

## Taming the beast: Update on Malaria Research

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**Abstract:** Malaria is a disease caused by an apicomplexan parasite, *plasmodium*. It can be life threatening and fatal if not treated promptly. 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. 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 (1, 2). 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. According to the 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.

**Key words:** Malaria Vaccine, Artemisinine, RTS, K13, AS01

### K13, Artemisinine and AS01

We have been struggling to develop an efficacious vaccine to strengthen our fight against malaria. Until now there is no malaria vaccine available commercially. 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. Antigens which are being used now days were discovered decades ago. However some progress has been made in the past several years toward developing malaria vaccine. The most advanced vaccine candidate is RTS,S/AS01 against the most deadly form of human malaria, *Plasmodium falciparum*. It is a recombinant protein candidate malaria vaccine that targets the *P. falciparum* circumsporozoite protein. 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 consider acceptable. However significant advances have been made with the completion of a Phase 3 Clinical trial of the RTS,S/AS01 candidate vaccine. In 2009 A phase 3 Clinical trial of the RTS,S/AS01 began in seven countries. It is completed and Phase 4 has been began now (3, 4).

A constant need to identify novel vaccine candidate as well as their characterization to explore their vaccine potential is the need of the hour. Erythrocyte binding antigen family, and the Rh protein family until now are the most promising candidates for development of blood stage vaccines. 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 (5). 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. In addition the approach to combine numerous antigens together to develop an effective vaccine has also been a successful strategy where the parasite is targeted at more than one pathways (6).

The malaria parasite life cycle involves two distinct hosts, mosquito and humans. Mosquito injects the sporozoites during a blood meal into the human host, which infect liver cells. Sporozoites undergo exo-erythrocytic schizogony, releasing merozoites into the blood stream. This is followed by erythrocytic schizogony where the parasite multiplies asexually in the erythrocytes. Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (7).

In absence of a successful vaccine, malaria control relies on the use of anti-malarial drugs. It is important to understand the enemy before designing the defense strategies. Therefore to recognize the complexities of parasite biology is the prerequisite to fight against malaria either through a vaccine or through antimalarials (8-10). 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 (11, 12). Phosphatidylinositol 4-Kinase (PI4K) has also shown to be an attractive target for malaria treatment (13). In addition targeting parasite specific pathways and enzymes is also important to avoid the off target effects of antimalarials(14).

Artemisinin-based combination therapies (ACTs) are the best available treatment for *P. falciparum* malaria. The impact of the use of ACTs for malaria treatment is proven by the fact that Miss TuYouyou was awarded the 2015 Nobel Prize for Medicine for the discovery of the malaria drug, Artemisinin. 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, Hailand and Viet Nam as observed in case of all previous anti-malarial drugs. 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)(15-20), 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 (21). 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*(21, 22). 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. 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 (23-26). 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. 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 (27-30). 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. 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.

### 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

1. Siddiqui FA. Malaria Control and Elimination: How Far we are: An Opinion Article. Journal of Biometrics & Biostatistics 2016. DOI: 10.4172/2155-6180.1000321
2. Ahmad S, Ahmad A, Aziz A. A Path Towards Eliminating Malaria: Drug Resistance A Major Roadblock? Sci J Biol. 2020;3(1): 001-009. <https://dx.doi.org/10.37871/sjb.id16>
3. WHO. 2018. Status report on artemisinin resistance and ACT efficacy. <https://www.who.int/malaria/publications/atoz/artemisinin-resistance-august2018/en/>. Accessed 17 July 2019.
4. "RTS,S malaria candidate vaccine reduces malaria by approximately one-third in African infants". malariavaccine.org. Malaria Vaccine Initiative Path. Archived from the original on 23 March 2013. Retrieved 19 March 2013.
5. Siddiqui FA, Dhawan S, Singh S, Singh B, Gupta P, Pandey A, Mohammed A, Gaur D, Chitnis CE. 2013. A thrombospondin structural repeat containing rhoptry protein from Plasmodium falciparum mediates erythrocyte invasion. Cell Microbiol 15:1341-1356. <https://doi.org/10.1111/cmi.12118>

