THE ROLES OF THAI AND ASEAN RESEARCHERS IN MALARIA CONTROL AND ELIMINATION

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ABSTRACT:

Malaria remains one of the major global public health problems in the tropics and subtropics including Southeast Asia. Despite progress made towards malaria research and control strategies, there remain daunting challenges, with the burden of malaria still very high in a number of ASEAN countries. Major critical issues that currently impede malaria control in the region include the development and spread of multidrug resistant *Plasmodium falciparum.* Researchers in each ASEAN country have been working in collaboration by networking to assist policy-making malaria control programmes in each country and the region. Different types of malaria research that support the control programme have been conducted by regional and international research institutions in each ASEAN country as well as within the collaborating networks. These include biology/biochemistry, molecular biology/genetics, pathophysiology, diagnosis, epidemiology/ control, pharmacology, drug resistance, entomology, clinical studies, immunology and vaccines, and health social sciences. Research aiming at enhancing malaria diagnosis and an increased understanding of host-parasite and parasite-vector interactions is also a major focus. With regards to Thailand, there are a number of achievements in the past that have made significant contributions to advancing basic knowledge, treatment, and malaria control and elimination in the ASEAN region and worldwide. Some of the key achievements that significantly contributed to basic knowledge and malaria control and elimination are highlighted in the article. Examples include the application of clinical pharmacology for dose optimization of antimalarial drugs in multidrug resistant *Plasmodium falciparum* and discovery and development of new antimalarial drug candidate P218 that targets malarial enzyme dihydrofolate reductase enzyme.

Keywords: Malaria, Southeast Asian, Drug resistance

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INTRODUCTION

Malaria remains one of the major global public health problems in the tropics and subtropics including Southeast Asia. The most recent World Malaria Report in 2012 revealed an estimated 3.4 billion people at risk, 207 million confirmed cases, and 627,000 deaths, of which 90% occurred in sub-Saharan Africa. Four major species of plasmodium commonly infect humans—*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. *P. falciparum* is the most dangerous and lethal form which accounts for most of the morbidity and mortality. The burden of *P. vivax* varies widely with World Health Organization estimating that it is responsible for approximately 70 to 80 million cases worldwide annually [1]. The disease is rarely life-threatening, but morbidity from a prolonged illness and the possibility of relapses from a persistent hepatic form (hypnozoite), which occurs more frequently with the tropical form of *P. vivax* found in ASEAN, is of major concern and cause considerable economic loss. Recently, humans infected with *Plasmodium knowlesi*, a simian malaria parasite, have been described in a number of ASEAN countries including Malaysia [2, 3], Singapore [4], Myanmar [5], Vietnam [6], Indonesia [7], the Philippines [8], and Thailand [9]. In Thailand, the first case of a human *P. knowlesi* infection was acquired in Prachuap Khiri Khan, a southern Thai province which was reported in 2004 [9]. No other humans infected with this species of *Plasmodium* were reported in Thailand until 2009, when 10 cases from Tak, Prachuap Khiri Khan, Chantaburi, Yala,

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and Narathiwat provinces were described [10]. These areas are located near the borders of Myanmar, Cambodia, and Malaysia. Recently, a total of 23 *P. knowlesi* infected patients have been reported in these border areas [11].

The Southeast Asian region harbors a significant proportion of the world's burden of malaria infection. In 2011, approximately 1.33 billion people, or 75% of the region's total population resided in areas that were at risk of malaria [1]. There were 2,144,849 confirmed malaria cases and 1,819 malaria deaths reported by the national malaria control programmes in the region in 2011. The distribution of each species varies from country to country and even within a country, but *P. falciparum* and *P. vivax* are the most common species of the malaria parasite. The malaria situation in Thailand improved significantly during the period 2000-2011. The annual parasite incidence *per* 1,000 population at risk was reduced from 2.05 in 2000 to 1.61 in 2011. The malaria mortality rate *per* 100,000 population at risk was reduced from 0.44 in 2000 to 0.14 in 2011, while the malaria case fatality rate was reduced from 0.45% in 2000 to 0.17% in 2011. Thirty-five percent of cases in 2011 were due to *P. vivax*. The primary vectors present in Thailand are *Anopheles dirus* and *An. minimus*. Thailand has two transmission peaks --June to August and October to November --when its rainy season leads to an increase in breeding by the primary vector. Malaria affects poor and vulnerable populations, particularly in areas where access to health services is a challenge, including those along international borders. Malaria transmission is particularly intense along international border areas. High-risk groups include workers in development projects, agroforestry, rubber plantations, and mining; subsistence farmers; ethnic communities; and settlers in forest and forest fringes. With the opening up of the Asian Economic Community (AEC) in 2015, nationals from all ASEAN countries will be able to move freely across international borders. This will have major repercussions for disease surveillance, control, and elimination, especially given the malaria challenges currently present at international borders [1].

Despite substantial improvements in the epidemiological situation, malaria remains one of the main public health concerns in Southeast Asia, particularly the Greater Mekong Sub-region (GMS), encompassing Cambodia, Laos, Thailand, Vietnam, Myanmar, and China (Figure 1). The Thai-Cambodian and Thai-Myanmar borders are known as major hotspots of multidrug resistant *P. falciparum* [12] and resistance containment remains a high

Figure 1 The Greater Mekong Sub-region (GMS), encompassing Cambodia, Laos, Thailand, Vietnam, Myanmar, and China

priority for malaria control. Since the 1960s, the Cambodia-Thailand border has been the global epicentre of emerging resistance to antimalarial drugs. It is in this region that parasite resistance to chloroquine was first documented, followed by resistance to sulfadoxine-pyrimethamine, which was later shown by molecular markers to have spread far outside the GMS, and finally to mefloquine [12]. Adoption of artemisinin-based combination therapies (ACTs) and other preventive interventions for *P. falciparum* treatment have aided in the decline of malaria deaths and remain important tools in the elimination of *P. falciparum.* However, recent emergence of artemisinin-resistant malaria is a great concern that could lead to the spread of resistant parasites to other parts of the world [13].

Apart from drug resistance in *P. falciparum*, the "sleeping giant" in the GMS is *P. vivax* malaria which has now become resistant to blood schizontocide chloroquine in some ASEAN countries, notably Indonesia [14, 15]. The estimated burden in the GMS is 260,000 cases based on the National Malaria Control Programme data but this is very likely to be a gross underestimation [16], especially given the huge numbers of people at risk of *P. vivax* malaria e.g., 129 million in Indonesia alone and 150 million for Southeast Asia [17]. Chloroquine and the tissue schizontocide primaquine have remained the mainstay treatment of *P. vivax* infection in Thailand and most of the ASEAN countries for more than 60 years, with conserved clinical efficacy of virtually 100% [18-20]. Chloroquine resistance is linked to increasing rates of anemia and may be an important factor in severe *P. vivax* malaria [15]. To date, there has been no clinical-parasitological evidence of

Table 1 Reported malaria cases by species and fatality in the eight ASEAN countries in 2012 [1]

Country	Number of malaria cases by species			Number of death cases
	P. falciparum	*P. vivax*	Other species	
Cambodia	4,639	4,451	0	45
Indonesia	199,977	187,583	981	252
Lao PDR	11,410	1,715	1	44
Malaysia	894	1,461	2,306	12
Myanmar	46,695	25,950	103	403
Philippines	4,744	2,189	57	16
Thailand	11,553	17,506	3,172	37
Vietnam	11,448	7,220	0	8

chloroquine resistant *P. vivax* in Thailand. Nevertheless, a trend in gradual decline of *in vitro* sensitivity to chloroquine has been documented in some areas of the country, particularly along the Thai-Myanmar border [21, 22].

Large-scale population movement from highly endemic areas to low endemic zones has contributed significantly to the spread of *P. falciparum* within and beyond the GMS [1]. The transmission of malaria across borders complicates surveillance and follow-up for national malaria control programmes. Thailand is the primary destination for migrant labour from neighboring countries. A well-known example of extensive migration leading to the spread of malaria is the movement of gem miners from Borai district, Trat province of Thailand, into the Pailin province of Cambodia. During the "Ruby Rush" of 1988 to 1992, a large influx of non-immune people entered Cambodia's Pailin province along the Thai-Cambodian border, which is the epicentre of drug-resistant malaria. During this period, it is estimated that 100,000 to 200,000 people crossed the border from Thailand to Cambodia, many of whom from the western Thai provinces bordering Myanmar. When the gem miners returned to their provinces, mefloquine-resistant *P. falciparum* spread westward. Estimates from clinics in Mae Sot district in Tak province in western Thailand along the Thai-Myanmar border were that 80% of malaria infections were acquired in eastern Thailand along the Thai-Cambodian border. Currently, the Thai-Myanmar border and the southern border of Yunnan, China, are the two main areas where frequent cross-border movement has exacerbated the malaria situation. The Mae Tao clinic in Mae Sot district, Tak province along the Thai-Myanmar border, which provides free health care for refugees and migrant workers, treated more than 8,000 cases of malaria in 2006, of which 75% were from Myanmar. The clinic reported 30 deaths from malaria the same year [23]. Furthermore, the unstable political situation and high degree of violence in the three southern provinces of Thailand, Yala, Songkhla, and Narathiwat, have severely disrupted the access to health services in these provinces. Confirmed malaria cases in Thailand have risen slightly since 2004, partly due to the escalation of civil conflict in these provinces. These three provinces accounted for more than 40% of Thailand's confirmed malaria cases in 2007 [23].

Critical challenges in malaria control

Through various elimination efforts, particularly by the WHO Global Malaria Eradication Programme, malaria has been completely eliminated in some areas with low levels of transmission and relatively good healthcare infrastructure [1]. Table 1 shows the number of malaria cases by species and fatality in the eight ASEAN countries. Early diagnosis and treatment with effective antimalarial drugs remain essential tools at all stages of the control programme. Since no effective vaccine is currently available, the key to addressing the challenge of reducing the burden of malaria is an integrated approach that combines preventative measures such as long-lasting insecticide-treated bed nets and indoor residual spraying (IRS) with DDT, improved access to personal protection measures, and improved access to effective antimalarial drugs. Rapid treatment of malaria cases with an effective antimalarial drug that quickly reduces the parasite load and is also strongly gametocytocidal can help reduce the rate of malaria transmission. Chemotherapy with effective antimalarial drugs plays an important role either in early control or in the attack phase to reduce transmission, or in the later stages to maintain interruption of transmission and prevent reintroduction of malaria [1].

