Comparison of Antibody Responses and Parasite Clearance in Artemisinin Therapeutic Efficacy Studies in the Democratic Republic of Congo and Asia

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Malaria remains a major cause of disease and death globally, with approximately 241 million cases and 627 000 deaths estimated in 2020, predominantly children living in sub-Saharan Africa [1]. While the scale-up of malaria control interventions, notably long-lasting insecticide nets and access to first-line artemisinin-based combination therapies (ACTs), have led to substantial reductions in the global burden of malaria over the last 2 decades [2,3], progress has now stalled, and malaria case incidence increased significantly from 2019 to 2020 [1].

Malaria control and elimination is threatened by the emergence of artemisinin-resistant Plasmodium falciparum. Artemisinin resistance is characterized by delayed parasite clearance following treatment with artemisinin derivatives. The slow-clearing phenotype is associated with nonsynonymous mutations in the propeller region of the P. falciparum kelch13 (K13) gene [4,5]. Currently, in therapeutic efficacy studies, delayed parasite clearance is defined by either a parasite clearance half-life (PC\textsubscript{½}) of ≥5 hours or persistent parasitemia by microscopy on day 3 after treatment [6]. Reports of artemisinin resistance first emerged in Western Cambodia in 2009, and over the following decade therapeutic efficacy studies...
revealed that artemisinin resistance continued to spread throughout the Greater Mekong subregion [5, 7–10] and west to West Bengal in India [11]. In African countries, despite a high diversity of nonsynonymous mutations present in kelch13 in *P. falciparum* parasites, the gene is not currently undergoing strong selection [12, 13]. More recently, however, de novo emergence of kelch13 mutations conferring resistance were identified in Rwandan and Ugandan patients [14–16]. If resistance becomes widespread in high-burden countries in Sub-Saharan Africa, it is estimated that ACT treatment failure could lead to an excess of 78 million cases and 116 000 deaths over a 5-year period [17]. It is therefore essential that the efficacy of artemisinin is accurately monitored to inform appropriate public health responses to prevent or contain the emergence and spread of artemisinin resistance in Africa and to preserve the life span of artemisinin derivatives.

While detection of artemisinin-resistant polymorphisms in *P. falciparum* infections is unequivocal, parasite clearance rates in response to ACT vary considerably between patients. Many factors contribute to this variability, including differences in splenic function, pharmacokinetics, parasite factors including the stage and synchronicity of the infection, and naturally acquired immunity [18–21]. Immunity to clinical malaria develops after repeated infections and predominantly targets the blood stages of malaria parasites [22]. It develops more quickly in high-transmission areas [23, 24], and it influences the epidemiology of malaria. In high-transmission areas such as sub-Saharan Africa, children bear the majority of the burden of symptomatic disease, whereas in low-transmission areas all ages are susceptible. Naturally acquired immunity is associated with reduced parasite densities [25], thus in higher-transmission settings immunity may mask the emergence of slow-clearing phenotypes, by reducing baseline parasitemia (a risk factor for delayed clearance) [20] or enhancing parasite clearance after treatment [26, 27]. Understanding the potential confounding effect of immunity on parasite clearance measures across a range of epidemiological settings is important to inform artemisinin resistance surveillance.

In a multinational therapeutic efficacy study (Tracking Resistance to Artemisinin Collaboration [TRAC]), we found that naturally acquired antibodies to *P. falciparum* varied within- and between-study sites in Southeast Asia, a region of relatively low malaria transmission where adults experience the greatest malaria burden, and antibody responses were associated with shorter PC_{50} following artemisinin treatment [26–28]. How immunity differs across the markedly different epidemiological contexts of Southeast Asia and sub-Saharan Africa is unknown, along with its impact on parasite clearance in therapeutic efficacy studies. Given the public health consequences of the emergence of artemisinin resistance in sub-Saharan Africa, we investigated antibody responses to selected *P. falciparum* antigens and artemisinin resistance indicators in children with acute uncomplicated malaria enrolled in the TRAC study in Kinshasa, Democratic Republic of Congo (DRC), an area of high perennial transmission. We compared these with results in the TRAC Asian sites, where malaria transmission is comparably low and heterogenous.

