



A Review: Artemisinin-Based Combination Therapies [ACTs] and K13 Polymorphism

Aqeel Ahmad¹ and Sultan Ahmad^{2*}

¹Department of Biotechnology, Jamia Millia Islamia, New Delhi, India

²Department of Microbiology, Shri Guru Ram Rai University, Dehradun, UK, India

ABSTRACT

Malaria is caused by four plasmodium species in humans (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*) which spread from one person to another via the bite of female Anopheles mosquito. *P. falciparum* causes most deaths from malaria [1] and is most prevalent on the African continent whereas *P. vivax* has a wider geographical distribution [2]. According to the latest WHO estimates, released in December 2015, there were 214 million cases of malaria in 2015 and 438,000 deaths [3]. Existing strategies to control malaria include vector control, chemoprevention and case management [4]. Without a fruitful antibody that would offer security against malaria, we have to depend on anti-malarial prescription to treat just as lessen the odds of getting the disease [5-8]. Artemisinin in mix with other moderate acting medications is suggested for the treatment of *P. falciparum* [9,10].

INTRODUCTION

Malaria is a disease caused by an apicomplexan parasite, plasmodium. It can be life threatening and fatal if not treated promptly [11]. Malaria does not confer sterile immunity that is why it is slightly different than other infectious diseases and it occurs mostly in poor tropical and subtropical areas of the world [12]. In many of the countries affected by malaria, it is a leading cause of illness and death especially in many developing countries, where young children and pregnant women are the groups most affected [13]. According to the World Health Organization's World Malaria Report 2017 nearly half the world's population lives in areas at risk of malaria transmission [14]. According to the WHO estimates, released in December 2015, there were 214 million cases of malaria in 2015 and 438,000 deaths [15]. Existing strategies to control malaria include vector control, chemoprevention and case management.

ART resistance phenotypes and K13

We have been struggling to develop an efficacious vaccine to strengthen our fight against malaria [16] until now there is no malaria vaccine available commercially [17]. Major bottlenecks for developing malaria vaccine are the high complexity of the malaria parasite, the lack of a traditional market, and the technical complexity of developing any vaccine against a parasite, lack of a sound understanding of complex immune response to malaria infection as malaria parasites are genetically complex [18]. Antigens which are being used nowadays were discovered decades ago [19]. However some progress has been made in the past several years toward developing malaria vaccine [20]. The most advanced vaccine candidate is RTS,S/AS01 against the most deadly form of human malaria, *Plasmodium falciparum* [21].

*Corresponding author

Sultan Ahmad, Department of Microbiology, Shri Guru Ram Rai University, Dehradun, UK, India

E-mail: sultan.sgrru@gmail.com

DOI: 10.37871/jels1124

Submitted: 27 July 2020

Accepted: 12 August 2020

Published: 13 August 2020

Copyright: © 2020 Ahmad A, et al. Distributed under Creative Commons CC-BY 4.0

OPEN ACCESS

Keywords

- Artemisinin
- K13
- Malaria vaccine
- RTS
- AS01

Subject: Biology

Topic(s): Biology, Virology

It is a recombinant protein candidate malaria vaccine that targets the *P. falciparum* circumsporozoite protein [22]. It is the only ongoing research vaccine against *P. falciparum*, which might still take 3 to 5 years to come in market, if safety and effectiveness are considered acceptable [23]. However, significant advances have been made with the completion of a Phase 3 Clinical trial of the RTS,S/AS01 candidate vaccine [24]. In 2009 a Phase 3 Clinical trial of the RTS,S/AS01 began in seven countries. It is completed and Phase 4 has been begun now [25]. A constant need to identify novel vaccine candidate as well as their characterization to explore their vaccine potential is the need of the hour [26]. Erythrocyte binding antigen family, and the Rh protein family until now are the most promising candidates for development of blood stage vaccines [27]. An important class of proteins performing crucial functions in the parasite and having blood stage vaccine potential are the Thrombospondin Structural Repeat (TSR) containing proteins [28]. Identifying the proteins involved in the invasion of red blood cell by the malaria parasite is commonly used to identify novel blood stage vaccine candidates [29]. The malaria parasite life cycle involves two distinct hosts, mosquito and humans [30]. Mosquito injects the sporozoites during a blood meal into the human host, which infect liver cells [2]. Sporozoites undergo erythrocytic schizogony, releasing merozoites into the blood stream. This is followed by erythrocytic schizogony where the parasite multiplies asexually in the erythrocytes [4]. Merozoites infect red blood cells [30]. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites [6]. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease [7,8]. The gametocytes, male [microgametocytes] and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal [9]. The parasites' multiplication in the mosquito is known as the sporogonic cycle [10]. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes [11]. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts [12]. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands [13]. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle [14]. In absence of a successful vaccine, malaria control relies on the use of anti-malarial drugs [15]. It is important to understand the enemy before designing the defense strategies [16,17]. Therefore to recognize the complexities of parasite biology is the prerequisite to fight against malaria either through a vaccine or through antimalarials [18,19]. Various signaling pathways involving small molecules like cAMP, cGMP are shown to be crucial regulators of parasite invasion and propagation, so inhibitors of their downstream effectors involving Protein Kinase A (PKA), Protein Kinase G (PKG), Calcium Dependent Protein kinases (CDPK1) like proteins can prove to be vital for antimalarial chemotherapy [20]. Phosphatidylinositol