Over the last decade, malaria burden in the Southeast Asian region has been reduced substantially. However, despite this progress, there are still daunting challenges, with the burden of malaria still very high in a number of ASEAN countries. Major critical issues that currently undermine malaria control in the region include: (i) development and spread of multidrug resistant

P. falciparum; (ii) prevalence of counterfeit and substandard antimalarial drugs and irrational drug use; (iii) low coverage of malaria control interventions among remote/ethnic populations and in some countries; and (iv) difficulty in accessing and using basic health services by migrant workers and ethnic minorities who are not citizens and therefore, not afforded the same social benefits. Infections in the population were undiagnosed until molecular detection methods could be adapted to the control programme available. Defining the limits and burden of *P. vivax*, *P. falciparum*, and the emerging *P. knowlesi* are essential for elimination and control efforts. A relative increase in *P. vivax* malaria has been identified in most endemic countries, and new foci of artemisinin resistant *P. falciparum* malaria are being detected. Parasite and vector resistance has rendered many insecticides and antimalarial drugs useless. The acquisition and spread of multidrug resistant *P. falciparum* is the key factor contributing to complexity in malaria control. It undermines malaria control and results in increased morbidity (e.g., anemia and low birth weight), economic costs, and mortality in several countries. In addition, it is the greatest challenge to malaria treatment, and is associated with the resurgence of malaria because of an increase in disease transmission due to returning treatment failures. The accumulating reports of chloroquine-resistant *P. vivax* in other parts of the world during the past three decades, particularly in ASEAN countries such as Indonesia [24], Papua New Guinea [25-28], Irian Jaya [29-32], Myanmar [33-35], and Vietnam [36], emphasize the need for closely and continuously monitoring clinical efficacy in conjunction with *in vitro* sensitivity *of P. vivax* isolates.

Activities towards malaria control and elimination: research and collaboration

Research and development (R&D) plays a major role in many enterprises. A typical ratio of R&D for an industrial company is about 3.5% of revenues while in some pharmaceutical companies this ratio may be several-fold higher. Although malaria control and elimination is not an industrial enterprise, most experts agree that efforts supported by evidence and new tools are more likely to be successful. In this context, R&D plays a pivotal role in shaping future malaria control and elimination activities. Much can be learned from successful programmes, but the heterogeneity of the malaria epidemiology between nations makes it essential to adapt programmes the current local needs.

It is apparent that several of the ASEAN nations fall below the ratio of researchers to population recommended by the United Nations Educational, Scientific and Cultural Organization (UNESCO). This significantly reduces R&D productivity in the region. The proportion of researchers to population is also much higher in the more developed nations (e.g., Singapore, Malaysia, and Thailand) compared to those which are less economically developed. Considering all of these public health challenges and hindrances to providing better quality of health in the ASEAN, activities towards the establishment of regional health innovation networks were initiated with the goal of enhancing product discovery and providing a sustainable essential health R&D through intraregional collaboration.

In addressing the previously described challenges in malaria control and elimination, the following should be addressed: (i) strengthening the health system including national and local capacities for malaria control and elimination; (ii) ensuring universal coverage of key interventions such as long-lasting insecticidal nets, indoor residual spraying, rapid diagnostic tests, microscopy, and artemisinin-based combination therapy (ACT) through multisectoral approaches; (iii) confining artemisinin resistance in the GMS, and preventing its resurgence in other areas; (iv) sustaining the efficacy of insecticides against the malaria vectors and preventing resurgence of malaria; (v) investing in research to develop tools to control outdoor transmission and test innovative mechanisms for delivering malaria control interventions to hard-to-reach populations at risk of malaria; (vi) sustaining political commitment and inter-country collaboration; and (vii) promoting both basic and translational scientific research to support the discovery and development of new promising tools for rapid diagnosis, disease prevention, and treatment. Monitoring and identifying factors contributing to antimalarial drug resistance is necessary for the country's future perspective of malaria control policy. There is a pressing need for systemic collaborative research and control of malaria in the Southeast Asian region. In addition, there is also an urgent need for operational research to assist program managers in the optimization, scaling up, and monitoring of interventions. Further work is needed to identify funding, prioritize research topics, strengthen capacity in endemic countries, and facilitate multi-country and institution collaborations.

The move towards malaria elimination in most endemic countries is on course. Regional response to these challenges has been developing in collaboration with member countries and partners. Efforts are being made to strengthen malaria control

and elimination activities along the Thai border areas and to prevent the spread of multidrug resistant *P. falciparum*. Thailand continues to enhance its malaria surveillance throughout the country and targets the most at-risk populations to achieve its elimination goals. In order to build on current successes and further reduce the malaria burden in the GMS, the six national malaria control programmes must overcome important challenges that characterize the Mekong Region. Researchers in each ASEAN country have been working in collaboration by networking to assist policy-making malaria control programmes in each country and the region. Thailand sits at the heart of Southeast Asia and is a major hub for collaborative research in the region. In each country, apart from the Ministry of Public Health, a number of research institutes are involved with malaria research. Each institute often works in collaboration with national and international research institutes in specific areas. With regards to Thailand, current malaria control activities in the country are supported by the Mekong Malaria Programme (MMP), which was established to coordinate malaria control and elimination activities in the countries of the Mekong Basin and is funded by the President's Malaria Initiative [37]. In addition, the malaria control program of Thailand is also supported by a Global Fund Round 7 grant that targets two population groups: migrant workers, their families, and the Thai communities that host them in the western and eastern border regions; and communities living in conflict zones in the southern provinces [38]. In 2011, additional funding was secured through a Global Fund Round 10 grant to continue targeting these at-risk groups [23]. Several international research collaborative networks have been established within the ASEAN region, with financial supports from the US, European countries, and Japan. Examples of key networks include: (i) Mekong Basin Disease Surveillance (MBDS) Initiative, (ii) Mekong Therapeutic Efficacy Study (TES) Network, (iii) Asia Pacific Malaria Elimination Network (APMEN), (iv) Southeast Asian Ministers of Education Organization and Tropical Medicine (SEAMEO TROPMED), (v) ASEAN Network for Drugs, Diagnostics, Vaccines and Traditional Medicines Innovation (ASEAN-NDI), (vi) South East Asia Research Network: The South East Asia Research Network (SEARN). Other supporting networks/collaborating centers include the South East Asia Infectious Disease Clinical Research Network (SEAICRN), the WorldWide Antimalarial Resistance Network (WWARN), and the WHO-TDR Clinical Coordination and Training Center (CCTC).

Research and collaboration: achievements in the past

In addition to various supported networks, different types of malaria research that support the control programme has been conducted by regional and international research institutions in each ASEAN country as well as within the collaborating networks. These include research focusing on epidemiology and control, drugs and drug resistance, entomology, clinical studies, biology/ chemistry, molecular/genetics, immunology/vaccines, pathophysiology, health social science/health policy, and diagnostics. Based on the results of a systemic research conducted for articles published from January 1990 to December 2009 by researchers in 11 Asia Pacific countries [39], malaria-related publications increased in a linear fashion over the last two decades, doubling between 1990 and 2009. Thailand had the highest output of malaria-related papers during 1990-2009 (n = 1,211), followed by Indonesia (n = 346), Malaysia (n=127), and the Philippines (n=107). The epidemiology and control of malaria accounted for 53% of publications, followed by drugs and drug resistance (47%), entomology (40%), clinical studies (35%), biology/chemistry (34%), molecular/genetics (32%), immunology/vaccines (28%), pathophysiology (17%), health social science/ health policy (11%), and diagnostics (10%). Overall there were more publications on *P. falciparum* compared with *P. vivax*, with the total ratio being 1.95 *P. falciparum* related articles for every *P. vivax* article. The trend of *P. falciparum* articles being dominant was apparent within each subject, with the exception of health social sciences/health policy, where the proportion of articles that did not relate specifically to either Plasmodium species was higher than the proportion that did. The majority of articles describing clinical trials related to *P. falciparum* (85%). In a more recent systemic review of research articles published by researchers in the GMS countries during 1993 to 2012 [40] also showed that publication numbers increased significantly over time. Ten research areas accounted for 2,264 (78.3%) publications: drug resistance 12.8% (n=371), entomology 11.42% (n=330), clinical trials 10.45% (n=302), pathophysiology 9.34% (n=270), epidemiology 8.96% (n=259), pharmacology 6.06% (n=175), parasite biology 5.19% (n=150), malaria control 4.88% (n=141), diagnosis/diagnostics 4.6% (n=133), and clinical studies 4.6% (n=133). Thailand produced the most publications with 1,684 (58.27%), followed by Viet Nam (365, 12.63%), Cambodia (139, 4.81%), Myanmar (132, 4.57%),

Yunnan Province, China (124, 4.3%), and Lao PDR (79, 2.73%).

With regards to malaria-related research in Thailand, there are a number of achievements in the past that have made significant contributions to advancing basic knowledge, treatment, and malaria control and elimination in the ASEAN region and worldwide. Key research institutes include: Faculty of Tropical Medicine and Mahidol Oxford Tropical Medicine Research Unit (MORU), Mahidol University; Faculty of Science, Mahidol University; Faculty of Science and Faculty of Medicine, Chulalongkorn University; Pramongkutklao College of Medicine; Chulabhorn International College of Medicine, Thammasat University; Armed Forces Research Institute of Medical Sciences (AFRIMS); and National Science and Technology Development Agency (NSTDA). Different aspects of malaria research have being conducted in these institutes including: biology/biochemistry, molecular biology/ genetics, pathophysiology, diagnosis, epidemiology/ control, pharmacology, drug resistance, entomology, clinical studies, immunology and vaccines, and health social sciences. This article highlights some of the key achievements in the past which contributed significantly to basic knowledge and malaria control and elimination.

