless, the definition of clinical resistance is affected by various factors like host immunity, drug concentration in blood or activity of partner drug in the artemisinin-based combination therapy (ACT) [34]. No matter how impressive the gains of the ARTs and ACTs, the emergence of ART resistance has been noted in Greater Mekong Subregion (GMS)(Laos, Cambodia, Thailand, Vietnam, and Mayanmar), which can lead to disastrous effects of malariaand an eventual spread to the African sub-continent.On the other hand, the risk of ART resistance

in the malarial isolates is a greater problem as compared to the failure of the chloroquine and sulphadioxinepyremethamine resistance in various parts of the worlddue to its emerging resistance to *falciparum* malaria where other drugs have failed[17, 22, 35, 36].

Parasite Clearance Rates

In order to elaborate the parasite clearance rates in Upper Mayanmar despite the presence of ART resistance, another study revealed the therapeutic effect of another ART derivative - dihydroartemisinin-piperquine (DP). Tun*et al.* evaluated the median half-life of the parasite and determined it to be less than 5 h (4.7 h) due to the frequent evaluation of parasitaemia indicating an intermediate resistance as compared to other types of mutations in the relevant region. Also, the importance of relation of site with the delayed parasite clearance rate[33, 37]. In a study by Amaratunga et al., the clearance of parasites took longer time than usual with a half-life of 11.28 h, indicating the widespread presence of ART-resistant phenotype outside Palin, Cambodia. It was also noted that some host factors accounted for the greater half-life while 40% half-life variation was due to parasite genetics. The identification of parasitic genetic clusterwas found corroborated with the genetic basis for the ART resistance phenotype [38].

The delayed parasite clearance rate and resistance has also been noted due to the presence of resistant parasitic hypnozoite reservoirs as observed in *P. falciparum* and *P. vivax*[39]. Other studies relate that the parasitic clearance half-life depends upon the susceptibility of the parasite to ART as well as on the developmental stage during ART treatment[17, 36].

However, ART resistance remains undetected due to the inefficacy of resistance phenotypes in drug susceptibility assays *in vitro*. Some success has been achieved using advanced in vitro assays providing an insight into the parasitic susceptibility at developmental ring stages in the erythrocytes. Moreover, artemisinin-resistant phenotypes have been reported with reduced susceptibility to ART in a T_0 [3H] hypoxanthine assay during the development of ring stage, prolonged resistant (ring) stage and reduced trophozoite stage during development. The extended resistant stages and temporary compression of the most susceptible developmental stage are observed to be highly associated with ART resistance. The altered pattern of development in the parasitic cell cycle is due to the increasing practicality of the ART-resistant parasites during ART exposure at ring stages. Such phenotype in the assayed samples indicates shortened asexual life cycle of the parasite. These novel phenotypes provide an opportunity to detect the function of mutations linked to ART resistance and determining molecular markers linked to ART clinical resistance[17].

Identification of K13 Mutations as a Molecular Markerof ART Resistance

In order to locate the gene responsible for artemisinin resistance, genome-wide association studies (GWAS) were carried out. The association of delayed clearance parasite rates with *P. falciparum* in Southeast Asia was indicated. After a single-nucleotide polymorphism (SNP) assay, Takala-Harrison *et al.,*via linkage-disequilibrium windows used as a marker of the decelerated clearance rate, recognized that four SNPs on chromosomes 10, 13, and 14 were related closely to the delayed parasite clearance. The SNPs on chromosome 10 and 13 indicated association with the genes involved in a DNA damage-tolerance pathway. These SNPs were later linked to the genes PF3D7_1343700 (Kelch 13) and PF3D7_1459600 (ENTH domain containing protein involved in clathrin mediated endocytosis) through an approach based on population genetics.The GWAS analysis has been utilized to highlight the heritable traits of clinical ART resistance and positive selection in geographical regions of ART resistance[40].

In recent studies, the K13-propeller mutations have been associated with artemisinin resistance *in vivo* and *in vitro* in Southeast Asia[36].In relevance with the previous research, clinical resistance to ARTs have shown delayed parasite clearance rate, parasite clearance half-life of >5h, presence of heritable Kelch propeller mutations in the Pf3D7_1343700 domain, and its rapid spread, as noted in Southeast Asia [22, 41].It has now been proved via screening of the ART-resistant *P. falciparum* with K13 mutations that the presence of mutated *P.falciparum* has passed beyond the classical western Thai-Cambodia border[42].

As simple as it seems, the genetic basis for ART resistance is challenged by the introduction of molecular markers, which revealed a complex mystery that remains unsolved. With the objective to identify the genetic basis of ACT traits for adaptation, Cheeseman *et al*. utilized a two-stage strategy to determine the genetic basis for underlying gene selection by comparing three geographical regions (Cambodia, Thailand and Laos). Screening and genotyping of 91 parasite clones determined single-nucleotide polymorphisms (SNPs) and copy number variations (CNVs). As a result, geographical differentiation and haplotype

structure at 6969 SNPs determined a region of strong selection on chromosome 13 which corresponds to the decelerated parasite clearance rates[43].

