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Malarial pathogenesis and interventions in Kelch mediated Artemisinin resistance in Plasmodium falciparum

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MALARIAL PATHOGENESIS AND INTERVENTIONS IN KELCH MEDIATED ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM*

by

KEERTHANA PITTALA

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Approved by

First Reader _____

Brigitte Ritter, Ph.D.
Assistant Professor of Biochemistry

Second Reader _____

Karen Symes, Ph.D.
Associate Professor of Biochemistry

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ABSTRACT

Malaria, a parasitic disease, was commonly associated with third world countries, with the highest mortality in nations in Sub-Saharan Africa and Asia. But, travel increases the risk of spread to more temperate regions, such as Western Europe and the United States where Malaria has been successfully eradicated. In the past 40 years, with a better understanding of the mosquito vector and the parasite itself, advancements in treatment and containment have been made.

Understanding the parasite as well as its pathogenesis is vital in formulating effective treatments. Following the incidences of *Plasmodium falciparum, knowlesi, vivax, malaria, ovale,* and less commonly *cynomolgi* and *simium* over time as well as region helps to better illuminate the methods of Malarial transmission, interplay with environmental factors, and methods of treatment. While each species of parasite is similar in terms of mode of infection, they differ slightly when considering incubation periods and diagnostic and treatment techniques.

Many drugs have been developed to treat Malaria and include Chloroquine, Primaquine and derivatives of Artemisinin. While the discovery of these drugs was a significant breakthrough that dramatically reduced incidence and deaths caused by

Malaria, improper administration of treatment has led to a recent increase in strains of the parasite which have developed drug resistance to Artemisinin Combination Therapies (ACT's). Of these species, *P. falciparum* and *P. vivax*, the most common causes of malaria, are also so far the only species to have developed drug resistance. The goal of this thesis is to explore popular interventions, both drug and public health based, and how research focus has now shifted to better understanding the mechanism of parasitic drug resistance, specifically linked to mutations found in the Kelch protein in *P. Falciparum*. The recent findings about Kelch mutations pave the way towards addressing the growing problem of anti-Malarial resistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACT ..... Artemisinin-based Combination Therapy

AIDS ..... Acquired Deficiency Syndrome

AL ..... Artemether + Lumefantrine

ASAQ ..... Artesunate Amodiaquine

CSP ..... Circumsporozoite Protein

DDT ..... dichloro-diphenyl-trichloroethane

DP, DHAPQ ..... Dihydroartemisinin-Piperaquine

G6PD ..... Glucose-6-Phosphate Deficiency

GMEP ..... Global Malaria Eradication Program

HIV ..... Human Immunodeficiency Virus

IPT ..... Intermittent Preventive Treatment

IPTc ..... Intermittent Preventive Treatment in Children

IPTi ..... Intermittent Preventive Treatment in Infants

IPTp ..... Intermittent Preventive Treatment of Malaria during Pregnancy

ITN ..... Insecticide-Treated Bed Nets

K13 ..... Kelch Protein on Chromosome 13

Pfcrt ..... *P. falciparum* chloroquine resistance transporter

PfMDR ..... *P. falciparum* multidrug resistant proteins

RAS ..... Radiation Attenuated Sporozoites

SE Asia ..... Southeast Asia

SMC ..... Seasonal Malaria Chemoprevention

SP+AQ ..... Sulfadoxine-Pyrimethamine + Amodiaquine

UPR.....Unfolded Protein Response

INTRODUCTION

Malaria is a treatable, preventable, parasitic disease with a high mortality rate and faces many obstacles when considering complete eradication of the disease. Over 219 million cases were reported in 2017 which was an increase from the 217 million cases reported the year before[1]. Efforts to address the burden of this historically devastating disease were the focus of many public health campaigns. The Global Malaria Eradication Program (GMEP), launched in 1955, sought to eradicate malaria through the use of dichloro-diphenyl-trichloroethane (DDT), an insecticide. Through this initiative Malaria, specifically caused by *P. falciparum* and *P. vivax*, was successfully eradicated in 37 countries with the use of drugs and DDT[2]. While total eradication was found to be possible, lack of funding and necessary infrastructure halted the GMEP's efforts. Worsened by states of poverty, the number of Malaria cases remain highest in Sub-Saharan Africa, especially Niger, as well as Asia, predominantly in India. In 2016, almost

Table 1. Estimated Malaria Death by WHO Region. 2015[2]

	Number of cases (000's)							
	AFR	AMR	EMR	EUR	SEAR	WPR	World	Outside sub-Saharan Africa
Lower	131 000	500	2 400	0	13 300	1 000	148 000	16 300
Estimated total	**191 000**	**800**	**3 800**	**0**	**14 400**	**1 200**	**212 000**	**18 100**
Upper	258 000	1 200	7 500	0	35 200	2 200	304 000	40 300
Lower	300	400	1100	0	3 400	500	6 600	5 800
Estimated *P. vivax*	**1 000**	**500**	**1 400**	**0**	**4 900**	**700**	**8 500**	**7 400**
Upper	2 100	800	1 700	0	6 800	900	10 800	9 300
% cases *P. vivax*	1%	69%	35%		34%	58%	4%	41%

AFR, WHO African Region; AMR, WHO Region of the Americas; EMR, WHO Eastern Mediterranean Region; SEAR, WHO South-East Asia Region; WPR, WHO Western Pacific Region

90% of cases recorded worldwide were found in Africa and resulted in 445,000 deaths worldwide[3–5] (Table 1). Groups at highest risk of infection include infants, children, pregnant women, and immunologically compromised individuals. Malarial infections often coincide with HIV and AIDS, diseases that are also predominantly associated with areas in which Malaria is endemic [5].

Treatment efforts for Malaria are two-fold: drugs which target the parasite and environmental interventions which target the mosquito vectors. Drug-based treatment includes various drugs that are administered upon diagnosis of Malaria, chemoprevention, and Malarial vaccines[6]. Drug development over the years has yielded multiple options for treatment including Quinine, Chloroquine, Artemisinin (and its derivatives), as well as others. While these drugs are administered after infection, there is also a possibility of preventative drug administration that would decrease development of Malaria all together or at least prevent progression into severe Malaria. The effectiveness of Chemoprevention is still being studied but may be a solution to reducing the mortality of Malaria[6]. Vaccines are also still under development and have yet to gain mainstream approval or use[5]. In the case of both chemoprevention and vaccines, the cost, benefits, and side effects of treatment play a major role in hindering a wider use of these interventions[5]. Efforts to decrease exposure to mosquitoes carrying the parasite include insecticide treated nets and spraying insecticides indoors. These environmental efforts combined with medical treatment has reduced morality significantly to the 445,000 deaths as of 2016[3].

Pathogenesis

Understanding the parasitic lifecycle has better illuminated points of intervention for drugs. Malarial parasites are transmitted from mosquito to human and vice versa; however, *P. Knowlesi*, a less common species, is an exception in terms of the vector and is transmitted from primates, specifically Macaques, to mosquitoes and then to humans[7,8]. The development of vaccines and drugs concentrates specifically on targeting *P. falciparum* because of its prevalence. In recent years, *P. falciparum* has developed the most robust drug resistance and is found in pan-tropical regions of Sub-Saharan Africa and South east (SE) Asia, see Table 2.

Table 2: **Geographical Distribution, Prevalence, Lethality and Drug Resistance Risk of the Human Malaria Parasites** (Adapated from Doolan et al. 2009)

Species	Range	Prevalence	Lethality risk	Drug resistance risk
P. falciparum	Pan-tropical	High	High	High
P. vivax	Pan-tropical, temperate	High	High (?)	High
P. malariae	Tropical	Low, focal	Low	Low
P. ovale	West Africa, Southeast Asia	Rare	Low	Low
P. knowlesi	Southeast Asia	Rare	High (?)	Low

Initially, gametophytes of the parasite in the female *Anopheles* mosquito slowly develop into sporozoites, a motile stage of the parasitic lifecycle. The sporozoites are passed from the mosquito vector to humans through a bite. These sporozoites first travel to the liver and begin to undergo mitosis. During an incubation period of 12-20 days, the sporozoites mature into schizonts which then rupture releasing merozoites into the bloodstream. Entry of these merozoites into the bloodstream causes the onset of typical Malarial symptoms such as fever, nausea, headaches, muscle pain and fatigue. The

merozoites invade red blood cells and become trophozoites resulting in ring shaped figures that can be seen in red blood cells through light microscopy, this stage is aptly named the ring stage, as shown in Figures 1 and 2[9,10]. After progression through the ring stage, more schizonts are formed within the red blood cells. These rupture once again