Clinical pharmacology and drug resistance: Considering the development of *P. falciparum* resistance successively to a number of antimalarial drugs, namely chloroquine, sulfadoxine-pyrimethamine, quinine, and mefloquine, Thailand is arguably the most experienced country in dealing with drug resistant malaria [12]. Resistance of *P. falciparum* to chloroquine was apparent in the late 1950s almost concurrently in Southeast Asia (along the Thai-Cambodian border) and South America. The first case of chloroquine-resistant *P. falciparum* in Thailand was reported in 1962 [41]. Chloroquine resistance was followed by reports of sulfadoxine-pyrimethamine and mefloquine resistance, both of which occurred in the Thai-Cambodian border in the mid-1960s and late 1980s, respectively [42, 43]. Due to a rising concern in drug resistance, Professor Harinasuta and her colleagues documented the efficacy of key antimalarial drugs in large groups of malarial patients, with detailed clinical, pharmacological, and parasitological assessments [44-49]. The introduction, development, and evaluation of artemisinin monotherapy and combination therapy for the treatment of uncomplicated and severe cases of *P. falciparum* malaria in Southeast Asia were also pioneered by her research group at the Hospital for Tropical Diseases in 1992. A series of clinical trials, in which artemisinin (qinghaosu) derivatives were in monotherapy or in combination with other drugs, was also conducted for evaluating the clinical efficacy and tolerability [45]. Results from these studies provided essential information which formed the basis for recommendations on malaria treatment policy of the Malaria Control Programme of Thailand especially in revising the dosage regimens or moving forward to a new alternative drug to overcome drug resistance.

"Clinical pharmacology" concerns the development of a scientifically disciplined system to determine rational, effective, and safe drug therapy. Most of the antimalarials now in clinical use were introduced before the modern era of dosage design based on pharmacokinetics and pharmacodynamics properties. The Clinical Pharmacology Unit was established at the Faculty of Tropical Medicine, Mahidol University, Thailand in 1989. Important advances in several of the clinical pharmacology research on malaria carried out at this Pharmacology Unit led to optimum dosing regimens for many antimalarial drugs which provided a more rational basis to cope with multidrug resistant *P. falciparum* malaria. Research in clinical pharmacology has shown that optimization of therapy by pharmacodynamics and pharmacokinetic monitoring not only improves efficiency and reduces the risk of adverse effects, but also delays the development of drug resistance. The pharmacokinetic profiles and biotransformation pathways of key antimalarial drugs (chloroquine, mefloquine, quinine, halofantrine, and artemisinins) were investigated in various groups of patients with *P. falciparum* or *P. vivax* infection (children, pregnant women, ethnics, patients with impaired renal and hepatic functions) and with different disease severity (acute uncomplicated and severe malaria) [44-49]. In addition, the bioavailabiliy and bioequivalence of various formulations of commonly used antimalarial drugs including pharmacokinetic drug interactions were investigated [44-49]. The information obtained has led to revisions in and dose optimization of the originally empirical or questionable dose recommendations, to ensure efficacy and safety of treatment regimens in patients.

Quinine is a good example which best illustrates the contribution of clinical pharmacology research to the treatment of malaria patients. The drug has been used mainly to treat severe malaria. The problems relating to clinical use of quinine in uncomplicated and severe malaria cases involve the maintenance of adequate plasma drug concentrations throughout the 7-days period of treatment, and concern about the toxicity (dysrhythmias, hypotension, blindness, or deafness) from quinine itself particularly following

parenteral administration [47]. Pharmacokinetic investigations showed that the pharmacokinetic properties of quinine was significantly altered in patients with malaria infection in proportion to the disease severity [47] and these changes result in a significant increase in plasma concentrations of quinine in malaria patients that may lead to toxicity. Optimal quinine dosage regimens and the value of loading doses were firmly established based on sound pharmacokinetics information obtained from patients with malaria. Loading dose twice of the maintenance dose (20 mg salt/kg) of quinine can be given safely to patients with severe symptoms by rate-controlled intravenous infusion or intramuscular injection without toxicity [48]. In uncomplicated falciparum malaria, based on the pharmacokinetic information, it is therefore recommended that in the area with quinine resistance, quinine should be given with tetracycline to improve therapeutic efficacy due to increased plasma quinine concentrations when given with tetracycline [49].

The quinolone-methanol antimalarial drug mefloquine was introduced for treatment of acute uncomplicated *P. falciparum* in Thailand following resistance of parasite strains to sulfadoxine-pyrimethamine in 1995 [12]. The drug can be used as single dose treatment as it has a long half-life. However, when used in highly endemic areas with no vector control, emergence of drug resistance developed rapidly, with subsequent need for larger doses. This has led to an increased incidence of vomiting and a lower cure rate [50]. The clinical pharmacokinetics of mefloquine suggest that therapeutic concentrations can be achieved with less vomiting if the drug is given as two divided doses [51].

Halofantrine appears to exhibit greater activity than mefloquine against multidrug resistant strains of *P. falciparum.* However, studies of its clinical pharmacology reveal several disadvantages. First, there is a wide intra- and inter-individual variation in oral absorption which results in unpredictable systemic drug concentrations wherein extremely poor absorption leads to therapeutic failure. Second, the drug exhibits concentration-dependent potential cardiotoxicity. Moreover, unpredictably high halofantrine plasma concentrations following standard doses results in a high incidence of QTc (corrected QT) interval prolongation of the electrocardiogram, which is associated with cardiovascular toxicity [52]. Halofantrine has thus, never been registered for clinical use in patients with malaria in Thailand.

The 8-aminoquinoline antimalarial primaquine is the only drug available for eradication of the hypnozoite stage of *P. vivax* and *P. ovale.* It is active against primary tissue stages in the liver of all human malaria and can be considered as a causal prophylactic agent [53, 54]. However, the dose needed for causal prophylaxis is very high and leads to acute intravascular hemolysis. The drug has therefore, never been routinely used for this purpose. Primaquine is rapidly absorbed (t_{max} 2 hours) and rapidly converted to carboxylic acid metabolite. Daily doses of 15 to 45 mg do not alter the pharmacokinetics of primaquine and accumulation of carboxy-primaquine after repeated doses is seen. The adverse effect of intravascular hemolysis occurs with daily doses (15 to 22.5 mg) for 14 days used for radical treatment of *P. vivax* and *P. ovale*. Patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency are particularly susceptible, due mainly to their enzyme deficiency status but not because of the difference in the pharmacokinetics in patients [55]. A dose of 45 mg per week for eight weeks is recommended for patients with G6PD deficiency. The use of primaquine as a gametocytocidal for blocking the transmission of *P. falciparum* malaria at a single dose of 45 mg is considered safe [53, 56].

In a race to combat the ever-increasing multidrug resistant *P. falciparum*, artemisinin, a natural product from the leafy portions of the Chinese medical plant Qinghao (*Artemisia annua* Linn.) and its sesquiterpene trioxane lactone derivatives artesunate, artemether, arteether, and dihydroartemisinin, or the so-called "first-generation artemisinins," have emerged as an effective group of antimalarial drugs [57]. The pharmacokinetics of artemisinin derivatives in various groups of patients when used as a single drug or as combination partner with other antimalarial drugs were investigated at the Hospital for Tropical Diseases, Bangkok, Thailand [45]. The disadvantage of artemisinins when used as monotherapeutic regimens is the relatively high recrudescence rates, varying from 3% to 50% depending on the dose and duration of treatment. This is explained by their pharmacokinetic property of having short half-lives (0.5-3 hours) [44]. In order to achieve radical cure, the drugs need to be given as a 7-day course, and this is associated with poor patient compliance. One solution to this problem is the introduction of ACTs--combination of artemisinins with long half-life antimalarial partners. With ACTs, the duration of treatment can be shortened to only 3 days, which promotes patient adherence, with the exposure of parasites to artemisinin monotherapy is minimized, reducing the likelihood of artemisinin resistance.

Monitoring and surveillance of drug resistance: In view of limited availability of effective antimalarial drugs in the pipeline, continued

monitoring and surveillance of drug therapy for *P. falciparum* and *P. vivax* has been a consistent control programme and research priority. The treatment of uncomplicated multidrug resistant *P. falciparum* malaria in Thailand has been modified regularly during the past 40 years to counter the rapid emergence and spread of drug resistance. The Thai National Malaria Control Guidelines have been revised periodically based on supporting evidence from therapeutic monitoring and surveillance studies for sensitivity of both *P. falciparum* and *P. vivax* in Thailand to first-line treatment. The system includes the monitoring of clinico-parasitological response (*in vivo*) and *in vitro* parasite sensitivity. Artemisinin-resistant malaria was first identified along the Cambodia-Thailand border with results from the surveillance sites showing an increasing failure rate of *P. falciparum* to ACTs (58-60). Decreasing therapeutic efficacy rates in sentinel sites coupled with prolonged parasite clearance time are serious concerns that could indicate the local emergence of *P. falciparum* strains in Thailand that are resistant to artemisinins. It is suspected that this resistant strain already spread from the Thai-Cambodian border to Thailand. A systemic study was conducted by a research group at Thammasat University and definite conclusion of "true resistance" of the *P. falciparum* isolates collected from Mae Sot, Tak province of Thailand (endemic area along Thai-Myanmar border) to artemisinin was confirmed with supporting pharmacokinetics (plasma/blood drug level) information [61]. The parasite clearance was not improved when the dose of artesunate was increased up to 8 mg/kg body weight/day or splitting the dose [62]. Artemisinin resistant P. falciparum is now prevalent across mainland Southeast Asia [63]. Nevertheless, despite the evidence of a low level decline in sensitivity of *P. falciparum* isolates to artemisinins in areas along the Thai-Myanmar border, in view of the limited effective alternative antimalarial drugs, ACTs would be expected to remain the key antimalarial drug for treatment of multidrug resistance *P. falciparum*. ACT has been associated with a reduction in malaria in the migrant population living on the Thai-Myanmar border [64]. Active monitoring and surveillance of clinical efficacy of ACT, including systematic approach for the identification of true artemisinin resistant parasites, is required for appropriate implementation of malaria control policy in this area [55]. Recently, the *PF_1343700 kelch* propeller domain (K13-propeller) and the *in vitro* ring-stage survival assay have been proposed as the valid tools for monitoring of artemisinin-resistant *P. falciparum* isolates [65-67]. For *P. vivax* malaria, although continued *in vitro* and *in vivo* monitoring suggest that the parasite strains in Thailand still retain sensitivity to the first-line drug chloroquine, continuous monitoring and surveillance is required.