Airey *et al*. conducted an expeditious study to analyze mutations in laboratory-adapted parasite clones selected for survival while receiving high ART dosage *in vitro*. The information yielded by such analysis can guide polymorphism analysis in ART-resistant parasitic samples from Cambodia. Sequencing an ART-sensitive F-32 Tanzania parasite line indicated artemisinin resistant K13 propeller mutations during DHA treatment in the RSA. The presence of linked-disequilibrium around genes indicated four K13 propeller mutations (Y493H, R539T, I543T, and C580Y) in the naturally-occurring parasites in Cambodia. These mutations were found associated with long parasite clearance half-life and higher frequency of survival rates in the RSA $^{0\text{-}3h}$. It was also found that different levels of K13 mutations present varying levels of ART resistance, which indicate the genetic background of the parasite influencing these levels. The need for a molecular marker persists to detect and control the widespread ART resistance. Thus, it was concluded that the prevalent mutations can be utilized as markers to determine the decelerated parasite clearance rates in malarial patients receiving ART treatment[36]. Similar studies were conducted by Ye *et al*., who identified the K13-propeller region of the *P. falciparum* gene as a molecular marker to detect artemisinin-resistant parasites *in vitro*. K13-propeller mutations are also able to identify parasitic clearance half-life (>5h) with 98.1% ART sensitivity and 88.4% host specificity [14].

Recent studies elaborate that the frequency of K13 mutations increase with ART usage, along with the purified selection working on the propeller region of the K-13 gene. The population of parasites unexposed to ACTs provide the fundamental information about K13-propeller gene behaving as a molecular marker of the ART resistance and elaborate a case of positive selection in the untreated propeller domain. Moreover, many contrasted K13-propeller mutations occur under ART pressure[44]. *In vitro* assays have determined decline in the *P. falciparum* susceptibility to ART, but no proof has yet been discovered. Feng and others accessed five mutations with three being recent. F446I mutation was predominant among the samples collected from China-Mayanmar border indicating the risks for emerging resistance in the Greater Mekong Subregion (GMS)[45]. Another molecular marker A578S was determined by Hawkes and associates in order to elaborate the genetic basis for the spread of malaria in the Ugandan Children. Being the severe case of malaria in Uganda, the nonsynonymous SNP A578S in the K13 gene may be another acknowledged marker, although not being directly associated with ART resistance, determining a delayed response and parasitic clearance rate to ART derivative [46]. Using the PCR method, DNA templates were derived from frozen samples for examination and measurement of the *Plasmodium* parasite by Tripura *et al.* The authors related the decreased ART component drug sensitivity with the K13 propeller mutant gene (C580Y) in an attempt to prevent, treat and eliminate infections caused by *P. falciparum* and *P. vivax*[39].

Another breakthrough regarding the K13-propeller mutation (C580Y) has raised a concern among various studies conferring to its widespread resistance to artemisinin[39, 47-51]. The C580Y is more prevalent than other ART resistant molecularmarkers, although it has not been observed as a consistent marker for ART resistance[33, 36, 37]. It is nonetheless a confirmed molecular marker of the K13 gene predominating along the Thailand-Mayanmar and Cambodia-Thailand border, while the F446I predominates along the Mayanmar-India and China-Mayanmar border [16]. Sequencing and genotyping of the *PfK13* of 98 *P. falciparum* isolates in Guyana by Chenet*et al*. determined the presence of K13 mutation (C580Y) in strong association with drug resistance[49]. Recently, a successful study expressed K13mutation (C580Y) in the genetically engineered clones of *P. falciparum* using the CRISPR-Cas9 system and demonstrated slow parasitic clearance rate. Through this study, direct link was established between K13 mutation and ART resistance[52].

Consequently, in a research conducted by Straimer*et al*., *P. falciparum* K13 locus were genetically modified using zinc-finger nuclease and the ring-survival rates were evaluated after drug exposure in vitro. These studies suggested the decrease in the parasitic survival rates after removal of K13 mutations from ART resistant Cambodian isolates. In contrast to relevant observations, Straimer and others detected higher resistance in some K13 mutations (M4761,R493H, I543T) than other K13 mutations (Y493H and C580Y), indicating the modest resistance of C580Y being predominant mutant allele in Cambodia. Also, it suggested that additional factors were involved in augmenting K13-mediated resistance in the Cambodian isolates[17].

K13 Polymorphism across the globe

Since the definite ART resistance phenotype is uncommon, the association of polymorphisms in marker genes is hard to relate with efficient results[53]. According to Mita*et al.,* 60 non-synonymous mutations have been identified in the K13 gene [44], while Fairhurst and Dondrop indicate that only 20 of 124 nonsynonymous K13 mutations can be associated with ART resistance (P441L, F446I, G449A, N458Y, C469Y, A481V, Y493H, S522C, G538V, R539T, I543T, P553L, R561H, V568G, P574L,

C580Y, D584V, F673I, A675V, and H719N). However, only four of these have been validated *in vivo* and *in vitro*: Y493H, R539T, I543T, and C580Y[16].

Recently, Arie*yet al*. exposed the association of K13-propeller polymorphisms with ART resistance. They showed the strong relation between ART resistance and four K13-propeller polymorphisms, i.eC580Y, Y493H, R539T, and M476L[36]. Similarly, many SNPs have been reported in K13-propeller gene along with multiple origins of the K13-propeller polymorphisms across the world, including Africa as well as Southeast Asia[53, 54]. As indicated by Tanabe *et al*., numerous SNPs along with haplotype (3D7 sequence) exist in the four continents being geographically distinct and continent specific [55]. The fundamental information about K13-propeller gene behaving as a molecular marker of the ART resistance provided a case of positive selection in the untreated propeller domain[44].