**METHODS**

**Study Design and Procedures**

Plasma samples were taken from 118 patients with uncomplicated *falciparum* malaria (of a total of 119) from Kinshasa, DRC, participating in the TRAC multicenter open-label drug-efficacy randomized control trial (Clinical Trials Registration NCT01350856). Antibody responses were compared with those in patients from Asian sites from the same trial, which have been published elsewhere [26]. Details of the original study design, inclusion criteria and procedures, as well as characterization of antibody responses in patients from Asian sites have been published elsewhere [5, 26]. Informed consent was obtained from all patients, and ethical approval was granted by the Oxford Tropical Research Ethics Committee (06/11), Alfred Hospital Committee for Ethics, Australia (485/12) the institutional review board, national ethics committee, or both for each site. Patients in the DRC were admitted to the hospital for supervised treatment with either artether-lumefantrine or artemesunate monotherapy at a dose of 4 mg/kg/d for 3 days, followed by a full course of artether-lumefantrine [5]. Body temperature, hematocrit, and blood smears were obtained for malaria parasite counts at 0, 4, 6, 8, and 12 hours and then every 6 hours until 2 consecutive counts were negative. The full sequence of kelch13 was determined for isolates collected at admission [5]. Herein, kelch13 mutations conferring resistance were defined as mutations after position 440. Patients infected with these parasites were compared with those with either wild-type infections or infections with kelch13 mutations before amino acid position 441.

**Measurement of Immunoglobulin G Antibodies to Parasite Antigens**

At enrollment, plasma concentrations of total antigen-specific immunoglobulin (Ig) G were measured by enzyme-linked immunosorbent assay (ELISA), as described elsewhere [26]; recombinant *P. falciparum* merozoite antigens [29], including merozoite surface protein (MSP) 1, C-terminal 19-kDa region (MSP1\_19), MSP2, FC27 allele (MSP2\_FC27); MSP2, 3D7 allele (MSP2\_3D7); MSP3; MSP6; MSP7; apical membrane antigen 1, reticulocyte-binding ligand homologue 2 (Rh2), erythrocyte-binding antigen (EBA) 175, region 2 (EBA175\_R2); EBA175, regions 3–5 (EBA175\_R3–5), and circumsporozoite protein (CSP) [30], were measured with high-throughput ELISA, as described elsewhere [26, 27]. All steps were performed on a Janus high-throughput liquid handling platform (Perkin Elmer), except...
for antigen coating. Antibody positivity was defined as optical density values higher than 2 standard deviations above the mean of nonexposed Melbourne controls. Antibody levels to variant surface antigens (VSAs) of *P. falciparum*-infected erythrocytes (3D7 strain) at the pigmented trophozoite stage were measured with flow cytometry [31].

**Statistical Analysis**

To investigate the association between antibodies and parasite clearance, the outcome variable PC½ (in hours) was derived using the WWARN parasite clearance estimator [19]. Multiple linear regression analysis was performed to determine the mean difference in PC½ between antibody response (seropositive/seronegative and continuous) for each antibody, adjusting for age and for whether patients had received artemether-lumefantrine or initial artesunate monotherapy. Median antibody levels and the proportion of IgG responders were compared for the DRC and Asian sites.

**RESULTS**

**Patient Characteristics, Artemisinin Resistance, and *P. falciparum* Antibody Profiles**

A total of 118 patients with uncomplicated falciparum malaria from Kinshasa, DRC, were included in the current study. All patients were children (median age [interquartile range (IQR)], 5 [3–6] years [3, 6]; 63 of 118 [53.4%] male), differing from patients enrolled in the Asian study sites, who were typically men of working age (Supplementary Table 1). At enrollment, the median (IQR) *P. falciparum* density in patients from DRC was 60 037/µL (IQR, 35 042–109 900/µL), which was similar to the median parasite densities in patients from Asian sites (Supplementary Table 1). In patients from Kinshasa, the median PC½ (IQR) was 2 (1.6–2.4) hours, with only 2 patients having a PC½ ≥5 hours and detectable parasitemia at day 3 and PC½ differed by treatment group (2.2 [1.7–2.5] hours for artemether-lumefantrine vs 1.85 [1.3–2.2] hours for artesunate [4mg/kg]).

The prevalence of kelch13 mutations in the propeller region was 2.5% (3 of 118), but none of these 3 children had PC½ ≥5 hours, and these markers (A578S, Q613E, and S522C) have not been associated with resistance elsewhere [32]. With the exception of patients in Ramu, Bangladesh, and Attapeu, Laos (≤2.6 hours), patients from most of the Asian sites had a higher median PC½ (range, 3–6.95 hours) and higher prevalence of kelch13 mutations (range, 9.1%–60.4%) (except for those from Ramu, Bangladesh; Attapeu, Laos; and Ratanakiri, Cambodia [≤0.8%]) (Supplementary Table 1) [5, 26].