Malaria biology/biochemistry: The *in vitro* cultivation system for *P. falciparum* was first established in Thailand at the Department of Biology, Faculty of Science, Chulalongkorn University in 1981. The established system was exploited for investigating basic biological characteristics of *P. falciparum* isolates collected from various malaria endemic areas of Thailand. A large collection of malaria parasites repertoire especially K1 and T9, became available for malaria parasite research worldwide. The collection formed the basis for the WHO Collaborating Centre on the Biological Characterization of Malaria Parasites at Chulalongkorn University in 1984. Over the years, database on susceptibility of *P. falciparum* isolates in Thailand to various drugs (*e.g.*, pyrimethamine, chloroquine, and quinine) have been accumulating. It was shown that the parasite isolates collected from malaria patients often contain a heterogeneous assortment of genetically distinct clones. Susceptibility to antimalarial drugs may therefore vary in cultures of uncloned isolates [68, 69].

The discovery of malarial protease enzyme as a requirement of the malaria parasite for the invasion of human red cells by merozoites of *P. falciparum* was reported by a researcher at the Department of Biochemistry, Faculty of Science, Mahidol University. Highly synchronous cultures of the erythrocyte stages of *P. falciparum* were used to determine the effects of a number of protease inhibitors (Leupeptin, N-tosyl-L-lysylchloromethylketone, and pepstatin) on parasite development and merozoite invasion. The results suggested that a chymotrypsin-like activity of the merozoite is important in the invasion process [70].

Malaria vector: Vector control has played an essential role in the reduction of malaria in the Southeast Asian region and is still indispensable to control malaria in endemic foci. Moreover, reducing transmission intensity is likely to slow the spread of drug resistance. The development of insecticide resistance may also jeopardize the vector control efforts. Hence, knowledge of vector resistance and changing trends of resistance in target species are basic requirements to guide insecticide use in malaria control programmes [71-73].

Species and evolutionary problems of the Anopheles have been thoroughly investigated at the Department of Biology, Faculty of Science, Mahidol University, which significantly contribute to a better understanding of their genetic differentiation and

speciation processes. The discovery of the sibling species complexes provides fundamental knowledge for further extensive studies on their biology (e.g., geographic distributions, bionomics, ecology, physiological adaptation, systematics, and phylogenetic relationships).

A method for heterologous gene expression in *Enterobacter amnigenus*, a potential host for the biological control of mosquito larvae was developed at the Department of Biochemistry, Faculty of Science, Mahidol University [74]. The integrative plasmid is now available to introduce any heterologous DNA into the *E. amnigenus* chromosome for the construction of promoter-probe vectors for the studies of gene regulation, or to construct plasmids suitable for the isolation of secretion signals. Immediate applications of this system will include the expression and secretion of crystal toxins from bacilli for the biological control of mosquito larvae infected with the bacterial host.

Antimalarial drug discovery and development: As antimalarial drug resistance compromises the effective treatment of the disease, there is a pressing need for ongoing drug discovery research that will provide effective, safe, and affordable antimalarial agents. Antimalarial drug development has been severely limited by the lack of interest of pharmaceutical companies in investing on the development of drugs for a disease of disadvantaged populations. Nearly all available antimalarials have been developed through government, including the US Military Research Programmes (chloroquine, primaquine, mefloquine, sulfadoxine-pyrimethamine, and halofantrine), which looked into the accidental identification of efficacy in a natural-product (quinine, and artemisinins) or the identification of antimalarial drugs marketed for other indications (folate antagonists, sulfas, antibiotics, and atovaquone). Until recently, there has been considerable progress in the development of new antimalarials, due in part to the availability of funding mechanisms for drug discovery research by different agencies in the form of public-private partnerships [*e.g.*, WHO/TDR, Medicines for Malaria Venture (MMV), The Bill and Melinda Gates Foundation, The Wellcome Trust, Irish Aid, and the United States Agency for International Development (USAID)]. Pharmaceutical companies like Bayer, GlaxoSmithKline (GSK), Roche, Novatis, have more roles in antimalarial drug development than before.

In the past, the major emphasis in antimalarial drug R&D was placed on the identification of molecular targets, with relatively few reports of novel lead compounds either from natural product sources or from rational design and synthesis. In recent years, with major advances in our understanding of malaria parasite biology coupled with the completion of the malaria genome, exciting opportunities have been presented for target-based antimalarial drug discovery. Malarial dihydrofolatereductase (DHFR) is the target of antifolate antimalarial drugs such as pyrimethamine and cycloguanil, the clinical efficacy of which have been compromised by resistance arising through mutations at various sites on the enzyme. Multiple mutations in *P. falciparum* DHFR (pfDHFR) was shown to emerge from stepwise selection of the single mutant at amino acid residue 108 (S108N) [75]. Further study demonstrated *P. falciparum* dihydrofolate reductase-thymidylate synthase (pfDHFR-TS) as an important target for antimalarial drugs [76]. The National Center for Genetic Engineering and Biotechnology (BIOTEC) research team, in collaboration with researchers from both local and international universities, has successfully developed new antimalarial drug candidate P218 that targets malarial enzyme DHFR and effectively kills both sensitive and resistant parasites. P218 compound was designed based-on 3-dimentional structures of the malarial DHFR in order to circumvent mutations that had led to pyrimethamine resistant, a safe and previously effective drug for treatment of malaria. This is the first novel antimalarial drug-compound discovered and developed in Thailand, to enter preclinical research studies through a joint venture between NSTDA and MMV. In the new phase of NSTDA-MMV joint research venture, the objective will be to gather evidence for proof-of-concept for human use of P218 compound fulfilling international GLP standard. If successful, it will be a great leap forward for the drug research and discovery programme of the country, as this will be Thailand's first example of novel design drug-compound to enter clinical trial. The manufacturing of P218 was scaled-up and batches were produced suitable for formal preclinical safety testing and initial clinical studies in humans. Initial safety testing indicated that P218 has a good safety margin between the toxicity in animals and the predicted effective human dose.

Apart from DHFR, *P. vivax* serine hydroxymethyltransferase (SHMT) was cloned and characterized for being further exploited as a promising dug target [77]. The enzyme that catalyzes the reversible reaction of serine and tetrahydrofolate to glycine and methylenetetrahydrofolate, is one of the three enzymes in dTMP synthesis pathway that is highly active during cell division and has been proposed as a potential chemotherapeutic target in infectious diseases and cancer. The nucleotide and amino acid sequences of SHMT from 12 *P. vivax*

isolates from Thailand revealed limited polymorphisms in three noncoding regions.

Natural-product derived compounds offer one of the promising approaches to chemotherapy. Indigenous plants play an important role in the treatment of many diseases and 80% of the people worldwide are estimated to use herbal remedies. One key feature of natural products is their enormous structural and chemical diversity. They represent a virtually inexhaustible reservoir of molecules, most of which are hardly explored and could constitute lead molecules for new antimalarial drugs, such as quinine (from the Peruvian Cinchona's bark) and artemisinin or qinghaosu (from the Chinese plant *Artemisia annua* Linn.). In endemic countries, accessible treatment against malaria is mainly based on the use of traditional herbal remedies [78]. Thailand is one of the countries in Southeast Asia where antimalarial drug development from natural products is a research area of interests for several research groups. Studies have been undertaken to evaluate the inhibitory effects of various plants extracts or isolated compounds on *P. falciparum* in various *in vitro* and animal models. Examples include mangosteen (*Garcinia mangostana* Linn.: pericarp) [78], plumbago (*Plumbago indica* Linn.) and its isolated compound plumbagin [79], Jun-Par (*Dracaena loureiri* Gagnep.), Hua-Kao-Yen (*Dioscorea membranacea* Pierre.), long pepper (*Piper chaba* Hunt.), nutmeg fruit (*Myristica fragrans* Houtt.), and aromatic ginger (*Kaempferia galanga* Linn.) [80].

Mechanisms of drug resistance and molecular markers of drug resistance: Understanding antimalarial drug resistance at its genetic background helps track and map the emergence and spread of drug resistant malaria. Both *in vivo* and *in vitro* methods for antimalarial drug resistance are not appropriate for large-scale studies by national malaria control programmes. Recent advances in molecular biology enable the identification of molecular markers that are linked to *P. falciparum* and *P. vivax* resistance to a number of antimalarial drugs. Several current molecular analyses of *P. falciparum* and *P. vivax* isolates provide evidence for possible link between the polymorphisms of several candidate genes and resistance of *P. falciparum* isolates in Thailand to commonly used antimalarial drugs. These include the genes encoding sarco/endoplasmic reticulum Ca2+-ATPase orthologue of *P. falciparum* (*pfatp6*), *P. falciparum* multidrug resistance 1 (*pfmdr1*), and *P. falciparum* multidrug resistance protein 1 (*pfmrp1*) [81]. Results from isolates collected from Thailand suggest that *pfmdr1* and *pfmrp1* appear to be the key genes that modulate multidrug resistance in *P. falciparum*. Copy number of *pfmdr1*, the gene which encodes an ATP—binding cassette (ABC) transporter, has been linked with resistance to mefloquine and *pfcrt* is linked to chloroquine resistance. *Pfmdr1* gene copy number is the key molecular marker of resistance of *P. falciparum* isolates in this area to artesunate-mefloquine (one of the ACTs) combination therapy [82].