4-Kinase (PI4K) has also shown to be an attractive target for malaria treatment [21]. In addition targeting parasite specific pathways and enzymes is also important to avoid the off target effects of antimalarials [22]. Artemisinin-Based Combination Therapies (ACTs) are the best available treatment for *P. falciparum* malaria [23]. The impact of the use of ACTs for malaria treatment is proven by the fact that Miss Tu Youyou was awarded the 2015 Nobel Prize for Medicine for the discovery of the malaria drug, Artemisinin [24]. But to the dismay of malaria research community all over the world, parasite resistance to artemisinins has been detected in 5 countries of the Greater Mekong subregion: Cambodia, Lao People's Democratic Republic, Myanmar, Thailand and Viet Nam as observed in case of all previous anti-malarial drugs [25]. The major concern now is the spread of multi-drug resistance to other regions with dire public health consequences. Many proteins have been identified to have role in ART resistance (e.g., ATG18, coronin, pfap2, falcipain 2a) [26], but the *P. falciparum* Kelch 13 [PfK13] protein is the validated marker for ART resistance so far, as mutations in its propeller domain have been associated with ART resistance [27]. The most critical benchmark in the study of ART resistance was the identification of single point mutations in the propeller region of *P. falciparum* kelch protein gene on chromosome 13 (PfK13) as a molecular marker associated with delayed parasite clearance *in vitro* and *in vivo* [28]. Numerous studies have been reported to determine the levels of polymorphisms of K13 in this region in order to map the spread and evolution of ART resistance [29]. Since K13 polymorphism is the only marker for ART resistance so far, it is important to screen for its genetic diversity in a region specific manner [30]. Very interestingly there have been some reports of slow parasite clearance rates even in absence of K13 mutant alleles suggesting the role of additional molecules in development of ART resistance in *P. falciparum*. It would be crucial to identify additional genetic loci involved in ART resistance [29]. In addition to *falciparum* malaria, it is inevitable to study the non-*falciparum* malaria, specially the *P. vivax* and also validate as well as screen for the drug resistance markers in *P. vivax*, especially in the endemic areas of higher *vivax* burden [29,30]. The pace at which the geographical extent of artemisinin resistance is spreading is faster than the rate at which control and elimination measures are being developed and introduced [31]. This emphasizes the fact that apart from understanding the current state and mechanisms of antimalarial drug resistance it is extremely essential at the same time to expand the current arsenal used against the parasite. This would include the identification and development of novel vaccine candidates and the anti-malarial drug targets for malaria [20-31].

CONCLUSION

A major challenge for the scientific community and funding agencies is to develop a successful and more effective malaria vaccine. Since a limited number of antigens

are being pursued as vaccine candidates so there is an urgent need to find more effective candidate vaccine antigens while testing the current candidates of malaria. In addition to identification of novel malaria vaccine candidates and validation of their vaccine potential, we need to identify new drug targets as well as new chemotherapies for malaria treatment to strengthen our arsenal in the fight against malaria. Understanding the parasite biology in further details will also broaden our strategies in its control and elimination worldwide.