IgG responses to multiple *P. falciparum* blood-stage antigens (merozoite and infected RBC) and the sporozoite antigen CSP, were quantified at enrollment, before antimalarial treatment (Figure 1). Both the levels and seroprevalence (27.1% [32 of 118]) of IgG responses to the sporozoite antigen CSP were lower in patients from Kinshasa (Supplementary Figure 1 and Supplementary Table 2), compared with the Asian sites (Supplementary Table 2). In patients from Kinshasa, seroprevalences of IgG responses to blood-stage antigens (MSP119, apical membrane antigen 1, MSP2fc27, MSP23D7, MSP3, MSP6, MSP7, EBA175RII, EBA175RIII−V, Rh2, and variant surface antigen, 3D7 strain (VSA3D7) ranged from 41.5% (49 of 118) to 89.0% (105 of 118). Both levels and seroprevalences to these antigens were broadly similar to responses in the Asian sites (Supplementary Figure 1 and Supplementary Table 2).

**Associations Between Antibody Responses and Treatment Efficacy**

We have previously reported that antibody responses are associated with shorter PC½ in Asian TRAC sites (mean difference in PC½ according to seropositivity, -0.16 to -0.65 hours overall and -0.07 to -0.52 hours in those with wild-type infections, depending on antigen) [26]. In contrast, there was no evidence for an association between antibody seropositivity or level for antibodies to *P. falciparum* antigens and shorter PC½ in patients from Kinshasa, DRC, after adjusting for age and treatment group (Table 1 and Supplementary Table 3 respectively). The mean PC½ for patients who were seronegative for antibodies to each of the *P. falciparum* antigens studied ranged from 1.91 to 2.15 hours, which was similar to the mean PC½ in seropositive patients (mean difference in PC½ close to zero) (Table 1). Similarly, there was no association between antibody levels and PC½ when antibody responses were analyzed as continuous variables; for every 2-fold increase in IgG level (optical density value), the change in PC½ was also close to zero (range, -0.07 to 0.15 hours) (Supplementary Table 3). Sensitivity analysis whereby the 3 cases with kelch13 mutants conferring resistance were removed (all of which had PC½ <5 hours) did not significantly alter results.

**DISCUSSION**

In the context of a multinational therapeutic efficacy study, we found that children with uncomplicated malaria from Kinshasa, DRC, had levels of antibodies to selected *P. falciparum* antigens that were broadly comparable to those seen in patients from Asian TRAC sites. In contrast to what was previously reported for pooled individual patient data from the TRAC sites in Asia [26], these antibody responses were not associated with faster parasite clearance. Thus, the substantially more rapid *P. falciparum* parasite clearance observed in Kinshasa compared with Asia was not explained by differences in the concentrations of the *P. falciparum* antibodies measured in this study. This may be because artesunate was still highly effective at killing drug-sensitive ring-stage parasites in DRC and mechanisms independent of the antibody-responses examined were sufficient for rapid parasite clearance.
Naturally acquired immunity to *P. falciparum* malaria, which protects against high-density infections and clinical symptoms, is acquired after repeated infections and underpins the age-dependent distribution of clinical disease in malaria-endemic areas [22]. Reflecting this, patients with uncomplicated clinical malaria enrolled in the TRAC study were young children in Kinshasa, DRC, an area of high perennial transmission, whereas in relatively low-transmission countries of Asia, patients were typically adults, a high-risk group in this region due to occupational exposure [33, 34]. Despite the different age profiles of the African and Asian participants, all infections had progressed to acute, uncomplicated clinical disease.