The mutations in the genes that encode dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) of *P. vivax* Thai isolates were shown to confer resistance to pyrimethamine and sulfadoxine, respectively [83- 85]. DHFR enzyme of *P. vivax* isolates in Thailand was characterized in relation to antifolate resistance [77]. The genes encoding the wild-type and six (five single and one double) mutant DHFR domains of the human malaria parasite, *P. vivax*, were cloned and expressed in *Escherichia coli*. Data on kinetic parameters and inhibitory constant suggest that the wild-type *P. vivax* is susceptible to antimalarial antifolates and that point mutations in the DHFR domain of *P. vivax* are responsible for antifolate resistance. The *P. vivax* multidrug resistance (*pvmdr*) and putative transporter protein (*pvcrt-o*), which are orthologous to *pfmdr1* and *pfcrt* genes, have been identified as chloroquine resistance markers in *P. vivax.* Results from a clinical study with *in vitro* sensitivity assay showed that the mutant alleles of both genes were associated with chloroquine resistance in *P. vivax* isolates in Southeast Asia [86].

Pathophysiology: Malaria causes disease through a number of pathways, which depend to a certain extent on the species. *P. falciparum* produces unique pathological effects due to its manipulation of the host's physiology. Over the last decade, there have been considerable advances in the understanding of the clinical pathophysiology of malaria, principally as a result of detailed clinical and laboratory studies. Host factors contributing to the growth and maturation of various *Plasmodium* species [87] and malaria disease severity [88] were investigated by various groups of Thai researchers. Host factors associated with severe *P. falciparum* malaria were identified. It was found that the release of a variety of toxins triggers the activation of host immune factors that include cytokines, such as tumor necrosis factor (TNF) and pro-inflammatory interleukins, oxygen free radicals, and nitric oxide, which result to the damaging of host endothelium and tissues [89, 90]. Cerebral malaria is one of the most severe manifestations of *P. falciparum* malaria. The sequestration of parasitized red blood cells to brain microvascular endothelium was shown to contribute to the pathophysiology of cerebral malaria [91, 92]. The interaction between malarial parasite molecules

and their receptors on endothelial cells that cause infected red blood cells to stick in small blood vessels of the brain and other organs was investigated by researchers at the Department of Microbiology and Immunology, Faculty of Science, Mahidol University [93]. The highlight of their work is the finding that malaria parasites can present more than one receptor (*e.g., P. falciparum* erythrocyte membrane protein-1: PfEMP-1) on the surface of the infected red blood cell and that infected red blood cells can bind to chondroitin sulfate A. This molecule is an important receptor for adherence in the placenta which might be responsible for severe complications of malarial infection during pregnancy. Peripheral and placental plasma as well as placental tissues were also collected from HIV and malaria co-infected pregnant patients to investigate the influence of HIV infection on cytokine profiles of malaria-infected pregnant individuals.

Host factors associated with treatment response and drug resistance: Treatment outcome following an antimalarial regimen depends on the interplay between the following three major components—host, malarial parasite, and antimalarial drug. "True resistance" must therefore be differentiated from treatment failure due to host and drug-related factors, although in practice, this is rather difficult to accomplish. Inadequately treated malaria could either be a result of the parasite load, or inappropriate prescription. With regards to host factor, the impacts of glucose-6-phosphate dehydrogenase (G6PD) status, and polymorphisms in human drug metabolizing enzymes --cytochrome P450 (CYP), haem oxygenase-1 (HO-1), and erythrocyte polymorphism, to treatment response and malaria disease severity were extensively explore.

G6PD deficiency, the most commonly known enzymopathy, is associated with neonatal jaundice and haemolytic anaemia usually after exposure to certain infections, foods, or medications.

The protective effect of a common G6PD deficiency variant in Southeast Asia-- the G6PD-Mahidol (487A) variant on *P. vivax* and *P. falciparum* infection and disease severity was investigated by a group of researchers at the Faculty of Medicine, Chulalongkorn University [94]. Results showed that G6PD-Mahidol (487A) variant reduces parasite density in patients with *P. vivax* but not *P. falciparum*. This may indicate that *P. vivax* is a driving force behind the strong selective advantage conferred by this mutation. Difference in the prevalence and distribution of G6PD gene variants among the Thai and Burmese populations in different malaria endemic areas were reported by our research group at the Chulabhorn International College of Medicine, Thammasat University [95]. Results suggest that dosage regimen of primaquine for treatment of both *P. falciparum* and *P. vivax* malaria may need to be optimized, based on endemic areas with supporting data on G6PD variants.

Human host genetic polymorphisms in CYP have been shown to contribute significantly to the antimalarial treatment response as seen with proguanil [57, 96]. Artesunate, an artemisinin derivative, which is the main component of ACTs, undergoes rapid and extensive conversion *via* CYP2A6 and, to a lesser extent, CYP2B6, CYP1A1, and CYP1A2. Results from the preliminary study by our research group provide evidence for the possible contribution of CYP2A6 and CYP2B6 genetic polymorphisms on clinico-parasitological treatment response following a three-day artesunate-mefloquine therapy [97].

HO-1 enzyme is proposed as one of the factors that may play a role in pathogenesis including susceptibility and severity of malaria disease. Difference in the expression of HO-1 genotype in different ethnic groups may contribute to different severity of malaria disease. The polymorphism of human HO-1 gene promoter is composed of the single nucleotide polymorphism (SNP) and (GT)n dinucleotide polymorphism which may contribute to the fine tuning of the transcription. Long (GT)n alleles have been found to link to resistance to cerebral malaria [88].

Erythrocyte polymorphisms are common in the Southeast Asian region. Host-dependent artemisinin resistance has been shown in *P. falciparum* infecting α-thalassemic hemoglobin (Hb) H alone (α-thal1/α-thal2) or Hb H with Hb Constant Spring (CS) (Hb H/Hb CS or α-thal1/Hb CS) erythrocytes [98- 101]. These genetically variant erythrocytes are capable of taking up a large portion of artemisinin drugs owing to a higher binding affinity of the drugs to Hb H in α-thalassemic erythrocytes than to Hb A, thereby reducing artemisinin effectiveness [86, 87]. In addition, oxidative stress exerted by erythrocytes deficient in glucose-6-phosphate dehydrogenase (G6PD) [102], has been shown to stimulate ring-stage parasites to upregulate their antioxidant defenses, thereby antagonizing the pro-oxidant activity of artemisinins and delaying parasite clearance by the spleen [103, 104].

Malaria diagnosis: Prompt and accurate diagnosis is critical to the effective management of malaria. The global impact of malaria has spurred interest in developing effective diagnostic strategies not only for resource-limited areas where malaria is a substantial burden on society, but also in developed countries, where malaria diagnostic expertise is often

lacking. Conventional methods include clinical diagnosis by history and physical examination, empirical/syndromic diagnosis (mainly the presence of fever in endemic areas), and use of light microscopy to examine stained peripheral blood smears. Nucleic acid amplification tests play almost no role in malaria diagnosis, as these assays are limited to a few large public health laboratories and are not available commercially. Reliable malaria-diagnostic tests were developed and introduced by different groups of Thai researchers, and some tests are commercially available, for example, latex agglutination assay [105] and cultivation of live malaria parasites [93], post-mortem organ diagnoses by investigating malaria parasites in tissue autopsy (*e.g.*, liver and spleen) [106], kidney [107], and brain [108]. Recently, loop-mediated isothermal amplification (LAMP) method, developed by a research group at AFRIMS for diagnosis of four human malaria parasites, was evaluated on a large scale at a remote clinic in Thailand where malaria is endemic [109]. The technique is claimed to be a simple and inexpensive molecular malaria-diagnostic test that detects the conserved 18S ribosome RNA gene of *P. falciparum*. Results from the study suggest that LAMP is more reliable and useful for routine screening for malaria parasites in regions where vector-borne diseases such as malaria, are endemic. It appears to be easy, sensitive, quick, and lower in cost than polymerase chain reaction (PCR).

Immunology and vaccine trials: The search for an effective vaccine for malaria prevention has progressed slowly during the past decades. One of the major challenges facing vaccine developers is the high level of naturally occurring polymorphism at several of the loci of the genes encoding surface proteins of *P. falciparum* and *P. vivax*. Immunologic studies at AFRIMS have helped to dissect the humoral and cellular immune responses to malaria, as well the immune suppression that is caused by acute falciparum malaria infections [110]. This interdisciplinary research on malaria continues with foci on mosquito biology and identification of new drug prophylaxis regimens; modeled on the Walter Reed Army Institute of Research (WRAIR) falciparum malaria challenge model, a human challenge model for *P. vivax* malaria is planned, to open a new dimension to the evaluation of drugs for treatment of *P. vivax* infection and vaccines.

CONCLUSION

Malaria remains one of the major global health problems in the tropics and subtropics including Southeast Asia. Despite substantial progress towards malaria control and elimination in the Southeast Asia region, important challenges still need to be addressed. Large-scale population movement from highly endemic areas to low endemic zones has contributed significantly to the spread of *P. falciparum* within and beyond the GMS. The move towards malaria elimination in most endemic countries is on course. In addressing these challenges, different types of malaria research that support the control programme has been conducted by regional and international research institutions in each ASEAN country as well as within the collaborating networks. Some of the key achievements of the Thai researchers that significantly contributed to basic knowledge and malaria control and elimination are highlighted in the article.