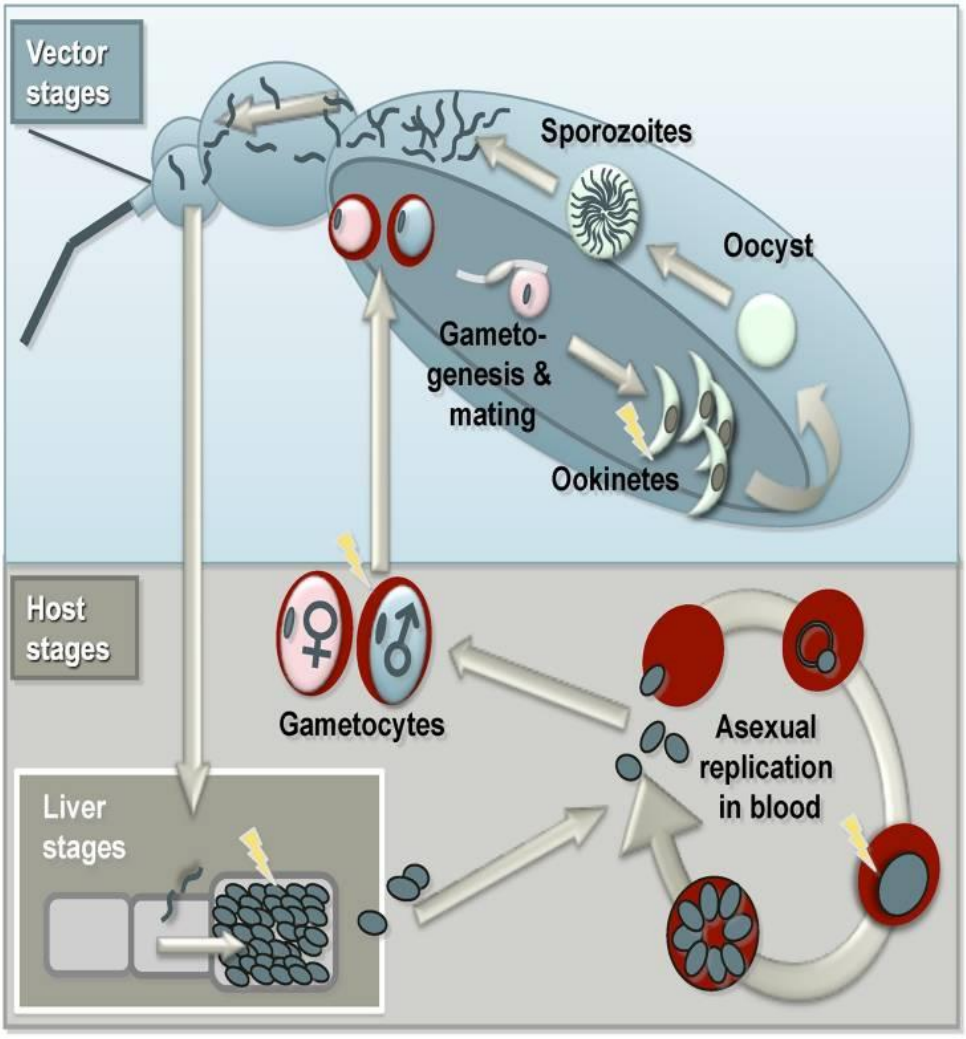

Figure 1: **Lifecycle of the Malarial Parasite** (Adapted from Reece et al. 2011) Progression of the parasite's lifecycle from a female *Anopheles* mosquito, into the blood of a host is shown. The production of gametophytes in the human host results in subsequent infection of other mosquitoes. Important steps of the cycle targeted by drugs are shown.

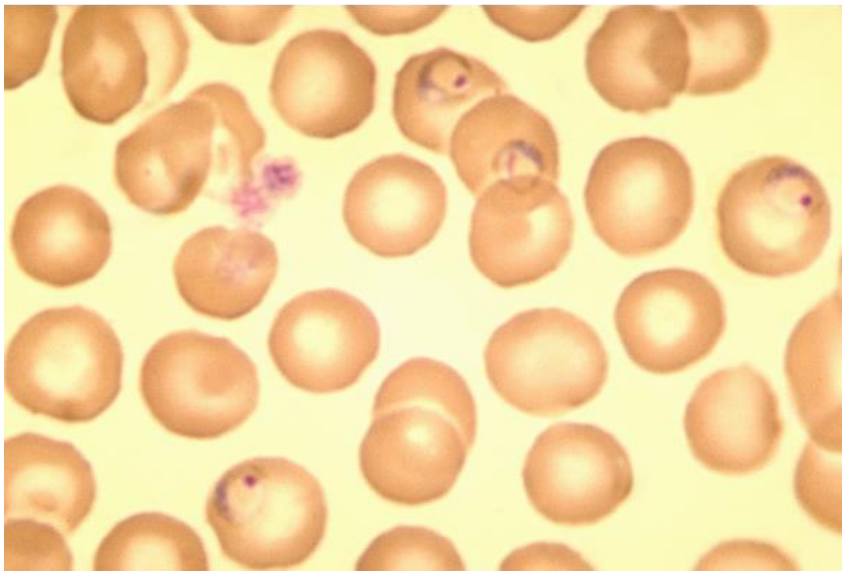

Figure 2: Plasmodium Malariae with Intracellular Ring Forms. (Adapted from Lawrence et al. 2002)

The Ring Stage in parasite development is marked with characteristic ring formations, caused by mature trophozoites, which can be seen inside the red blood cells with light microscopy. This sign is commonly used to confirm diagnosis of Malaria.

releasing more merozoites for infection of more red blood cells as well as possibly passing on the merozoites into any mosquitoes that feed off infected individuals. Rupture of and subsequent infection of other red blood cells exponentially increases parasitemia while also putting the patient at risk of anemia.

P. Vivax and *P. Ovale* have a unique dormancy stage in their parasitic progression[11,12]. Sustained dormant hypnozoites, derived from the same sporozoites, in the liver can lead to malarial relapse weeks, months, or even years later[7,13,14]. With progression of the disease, especially if left untreated, there is a greater chance of development of hypoglycemia, excessive electrolyte and fluid loss, ketoacidosis, anemia, and in the most severe cases cerebral malaria[3,5]. Each stage of infection presents a

different area of attack for either drugs or vaccines. Along with stages of infection, specific molecular mechanisms can be targeted with chemotherapy. Changes within these mechanisms can also cause the development of resistance to chemotherapy interventions. Kelch is one such protein that has been linked to antimalarial drug resistance. Many studies have found a positive correlation between mutated forms of Kelch and resistance to ACT's. The interplay between chemotherapy resistance and Kelch protein mutations is, currently, a vital method of tracking the progression of resistance over time and geographically. Understanding the mechanism of Kelch mutation related Artemisinin resistance and current research into the region-specific prevalence of such mutations, can help shape future anti-Malarial efforts. Current interventions are effective but not foolproof. There are many challenges that health professionals must overcome in order to work towards total Malaria eradication.

LITERATURE REVIEW

CURRENT INTERVENTIONS

Chemotherapy

Youyou Tu, a Chinese scientist studying herbal remedies for Malaria, was awarded the Nobel Prize in Physiology and Medicine for her discovery of Artemisinin in 1979[15]. Tu turned to traditional Chinese herbal medicine during efforts to find a treatment for Malaria which killed many soldiers fighting in the Vietnam War. Extracted from Sweet Wormwood, Artemisinin became one of the first medications that effectively treated Malaria[16]. Recent research also supports anticancer properties of Artemisinin along with its effects on parasitic diseases like Malaria and Schistosomiasis[8,17].

Nowadays, Artemisinins refer to a group of derivatives of the original Artemisnin compound including Artesunate, Artemether, Artemisone, and Dihydroartemisinin. These Artemisinins are widely used in treating Malaria around the world but have not been approved for use in the United States[18]. Artesunate causes arrhythmias and therefore is not allowed for use in the United States but is still a viable option around the world. It is also one of the few anti-malarials that is given intravenously rather than orally in severe cases of Malaria[19].