References

- Siddiqui FA. Malaria control and elimination: How far we are: An Opinion Article. *J Biom Biostat.* 2016; 7: 321. DOI: 10.4172/2155-6180.1000321
- Ahmad S, Ahmad A, Aziz A. A path towards eliminating malaria: Drug resistance a major roadblock? *Sci J Biol.* 2020; 3: 001-009. DOI: <https://dx.doi.org/10.37871/sjb.id16>
- WHO. 2018. Status report on artemisinin resistance and ACT efficacy. 2019. <https://tinyurl.com/y45gkx8r>
- RTS malaria candidate vaccine reduces malaria by approximately one-third in African infants". Malaria vaccine org. Malaria Vaccine Initiative Path. 2013. <https://tinyurl.com/yhvhhl0f>
- Siddiqui FA, Dhawan S, Singh S, Singh B, Gupta P, Pandey A, et al. A thrombospondin structural repeat containing rho protein from *Plasmodium falciparum* mediates erythrocyte invasion. *Cell Microbiol.* 2013; 15: 1341-1356. DOI: <https://doi.org/10.1111/cmi.12118>
- Pandey AK, Reddy KS, Sahar T, Gupta S, Singh H, Reddy EJ, et al. Identification of a potent combination of key *Plasmodium falciparum* merozoite antigens that elicit strain-transcending parasite-neutralizing antibodies. *Infect Immun.* 2013; 81: 441-451. DOI: 10.1128/IAI.01107-12
- Cowman AF, Berry D, Baum J. The cellular and molecular basis for malaria parasite invasion of the human red blood cell. *JCB.* 2012; 198: 961-971. DOI: <https://doi.org/10.1083/jcb.201206112>
- Arnott A, Barry AE, Reeder JC. Understanding the population genetics of *Plasmodium vivax* is essential for malaria control and elimination. *Malar J.* 2012; 11: 14. DOI: 10.1186/1475-2875-11-14.
- Dawn A, Singh S, More KR, Siddiqui FA, Pachikara N, Ramdani G, et al. The central role of cAMP in regulating *Plasmodium falciparum* merozoite invasion of human erythrocytes. *PLoS Pathog.* 2014; 10: e1004520. DOI: <https://doi.org/10.1371/journal.ppat.1004520>
- Alam MM, Solyakov L, Bottrill AR, Flueck C, Siddiqui FA, Singh S, et al. Phosphoproteomics reveals malaria parasite. Protein Kinase G as a signalling hub regulating egress and invasion. *Nat. Commun.* 2015; 6: 7285. <https://tinyurl.com/y39c5cv8>
- McNamara CW, Lee MC, Lim CS, Lim SH, Roland J, Simon O, et al. Targeting *Plasmodium* PI(4)K to eliminate malaria. *Nature.* 2013; 504: 248-253. DOI: 10.1038/nature12782.
- Hati S, Madurkar SM, Bathula C, Thulluri C, Agarwal R, Siddiqui FA, et al. Design, synthesis and biological evaluation of small molecules as potent glucosidase inhibitors. *Eur J Med Chem.* 2015; 100: 188-196. DOI: <https://doi.org/10.1016/j.ejmech.2015.04.059> PMID:26087029.
- Hassett MR, Sternberg AR, Roepe PD. Inhibition of human class I vs class III phosphatidylinositol 3'-kinases. *Biochemistry.* 2017; 56: 4326-4334. DOI: <https://doi.org/10.1021/acs.biochem.7b00413>
- Rocamora F, Zhu L, Liong KY, Dondorp A, Miotto O, Mok S, et al. Oxidative stress and protein damage responses mediate artemisinin resistance in malaria parasites. *PLoS Pathog.* 2018; 14: e1006930. DOI: <https://doi.org/10.1371/journal.ppat.1006930>
- Breglio KF, Amato R, Eastman R, Lim P, Sa JM, Guha R, et al. A single nucleotide polymorphism in the *Plasmodium falciparum* atg18 gene associates with artemisinin resistance and confers enhanced parasite survival under nutrient deprivation. *Malar J.* 2018; 17: 391. DOI: <https://doi.org/10.1186/s12936-018-2532-x>
- Demas AR, Sharma AI, Wong W, Early AM, Redmond S, Bopp S, et al. Mutations in *Plasmodium falciparum* actin-binding protein coronin confer reduced artemisinin susceptibility. *Proc Natl Acad Sci.* 2018; 115: 12799-12804. DOI: <https://doi.org/10.1073/pnas.1812317115>