The K13 propeller polymorphisms are known to exist widely around the world. According to a report by Edwards *et al.,* the dispersion of malarial infection becomes widespread due to the shifting populations of higher transmission areas to lower transmission areas, which in turn retard the control and elimination of the dreadful disease by importing the infection and spreading drug resistance. Such has been demonstrated in the cross Cambodian border, the French Island of Mayaotte, and China, where the malarial infections are mainly imported from other regions or transmitted locally[45, 56, 57].

Reports of independent global emergence of K13 mutation in a variety of locations like Mayotte(N490H, F495L, N554H/K, and E596G) and Guyana(C580Y) have been received recently, although no information on the clinical or phenotypic resistance has been noted in these isolates [49, 57].To mark a standard for the spread of K13 polymorphisms, a novel study sequenced and genotyped 581 *P. falciparum* K13-propeller isolates from Asia, Africa, Maleneia and South America collected before and after ACT intervention. The population of isolates exposed to drugs showed higher frequencies of mutations, nucleotide and haplotype diversity as compared to the unexposed parasite population. Further indications included the prevalence of C580Y mutations earlier than that of the first report of ART resistance in 2007[2, 21, 44].

A global analysis was conducted by Menard et al to map the K13-propeller polymorphisms. The authors utilized 14,037 samples from 59 countries and sequenced K13-propeller polymorphisms to evaluate the emergence and dissemination of mutations by haplotyping neighboring loci. Isolates having a similar K13 mutation were related genetically by evaluating two adjacent loci. Such phenomenon revealed the emergence of events beside the spread of mutations for ART resistance. Also, the difference of mutations and haplotypes in the two resistance regions in Asia suggested selection pressure in the relative areas due to the usage of ACTsmainly. The ratio of heterogenous nonsynonymous K13 mutations in Asia ranged from fixed to high in western Cambodia, intermediate in Mayanmar and Vietnam, moderate in eastern Cambodia, Thailand, China and Laos, and low everywhere else. K13 mutations were reported uncommon in South America, Oceania and Africa except a few African nations[58]. It is however interesting to learn that the K13 polymorphisms associated with ART resistance in Southeast Asia (Y493H, R539T, I543Tand C580Y)have been absent in the sub-Saharan regions and in contrast to it, the other non-synonymous SNPs identified in sub-Saharan regions have not been observed in the Southeast Asian *P*. *falciparum* isolates. Thus, it is proposed that the K13 polymorphisms can vary geographically and determination of K13 propeller genetic studies can reveal and monitor the global emergence of resistance to ART [34, 59, 60].

In relevance to the previous studies, other K13 polymorphisms were studied by Tacoli et al. Two K13 polymorphisms (P574L and A675V) are ubiquitously present in Southeast Asia and associated with decelerated clearance rate. The relevant inquiry also reports that the K13 polymorphism P574L was observed for the first time in Rwanda, suggesting their unique presence with the inclusion of strains linked to ART resistance [61]. However, the low prevalence of K13 propeller in Africa was confirmed by Torrentino-Madamet et al while identifying K13 propeller polymorphisms in the *P. falciparum* isolates collected from 29 patients receiving AL treatment on the French Island of Mayotte in 2013-2014[57]. A study by Duru and colleagues indicated the limited genetic diversity of the parasites showing the presence of K13 polymorphisms in almost every parasite isolate from Cambodia [32]. However, the ART resistance was noted to be confined to Southeast Asia and China. Since ART-resistant K13 mutation has not been prevalent in Africa, the abundant presence of K13-propeller polymorphism A578S and others have been reported by various studies which pose serious threat to K13 propeller functioning[58, 62, 63].

Though it is crucial to urgently develop and implement targeted interventions to contain and eliminate ART resistance to its current locations[42], what is more alarming is the independent emergence of K13 mutations in multiple geographic locations suggesting that efforts to eliminate artemisinin-resistant malarial parasites in one region may have a limited impact on the emergence of resistance in neighboring regions.It further highlights the need to map K13 mutations throughout the malaria-endemic world. This report is consistent in the studies determining polymorphisms in Haiti [59], Uganda[46, 64, 65], Angola

and Mozambique[34], Rwanda[61], Kenya [66, 67], Ethiopia [68, 69], Senegal[70], Mayotte [57], Southeast Asia [40], Cambodia [36], Vietnam [71], Bangladesh[72], China [51][73].

Mechanism of ART resistance and K13 Polymorphism

Since K13-propeller mutations are highly prognostic of resistance, the knowledge of underlying mechanisms that yields ART-resistant*P.falciparum* remains unknown [74]. It has been reported that the range of K13 mutations and development of ART resistance by single mutations point towards the declining functionality of the K13 protein. Previous research has indicated the function of human kelch-containing proteins as adapters bringing substrates into ubiquitination complexes [75]. Nonetheless, as compared to human kelch proteins (Keap 1), K13 belongs to the kelch super family of proteins, constituting of a particular *Plasmodium* domain and an N-terminal domain, a BTB/POZ domain and a six-blade C-terminal propeller domain made up of basic kelch motifs [76, 77].