The most common theory of anti-malarial activity is that Artemisins are activated when bound to the heme in red blood cells which opens the ring and generates reactive oxygen species and free radicals[20,21]. An endoperoxide bridge, highlighted in yellow in all four Artemisinin derivatives depicted in Figure 3, is hypothesized to be activated by a

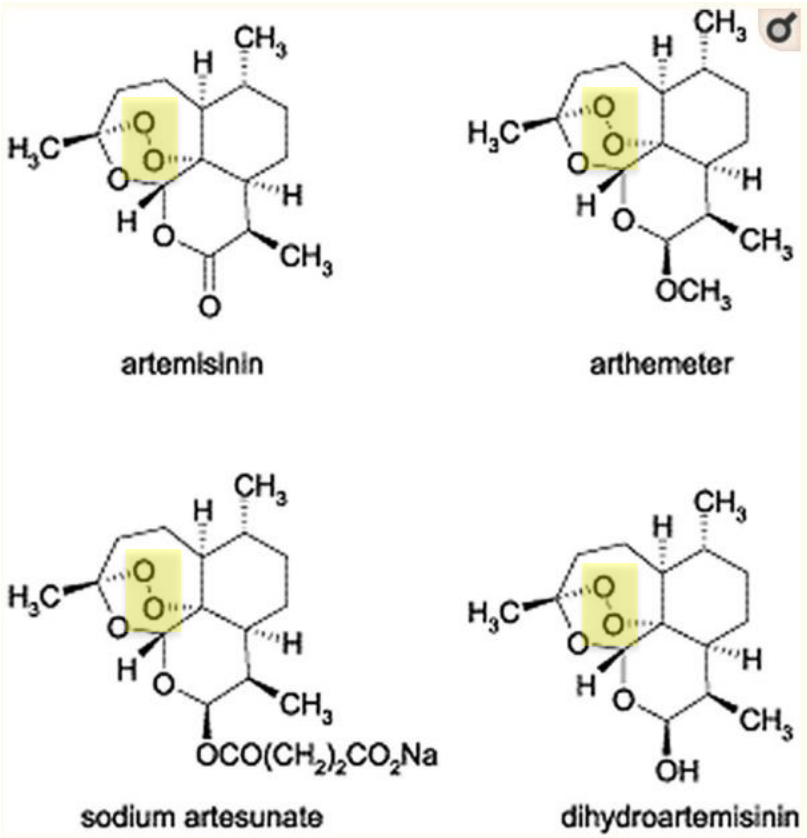

Figure 3: Artemisinin and Derivatives Structures.[1]

While the structures of all four Artemisinin derivatives differ slightly, they all possess the distinctive endoperoxide bridge (highlighted in yellow for each molecule). This is the distinctive motif that makes Ferrous ions interact with in Artemisinins that make this specific chemical family so effective in creating free radicals that attack malarial parasites.

ferrous ion which then leads to the generation of free radicals upon interaction with heme[1]. These free radicals directly destroy the parasites intracellular structures through

oxidation and alkylation[22]. It is proposed that *P. falciparum* parasites which consume hemoglobin, in the blood-stage of the lifecycle, then are vulnerable to Artemisinins. While a specific mechanism for the anti-malarial function of Artemisinin remains ill-defined, current research points to this mechanism as the key behind ACT's anti-malarial effects[17,21].

ACT's are the most popular and recommended treatment for Malaria and pair Artemisinin derivatives with Cinchona Alkaloids like Quinine and Quinidine. Pairing Artemisinins with other drugs is recommended in order to decrease the chance of resistance development. Artemisinins act on both the asexual and sexual parasitic stages but with a short half-life (about 1 hour) and lack the ability to prevent infection. While effective in quickly treating severe malaria, Artemisinins' short half-life increases chance of relapse. Therefore, Artemisinins are paired with longer-lasting drugs in ACT[23].

Other Artemisinin based drugs include Chloroquine and Primaquine. Both of these options are effective interventions but have other side effects and challenges. Chloroquine can be used for prevention and treatment of Malaria. It's mechanism of action prevents protein synthesis by binding to the parasitic DNA[24]. The malarial parasite attacks Hemoglobin within red blood cells, during a later stage of its life cycle, producing toxic hemazoin crystals. Chloroquine disrupts the production of vital proteins necessary for the breakdown of these crystals in vacuoles and causes a buildup of toxins which kills the parasite[25]. The malfunction of the vacuole and a lack of proteins necessary for the degradation of hemazoin crystals slows down the breakdown of Chloroquine and increases its anti-malarial effects. While very successful in treating Malaria, overuse of

Chloroquine has led to *P. falciparum* and *P. vivax* developing resistance over the years and this resistance has spread to every region where malaria is endemic[25].

Primaquine, or Primaquine phosphate, is another successful treatment against Malaria. It has the unique ability to target 'dormant' parasites that cause relapses and is especially effective against *P. vivax*[26]. Unfortunately, Primaquine has many side effects that must be considered before use, these side effects limit safe usage of the drug. Anemia is a significant issue with any anti-malarial treatment since targeted therapy can result in the death of red blood cells and is especially dangerous in children. But, Primaquine use in individuals with Glucose-6-Phosphate Deficiency (G6PD) can lead to Hemolytic Anemia. G6PD, a lack of the enzyme Glucose-6-Phospahte Dehydrogenase, renders patients unable to make NADPH, an energy molecule generated in the Pentose Phosphate pathway that is vital for the synthesis of Ribose-5-Phosphate, which makes up the backbone of RNA and subsequently DNA. NADPH, a by-product of this pathway, is essential for reacting with reactive oxygen species in red blood cells and decreasing their toxicity to the cell. A deficiency of Glucose-6-Phosphate Dehydrogenase in red blood cells, the enzyme necessary for the first step of the Pentose Phosphate Pathway, causes a buildup of reactive oxygen species that eventually results in cell death or Hemolytic Anemia[27]. Before receiving treatment, individuals must be tested for G6PD, to avoid hemolytic anemia down the line. Limited testing is available for those who might have G6PD, therefore in regions where adequate testing is not an option, other support for anemia must be readily available. In moderate cases of Malaria, Primaquine can be given

to individuals with G6PD but with careful monitoring and consideration of benefits versus side effects[27,28].

P. falciparum's and *vivax*'s resistance to Artemisinins and more broadly ACT's is of growing concern considering the incidence rate of Malaria[29]. In recent years, there has been a recorded increase in resistant strains, especially in Africa, which threaten to undo progress made in reducing mortality. This "evolutionary arms race" between parasite, vector, and patient has therefore made advancements in understanding the mechanism behind Artemisinin resistance incredibly important in order to continue progress in hopefully eradicating Malaria[8].

Chemoprevention

Seasonal Malarial Chemoprevention (SMC) or Intermittent Preventive Treatment (IPT) is an effective prevention of Malaria for high risk populations and provides different options for children and pregnant women. Intermittent Preventive Treatment of Malaria in children (IPTc) or in infants (IPTi) is a targeted therapy specifically for children in high prevalence regions. Medication with Sulfadoxine-Pyrimethamine and Amodiaquine (SP+AQ) given before and during the Malarial season maintain high concentrations of anti-malarial drug in the blood in the hopes of preventing infection[30–32]. Studies have found that IPTi and IPTc with SP+AQ administered monthly to children under five, the demographic at the greatest risk for infection, in the Sub-Saharan African region reduce the rate of infection and overall mortality[31]. However, widespread

application of such a treatment faces structural limitations that must be considered in terms of distribution of the drug to at risk populations, consistent administration during the Malarial season, and cost. Intermittent Preventive Treatment of Malaria during Pregnancy (IPTp) faces the same limitations as IPTc but focuses on combining Malarial treatment with antenatal care[33]. Both the mother and the fetus are at risk of infection and infection could cause spontaneous abortion, maternal death, and neonatal death[34]. Dihydroartemisinin-piperaquine (DHAP), another option for chemoprevention especially in IPTp, is an alternative for multi-drug resistant *P. falciparum* and in regions where the risk of infection is high all year around, such at SE Asia[32,35]. Again, monthly administration is important in maintaining appropriate levels of the drugs in the system[35]. To prevent relapses, especially in *P. vivax*, Primaquine is a prime candidate because of its focused attack on parasites that stay dormant within hepatic tissues[36]. Unfortunately, Primaquine is not safe for pregnant women and has been linked to DNA damage and genetic mutations in the fetus and possible negative effects on fertility[27,36].

As with regular chemotherapy upon presentation of Malaria symptoms, SMC and IPT can also lead to the development of drug-resistance[37]. For populations with a high incidence of Malaria, chemoprevention is not the preferred method of treatment. Even if the steep cost of SP+AQ and DHAP was overcome, there still would be an issue of noncompliance when following drug regimens, sharing of medication, and a lack of sustainability in the long-term for any of those previous reasons[38].

Natural Immunity

Natural or Acquired Immunity is not well understood, and ongoing research aims to better understand the mechanisms of natural immunity in order to develop vaccines and directed medication. Historically, European travelers to tropical regions who had no previous exposure to Malaria, considered malaria-naïve individuals, suffered the greatest. On the other hand, adults native to those lands did not face such adverse or immediate infection which led to the question of innate immunity. In regions where Malaria is endemic, like Sub-Saharan/Sahelian Africa and SE Asia, consistent and continuous exposure and infection by *P. falciparum* aids in the acquisition of immunity. Such immunity is found to fade with decreased exposure to the parasite[39].

While healthy individuals of a population where Malaria is endemic benefit from natural immunity, vulnerable individuals like pregnant women and children are at higher risk of serious infection. Maternal transmission of immunity is effective in preventing infection in children before the age of 6 months[39]. The transfer of antibodies, specifically IgG and IgA in utero and through breast milk respectively, jumpstart infants' immune response to parasitemia exposure. During these vital first six months, infants are at the highest risk with risk of malarial mortality decreasing significantly after five years of age[39,40]. Despite high risk of infection in infancy, children also benefit from early exposure through the development of their immunity which is sustained as they continue to live in the region. As is the case in areas in which Malaria is endemic, many individuals may carry the parasites and show no symptoms, which helps maintain natural immunity but can unknowingly spread region-specific parasites to other vulnerable

populations[41]. Natural immunity can therefore prevent infection in those who are carriers but maintains the level of parasites in a community which increases the chance of transmission to those more susceptible.