- Henriques G, van Schalkwyk DA, Burrow R, Warhurst DC, Thompson E, Baker DA, et al. The Mu subunit of *Plasmodium falciparum* clathrin-associated adaptor protein 2 modulates in vitro parasite response to artemisinin and quinine. *Antimicrob Agents Chemother.* 2015; 59: 2540-2547. DOI: <https://doi.org/10.1128/AAC.04067-14>
- Siddiqui FA, Cabrera M, Wang M, Brashear A, Kemirembe K, Wang Z, Miao J, et al. *Plasmodium falciparum* falcipain-2a polymorphisms in Southeast Asia and their association with artemisinin resistance. *J Infect Dis.* 2018; 218: 434-442. DOI: <https://doi.org/10.1093/infdis/jiy188>
- Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois A-C, Khim N, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature.* 2014; 505: 50. <https://tinyurl.com/y253zj8p>
- Siddiqui FA, Boonhok R, Cabrera M, Mbenda HGN, Wang M, Min H, et al. Role of *Plasmodium falciparum* Kelch 13 Protein Mutations in *P. falciparum* Populations from Northeastern Myanmar in Mediating Artemisinin Resistance. *Mbio:* 2020; 11: e01134-1119. DOI: <https://doi.org/10.1128/mBio.01134-19> PMID:32098812
- Takala-Harrison S, Clark TG, Jacob CG, Cummings MP, Miotto O, Dondorp AM, et al. Genetic loci associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment in Southeast Asia. *Proc Natl Acad Sci USA.* 2013; 110: 240-245. DOI: <https://doi.org/10.1073/pnas.1211205110>
- Tun KM, Imwong M, Lwin KM, Win AA, Hlaing TM, Hlaing T, et al. Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: A cross-sectional survey of the K13 molecular marker. *Lancet Infect Dis.* 2015; 15: 415-421. DOI: [https://doi.org/10.1016/S1473-3099\(15\)70032-0](https://doi.org/10.1016/S1473-3099(15)70032-0)
- Zhang J, Li N, Siddiqui FA, Xu S, Geng J, Zhang J, et al. *In vitro* susceptibility of *Plasmodium falciparum* isolates from the China-Myanmar border area to artemisinins and correlation with K13 mutations. *Int J for Parasitol Drugs Drug Resist.* 2019; 10: 20-27. DOI: 10.1016/j.ijpddr.2019.04.002
- Zhao Y, Ziling Liu, Soe MT, Wang L, Soe TN, Wei H, et al. Genetic Variations Associated with Drug Resistance Markers in Asymptomatic *Plasmodium falciparum* Infections in Myanmar. *Genes.* 2019; 10: 692. DOI: 10.3390/genes10090692
- Wang M, Siddiqui FA, Fan Q, Luo E, Cao Y, Cui L. Limited genetic diversity in the PvK12 Kelch protein in *Plasmodium vivax* isolates from Southeast Asia. *Malar J* 2016; 15: 537. DOI: <https://doi.org/10.1186/s12936-016-1583-0>
- Mbenda HGN, Zeng W, Bai Y, Siddiqui FA, Yang Z, Cui L. Genetic diversity of the *Plasmodium vivax* phosphatidylinositol 3-kinase gene in two regions of the China-Myanmar border. *Infect Genet Evol.* 2018; 61: 45-52. <https://tinyurl.com/yhnhcnyo2>
- Li J, Zhang J, Li Q, Hu Y, Ruan Y, Tao Z, et al. *Ex vivo* susceptibilities of *Plasmodium vivax* isolates from the China-Myanmar border to antimalarial drugs and association with polymorphisms in Pvdml1 and Pvcrt-o genes. *PLOS Neglected Tropical Diseases.* 2020; 14: e0008255. DOI: <https://doi.org/10.1016/j.megid.2018.02.018>
- Mbenda HGN, Wang M, Guo J, Siddiqui FA, Hu Y, Yang Z, et al. Evolution of the *Plasmodium vivax* multidrug resistance 1 gene in the Greater Mekong Subregion during malaria elimination. *Parasites & vectors.* 2020; 13: 67. DOI: 10.1186/s13071-020-3934-5.

How to cite this article: Ahmad A, Ahmad S. A Review: Artemisinin-Based Combination Therapies [ACTs] and K13 Polymorphism. J Environ Life Sci. 2020; 6: 088-091. DOI: <https://dx.doi.org/10.37871/jels1124>