The propeller domain entertains many protein-protein sites and intercedes cellular functions like ubiquitin-regulated protein degradation and oxidative stress responses. It is suggested that the Fe-dependent generation of reactive oxygen species (ROS) mediates the potential antimalarial effect of the ART and its derivatives, inducing alteration in the redox balance, and hence damages the cellular targets. It seems interesting that the toxicity of ART derivatives depends upon their pro-oxidant activity, in contrast to their involvement in the regulation of cytoprotective and protein degradation responses to outside stress[36]. However, this hypothesis further supports the evidence that K13 is highly homologous to the human Kelch protein (Keap 1), which is required in cell adaptation to oxidative stress [78]. The human kelch protein (Keap 1) is a negative regulator of the inducible cytoprotective response dependent on the nuclear erythroid 2-related factor 2 (Nrf2)[79]. The Nrf2 binds to the antioxidant response element (ARE) present in the gene promoters involved in phase II detoxification and oxidative stress responses. The Nrf2 is degraded by the Keap 1, which targets it through the cullin 3 ligase complex for ubiquilination[80]. Therefore, it is presumed that the K13 propeller performs similar functions in the *Plasmodium*, i.e directing the transcription factors incorporated in anti-oxidant responses through ligase complex. No orthologues of Nrf2 have been determined in the parasitic genome [36]. On the contrary, many suggested hypotheses have elaborated the K13 polymorphism role in regulating artemisinin resistance in *P. falciparum* isolates.

The drug responses of Cambodian wild-type K13 and mutated samples of *P. falciparum*, Dogovski et al indicated the inducement of drug retardation and accumulation of ubiquinated proteins by the ART. This action contributes to cellular stress response. The decelerated protein ubiquitination and delayed early apoptosis after drug exposure is exhibited by the resistant parasite strains, which indicates higher levels of cellular stress response. Due to its similarity to substrate adapters for cullin3 ubiquitin ligases, the role of K13 is determined in reducing the level of ubiquitinated proteins[80, 81].

Furthermore, Mbengue*et al.,* recently reported that artemisinins are potent inhibitors of P. falciparum phosphatidylinositol-3-kinase (PfPI3K). PfPI3K phosphorylates phosphatidylinositol (PI) to produce phosphatidylinositol 3-phosphate (PI3P) which promotes cell signaling for parasite survival, such as inhibition of apoptosis.Hence, inhibition of PfPI3K activity by DHA causes a reduction in PI3P level and subsequently leads to parasite death. They further showed that PfPI3K interacts with K13 and the K13 mutationshinder this interaction resulting in reduced polyubiquitination of PfPI3K, leading to the accumulation of PfPI3K, as well as its lipid product phosphatidylinositol-3-phosphate (PI3P). Thereby the authors concluded that levels of PI3P can be used as an additional marker for prediction of artemisinin resistance.Buthow the elevated PI3P leads to resistance needs to be further evaluated [50, 65].

To elaborate the mechanism of ART further, Mok et al emphasized the comprehensive changes of the parasite transcriptional program altering its physiology as a reason for ART resistance. Later, the authors carried out the transcriptome analyses of 1043 *P. falciparum* isolates to uncover the underlying mechanism of artemisinin resistance. They found that ART resistance was highly correlated with up-regulated genes incorporated in protein process, and since these pathways participate in unfolded protein response (UPR) involving the major *Plasmodium* reactive oxidative stress complex (PROSC) and TCP-1 ring complex (TRiC) chaperone complexes, they may serve as the major intermediate for ART resistance caused by K13 mutation in *P. falciparum*and mitigate protein damage caused by artemisinin [82]. It has been proposed that the K13 mutations mediate ART resistance by limiting their effects on particular targets at ring stage. Recent studies have provided evidence that the phosphatidylinositol-3-kinase (PfPI3K) of the *P. falciparum* is targeted specifically by the artemisinins and its levels are increased with K13 mutations in parasites[50]. Thus screening of K13 and PI3K proteins in *Plasmodium vivax* may help us extrapolate our current knowledge of drug resistance to Vivax parasites [83-85].Another report by Wang and others proposes that the parasites with K13-propeller mutations are able to overcome protein damage due to the drug modifications by

activating the stress response; thus, they are selected as they have a higher capability to survive the drug treatment at the early ring stage, at which point drug activation and drug pressure are relatively low; thereby enriching these mutations in the parasitepopulation[33].

Other proteins have been conferred in mediating the ART resistance in the absence of K13 mutations. TRAC studies have revealed that nonsynonymous polymorphisms in multidrug-resistance protein 2, apicoplast ribosomal protein S10 , chloroquine-resistance transporter (pfcrt), and ferredoxin determine the genetic background for the K13 mutations to arise [86]. It would also be important to decipher the K13-independent mechanisms of ART resistance [87][88, 89, 98]

The role of these proteins and pathways in artemisinin resistance is plausible, but needs further evaluation. It would be very interesting to delineate the normal function of K13 and the effect of various mutations found in the propeller domain of K13. Furthermore, it would also be interesting to decipher the identity of putative K13 targets and their association with ubiquitin ligase activity. K13-molecular targetswould give the critical insight forinterrogatingits role in the underlying mechanism of ART resistance.Currently K13-propeller polymorphisms appear to be the only useful molecular marker for chasing the emergence and spread of ART resistance in *P. falciparum*.

Conclusion and Future Implications

The spread of malaria and the threat to the efficacy of antimalarial drugs have raised a global concern. Artemisinin has been used as a potential anti-malarial in combination with less potent drugs, but it has confronted resistance in the malaria species. The supposed ART resistance is likely to be defined as the increased rate of decelerated parasite clearance phenotype or the K13-propeller mutations, while the confirmed ART resistance delivers the slow clearing parasite phenotype along with K13 mutations associated with ART resistance[16].