The mechanism of natural immunity is complicated but is assumed to develop in the blood stage of malarial infection. Both antibodies that target infected red blood cells and T-cells are activated during the blood-stage of the malarial lifecycle, thereby preventing the development and spread of the parasite to hepatocytes[42]. But, such natural immunity requires sustained exposure to continue the production of targeted antibodies and T-cells, which still leave adults susceptible to infection. Conversely, vaccines and other interventions that strive to limit exposure to the parasite can interfere with innate immunity. If efforts to eradicate mosquito vector populations are not thorough, it could do more harm than good by negatively impacting the development of a population's innate immunity. Therefore, sterilizing immunity–or permanent immunity–to Malaria is the best option; however, it has never been achieved neither innately nor through the use of vaccines and remains a major focus of current research.

Vaccines

Vaccines developed in response to Malaria are inspired by natural immunity that some individuals possess or can develop therefore vaccines are seen as induced immunity. As is the case in both types of immunity, there are many challenges when considering development, sustainability, and administration. Initial development of a

vaccine relied on the application of Radiation Attenuated malarial Sporozoites (RAS) which acted at the blood-stage of infection through activation of antibodies and T-cells that targeted surface proteins of infected red blood cells, which were subsequently destroyed in the spleen[42]. RAS mimic natural immunity but work by strengthening the body's usual response to the first stages of infection. This led to the development of the multiple RAS-derived vaccines including one in use today, RTS,S.

Approved in 2015, the Malarial vaccine RTS,S was found to be effective in preventing infection by *P. falciparum*[43]. RTS,S shares the same effects as RAS in its induction of antibody production and T-cell activation but specifically acts on the liver stage of the parasite's lifecycle and targets the most common surface protein of sporozoites: the circumsporozoite protein (CSP)[42]. CSP is said to play a significant role in the attachment of parasitic sporozoites to hepatocytes, hence targeting this particular surface protein with antibodies might prevent migration of the parasite into hepatocytes, see Figure 4[44].

High circulating levels of targeted antibodies and T-cells as a result of the vaccine ensure that infection can be fought off. Unfortunately, as with natural immunity where sustained exposure is required, the vaccine must be administered repeatedly to also maintain a consistent and specific pool of memory B cells in a patient's body. The relative efficacy of the vaccine is therefore low, at about 30% protection over the course of four years and lacks sufficient data to support widespread use in all areas afflicted by Malaria[42,43]. Based on the relative success of RTS,S, future vaccines will hopefully sustainably raise antibody levels and eradicate parasites in the blood and liver stage.

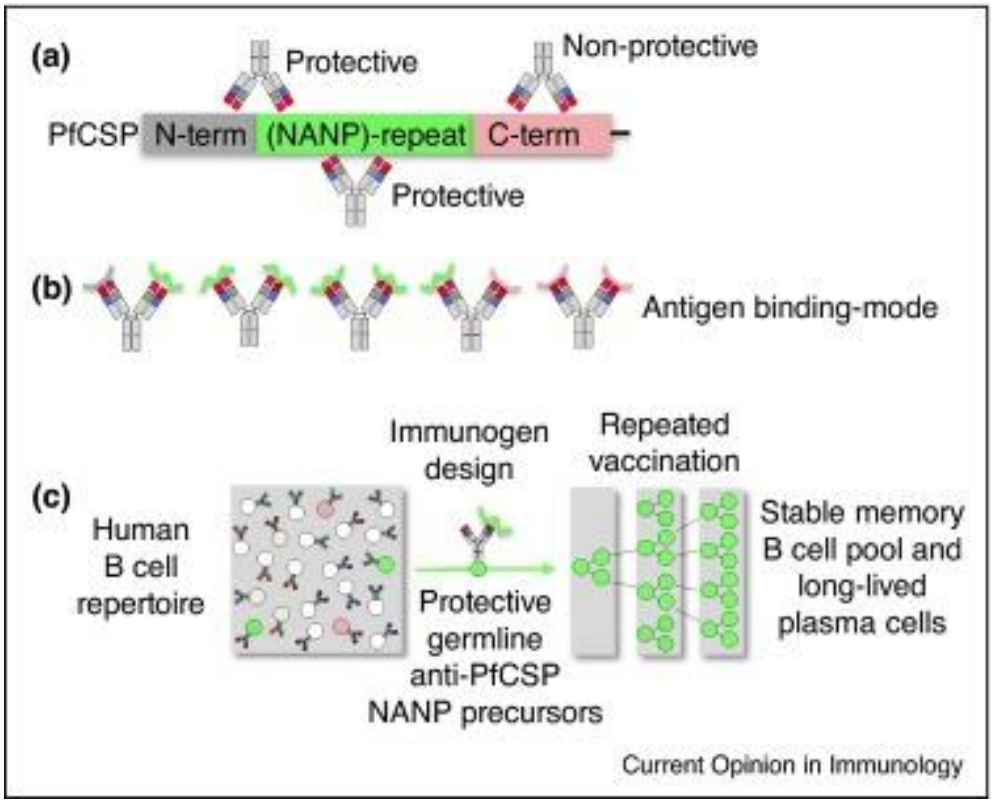

Figure 4 Next-Generation PfCSP Malaria Vaccine Design Strategy. (Adapted from Wardemann et al., 2018)

(a) Shown are three separate regions of CSP targeted by antibodies. The NANP-repeat segment is the most highly conserved section across parasites. Antibodies that have been developed to target this NANP-repeat segment of CSP have been found to be the most effective.
(b) and (c) show how B cell induction results in antibody production, and subsequent creation of a long-term pool of plasma cells which generate and maintain immunity. But, due to the short lived nature of vaccines, repeated vaccines are necessary to develop and maintain an immune response.

As of 2019, the vaccine will be available to children only in Ghana, Kenya, and Malawi as it is still in its trial phase[45]. The vaccine is generally well accepted by recipients but there are many other cultural and societal limitations including lack of knowledge or fear of vaccines, low quality of healthcare and healthcare facilities, and geographical distance to such care[45]. Such structural issues call for action outside of just

providing vaccines or healthcare and require a better understanding of communities in Ghana, Kenya, and Malawi. Initiatives must encompass educating populations about the benefits of vaccines, providing vaccines for free to combat financial restrictions, and keeping lines of communications with the target populations open in order to consistently administer the vaccine, track its efficacy, and also to learn of other challenges[45,46].

Public Health Interventions

Aside from chemoprevention and vaccines, public health interventions focus on preventing and controlling the rate of infection through cost-effective and sustainable methods of containing the vector, mosquitoes. Each intervention poses limitations in terms of cost, durability, and amount of time for which it is effective.

One option is the use of Insecticide-Treated Bed Nets (ITN), which are treated with long-lasting insecticides, usually pyrethroids. These nets provide protection to all demographics including those most susceptible: children under the age of five and pregnant women[38]. ITN's require an initial investment but are easily installed and cost-effective, the nets last for years, although they require reapplication of the insecticide over time, and therefore are seen as an effective intervention not only in Sub-Saharan Africa but across Southern Asia. Another less cost-effective option is investing initially in higher quality nets that do not require reapplication of insecticide, which incurs a greater initial investment but avoids concerns over reapplying the repellant in a timely manner. Through the diffuse use of ITN's, rate of infection drops drastically and

mosquito to human and human to mosquito transmission decreases as well. With a drop in overall incidence of malaria the whole community benefits[38].

Pyrethroids can be used independently of nets and applied to clothing, buildings, and tents as a repellant. For refugees or in areas where ITN's are not convenient, the more versatile repellant sprays are preferred. But, better understanding the needs of a community can help determine the best intervention. For example, instead of distributing ITN's Rowland et al. chose instead to impregnate blankets, or *chaddars,* worn by the Afghan refugees displaced from their homes after a Soviet invasion, with pyrethroids to repel mosquitoes[47]. The camps established in Pakistan lacked adequate resources or housing therefore ITNs were not an ideal choice of intervention. Post treatment of *chaddars* and topsheets, the number of reported cases of infection dropped by 64% for children and 38% in adults under the age of 20 versus groups without treated blankets or sheets[47,48]. Through treatment of both topsheets and *chaddars* in the Afghani population, materials that the refugees already possessed or were provided and use regularly, there was no need for the introduction of ITNs. In such a case, treatment of existing materials with insecticide is an effective intervention.