REFERENCES

1. World Health Organization [WHO]. World malaria report. Geneva: World Health Organization; 2013.
2. Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet. 2004 Mar; 363(9414): 1017-24.
3. Cox-Singh J, Singh B. Knowlesi malaria: newly emergent and of public health importance? Trends Parasitol. 2008 Sep; 24(9): 406-10.
4. Ng OT, Ooi EE, Lee CC, Lee PJ, Ng LC, Pei SW, et al. Naturally acquired human *Plasmodium knowlesi* infection, Singapore. Emerg Infect Dis. 2008 May; 14(5): 814-6.
5. Jiang N, Chang Q, Sun X, Lu H, Yin J, Zhang Z, et al. Co-infections with *Plasmodium knowlesi* and other malaria parasites, Myanmar. Emerg Infect Dis. 2010 Sep; 16(9): 1476-8.
6. Van den Eede P, Van HN, Van Overmeir C, Vythilingam I, Duc TN, Hung le X, et al. Human *Plasmodium knowlesi* infections in young children in central Vietnam. Malar J. 2009; 8: 249. doi: 10.1186/ 1475-2875-8-249.
7. Figtree M, Lee R, Bain L, Kennedy T, Mackertich S, Urban M, et al. *Plasmodium knowlesi* in human, Indonesian Borneo. Emerg Infect Dis. 2010 Apr; 16(4): 672-4.
8. Luchavez J, Espino F, Curameng P, Espina R, Bell D, Chiodini P, et al. Human Infections with *Plasmodium knowlesi*, the Philippines. Emerg Infect Dis. 2008 May; 14(5): 811-3.
9. Jongwutiwes S, Putaporntip C, Iwasaki T, Sata T, Kanbara H. Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. Emerg Infect Dis. 2004 Dec; 10(12): 2211-3.
10. Putaporntip C, Hongsrimuang T, Seethamchai S, Kobasa T, Limkittikul K, Cui L, et al. Differential prevalence of Plasmodium infections and cryptic *Plasmodium knowlesi* malaria in humans in Thailand. J Infect Dis. 2009 Apr; 199(8): 1143-50.
11. Jongwutiwes S, Buppan P, Kosuvin R, Seethamchai S, Pattanawong U, Sirichaisinthop J, et al. *Plasmodium knowlesi* Malaria in humans and macaques, Thailand. Emerg Infect Dis. 2011 Oct; 17(10): 1799-806.

12. Na-Bangchang K, Congpuong K. Current malaria status and distribution of drug resistance in East and Southeast Asia with special focus to Thailand. Tohoku J Exp Med. 2007 Feb; 211(2): 99-113.
13. Na-Bangchang K, Karbwang J. Emerging artemisinin resistance in the border areas of Thailand. Expert Rev Clin Pharmacol. 2013 May; 6(3): 307-22.
14. Ratcliff A, Siswantoro H, Kenangalem E, Wuwung M, Brockman A, Edstein MD, et al. Therapeutic response of multidrug-resistant *Plasmodium falciparum and P. vivax* to chloroquine and sulfadoxine-pyrimethamine in southern Papua, Indonesia. Trans R Soc Trop Med Hyg. 2007 Apr; 101(4): 351-9.
15. Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, Karyana M, et al. Multidrug-resistant *Plasmodium vivax* associated with severe and fatal malaria: a prospective study in Papua, Indonesia. PLoS Med. 2008 Jun; 5(6): e128. doi: 10.1371/journal.pmed. 0050128.
16. Presiden's Malaria Initiativ Greater Mekong Subregion Malaria Operational Plan FY 2015. Available from http://pmi.gov/countries/mops/fy12/mekong_mop_fy12. pdf
17. Elyazar IR, Gething PW, Patil AP, Rogayah H, Sariwati E, Palupi NW, et al. *Plasmodium vivax* malaria endemicity in Indonesia in 2010. PLoS One. 2012; 7(5): e37325. doi: 10.1371/journal.pone.0037325
18. Tasanor O, Ruengweerayut R, Sirichaisinthop J, Congpuong K, Wernsdorfer WH, Na-Bangchang K. Clinical-parasitological response and *in-vitro* sensitivity of *Plasmodium vivax* to chloroquine and quinine on the western border of Thailand. Trans R Soc Trop Med Hyg. 2006 May; 100(5): 410-8.
19. Vijaykadga S, Rojanawatsirivej C, Cholpol S, Phoungmanee D, Nakavej A, Wongsrichanalai C. In vivo sensitivity monitoring of mefloquine monotherapy and artesunate-mefloquine combinations for the treatment of uncomplicated falciparum malaria in Thailand in 2003. Trop Med Int Health. 2006 Feb; 11(2): 211-9.
20. Muhamad P, Ruengweerayut R, Chacharoenkul W, Rungsihirunrat K, Na-Bangchang K. Monitoring of clinical efficacy and *in vitro* sensitivity of *Plasmodium vivax* to chloroquine in area along Thai Myanmar border during 2009-2010. Malar J. 2011; 10: 44. doi: 10.1186/ 1475-2875-10-44.
21. Tan-ariya P, Na-Bangchang K, Tin T, Limpaibul L, Brockelman CR, Karbwang J. Clinical response and susceptibility *in vitro* of *Plasmodium vivax* to the standard regimen of chloroquine in Thailand. Trans R Soc Trop Med Hyg. 1995 Jul-Aug; 89(4): 426-9.
22. Congpuong K, Na-Bangchang K, Thimasarn K, Tasanor U, Wernsdorfer WH. Sensitivity of *Plasmodium vivax* to chloroquine in Sa Kaeo Province, Thailand. Acta Trop. 2002 Aug; 83(2): 117-21.
23. Ministry of Health, Department of Disease Control, Bureau of Vector Borne Diseases. National strategic plan for malaria control and elimination in Thailand. Ministry of Health; 2011.
24. Schwartz IK, Lackritz EM, Patchen LC. Chloroquine-resistant *Plasmodium vivax* from Indonesia. N Engl J Med. 1991 Mar; 324(13): 927. doi: 10.1056/NEJM 199103283241317
25. Rieckmann KH, Davis DR, Hutton DC. *Plasmodium vivax* resistance to chloroquine? Lancet. 1989 Nov; 2(8673): 1183-4.
26. Schuurkamp GJ, Spicer PE, Kereu RK, Bulungol PK. A mixed infection of vivax and falciparum malaria apparently resistant to 4-aminoquinoline: a case report. Trans R Soc Trop Med Hyg. 1989 Sep-Oct; 83(5): 607-8.
27. Whitby M, Wood G, Veenendaal JR, Rieckmann K. Chloroquine-resistant *Plasmodium vivax*. Lancet. 1989 Dec; 2(8676): 1395. doi:10.1016/S0140-6736(89) 92002-3.
28. Collignon P. Chloroquine resistance in *Plasmodium vivax*. J Infect Dis. 1991 Jul; 164(1): 222-3.
29. Baird JK, Basri H, Purnomo, Bangs MJ, Subianto B, Patchen LC, et al. Resistance to chloroquine by *Plasmodium vivax* in Irian Jaya, Indonesia. Am J Trop Med Hyg. 1991 May; 44(5): 547-52.
30. Murphy GS, Basri H, Purnomo, Andersen EM, Bangs MJ, Mount DL, et al. Vivax malaria resistant to treatment and prophylaxis with chloroquine. Lancet. 1993 Jan; 341(8837): 96-100.
31. Fryauff DJ, Soekartono, Tuti S, Leksana B, Suradi, Tandayu S, et al. Survey of resistance *in vivo* to chloroquine of *Plasmodium falciparum* and *P. vivax* in North Sulawesi, Indonesia. Trans R Soc Trop Med Hyg. 1998 Jan-Feb; 92(1): 82-3.
32. Sumawinata IW, Bernadeta, Leksana B, Sutamihardja A, Purnomo, Subianto B, et al. Very high risk of therapeutic failure with chloroquine for uncomplicated *Plasmodium falciparum* and *P. vivax* malaria in Indonesian Papua. Am J Trop Med Hyg. 2003 Apr; 68(4): 416-20.
33. Marlar T, Myat Phone K, Aye Yu S, Khaing Khaing G, Ma S, Myint O. Development of resistance to chloroquine by *Plasmodium vivax* in Myanmar. Trans R Soc Trop Med Hyg. 1995 May-Jun; 89(3): 307-8.
34. Myat Phone K, Myint O, Myint L, Thaw Z, Kyin Hla A, Nwe Nwe Y. Emergence of chloroquine-resistant *Plasmodium vivax* in Myanmar (Burma). Trans R Soc Trop Med Hyg. 1993; 87: 687.
35. Guthmann JP, Pittet A, Lesage A, Imwong M, Lindegardh N, Min Lwin M, et al. *Plasmodium vivax* resistance to chloroquine in Dawei, southern Myanmar. Trop Med Int Health. 2008 Jan; 13(1): 91-8.
36. Phan GT, de Vries PJ, Tran BQ, Le HQ, Nguyen NV, Nguyen TV, et al. Artemisinin or chloroquine for blood stage *Plasmodium vivax* malaria in Vietnam. Trop Med Int Health. 2002 Oct; 7(10): 858-64.
37. World Health Organization [WHO]. Containment of malaria multi-drug resistance on the Cambodia-Thailand border. Geneva: World Health Organization; 2007.
38. National Malaria Control Programme. Round 7: Partnership towards malaria reduction in migrants and conflict-affected populations in Thailand. Thailand: Global Fund to Fight AIDS, Tuberculosis and Malaria; 2007.
39. Andersen F, Douglas NM, Bustos D, Galappaththy G, Qi G, Hsiang MS, et al. Trends in malaria research in 11 Asian Pacific countries: an analysis of peer-reviewed publications over two decades. Malar J. 2011; 10: 131. doi: 10.1186/1475-2875-10-131.