K13 polymorphism has proved to be the only crucial molecular marker available for tracking the ART resistance. It may be speculated that mutations in K13 may also come with a cost to parasite fitness, and might be lost rapidly in populations in the absence of artemisinin selection. Most critical in this direction would be to determine the exact physiological roles of K13 in the parasite and the effect of these polymorphisms on its function.Very interestingly, there have been some reports of slow parasite clearance rates even in the absence of K13 mutant alleles suggesting the role of additional molecules in development of ART resistance in *P. falciparum*. It would be crucial to identify additional genetic loci involved in ART resistance[14, 21, 86].Novel methodologies like GWAS[40, 43], click chemistry [33], genetic tools [17, 52], transcriptomics[82] and chemogenic profiling [90]can prove to be vital for solving this mystery of parasite clever escape from the currently usedantimalarialdrugs.Apart from understanding the current state and mechanisms of antimalarial drug resistance, it is also extremely essential to broaden understanding of this intelligent parasite [91-93]and at the same time to expand the current arsenal used against the parasite[94-98].

All in all, the artemisinin resistance still remains a gray area, about which not much is known. Strategies for regular monitoring and extensive surveillance of K13 prevalence should be implemented. The national drug policies should be viewed carefully and altered in a timely fashion according to the frequency of spreading resistance. The discovery and identification of infection phenotypes should be monitored in the malaria endemic regions and the research for the mechanism and intervention for the prevailing resistance needs to be urgently investigated.

References

1. WHO, World Malaria Report 2014. 2014, WHO: Geneva.

2. Fairhurst, R.M., et al., Artemisinin-resistant malaria: research challenges, opportunities, and public health implications. The American journal of tropical medicine and hygiene, 2012. **87**(2): p. 231-241.

3. Siddiqui. F.A., Malaria Control and Elimination: How Far we are: An Opinion Article. Journal of Biometrics & Biostatistics 2016. **10**(October): p. DOI: 10.4172/2155-6180.1000321.

4. Wangdahl, A., et al., Severity of Plasmodium falciparum and Non-falciparum Malaria in Travelers and Migrants: A Nationwide Observational Study Over 2 Decades in Sweden. J Infect Dis, 2019. **220**(8): p. 1335-1345.

5. Brashear, A.M., et al., A glance of the blood stage transcriptome of a Southeast Asian Plasmodium ovale isolate. PLoS Negl Trop Dis, 2019. **13**(11): p. e0007850.

6. Draper, S.J., et al., Malaria Vaccines: Recent Advances and New Horizons. Cell Host Microbe, 2018. **24**(1): p. 43-56.

7. Pandey, A.K., et al., Identification of a potent combination of key Plasmodium falciparum merozoite antigens that elicit strain-transcending parasite-neutralizing antibodies. Infect Immun, 2013. **81**(2): p. 441-51.

8. Siddiqui, F.A., et al., A thrombospondin structural repeat containing rhoptry protein from Plasmodium falciparum mediates erythrocyte invasion. Cell Microbiol, 2013. **15**(8): p. 1341-56.

9. White, N.J., Antimalarial drug resistance. The Journal of clinical investigation, 2004. **113**(8): p. 1084-1092.

10. WHO, Global Report on Antimalarial Efficacy and Drug Resistance: 2000-2010. 2010, WHO: Geneva. p. 9-10.

11. Yeung, S., et al., Antimalarial drug resistance, artemisinin-based combination therapy, and the contribution of modeling to elucidating policy choices. The American journal of tropical medicine and hygiene, 2004. **71**(2 suppl): p. 179-186.

12. Klayman, D.L., Qinghaosu (artemisinin): an antimalarial drug from China. Science, 1985. **228**(4703): p. 1049-1055.

13. White, N.J., Qinghaosu (artemisinin): the price of success. Science, 2008. **320**(5874): p. 330-334.

14. Ye, R., et al., Distinctive origin of artemisinin-resistant Plasmodium falciparum on the China-Myanmar border. Scientific reports, 2016. **6**.

15. Davis, T., H.A. Karunajeewa, and K.F. Ilett, Artemisinin-based combination therapies for uncomplicated malaria. Med J Aust, 2005. **182**(4): p. 181-5.

16. Fairhurst, R.M. and A.M. Dondorp, Artemisinin-resistant Plasmodium falciparum malaria. Microbiology spectrum, 2016. **4**(3).

17. Straimer, J., et al., K13-propeller mutations confer artemisinin resistance in Plasmodium falciparum clinical isolates. Science, 2015. **347**(6220): p. 428-431.

18. Golenser, J., et al., Current perspectives on the mechanism of action of artemisinins. International journal for parasitology, 2006. **36**(14): p. 1427-1441.

19. O'Neill, P.M., et al., Enantiomeric 1, 2, 4-Trioxanes Display Equivalent in vitro Antimalarial Activity Versus Plasmodium falciparum Malaria Parasites: Implications for the Molecular Mechanism of Action of the Artemisinins. ChemBioChem, 2005. **6**(11): p. 2048-2054.

20. O'Neill, P.M. and G.H. Posner, A medicinal chemistry perspective on artemisinin and related endoperoxides. Journal of medicinal chemistry, 2004. **47**(12): p. 2945-2964.

21. Ashley, E.A., et al., Spread of artemisinin resistance in Plasmodium falciparum malaria. New England Journal of Medicine, 2014. **371**(5): p. 411-423.

22. Hott, A., et al., Artemisinin-resistant Plasmodium falciparum parasites exhibit altered patterns of development in infected erythrocytes. Antimicrobial agents and chemotherapy, 2015. **59**(6): p. 3156-3167.