Insecticides, like DDT, can also be sprayed over larger areas to control vector populations especially during the rainy season and in tropical regions where mosquitoes reproduce easily. Stagnant waters provide ample breeding grounds for *Anopheles* mosquitoes and targeting such areas with diffuse insecticide could cause greater repercussions on the environment. But, in cases where mortality and morbidity are too high, insecticides can quickly kill off large populations of mosquitoes[38,49]. With growing

knowledge of environmental treatments, it may be possible in the future to target breeding grounds by draining areas of stagnant water and disrupting the development of mosquito larvae[38].

It is important as well to recall the importance of maintaining regular exposure to the parasite for native populations to develop and retain their natural immunity. So while ITNs, insecticide sprayings, and repellants are beneficial to decreasing the chance of infection in vulnerable populations, there is still a tradeoff in terms of natural immunity. As Phommasone et al. found in Laos that 20% of the 888 citizens who were tested were infected with P. falciparum but showed no symptoms[50]. With such a high level of infection, especially with proof of anti-malarial resistance in 75% of the infected individuals, their one recommendation was a greater focus on "rapid elimination" of the parasite and the vector[50]. Such asymptomatic individuals are capable of passing on anti-malarial resistant parasites to those more susceptible. Individuals most vulnerable, apart from infants and pregnant women, also include malaria-naïve individuals, especially immigrants and refugees. With no previous exposure to malaria, immigrants and refugees to areas endemic for malaria struggle with appropriate shelter, a lack of acquired immunity to Malaria to fight off infection, and limited resources for preventing infection like ITNs.

ARTEMISININ COMBINATION THERAPIES (ACT's)

Resistance to Artemisinin and its derivatives is slowly growing and is prevalent in Southern Asian countries but harder to find in African regions[51]. Since the burden of Malaria is greatest in the Sub-Saharan African region, ACT is invaluable in treating patients. Resistance development to ACT could prove to be a significant issue as new drugs are still in the developmental phase. With few alternatives, it is vital that Artemisinin resistance is better understood and tracked in order to develop alternative drugs and to find a solution to this growing issue. Current combinations for ACT therapy include Artemether-Lumefantrine, Artesunate-Amodiaquine, Artesunate-Mefoquine, Dihydroartemisinin-Piperaquine, and Artesunate-Sulfadoxine-Pyrimethamine. Artemisinins, a drug with a short half-life, are paired with longer lasting drugs to be most effective in parasite elimination[52]. Every variation of ACT therapy is susceptible to resistance. Different markers of the parasite are associated with slower responses to single drug treatments, like Primaquine and Piperaquine. But once paired with Artemisinins or Chloroquine, the parasite's half-life is significantly reduced[52]. Therefore, to combat the decrease in efficacy of ACT, triple combination treatments are being developed to target parasites with multiple anti-malarial resistant mutations[52,53]. Thus, focusing on resistance development is vital to addressing the growing issue of Artemisinin resistance that could halt efforts of Malaria eradication.

CHLOROQUINE RESISTANCE

Cholorquine, a Cinchona Alkaloid, is one option for pairing with Artemisinins for ACT and development of Chloroquine resistance is linked to Artemisinin resistance. Within the past two decades there has been an increase in resistance of *P. falciparum* and *P. vivax* to Chloroquine. Buppan et al. focused on multiple new mutations found in the gene for the Chloroquine resistance transporter and sought to explore the effects of "antimalarial selective pressure"[54]. Blood samples collected from infected individuals in Thailand from 1991 through 2016 were evaluated to track the progression and prevalence of mutations. A point mutation in the P. falciparum chloroquine resistance transporter (*Pfcrt*) found on the membranes of vacuoles is a main feature of resistance. The known mechanism of the effect of Chloroquine on the parasite involves degradation of toxins in the parasite. A mutation in *Pfcrt* would prevent the transport and buildup of Chloroquine inside vacuoles which leads to resistance[25]. Treatment of ACT differs between a 2-day and 3-day regimen, with improved prognosis and decreased presence of plasmodia for patients who had undergone the 3-day regimen. Through sequencing, they found 21 unique genotypes of *Pfcrt* which persisted in Thailand over the years but also were similar to genotypes found in Ghana which pointed at the spread of *P. falciparum's* resistance across borders. Through sequencing, 26 nucleotide changes that resulted in 21 amino acid changes, mostly in the transmembrane domain coding segment of the *Pfcrt* gene, were found. A greater number of these haplotypes of *P. falciparum* were found in

patients who had undergone the 3-day ACT regimen. It is hypothesized that the greater concentrations of drugs over a longer period of time forces more mutations in the plasmodia reinforcing the theory of how antimalarials encourage mutations[55]. Buppan et al. also showed that the sustained incidence of certain haplotypes over the course of two decades implied that those mutations were particularly successful in resisting Chloroquine in ACT regimens[54]. As these mutations in the vacuole transporter gene may not definitively point to Chloroquine resistance, it has been shown that these mutated plasmodia concurrently resist Artemisinins and other drugs that are used in ACT. With a decrease in treatment with Chloroquine as a result of studies that proved the rise in resistance, chloroquine-sensitive plasmodia are now less common. This could encourage use of Chloroquine once again as an effective antimalarial especially within ACT. However, an increased use of 3-day ACT regimens again leads to the developed of Artemisinin resistance. With an overlap in the mutations' effects on Chloroquine and Artemisinin metabolism, the pressure to find new drugs is growing.

THE KELCH PROTEIN

Originally found in *drosophilia*, The Kelch protein located on chromosome 13 (K13) in *P. falciparum* has shown multiple polymorphisms in Artemisinin-resistant strains of the parasite, specifically in the Kelch propeller domain (Figure 5)[56]. Changes in this domain serve as markers for Artemisinin resistance and help to trace the development and progression of resistance in different regions. The Kelch protein is part of a larger family of proteins that possess similar motifs, namely the repeating Kelch

domains which fold in to a propeller[57]. The Cullin3-binding site, marked in Figure 5, interacts with ligases and links Kelch to the ubiquitination process[58].

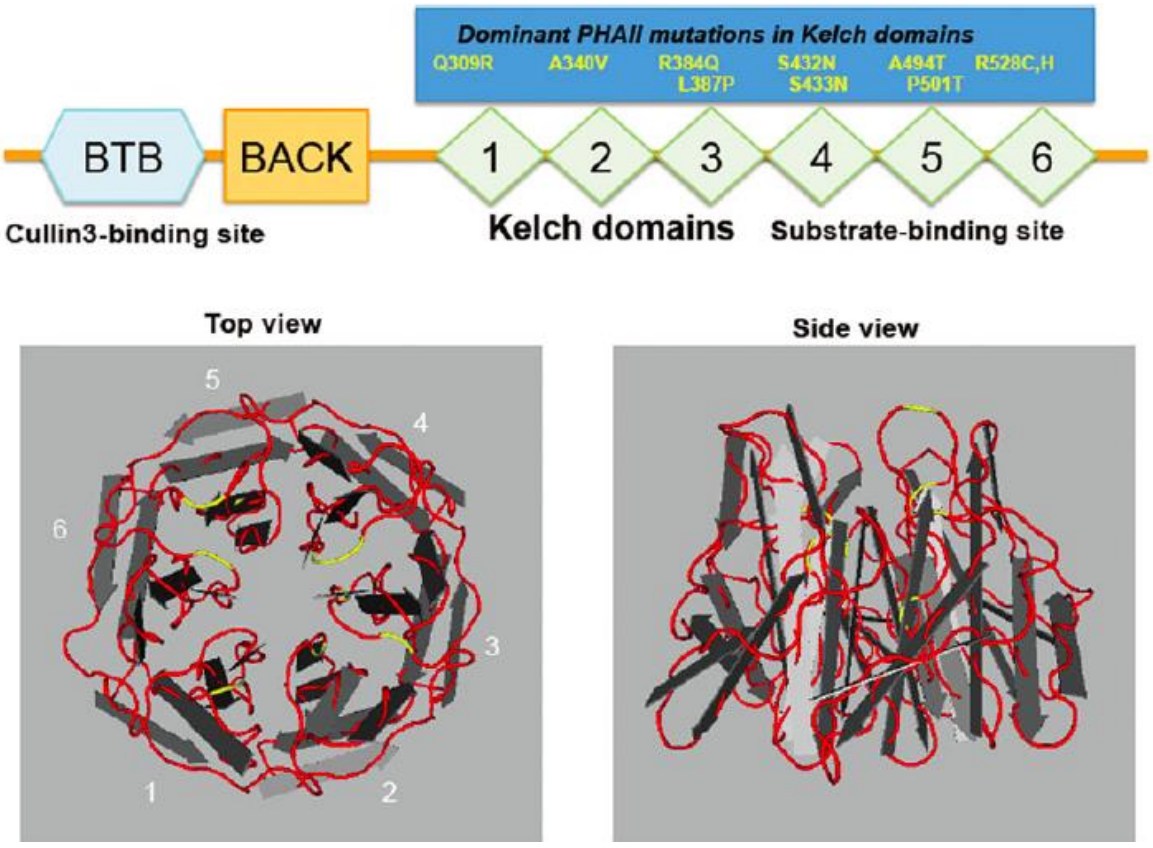

Figure 5: Kelch Propeller Domain (Uchida et al., 2014)

Each repeating Kelch domain folds in a β-sheet which makes up a single "blade" of the "propeller." This propeller region forms a substrate binding site important in initiating signal cascades. The Cullin2-binding site (labeled BTB) is also important and serves as the link between the Kelch protein and the Ubiquitination of misfolded proteins.