6. Pandey AK, Reddy KS, Sahar T, Gupta S, Singh H, Reddy EJ, Asad M, Siddiqui FA, Gupta P, Singh B, More KR, Mohammed A, Chitnis CE, Chauhan VS, Gaur D. 2013. Identification of a potent combination of key Plasmodium falciparum merozoite antigens that elicit strain-transcending parasite-neutralizing antibodies. *Infect. Immun.* 81:441-451. doi:10.1128/IAI.01107-12
7. 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;81:441-51
8. Cowman AF, Berry D, Baum J (2012). "The cellular and molecular basis for malaria parasite invasion of the human red blood cell". *Journal of Cell Biology.* 198 (6): 961-71.
9. Arnott A, Barry AE, Reeder JC (2012). "Understanding the population genetics of Plasmodium vivax is essential for malaria control and elimination". *Malaria Journal.* 11: 14.
10. Liang X, Hart KJ, Dong G, Siddiqui FA, Sebastian A, Li X, Albert I, Miao J, Lindner SE, Cui L. 2018. Puf3 participates in ribosomal biogenesis in malaria parasites. *J Cell Sci* 131:jcs212597. <https://doi.org/10.1242/jcs.212597>.
11. Brashear AM, Roobsoong W, Siddiqui FA, Nguitrugool W, Sattabongkot J, López-Urbe MM, et al. (2019) A glance of the blood stage transcriptome of a Southeast Asian Plasmodium ovale isolate. *PLoS Negl Trop Dis* 13(11): e0007850. <https://doi.org/10.1371/journal.pntd.0007850>
12. Dawn A, Singh S, More KR, Siddiqui FA, Pachikara N, Ramdani G, Langsley G, Chitnis CE. 2014. The central role of cAMP in regulating Plasmodium falciparum merozoite invasion of human erythrocytes. *PLoS Pathog* 10:e1004520 <https://doi.org/10.1371/journal.ppat.1004520>
13. Alam MM, Solyakov L, Bottrill AR, Flueck C, Siddiqui FA, Singh S, Mistry S, Viskaduraki M, Lee K, Hopp CS, Chitnis CE, Doerig C, Moon RW, Green JL, Holder AA, Baker DA, and Tobin AB. (2015) Phosphoproteomics reveals malaria parasite Protein Kinase G as a signalling hub regulating egress and invasion. *Nat. Commun.* 6, 7285
14. McNamara CW, Lee MC, Lim CS, Lim SH, Roland J, Simon O, Yeung BK, Chatterjee AK, McCormack SL, Manary MJ, Zeeman AM, Dechering KJ, Kumar TS, Henrich PP, Gagaring K, Ibanez M, Kato N, Kuhlen KL, Fischli C, Nagle A, Rottmann M, Plouffe DM, Bursulaya B, Meister S, Rameh L, Trappe J, Haasen D, Timmerman M, Sauerwein RW, Suwanarusk R, Russell B, Renia L, Nosten F, Tully DC, Kocken CH, Glynn RJ, Bodenreider C, Fidock DA, Diagana TT, Winzeler EA. Targeting Plasmodium PI(4)K to eliminate malaria. *Nature.* 2013 Dec 12;504(7479):248-253. doi: 10.1038/nature12782. Epub 2013 Nov 27.
15. Hati S, Madurkar SM, Bathula C, Thulluri C, Agarwal R, Siddiqui FA, Dangi P, Adepally U, Singh A, Singh S, Sen S. Design, synthesis and biological evaluation of small molecules as potent glucosidase inhibitors. *Eur J Med Chem.* 2015; 100:188-196. <https://doi.org/10.1016/j.ejmech.2015.04.059> PMID:26087029
16. Hassett MR, Sternberg AR, Roepe PD. 2017. Inhibition of human class I vs class III phosphatidylinositol 3-kinases. *Biochemistry* 56:4326 - 4334. <https://doi.org/10.1021/acs.biochem.7b00413>.
17. Rocamora F, Zhu L, Liang KY, Dondorp A, Miotto O, Mok S, Bozdech Z. 2018. Oxidative stress and protein damage responses mediate artemisinin resistance in malaria parasites. *PLoS Pathog* 14:e1006930. <https://doi.org/10.1371/journal.ppat.1006930>.
18. Breglio KF, Amato R, Eastman R, Lim P, Sa JM, Guha R, Ganesan S, Dorward DW, Klumpp-Thomas C, McKnight C, Fairhurst RM, Roberts D, Thomas C, Simon AK. 2018. A single nucleotide polymorphism in the Plasmodium falciparum atg18 gene associates with artemisinin resistance and confers enhanced parasite survival under nutrient deprivation. *Malar J* 17:391. <https://doi.org/10.1186/s12936-018-2532-x>.
19. Demas AR, Sharma AI, Wong W, Early AM, Redmond S, Bopp S, Neafsey DE, Volkman SK, Hartl DL, Wirth DF. 2018. Mutations in Plasmodium falciparum actin-binding protein coronin confer reduced artemisinin susceptibility. *Proc Natl Acad Sci USA* 115:12799 -12804. <https://doi.org/10.1073/pnas.1812317115>.