23. White, N., et al., Averting a malaria disaster. The Lancet, 1999. **353**(9168): p. 1965-1967.

24. von Seidlein, L., et al., Treatment of African children with uncomplicated falciparum malaria with a new antimalarial drug, CGP 56697. Journal of infectious diseases, 1997. **176**(4): p. 1113-1116.

25. Tjitra, E., et al., Therapy of uncomplicated falciparum malaria: a randomized trial comparing artesunate plus sulfadoxine-pyrimethamine versus sulfadoxine-pyrimethamine alone in Irian Jaya, Indonesia. The American journal of tropical medicine and hygiene, 2001. **65**(4): p. 309-317.

26. Adjuik, M., et al., Amodiaquine-artesunate versus amodiaquine for uncomplicated Plasmodium falciparum malaria in African children: a randomised, multicentre trial. The Lancet, 2002. **359**(9315): p. 1365-1372.

27. Barennes, H., et al., A randomized trial of amodiaquine and artesunate alone and in combination for the treatment of uncomplicated falciparum malaria in children from Burkina Faso. Tropical Medicine & International Health, 2004. **9**(4): p. 438-444.

28. Staedke, S.G., et al., Combination treatments for uncomplicated falciparum malaria in Kampala, Uganda: randomised clinical trial. The Lancet, 2004. **364**(9449): p. 1950-1957.

29. Awab, G.R., et al., Clinical trials of artesunate plus sulfadoxine-pyrimethamine for Plasmodium falciparum malaria in Afghanistan: maintained efficacy a decade after introduction. Malaria journal, 2016. **15**(1): p. 1.

30. Dondorp, A.M., et al., Artemisinin resistance in Plasmodium falciparum malaria. New England Journal of Medicine, 2009. **361**(5): p. 455-467.

31. WHO, Status report on artemisinin and ACT resistance - September 2015. 2015 WHO: Geneva.

32. Duru, V., et al., Plasmodium falciparum dihydroartemisinin-piperaquine failures in Cambodia are associated with mutant K13 parasites presenting high survival rates in novel piperaquine in vitro assays: retrospective and prospective investigations. BMC medicine, 2015. **13**(1): p. 1.

33. Wang, Z., et al., Prevalence of K13-propeller polymorphisms in Plasmodium falciparum from China-Myanmar border in 2007–2012. Malaria journal, 2015. **14**(1): p. 1.

34. Escobar, C., et al., Polymorphisms in Plasmodium falciparum K13-Propeller in Angola and Mozambique after the Introduction of the ACTs. PLoS One, 2015. **10**(3): p. e0119215.

35. Liu, H., et al., Investigation and control of a Plasmodium falciparum malaria outbreak in Shan Special Region II of Myanmar along the China-Myanmar Border from June to December 2014. Infectious diseases of poverty, 2016. **5**(1): p. 1.

36. Ariey, F., et al., A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature, 2014. **505**(7481): p. 50-55.

37. Tun, K.M., et al., Parasite clearance rates in Upper Myanmar indicate a distinctive artemisinin resistance phenotype: a therapeutic efficacy study. Malaria journal, 2016. **15**(1): p. 1.

38. Amaratunga, C., et al., Artemisinin-resistant Plasmodium falciparum in Pursat province, western Cambodia: a parasite clearance rate study. The Lancet infectious diseases, 2012. **12**(11): p. 851-858.

39. Tripura, R., et al., Persistent Plasmodium falciparum and Plasmodium vivax infections in a western Cambodian population: implications for prevention, treatment and elimination strategies. Malaria journal, 2016. **15**(1): p. 1.

40. Takala-Harrison, S., et al., Genetic loci associated with delayed clearance of Plasmodium falciparum following artemisinin treatment in Southeast Asia. Proceedings of the National Academy of Sciences, 2013. **110**(1): p. 240-245.

41. O'Brien, C., et al., Recent clinical and molecular insights into emerging artemisinin resistance in Plasmodium falciparum. Current opinion in infectious diseases, 2011. **24**(6): p. 570.

42. Bosman, P., et al., Plasmodium prevalence and artemisinin-resistant falciparum malaria in Preah Vihear Province, Cambodia: a cross-sectional population-based study. Malaria journal, 2014. **13**(1): p. 1.

43. Cheeseman, I.H., et al., A major genome region underlying artemisinin resistance in malaria. Science, 2012. **336**(6077): p. 79-82.

44. Mita, T., et al., Little polymorphism at the K13 propeller locus in worldwide Plasmodium falciparum populations prior to the introduction of artemisinin combination therapies. Antimicrobial agents and chemotherapy, 2016. **60**(6): p. 3340-3347.

45. Feng, J., et al., Evaluation of antimalarial resistance marker polymorphism in returned migrant workers in china. Antimicrobial agents and chemotherapy, 2015 a. **59**(1): p. 326-330.

46. Hawkes, M., et al., Slow clearance of Plasmodium falciparum in severe pediatric Malaria, Uganda, 2011–2013. Emerging infectious diseases, 2015. **21**(7): p. 1237.

47. Kite, W.A., et al., Alternative methods for the Plasmodium falciparum artemisinin ring-stage survival assay with increased simplicity and parasite stage-specificity. Malaria journal, 2016. **15**(1): p. 1.

48. Alareqi, L.M., et al., Molecular markers associated with resistance to commonly used antimalarial drugs among Plasmodium falciparum isolates from a malaria-endemic area in Taiz governorate—Yemen during the transmission season. Acta Tropica, 2016. **162**: p. 174-179.