MECHANISM OF KELCH RELATED ARTEMISININ RESISTANCE

While many studies support the significance of Kelch in Artemisinin resistance it is also important to explore why mutations in this specific protein cause resistance. Kelch proteins are universally associated with ubiquitination of misfolded or non-functional proteins[59]. Ubiquitination marks misfolded proteins for breakdown in order to maintain homeostasis within in a cell. Kelch mutations impair the initiation of ubiquitination and lead to a buildup of misfolded proteins, which upregulates the Unfolded Protein Response (UPR) response[53].

Mok et al. found that Artemisinin resistant parasites showed increased activity of the unfolded protein response, linked to Kelch polymorphisms, which maintains these parasites at a "younger" stage in their lifecycle[22]. The UPR is an intracellular cascade which takes action against misfolded proteins and maintains homeostasis. Because of its vital function, UPR also controls apoptosis. Cancer cells have been shown to take advantage of this "cyto-protective" quality of the UPR in order to maintain their growth[59]. This cancer-like quality aids the resistance of the mutated parasite to Artemisinin. Conversely in terms of this shared quality to cancer cells, DNA replication in these parasites was slowed down reaffirming their resistance to developing or aging like wild-type parasites. During the ring stage, while parasites are inside red blood cells, the step at which Artemisinin attacks, these young parasites are able to endure the antimalarial effects more effectively and survive[22]. Hott et al. proved that this prolonged ring stage was positively correlated with parasites that showed resistance to Artemisinin[60]. By using transcriptome analyses, Mok et al. found that slowing down the

aging process in the ring-stage led to decreased digestion of heme which is a prime target of Artemisinin[22]. Decreased digestion of heme means that Artemisinins create fewer free radicals. The limited free radicals that are produced are processed through the increased UPR before inflicting substantial damage to the parasite. Therefore, Kelch mutations that upregulate the UPR lead to Artemisinin resistance and require alternative interventions.

MUTATIONS OF KELCH

There has been a noticeable pattern in polymorphisms of Kelch arising in areas with recorded anti-malarial resistance. Known mutations that are markers of Artemisinin resistance include C508Y, Y493H, R539T, and I543T[51,56,61]. These specific mutations were established as markers based on the increase in parasite half-life[62]. The most common mutations that have been found to be associated with Artemisinin resistance are C508Y, F446I and more recently A578S and N585K. The A578S mutation is newer, located close to the C508Y mutation, and has been linked to slower parasitic clearance but the direct effect of this mutation is not well understood[63].

Each mutation is currently associated with different regions. C508Y is a significant problem in SE Asia and is spreading to other Malaria ridden areas by way of immigrants and refugees[64]. Site specific mutations in Kelch confirm that specific polymorphisms, especially C508Y, are involved in Artemisinin resistance[65]. Sá et al. used a monkey malaria model and found that parasites with the C508Y mutation survived the ring stage even with the administration of Artemisinins[66]. The prevalence of C508Y related resistance just within in the SE Asian countries, like Cambodia and Malaysia,

with limited exposure in other areas may point to issues that are hindering the spread of resistance[67]. According to a longitudinal study conducted by Cerqueira et al., a mismatch between host genetics and immune systems between individuals native to SE Asia versus Africa may be impeding the spread of specifically the C508Y mutation[68]. Historically, SE Asia has higher rates of infection and resistance to ACT, which require higher doses of medication that results in more and stronger resistance mutations. This trend was clear when Cerqueira et al. compared 194 isolates from 2001 to 2014; clearance rate of parasites increased three-fold[68].

The F446I mutation is also concentrated in SE Asia but has also been found in African countries. A578S is not as well researched but appears to be a mutation unique to Africa[51]. The N585K mutation, a recently identified mutation, was found only in parasites after treatment with ACT. Ingasia et al. hypothesizes that the new mutation might represent the selective pressure placed on the parasite through treatment with Artemisinins[69]. Whether this mutation causes Artemisinin resistance or not is unknown at this point. All these mutations are localized on the repeating Kelch domains of the protein, which makeup the blades of the propeller. The C508Y mutation substitutes a Cysteine for a Tyrosine, both of which are polar amino acids. The F446I mutation substitutes a Phenylalanine for an Isoleucine which are both non polar amino acids. The A578S mutation has the most significant change with an Alanine being replaced with a Serine, a polar amino acid replaced with a nonpolar. These amino acids are vital in the formation of the β-sheets that make up a single blade. Disrupting the formation of the blade changes the functionality of the Kelch protein itself. There is a plethora of various

recorded mutations aside from the three listed above, see Table 3, but these other mutations lack sufficient evidence to be used as an Artemisinin resistant marker and do not necessarily cause Artemisinin resistance.

All studies which compared the development of resistance *in vitro* and *in vivo* found antimalarial selective pressure to play a significant role[54]. Tyagi et al. compared the development of resistance in red blood cells and in a mouse model[70]. High doses of Artesunate were administered exceeding the dosage typically given to patients suffering from severe Malaria. This concentration in both red blood cells and in mice resulted in high resistance parasites, measured by rate of parasite survival and transmission. This data proposes that the higher the concentration of Artesunate administered, the greater the resistance to the drug and level of parasitemia in the mice. While such anti-malarial resistant parasites are not present in the field, Tyagi et al. postulated that this could be the future of malaria[70].

These studies point to the continual development and adaptation of the parasite in response to current treatment options. While chemotherapy, vaccines, and chemoprevention options are effective for now, there is a race to address the changing landscape of Malaria as resistance grows. Understanding the mechanism of resistance is just the one aspect of this public health issue. Tracking the incidence of Kelch mutation development is also vital to understanding the development of mutations as well as incidence of resistance globally.

DISCUSSION OF CURRENT LITERATURE

There are many current studies on the prevalence of specific mutations in Kelch. Of the 80 articles that covered Kelch mutations in relation to Malaria there were 30 region specific studies of polymorphisms. Organized by region, in table 3, the studies ultimately recorded the incidence of certain polymorphisms, while taking note of novel mutations.

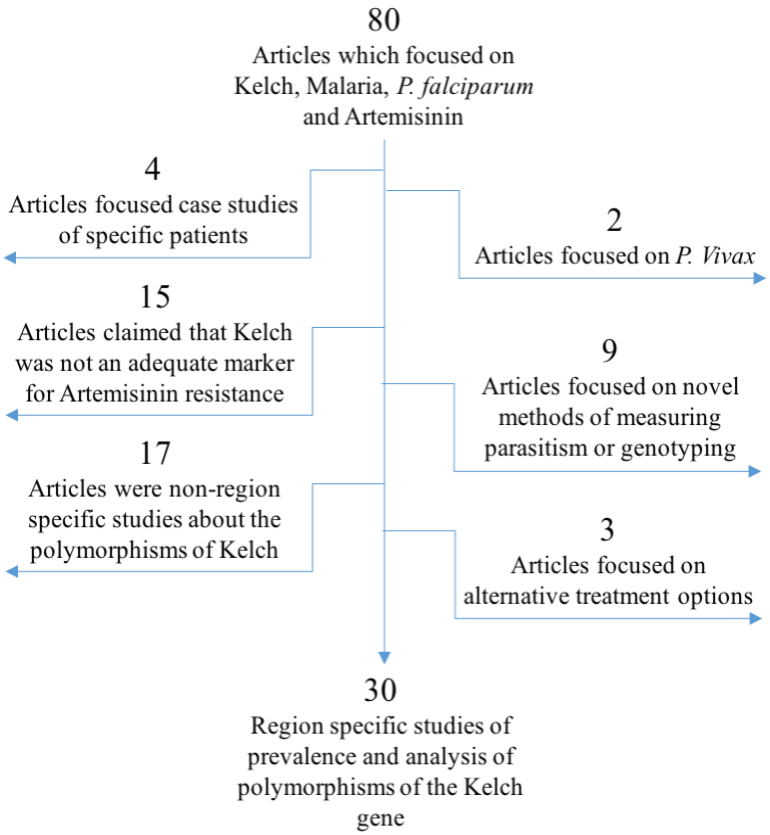

Figure 6: Articles Chosen for Analysis
Of the 80 articles that pertained to Malaria drug resistance, *P.Falciparum*, and the Kelch protein only 30 studies focused specifically on tracking the prevalence of various Kelch mutations in a chosen region where Malaria was endemic. The structure of the studies varied. Some studies focused on tracking which mutations persisted after administration of Antimalarials while other studies were purely observational and were purely researching mutation prevalence in a chosen population.