40. Taylor WR. Malaria research in the Greater Mekong Subregion: an overview. Southeast Asian J Trop Med Public Health. 2013; 44(Suppl 1): 231-48; discussion 306-7.
41. Harinasuta T, Suntharasamai P, Viravan C. Chloroquine-resistant falciparum malaria in Thailand. Lancet. 1965 Oct; 2(7414): 657-60.
42. Malikul S. The current situation of the anti-malaria programme in Thailand. Southeast Asian J Trop Med Public Health. 1988 Sep; 19(3): 355-9.
43. Boudreau EF, Webster HK, Pavanand K, Thosingha L. Type II mefloquine resistance in Thailand. Lancet. 1982 Dec; 320(8311): 1335. doi:10.1016/S0140-6736(82) 91532-X.
44. Karbwang J, Na-Bangchang K. Clinical pharmacology of mefloquine. In: Karbwang J, Wernsdorfer WH, eds. Clinical pharmacology of antimalarials. Bangkok: Clinical Pharmacology Unit, Faculty of Tropical Medicine, Mahidol University; 1993. p.167-224.
45. Karbwang J, Na-Bangchang K. Clinical pharmacology of artemisinin compounds. In: Karbwang J, Wernsdorfer WH, eds. Clinical pharmacology of antimalarials. Bangkok: Clinical Pharmacology Unit, Faculty of Tropical Medicine, Mahidol University; 1993. p. 263-304.
46. Karbwang J, Na-Bangchang K. Clinical pharmacology of halofantrine. In: Karbwang J, Wernsdorfer WH, eds. Clinical pharmacology of antimalarials. Bangkok: Clinical Pharmacology Unit, Faculty of Tropical Medicine, Mahidol University; 1993. p.225-61.
47. Na-Bangchang K, Karbwang J. Clinical pharmacology of quinine. In: Karbwang J, Wernsdorfer WH, eds. Clinical pharmacology of antimalarials. Bangkok: Clinical Pharmacology Unit, Faculty of Tropical Medicine, Mahidol University; 1993. p.127-65.
48. Wattanagoon Y, Phillips RE, Warrell DA, Silamut K, Looareesuwan S, Nagachinta B, et al. Intramuscular loading dose of quinine for falciparum malaria: pharmacokinetics and toxicity. Br Med J (Clin Res Ed). 1986 Jul; 293(6538): 11-3.
49. Karbwang J, Molunto P, Bunnag D, Harinasuta T. Plasma quinine levels in patients with falciparum malaria when given alone or in combination with tetracycline with or without primaquine. Southeast Asian J Trop Med Public Health. 1991 Mar; 22(1): 72-6.
50. Karbwang J, Bangchang KN, Bunnag D, Harinasuta T. Pharmacokinetics and pharmacodynamics of mefloquine in Thai patients with acute falciparum malaria. Bull World Health Organ. 1991; 69(2): 207-12.
51. Karbwang J, Na-Bangchang K. Clinical application of mefloquine pharmacokinetics in the treatment of *P falciparum* malaria. Fundam Clin Pharmacol. 1994; 8(6): 491-502.
52. Karbwang J, Na Bangchang K, Bunnag D, Harinasuta T, Laothavorn P. Cardiac effect of halofantrine. Lancet. 1993 Aug; 342(8869): 501-4.
53. White NJ. Primaquine to prevent transmission of falciparum malaria. Lancet Infect Dis. 2013 Feb; 13(2): 175-81.
54. Pasaribu AP, Chokejindachai W, Sirivichayakul C, Tanomsing N, Chavez I, Tjitra E, et al. A randomized comparison of dihydroartemisinin-piperaquine and artesunate-amodiaquine combined with primaquine for radical treatment of vivax malaria in Sumatera, Indonesia. J Infect Dis. 2013 Dec; 208(11): 1906-13.
55. Bangchang KN, Songsaeng W, Thanavibul A, Choroenlarp P, Karbwang J. Pharmacokinetics of primaquine in G6PD deficient and G6PD normal patients with vivax malaria. Trans R Soc Trop Med Hyg. 1994 Mar-Apr; 88(2): 220-2.
56. Hanboonkunupakarn B, Ashley EA, Jittamala P, Tarning J, Pukrittayakamee S, Hanpithakpong W, et al. Open-label crossover study of primaquine and dihydroartemisinin-piperaquine pharmacokinetics in healthy adult thai subjects. Antimicrob Agents Chemother. 2014 Dec; 58(12): 7340-6.
57. Visser BJ, van Vugt M, Grobusch MP. Malaria: an update on current chemotherapy. Expert Opin Pharmacother. 2014 Oct; 15(15): 2219-54.
58. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM, et al. Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med. 2008 Dec; 359(24): 2619-20.
59. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2009 Jul; 361(5): 455-67.
60. Amaratunga C, Sreng S, Suon S, Phelps ES, Stepniewska K, Lim P, et al. Artemisinin-resistant Plasmodium falciparum in Pursat province, western Cambodia: a parasite clearance rate study. Lancet Infect Dis. 2012 Nov; 12(11): 851-8.
61. Na-Bangchang K, Muhamad P, Ruaengweerayut R, Chaijaroenkul W, Karbwang J. Identification of resistance of Plasmodium falciparum to artesunate-mefloquine combination in an area along the Thai-Myanmar border: integration of clinico-parasitological response, systemic drug exposure, and in vitro parasite sensitivity. Malar J. 2013; 12: 263. doi: 10.1186/1475-2875-12-263.
62. Das D, Tripura R, Phyo AP, Lwin KM, Tarning J, Lee SJ, et al. Effect of high-dose or split-dose artesunate on parasite clearance in artemisinin-resistant falciparum malaria. Clin Infect Dis. 2013 Mar; 56(5): e48-58.
63. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med. 2014 Jul; 371(5): 411-23.
64. Carrara VI, Lwin KM, Phyo AP, et al. Malaria burden and artemisinin resistance in the mobile and migrant population on the Thai-Myanmar border, 1999-2011: an observational study. PLoS Med. 2013; 10(3): e1001398. doi: 10.1371/journal.pmed.1001398.
65. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature. 2014 Jan 2; 505(7481): 50-5.
66. Straimer J, Gnadig NF, Witkowski B, Amaratunga C, Duru V, Ramadani AP, et al. K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. Science. 2014. doi: 10.1126/science. 1260867.
67. Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, et al. Independent emergence of

artemisinin resistance mutations among plasmodium falciparum in Southeast Asia. J Infect Dis. 2014. doi: 10.1093/infdis/jiu491

68. Thaithong S. Clones of different sensitivities in drug-resistant isolates of *Plasmodium falciparum*. Bull World Health Organ. 1983; 61(4): 709-12.
69. Thaithong S, Beale GH, Chutmongkonkul M. Variability in drug susceptibility amongst clones and isolates of *Plasmodium falciparum*. Trans R Soc Trop Med Hyg. 1988; 82(1): 33-6.
70. Dejkriengkraikhul P, Wilairat P. Requirement of malarial protease in the invasion of human red cells by merozoites of *Plasmodium falciparum*. Z Parasitenkd. 1983; 69(3): 313-7.
71. Rattanarithikul R, Harrison BA, Harbach RE, Panthusiri P, Coleman RE, Panthusiri P. Illustrated keys to the mosquitoes of Thailand. IV. Anopheles. Southeast Asian J Trop Med Public Health. 2006; 37(Suppl 2): 1-128.
72. Surachetpong W, Singh N, Cheung KW, Luckhart S. MAPK ERK signaling regulates the TGF-beta1-dependent mosquito response to *Plasmodium falciparum*. PLoS Pathog. 2009 Apr; 5(4): e1000366. doi: 10.1371/journal.ppat.1000366
73. Surachetpong W, Pakpour N, Cheung KW, Luckhart S. Reactive oxygen species-dependent cell signaling regulates the mosquito immune response to *Plasmodium falciparum*. Antioxid Redox Signal. 2011 Mar 15; 14(6): 943-55.
74. Nantapong N, Tanapongpipat S, Cole J, Panyim S. Development of a method for heterologous gene expression in *Enterobacter amnigenus*, a potential host for the biological control of mosquito larvi. J Microbiol Methods. 2002 May; 49(3): 329-34.
75. Sirawaraporn W, Sathitkul T, Sirawaraporn R, Yuthavong Y, Santi DV. Antifolate-resistant mutants of *Plasmodium falciparum* dihydrofolate reductase. Proc Natl Acad Sci USA. 1997 Feb; 94(4): 1124-9.
76. Yuvaniyama J, Chitnumsub P, Kamchonwongpaisan S, Vanichtanankul J, Sirawaraporn W, Taylor P, et al. Insights into antifolate resistance from malarial DHFR-TS structures. Nat Struct Biol. 2003 May; 10(5): 357-65.
77. Leartsakulpanich U, Kongkasuriyachai D, Imwong M, Chotivanich K, Yuthavong Y. Cloning and characterization of *Plasmodium vivax* serine hydroxymethyltransferase. Parasitol Int. 2008 Jun; 57(2): 223-8.
78. Bunyong R, Chaijaroenkul W, Plengsuriyakarn T, Na-Bangchang K. Antimalarial activity and toxicity of *Garcinia mangostana* Linn. Asian Pacific Journal of Tropical Medicine. 2014; 7(9): 693-8.
79. Sumsakul W, Plengsuriyakarn T, Chaijaroenkul W, Viyanant V, Karbwang J, Na-Bangchang K. Antimalarial activity of plumbagin in vitro and in animal models. BMC Complement Altern Med. 2014; 14: 15. doi: 10.1186/1472-6882-14-15.
80. Thiengsusuk A, Chaijaroenkul W, Na-Bangchang K. Antimalarial activities of medicinal plants and herbal formulations used in Thai traditional medicine. Parasitol Res. 2013 Apr; 112(4): 1475-81.
81. Picot S, Olliaro P, de Monbrison F, Bienvenu AL, Price RN, Ringwald P. A systematic review and meta-analysis of evidence for correlation between molecular markers of parasite resistance and treatment outcome in falciparum malaria. Malar J. 2009; 8: 89. doi: 10.1186/1475-2875-8-89.
82. Na-Bangchang K, Ruengweerayut R, Mahamad P, Ruengweerayut K, Chaijaroenkul W. Declining in efficacy of a three-day combination regimen of mefloquine-artesunate in a multi-drug resistance area along the Thai-Myanmar border. Malar J. 2010; 9: 273. doi: 10.1186/1475-2875-9-273.
83. Rungsihirunrat K, Sibley CH, Mungthin M, Na-Bangchang K. Geographical distribution of amino acid mutations in *Plasmodium vivax* DHFR and DHPS from malaria endemic areas of Thailand. Am J Trop Med Hyg. 2008 Mar; 78(3): 462-7.
84. Hawkins VN, Joshi H, Rungsihirunrat K, Na-Bangchang K, Sibley CH. Antifolates can have a role in the treatment of *Plasmodium vivax*. Trends Parasitol. 2007 May; 23(5): 213-22.
85. Hawkins VN, Auliff A, Prajapati SK, Rungsihirunrat K, Hapuarachchi HC, Maestre A, et al. Multiple origins of resistance-conferring mutations in *Plasmodium vivax* dihydrofolate reductase. Malar J. 2008; 7: 72. doi: 10.1186/1475-2875-7-72.
86. Suwanarusk R, Russell B, Chavchich M, Chalfein F, Kenangalem E, Kosaisavee V, et al. Chloroquine resistant Plasmodium vivax: in vitro characterisation and association with molecular polymorphisms. PLoS One. 2007; 2(10): e1089. doi: 10.1371/journal.pone.0001089
87. Pattanapanyasat K, Sratongno P, Chimma P, Chitjamnongchai S, Polsrila K, Chotivanich K. Febrile temperature but not proinflammatory cytokines promotes phosphatidylserine expression on *Plasmodium falciparum* malaria-infected red blood cells during parasite maturation. Cytometry A. 2010; 77 (6): 515-23.
88. Kuesap J, Hirayama K, Kikuchi M, Ruangweerayut R, Na-Bangchang K. Study on association between genetic polymorphisms of haem oxygenase-1, tumour necrosis factor, cadmium exposure and malaria pathogenicity and severity. Malar J. 2010; 9: 260. doi: 10.1186/1475-2875-9-260.
89. Ubalee R, Suzuki F, Kikuchi M, Tasanor O, Wattanagoon Y, Ruangweerayut R, et al. Strong association of a tumor necrosis factor-α promoter allele with cerebral malaria in Myanmar. Tissue Antigens. 2001 Dec; 58(6): 407-10.
90. Udomsangpetch R, Chivapat S, Viriyavejakul P, Riganti M, Wilairatana P, Pongponratin E, et al. Involvement of cytokines in the histopathology of cerebral malaria. Am J Trop Med Hyg. 1997 Nov; 57(5): 501-6.
91. Udomsangpetch R, Sueblinvong T, Pattanapanyasat K, Dharmkrong-at A, Kittikalayawong A, Webster HK. Alteration in cytoadherence and rosetting of *Plasmodium falciparum*-infected thalassemic red blood cells. Blood. 1993 Dec; 82(12): 3752-9.
92. Udomsangpetch R, Pipitaporn B, Silamut K, Pinches R, Kyes S, Looareesuwan S, et al. Febrile temperatures induce cytoadherence of ring-stage *Plasmodium falciparum*-infected erythrocytes. Proc Natl Acad Sci USA. 2002 Sep; 99(18): 11825-9.
93. Chaiyaroj SC, Angkasekwinai P, Buranakiti A,