49. Chenet, S.M., et al., Independent emergence of the Plasmodium falciparum kelch propeller domain mutant allele C580Y in Guyana. Journal of Infectious Diseases, 2015: p. jiv752.

50. Mbengue, A., et al., A molecular mechanism of artemisinin resistance in Plasmodium falciparum malaria. Nature, 2015. **520**(7549): p. 683-687.

51. Feng, J., et al., Amplification of pfmdr1, pfcrt, pvmdr1, and K13 propeller polymorphisms associated with Plasmodium falciparum and Plasmodium vivax isolates from the China-Myanmar border. Antimicrobial agents and chemotherapy, 2015 b. **59**(5): p. 2554-2559.

52. Ghorbal, M., et al., Genome editing in the human malaria parasite Plasmodium falciparum using the CRISPR-Cas9 system. Nature biotechnology, 2014. **32**(8): p. 819-821.

53. Chatterjee, M., et al., No polymorphism in Plasmodium falciparum K13 propeller gene in clinical isolates from Kolkata, India. Journal of pathogens, 2015. **2015**.

54. Huang, B., et al., Polymorphisms of the artemisinin resistant marker (K13) in Plasmodium falciparum parasite populations of Grande Comore Island 10 years after artemisinin combination therapy. Parasites & vectors, 2015. **8**(1): p. 1.

55. Tanabe, K., et al., Spontaneous mutations in the Plasmodium falciparum sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (PfATP6) gene among geographically widespread parasite populations unexposed to artemisinin-based combination therapies. Antimicrobial agents and chemotherapy, 2011. **55**(1): p. 94-100.

56. Edwards, H.M., et al., Novel cross-border approaches to optimise identification of asymptomatic and artemisinin-resistant Plasmodium infection in mobile populations crossing Cambodian borders. PloS one, 2015. **10**(9): p. e0124300.

57. Torrentino-Madamet, M., et al., K13-propeller polymorphisms in Plasmodium falciparum isolates from patients in Mayotte in 2013 and 2014. Antimicrobial agents and chemotherapy, 2015. **59**(12): p. 7878-7881.

58. Ménard, D., et al., A worldwide map of Plasmodium falciparum K13-propeller polymorphisms. New England Journal of Medicine, 2016. **374**(25): p. 2453-2464.

59. Carter, T.E., et al., Artemisinin resistance-associated polymorphisms at the K13-propeller locus are absent in Plasmodium falciparum isolates from Haiti. The American journal of tropical medicine and hygiene, 2015. **92**(3): p. 552-554.

60. Siddiqui, F.A., et al., Role of Plasmodium falciparum Kelch 13 Protein Mutations in P. falciparum Populations from Northeastern Myanmar in Mediating Artemisinin Resistance. mBio, 2020. **11**(1).

61. Tacoli, C., et al., Artemisinin Resistance–Associated K13 Polymorphisms of Plasmodium falciparum in Southern Rwanda, 2010–2015. The American Journal of Tropical Medicine and Hygiene, 2016: p. 16-0483.

62. Taylor, S.M., et al., Absence of putative artemisinin resistance mutations among Plasmodium falciparum in sub-Saharan Africa: a molecular epidemiologic study. Journal of Infectious Diseases, 2015. **211**(5): p. 680-688.

63. Kamau, E., et al., K13-propeller polymorphisms in Plasmodium falciparum parasites from sub-Saharan Africa. Journal of Infectious Diseases, 2014: p. jiu608.

64. Cooper, R.A., et al., Lack of artemisinin resistance in Plasmodium falciparum in Uganda based on parasitological and molecular assays. Antimicrobial agents and chemotherapy, 2015. **59**(8): p. 5061-5064.

65. Conrad, M.D., et al., Polymorphisms in K13 and falcipain-2 associated with artemisinin resistance are not prevalent in Plasmodium falciparum isolated from Ugandan children. PloS one, 2014. **9**(8): p. e105690.

66. Muwanguzi, J., et al., Lack of K13 mutations in Plasmodium falciparum persisting after artemisinin combination therapy treatment of Kenyan children. Malaria journal, 2016. **15**(1): p. 1.

67. Borrmann, S., et al., Genome-wide screen identifies new candidate genes associated with artemisinin susceptibility in Plasmodium falciparum in Kenya. Scientific reports, 2013. **3**: p. 3318.

68. Bayih, A.G., et al., A Unique Plasmodium falciparum K13 Gene Mutation in Northwest Ethiopia. The American journal of tropical medicine and hygiene, 2016. **94**(1): p. 132-135.

69. Heuchert, A., et al., Molecular markers of anti-malarial drug resistance in southwest Ethiopia over time: regional surveillance from 2006 to 2013. Malaria journal, 2015. **14**(1): p. 1.

70. Boussaroque, A., et al., Emergence of Mutations in the K13 Propeller Gene of Plasmodium falciparum Isolates from Dakar, Senegal, in 2013-2014. Antimicrobial agents and chemotherapy, 2016. **60**(1): p. 624-627.

71. Thriemer, K., et al., Delayed parasite clearance after treatment with dihydroartemisinin-piperaquine in Plasmodium falciparum malaria patients in central Vietnam. Antimicrobial agents and chemotherapy, 2014. **58**(12): p. 7049-7055.

72. Mohon, A.N., et al., Mutations in Plasmodium falciparum K13 propeller gene from Bangladesh (2009–2013). Malaria journal, 2014. **13**(1): p. 1.

73. Zhang, J., et al., In vitro susceptibility of Plasmodium falciparum isolates from the China-Myanmar border area to artemisinins and correlation with K13 mutations. Int J Parasitol Drugs Drug Resist, 2019. **10**: p. 20-27.