Table 3: Compilation of 30 Region-Specific Studies on Kelch Polymorphisms

Author	Area	Samples	Treatments	Mutations Found
AFRICA				
Djaman et al.	Cameroon, Cote D'Ivoire	47	-	E602D
Guerra et al.	Continental Equatorial Guinea	144	ACT	E556K, P574L
Menegon et al.	Eritrea	180	-	0
Heuchert et al.	Ethiopia	177	AL	0
Nguetse et al.	Gabon, Ghana, Kenya	287	ACT	0
Ingasia et al.	Kenya	315	ACT	**A578S**
Ouattara et al.	Mali	87	ACT	**F446I**
Oyebola et al.	Nigeria	89	AL	0
Bayih et al.	NW Ethiopia	148	ACT	R622I, A621A, G690G
Tacoli et al.	S Rwanda	222	-	P574L, A675V
Boussarogue et al.	Senegal	92	-	**A578S**, N554H, Q613H
Talundzic et al.	Senegal	207	-	Q468Q, C469C
Dieye et al.	Senegal, Mali, Gambia	463	AL	0
Kakowla et al.	Tanzania	328	AL, ASAQ, DHAPQ	0
Hawkes et al.	Uganda	78	Artesunate	**A578S**
SOUTH ASIA				
Mishra et al.	NE India	254	-	**F446I**, Y588C, D641G
EAST ASIA				
Huang et al.	S China	218	Artesunate, DHAPQ	**F446I**
Sun et al.	Yunnan Province, China	190	-	**F446I**, **A578S**, E556D
MIDDLE EAST				
Alaregi et al.	Yemen	50	Aretsunate and SP	0
SOUTHEAST ASIA				
Kheang et al.	Cambodia	76	Artesunate	**C508Y**, Y493H, Wild
Parobek et al.	Cambodia	78	-	**C580Y**
Grigg et al.	Malaysia	49	Artesunate	**F446I**
Tun et al.	Myanmar	116	DP, Primaquine	**F446I**
Win et al.	Myanmar	206	AL	**F446I**
Bonnington et al.	Myanmar	35	Artesunate + ACT	**F446I**, R561H, C508Y
Nyunt et al.	Myanmar	1160	ACTDP, AL, AM	**C580Y**, R561H
Phommasone et al.	Laos	888	-	**C508Y**
Takala-harrison et al.	SE Asia	123	-	**C580Y**
SOUTH AMERICA				
Chenet et al.	Guyana	98	-	**C580Y**
AUSTRALIA				
Prosser et al.	New South Wales Province	153	AL, and others	**C508Y**

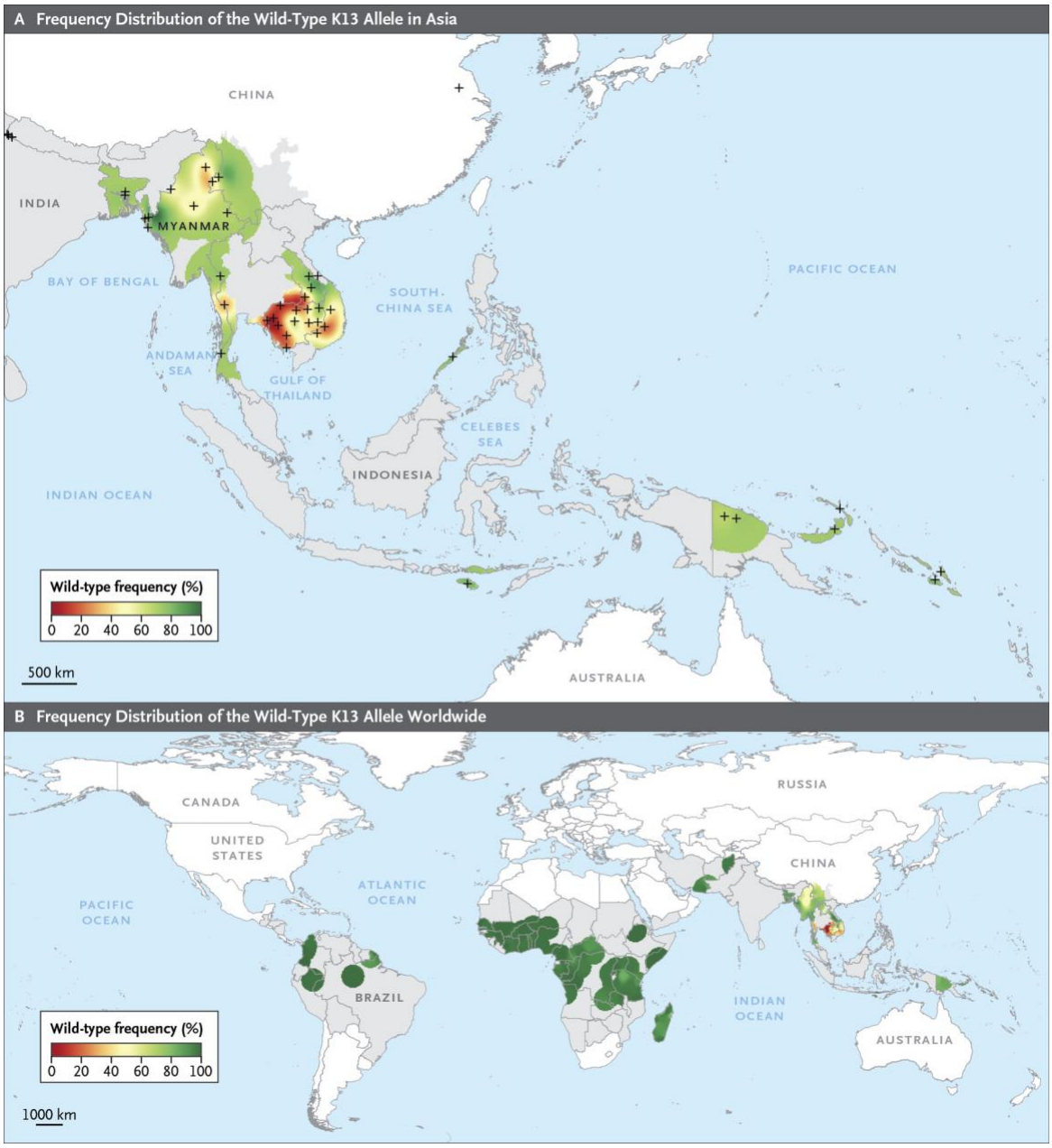

Figure 7: Frequency Distribution of Wild-Type K13 Allele (Ménard et al. (2017)) The wild-type frequency is shown in green, with a majority of SE Asia marked in yellows and reds to show the prevalence of mutated parasites in the region. Countries in grey are where Malaria is endemic. This map was generated based on studies done before 2016 therefore the data might differ from what is represented in Table 3.

The map of haplotypes in the map in figure 7 visually represents and confirms data in table 3[51]. While data from table 3 and the map of figure 7 have many similarities, data collected by Ménard et al. to generate the visual is limited to 2016, and many studies had been conducted and published since then[51]. While Ménard et al. claims that mutations in Kelch were limited to SE Asia, studies have proven that mutations and resistance has begun to spread to parts of Africa[51].

The data chosen for table 3 was limited to number of participants, treatment administered, and polymorphisms found. Demographics that were tested and relative incidence of mutations was not included based on different methods of measurement and data compilation that made it difficult to compare results. These parameters, however, are important to return to when analyzing and comparing all 30 studies.

Of the 30 chosen studies, the 11 studies, throughout all 7 regions outlined below, that did not administer any type of treatment to individuals concentrated efforts on finding polymorphisms of Kelch that were already known to cause resistance to Artemisinins. The other 19 studies followed the general outline of administering medication, following up with more medication as seen fit, and recording rates of parasitemia. Higher counts of parasitemia post treatment with anti-malarials, which were all Artemisinin based, pointed to anti-malarial resistance. Ultimately, all these individuals' blood was drawn and analyzed to find which mutations were present. While anti-malarial selective pressure was a large determinant in the development and incidence of mutations especially in Cambodia, other recorded incidences had varied causes. Prosser et al. found a single case of C508Y mutations in parasites from the blood of an

individual who had recently traveled to Papua New Guinea[64]. Every study in SE Asia, including Malaysia and Cambodia and surrounding countries showed prevalence of the C508Y mutation. This mutation has been shown to cross borders as Mishra et al. found while testing individuals in Arunachal Pradesh in the Northern part of India that shares its border with Myanmar[71]. The most significant trend, however, was the lack of mutations when one moves further away from SE Asian countries. An exception to this trend are Chenet et al.'s findings which highlight how anti-malarial pressure can cause mutations[72]. The C508Y mutation found in Guyana was determined to be independent of the C508Y mutation native to SE Asia. Microsatellite differences between these two similar but different mutations provide necessary proof that the mutation in Guyana was developed independently and not acquired originally in SE Asia[72]. Despite significant concerns about resistance, Kheang et al. postulates resistance might not be as strong as originally assumed[73]. In their study in provinces of Cambodia, 84% of the participants has the C508Y mutation but all but 1 of them recovered fully under ACT. This makes Artemisinin resistance an even more complicated issue considering the variance in resistance in different individuals, populations, and demographics.