Looareesuwan S, Rogerson SJ, Brown GV. Cytoadherence characteristics of *Plasmodium falciparum* isolates from Thailand: evidence for chondroitin sulfate a as a cytoadherence receptor. Am J Trop Med Hyg. 1996 Jul; 55(1): 76-80.

94. Louicharoen C, Patin E, Paul R, Nuchprayoon I, Witoonpanich B, Peerapittayamongkol C, et al. Positively selected G6PD-Mahidol mutation reduces *Plasmodium vivax* density in Southeast Asians. Science. 2009 Dec; 326(5959): 1546-9.
95. Phompradit P, Kuesap J, Chaijaroenkul W, Rueangweerayut R, Hongkaew Y, Yamnuan R, et al. Prevalence and distribution of glucose-6-phosphate dehydrogenase (G6PD) variants in Thai and Burmese populations in malaria endemic areas of Thailand. Malar J. 2011; 10: 368. doi: 10.1186/1475-2875-10-368.
96. Na-Bangchang K, Karbwang J. Current status of malaria chemotherapy and the role of pharmacology in antimalarial drug research and development. Fundam Clin Pharmacol. 2009 Aug; 23(4): 387-409.
97. Phompradit P, Muhamad P, Cheoymang A, Na-Bangchang K. Preliminary investigation of the contribution of CYP2A6, CYP2B6, and UGT1A9 polymorphisms on artesunate-mefloquine treatment response in Burmese patients with *Plasmodium falciparum* malaria. Am J Trop Med Hyg. 2014 Aug; 91(2): 361-6.
98. Yuthavong Y, Butthep P, Bunyaratvej A, Fucharoen S. Decreased sensitivity of artesunate and chloroquine of *Plasmodium falciparum* infecting hemoglobin H and/or hemoglobin constant spring erythrocytes. J Clin Invest. 1989 Feb; 83(2): 502-5.
99. Kamchonwongpaisan S, Chandra-ngam G, Avery MA, Yuthavong Y. Resistance to artemisinin of malaria parasites *(Plasmodium falciparum)* infecting alpha-thalassemic erythrocytes *in vitro*. Competition in drug accumulation with uninfected erythrocytes. J Clin Invest. 1994 Feb; 93(2): 467-73.
100. Vattanaviboon P, Wilairat P, Yuthavong Y. Binding of dihydroartemisinin to hemoglobin H: role in drug accumulation and host-induced antimalarial ineffectiveness of α-thalassemic erythrocytes. Mol Pharmacol. 1998 Mar; 53(3): 492-6.
101. Charoenteeraboon J, Kamchonwongpaisan S, Wilairat P, Vattanaviboon P, Yuthavong Y. Inactivation of artemisinin by thalassemic erythrocytes. Biochem Pharmacol. 2000 Jun; 59(11): 1337-44.
102. Laosombat V, Sattayasevana B, Chotsampancharoen T, Wongchanchailert M. Glucose-6-phosphate dehydrogenase variants associated with favism in Thai children. Int J Hematol. 2006 Feb; 83(2): 139-43.
103. Chotivanich K, Udomsangpetch R, Dondorp A, Williams T, Angus B, Simpson JA, et al. The mechanisms of parasite clearance after antimalarial treatment of *Plasmodium falciparum* malaria. J Infect Dis. 2000 Aug; 182(2): 629-33.
104. Chotivanich K, Udomsangpetch R, McGready R, Proux S, Newton P, Pukrittayakamee S, et al. Central role of the spleen in malaria parasite clearance. J Infect Dis. 2002 May; 185(10): 1538-41.
105. Polpanich D, Tangboriboonrat P, Elaissari A, Udomsangpetch R. Detection of malaria infection via latex agglutination assay. Anal Chem. 2007 Jun 15; 79(12): 4690-5.
106. Prommano O, Chaisri U, Turner GD, Wilairatana P, Ferguson DJ, Viriyavejakul P, et al. A quantitative ultrastructural study of the liver and the spleen in fatal falciparum malaria. Southeast Asian J Trop Med Public Health. 2005 Nov; 36(6): 1359-70.
107. Nguansangiam S, Day NP, Hien TT, Mai NT, Chaisri U, Riganti M, et al. A quantitative ultrastructural study of renal pathology in fatal *Plasmodium falciparum* malaria. Trop Med Int Health. 2007 Sep; 12(9): 1037-50.
108. Sachanonta N, Medana IM, Roberts R, Jones M, Day NP, White NJ, et al. Host vascular endothelial growth factor is trophic for Plasmodium falciparum-infected red blood cells. Asian Pac J Allergy Immunol. 2008 Mar; 26(1): 37-45.
109. Sattabongkot J, Tsuboi T, Han ET, Bantuchai S, Buates S. Loop-mediated isothermal amplification assay for rapid diagnosis of malaria infections in an area of endemicity in Thailand. J Clin Microbiol. 2014 May; 52(5): 1471-7.
110. Brown A, Nitayaphan S. The Armed Forces Research Institute of Medical Sciences: five decades of collaborative medical research. Southeast Asian J Trop Med Public Health. 2011 May; 42(3): 477-90.

LIST OF ABBREVIATIONS

AEC	Asian Economic Community
AFRIMS	Armed Forces Research Institute of Medical Sciences
ASEAN	Association of Southeast Asian Nations
ASEAN-NDI	ASEAN Network for Drugs, Diagnostics, Vaccines and Traditional Medicines Innovation
APMEN	Asia Pacific Malaria Elimination Network
ATCs	Artemisinin Combination Therapies
BIOTEC	National Center for Genetic Engineering and Biology
CS	(Hemoglobin) Constant Spring
CYP	cytochrome P450
GMS	Greater Mekong Sub-region
G6PD	Glucose-6-phosphate dehydrogenase
GSK	GlaxoSmithKline

Hb	Hemoglobin
HO-1	Hemeoxygenase-1
IRS	Indoor residual spraying
LAMP	Loop-mediated isothermal amplification
MBDS	Mekong Basin Disease Surveillance
MMP	Mekong Malaria Porgramme
MORU	Mahidol Oxford Tropical Medicine Research Unit
NSTDA	National Science and Technology Development Agency
PCR	Polymerase chain reaction
P. falciparum	*Plasmodium falciparum*
P. vivax	*Plasmodium vivax*
P. ovale	*Plasmodium ovale*
P. malariae	*Plasmodium malariae*
P. knowlesi	*Plasmodium knowlesi*
pfmdr1	*Plasmodium falciparum* multidrug resistance 1
pfatp6	*Plasmodium falciparum* Ca^{2+}-ATPase
R&D	Research and development
SEARN	South East Asia Research Netowork
SEMEO TROPMED	Southeast Asian Ministers of Education Organization Tropical Medicine
SHMT	Serine hydroxymethyltransferase
SNP	Single nucleotide polynorphism
TNF	Tumor necrosis factor
UNESCO	United Nations Educational, Scientific and Cultural Organization
USAID	United States Agency for International Development
WHO/TDR	World Health Organization/Tropical Disease Research
WRAIR	Walter Reed Army Institute of Research