74. Mok, S., et al., Drug resistance. Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science, 2015. **347**(6220): p. 431-5.

75. Woodrow, C.J. and N.J. White, The clinical impact of artemisinin resistance in Southeast Asia and the potential for future spread. FEMS Microbiology Reviews, 2016: p. fuw037.

76. Adams, J., R. Kelso, and L. Cooley, The kelch repeat superfamily of proteins: propellers of cell function. Trends in cell biology, 2000. **10**(1): p. 17-24.

77. Prag, S. and J.C. Adams, Molecular phylogeny of the kelch-repeat superfamily reveals an expansion of BTB/kelch proteins in animals. BMC bioinformatics, 2003. **4**(1): p. 1.

78. Mitsuishi, Y., H. Motohashi, and M. Yamamoto, The Keap1-Nrf2 system in cancers: stress response and anabolic metabolism. Frontiers in oncology, 2011. **2**: p. 200-200.

79. Velichkova, M. and T. Hasson, Keap1 regulates the oxidation-sensitive shuttling of Nrf2 into and out of the nucleus via a Crm1-dependent nuclear export mechanism. Molecular and cellular biology, 2005. **25**(11): p. 4501-4513.

80. Villeneuve, N.F., A. Lau, and D.D. Zhang, Regulation of the Nrf2–Keap1 antioxidant response by the ubiquitin proteasome system: an insight into cullin-ring ubiquitin ligases. Antioxidants & redox signaling, 2010. **13**(11): p. 1699-1712.

81. Dogovski, C., et al., Targeting the cell stress response of Plasmodium falciparum to overcome artemisinin resistance. PLoS Biol, 2015. **13**(4): p. e1002132.

82. Mok, S., et al., Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science, 2015. **347**(6220): p. 431-435.

83. Ngassa Mbenda HG, Zeng W, Bai Y, Siddiqui FA, Yang Z, Cui L. Genetic diversity of the Plasmodium vivax phosphatidylinositol 3-kinase gene in two regions of the China–Myanmar border. Infect Genet Evol. 2018;61:45–52.

84. Ngassa Mbenda, H.G., Wang, M., Guo, J. et al. Evolution of the Plasmodium vivax multidrug resistance 1 gene in the Greater Mekong Subregion during malaria elimination. Parasites Vectors 13, 67 (2020).https://doi.org/10.1186/s13071-020-3934-5.

85. Wang, M., Siddiqui, F.A., Fan, Q. et al. Limited genetic diversity in the PvK12 Kelch protein in Plasmodium vivax isolates from Southeast Asia. Malar J 15, 537 (2016). https://doi.org/10.1186/s12936-016-1583-0

86. Miotto, O., et al., Genetic architecture of artemisinin-resistant Plasmodium falciparum. Nature genetics, 2015. **47**(3): p. 226-234.

87. Mukherjee, A., et al., Artemisinin resistance without pfkelch13 mutations in Plasmodium falciparum isolates from Cambodia. Malar J, 2017. **16**(1): p. 195.

88. Siddiqui, F.A., et al., Plasmodium falciparum Falcipain-2a Polymorphisms in Southeast Asia and Their Association With Artemisinin Resistance. J Infect Dis, 2018. **218**(3): p. 434-442.

89. Zhao, Y., et al., Genetic Variations Associated with Drug Resistance Markers in Asymptomatic Plasmodium falciparum Infections in Myanmar. Genes (Basel), 2019. **10**(9).

90. Pradhan, A., et al., Chemogenomic profiling of Plasmodium falciparum as a tool to aid antimalarial drug discovery. Scientific reports, 2015. **5**.

91. Alam, M.M., et al., Phosphoproteomics reveals malaria parasite Protein Kinase G as a signalling hub regulating egress and invasion. Nat Commun, 2015. **6**: p. 7285.

92. Dawn A, Singh S, More KR, Siddiqui FA, Pachikara N, et al. (2014) The Central Role of cAMP in Regulating Plasmodium falciparum Merozoite Invasion of Human Erythrocytes. PLoS Pathog 10(12): e1004520. doi:10.1371/journal.ppat.1004520.

93. Liang, X., et al., Puf3 participates in ribosomal biogenesis in malaria parasites. J Cell Sci, 2018. **131**(6).

94. Balaich, J.N., et al., The Nonartemisinin Sesquiterpene Lactones Parthenin and Parthenolide Block Plasmodium falciparum Sexual Stage Transmission. Antimicrobial agents and chemotherapy, 2016. **60**(4): p. 2108-2117.

95. Mott, B.T., et al., High-throughput matrix screening identifies synergistic and antagonistic antimalarial drug combinations. Scientific reports, 2015. **5**.

96. Baragaña, B., et al., A novel multiple-stage antimalarial agent that inhibits protein synthesis. Nature, 2015. **522**(7556): p. 315-320.

97. Hati, S., et al., Design, synthesis and biological evaluation of small molecules as potent glucosidase inhibitors. Eur J Med Chem, 2015. **100**: p. 188-96.

98. Li J, Zhang J, Li Q, Hu Y, Ruan Y, Tao Z,et al. (2020) Ex vivo susceptibilities of Plasmodium vivax isolates from the China-Myanmar border to antimalarial drugs and association with polymorphisms in Pvmdr1 and Pvcrt-o genes.PLoS Negl Trop Dis 14(6): e0008255. https://doi.org/10.1371/journal.pntd.0008255