Venturing further out to Africa, there is a lack of substantial artemisinin resistance. The difference in mutations recorded in these regions can also be attributed to the ACT regimens that are readily available. Artesunate is preferred for treatment in SE Asia while Sub-Saharan Africa relies on other combinations of Artemether and DHAPQ[19]. The F446I mutation, the most diffuse mutation with incidence in Africa and parts of Asia, was found to be relatively common and linked to Artemisinin resistance[74–

[76]. Kakowla et al. found only 7 mutated parasites out of the 328 samples collected in Tanzania[77]. While parasitism persisted longer than others that underwent the AL/ASAQ and DHAPQ treatment, by the third day there were complete clearance of the parasites. A specific mutation was not mentioned, but the 7 mutations found were probably not strong enough to resist Artemisinin treatment altogether. While Bayih et al. managed to isolate 3 novel mutations, with R622I showing significant Artemisinin resistance, only three out of the 148 samples taken from Northwest Ethiopia showed mutations[78].

The sudden rise in Artemisinin resistance in Sub-Saharan Africa is clear through Tacoli et al.'s findings that showed growth in polymorphisms recorded[79]. Incidence in a pool of 220 participants went from 0 to 2.5% to 4.5% from 2010 to 2014 to 2015 respectively. The P574L mutation and A657V mutation originally associated only with SE Asia were also found in Rwanda further supporting the pressure that anti-malarials place on parasites that leads to these novel mutations in regions with limited resistance[79].

Uniquely, individuals recruited by Phommasome et al. in Laos, an area where Malaria is endemic, had constant levels of parasitemia but no actual symptoms of infection[50]. 75% of this population possessed the C508Y mutation. This poses a distinctive problem, as people living in these districts serve as a pool for sustaining parasite populations while also protecting the C508Y mutation. *P. falciparum*, that do not already carry the parasites, can easily be infected by feeding on individuals with no serious repercussions because of their innate immunity. As is the case in high incidence areas, Phommasome et al. advocates for focus on elimination of the vector over treatment[50].

The E602D mutation, listed in table 3, that Djaman et al. found in the Côte D'Ivoire was not linked to artemisinin resistance but still aids in tracking the progression of region-specific mutation development[80]. Long-term studies that track the development of mutations to wild-type *P. falciparum* provide the best evidence of the effects of anti-malarial medications. Guerra et al. compared results over the course of 8 years and discovered novel mutations in Continental Equatorial Guinea[81]. While Kelch polymorphisms are a reliable marker for Artemisinin resistance, they are not foolproof.

As Kakowla et al. found, complete clearance of the mutated parasite with Artemisinins showed that Kelch polymorphisms may not be the best marker for resistance[77]. The *P. falciparum* multidrug resistant proteins (*PfMDR*) and P. falciparum Ferrodoxin might be promising new markers that can strengthen identification of resistant parasites[82]. Evidence already confers the effectiveness of these two markers for example Nguetse et al. found *PfMDR1* to be associated with high levels of parasitemia in East African children[83]. The popular AL treatment preferred in African nations has also been linked to mutations in the *PfMDR* gene[84].

Artemisinins, because of their use in ACT, have led to the most robust resistance due to their diffuse use. This poses a problem for many nations and populations that rely on ACT's as primary treatment for infection. There may be other drugs in the market but the effectiveness of the drug must outweigh its price and side effects. Therefore, research about Kelch polymorphisms that serve as a marker for ACT resistance in *P. falciparum* is invaluable. Tracking the progression of Kelch haplotypes and comparing microsatellite markers can show how anti-malarial drugs place pressure on *P. falciparum* and lead to

the development of novel mutations in an effort to survive ACT. These markers also point out that anti-malarial resistance development can happen independently and parasites do not necessarily need to be transported in a human host. With a better understanding of these mutations, addressing the rise in Artemisinin resistance will hopefully become easier.

CONCLUSION

With such a significant burden worldwide and throughout history, Malaria is a prime concern for health organizations and professionals. Though efforts have concentrated on complete eradication in the past, doing so successfully in North America and Europe, Malaria still remains endemic to a majority of African and tropical nations. Since their discovery in the late 1970s, Artemisinins have played a key role in combating this Malarial burden. These drugs effectively shed light on the mechanism of the parasite's attack on the human system while intervening at the blood stage and preventing further infection. Unfortunately, the slow and steady development of *P. falciparum*'s resistance to Artemisinins and its derivatives seems to have locked in the future of Malaria treatment. Few other options exist for treatment and there has been resistance development to these other popular drugs, like Chloroquine and Primaquine.

The development of multi-drug resistant Malaria has, in recent years, shifted the focus to more long-term solutions that concentrate on prevention rather than treatment. Public health interventions, chemoprevention, and vaccines form the foundation of these efforts to combat the steady mortality of Malaria. Public health interventions concentrating on educating populations at highest risk of infection are the most successful. Encouraging populations to actively learn about the disease and its prevention will hopefully help individuals gain a better understanding of the pathogenesis and allow them to take control of medication and interventions that are available. Rowland et al. (1999)'s efforts to impregnate chaddars of Afghan immigrants with insecticide were done so in reaction to news that individuals given mosquito nets would trade those nets in the

camp for other necessities. With little other resources or sources of income, the nets turned into currency and lost their true intention for those who truly needed them. Finding a solution to such infrastructural issues is paramount and presents a new challenge for every population. Impregnating the chaddars which were already used every day and culturally significant points towards public health interventions being led by native professionals who have an understanding of a population's cultures and customs or for research teams to invest more time into understanding these infrastructural issues and using them to their advantage. Educating individuals also helps to explain the danger of sharing medication or not completing regimens are prescribed. Hopefully, such information would help slow the development of resistance to Artemisinins and ACTs.

Chemoprevention faces the same limitations as chemotherapy in terms of patients following regimens but also financial limits. While Chloroquine is cheaper than ACT, both are still expensive when faced with the sheer burden of malaria. That financial burden is then either placed on families or on a country's government, regardless it serves as another obstacle in the fight against Malaria. The consistent cost of medications pushes organizations and medical professionals to find more effective solutions that cost less and are more long-term. ITNs are one such investment that last for years and can be re-impregnated with insecticide as needed. With a range of options available that differ in upfront cost, the nets require a hefty initial investment but pay themselves off over years of use. Higher quality nets that do not require re-application of the insecticide and are higher in price may seem like a logical intervention such an investment may be too much for high-risk families who live in poverty.

Concentrating on the elimination of the mosquito vector is another option that has the potential to be successful but requires a closer look at the environmental toll it would have. Cost also becomes an issue here with eradication efforts with insecticide requiring consistent investment with no certain guarantees of results. There is also proof of mosquitoes developing resistance to insecticides because of their continued exposure to common insecticides. Environmental interventions may be an option as most areas in which Malaria is endemic are tropical and therefore provide plenty of stagnant pools of water that serve as breeding grounds year-round. Draining these pools and decreasing breeding grounds has also been presented as an option and would help decrease the population of the vector itself. Decreasing the vector decreases the chance of infection which is a positive but can also hinder the development of natural immunity of these populations. Individuals that maintain a constant level of parasitemia retain a constant level of antibodies and T cells that fight against the parasite. Adults that possess this acquired natural immunity to the parasite are protected through infection but this leaves children, pregnant women, and immuno-compromised individuals at risk. This tradeoff must also be considered when weighing the benefits and costs of mosquito control. Outright elimination of the vector was highly encouraged by many studies conducted in SE Asian countries but this must be weighed against the natural immunity that infection grants. These public health interventions and efforts to prevent Malaria must be partnered with chemotherapy. Infection is constant and therefore, resources are spread thin when nations try to balance both the treatment and prevention of the disease. With

chemotherapy resistance becoming a bigger issue, it is natural that so much time and effort is being invested into finding out the cause and mechanism of resistance.

The need for effective chemotherapy against Malaria is, therefore, a primary concern of the World Health Organization. ACT's that combine three different drugs to ensure that even multi-drug resistance parasites are targeted effectively might be a growing option. Unfortunately, drug resistance will always be an issue as long as chemotherapy is necessary against Malaria. But, this new understanding of the mechanism of resistance will hopefully encourage changes in public health interventions, education about Malaria and the development of new treatments that target multi-drug resistant strains of the parasite and the actual mechanism behind the drug resistance itself.

LIST OF JOURNAL ABBREVIATIONS

AJTMH	The American Journal of Tropical Medicine and Hygiene
AmAC	Antimicrobial Agents and Chemotherapy
MJ	Malaria Journal
NEJM	New England Journal of Medicine
PNAS	Proceedings of the National Academy of Science of the United States of America

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