20. Henriques G, van Schalkwyk DA, Burrow R, Warhurst DC, Thompson E, Baker DA, Fidock DA, Hallett R, Flueck C, Sutherland CJ. 2015. The Mu subunit of Plasmodium falciparum clathrin-associated adaptor protein 2 modulates in vitro parasite response to artemisinin and quinine. *Antimicrob Agents Chemother* 59:2540 –2547. <https://doi.org/10.1128/AAC.04067-14>.
21. Siddiqui FA, Cabrera M, Wang M, Brashear A, Kemirembe K, Wang Z, Miao J, Chookajorn T, Yang Z, Cao Y, Dong G, Rosenthal PJ, Cui L. 2018. Plasmodium falciparum falcipain-2a polymorphisms in Southeast Asia and their association with artemisinin resistance. *J Infect Dis* 218:434 – 442. <https://doi.org/10.1093/infdis/jiy188>.
22. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois A-C, Khim N, Kim S, Duru V, Bouchier C, Ma L, Lim P, Leang R, Duong S, Sreng S, Suon S, Chuor CM, Bout DM, Ménard S, Rogers WO, Genton B, Fandeur T, Miotto O, Ringwald P, Le Bras J, Berry A, Barale J-C, Fairhurst RM, Benoit-Vical F, Mercereau-Puijalon O, Ménard D. 2014. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. *Nature* 505:50 –55.
23. Siddiqui FA, Boonhok R, Cabrera M, Mbenda HGN, Wang M, Min H, Liang X, Qin J, Zhu X, Miao J, Cao Y, Cui L. Role of Plasmodium falciparum Kelch 13 Protein Mutations in P. falciparum Populations from Northeastern Myanmar in Mediating Artemisinin Resistance. *Mbio*:2020;11:e01134–19. <https://doi.org/10.1128/mBio.01134-19> PMID:32098812
24. Takala-Harrison S, Clark TG, Jacob CG, Cummings MP, Miotto O, Dondorp AM, Fukuda MM, Nosten F, Noedl H, Imwong M, Bethell D, Se Y, Lon C, Tyner SD, Saunders DL, Socheat D, Ariey F, Phyo AP, Starzengruber P, Fuehrer HP, Swoboda P, Stepniewska K, Flegg J, Arze C, Cerqueira GC, Silva JC, Ricklefs SM, Porcella SF, Stephens RM, Adams M, Kenefic LJ, Campino S, Auburn S, MacInnis B, Kwiatkowski DP, Su XZ, White NJ, Ringwald P, Plowe CV. 2013. Genetic loci associated with delayed clearance of Plasmodium falciparum following artemisinin treatment in Southeast Asia. *Proc Natl Acad Sci USA* 110:240 –245. <https://doi.org/10.1073/pnas.1211205110>.
25. Tun KM, Imwong M, Lwin KM, Win AA, Hlaing TM, Hlaing T, Lin K, Kyaw MP, Plewes K, Faiz MA, Dhorda M, Cheah PY, Pukrittayakamee S, Ashley EA, Anderson TJ, Nair S, McDew-White M, Flegg JA, Grist EP, Guerin P, Maude RJ, Smithuis F, Dondorp AM, Day NP, Nosten F, White NJ, Woodrow CJ. 2015. Spread of artemisinin-resistant Plasmodium falciparum in Myanmar: a cross-sectional survey of the K13 molecular marker. *Lancet Infect Dis* 15:415– 421. [https://doi.org/10.1016/S1473-3099\(15\)70032-0](https://doi.org/10.1016/S1473-3099(15)70032-0).
26. Zhang J, Li N, Siddiqui FA, Xu S, Geng J, Zhang J, He X, Zhao L, Pi L, Zhang Y, Li C, Chen X, Wu Y, Miao J, Cao Y, Cui L, Yang Z. In vitro susceptibility of Plasmodium falciparum isolates from the China-Myanmar border area to artemisinins and correlation with K13 mutations. 2019 *International Journal for Parasitology: Drugs and Drug Resistance*. DOI: 10.1016/j.ijpddr.2019.04.002
27. Zhao Y, Ziling Liu, Soe MT, Wang L, Soe TN, Wei H, Than A, Aung PL, Li Y, Zhang X, Hu Y, Wei H, Zhang Y, Burgess J, Siddiqui FA, Menezes L, Wang Q, Kyaw MP, Cao Y, Cui L. Genetic Variations Associated with Drug Resistance Markers in Asymptomatic Plasmodium falciparum Infections in Myanmar. 2019 *Genes* 10 (9), 692. DOI: 10.3390/genes10090692
28. 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. <https://doi.org/10.1186/s12936-016-1583-0>
29. Ngassa Mbenda HG, Zeng W, Bai Y, Siddiqui FA, Yang Z, Cui L. Genetic diversity of the Plasmodium vivax phosphatidylinositol 3-kinase gene in two regions of the China–Myanmar border. *Infect Genet Evol*. 2018;61:45–52.
30. Li J, Zhang J, Li Q, Hu Y, Ruan Y, Tao Z, Xia H, Qiao J, Meng L, Zeng W, Li C, He X, Zhao L, Siddiqui FA, Miao J, Yang Z, Fang Q, Cui L. Ex vivo susceptibilities of Plasmodium vivax isolates from the China-Myanmar border to antimalarial drugs and association with polymorphisms in Pvm-dr1 and Pvcrt-o genes. 2020 *PLOS Neglected Tropical Diseases* 14 (6), e0008255
31. Mbenda HGN, Wang M, Guo J, Siddiqui FA, Hu Y, Yang Z, Kittichai V, Sattabongkot J, Cao Y, Jiang L, Cui L. Evolution of the Plasmodium vivax multidrug resistance 1 gene in the Greater Mekong Subregion during malaria elimination. 2020. *Parasites & vectors* 13 (1), 67.