**Figure 1.** Immunoglobulin (Ig) G responses to *Plasmodium falciparum* antigens in the Tracking Resistance to Artemisinin Collaboration (TRAC) study participants from Kinshasa, Democratic Republic of Congo. *A*, Dots represent individual IgG responses to selected recombinant *P. falciparum* antigens; black horizontal lines, medians; and whiskers, interquartile ranges. Abbreviation: MFI, mean fluorescence intensity; OD, optical density. *B*, Seroprevalences of IgG antibody responses to each antigen. Sporozoite antigen is shown in orange, merozoite surface proteins (MSPs) in dark green and rhoptry/microneme proteins in light green (measured by enzyme-linked immunosorbent assay), and variant surface antigens on 3D7-infected erythrocytes in blue (measured by flow cytometry). Seropositivity cutoffs for each antigen were as follows: circumsporozoite protein (CSP), 0.40; MSP1<sub>19</sub>, 0.27; MSP2<sub>FC27</sub>, 0.03; MSP2<sub>3D7</sub>, 0.07; MSP2, 0.14; MSP6, 1.15; MSP7, 0.17; apical membrane antigen 1 (AMA1), 0.07; erythrocyte-binding antigen (EBA) 175, region 2 (EBA175<sub>RII</sub>), 0.11; EBA 175, regions 3–5, EBA175<sub>RIII</sub>-, 0.07; reticulocyte-binding ligand homologue 2 (Rh2), 0.30; and VSA 3D7 336.1. Abbreviation: CI, confidence interval.
and all met the same study inclusion criteria; therefore, by definition, both groups lacked sufficient disease controlling activity for that infection. In these patients, children from DRC had levels of antibodies to relatively conserved merozoite antigens that were comparable to those of patients from Asian sites, who were mostly adults, suggesting that they may have had similar cumulative exposure.

While we assessed total IgG responses specific for a range of \textit{P. falciparum} antigens, including relatively conserved antigens and those genetically diverse across parasite populations, considerable variation was observed in responses to individual antigens both within and across populations and the potential role of functional antibody responses was not investigated. Functional antibody responses, including parasite opsonization to promote phagocytosis and merozoite lysis [41, 42].

Consistent with this hypothesis, we previously reported that among adults from the Asian TRAC sites, the magnitude of effect of antibody positivity on \( \text{PC}_{50} \) was larger in patients infected with parasites containing \textit{kelch13} mutations conferring resistance than in those with wild-type parasites or parasites with other \textit{kelch13} mutations. However, the association between antibodies and \( \text{PC}_{50} \) was still evident in Asian patients with wild-type parasites, unlike what was observed in DRC patients [26]. This may be related to the younger age of the African patients. In Malian children receiving oral arsensuate for treatment of uncomplicated malaria, antibody responses to autologous infected erythrocytes were positively correlated with age and negatively correlated with peak pitting rate [39, 43]. This immune-independent parasite clearance mechanism may partially explain why, overall, DRC patients had substantially more rapid \textit{P. falciparum} parasite clearance than Asian patients, despite some children having been treated with artemether-lumefantrine, which was associated with longer parasite clearance time (median, 2.2 hours vs 1.85 hours with

### Table 1. Associations Between Antibody Seropositivity and Parasite Clearance Half-Life in 118 Patients from Kinshasa, Democratic Republic of Congo, With Adjustment for Age and Treatment

| Antigen | No. (%) Seropositive | \( \text{PC}_{50} \) in Seronegative Patients, Mean, h | Difference in \( \text{PC}_{50} \) if Seropositive, Mean (95% CI), h | \( P \) Value |
|---------|----------------------|-----------------------------------------------|------------------------------------------------|----------------|
| CSP     | 32 (27.1)            | 2.10                                          | –.11 (–.48 to .26)                              | .56            |
| MSP119  | 82 (69.5)            | 2.14                                          | –.09 (–.44 to .27)                              | .64            |
| MSP2FC27| 106 (89.0)           | 2.15                                          | –.10 (–.63 to .44)                              | .72            |
| MSP23D7 | 104 (88.1)           | 2.05                                          | –.002 (–.53 to .52)                             | >.99           |
| MSP3    | 80 (67.8)            | 2.02                                          | –.11 (–.25 to .46)                              | .56            |
| MSP6    | 49 (41.5)            | 2.13                                          | –.14 (–.47 to .20)                              | .43            |
| MSP7    | 91 (77.1)            | 2.07                                          | –.01 (–.40 to .42)                              | .95            |
| AMA1    | 94 (79.7)            | 2.05                                          | –.03 (–.40 to .45)                              | .91            |
| EBA175RII| 52 (44.1)           | 1.91                                          | .40 (.05–.75)                                   | .03            |
| EBA175RIILV| 93 (78.8)      | 2.03                                          | –.02 (–.40 to .44)                              | .93            |
| Rh2     | 78 (66.1)            | 1.95                                          | .17 (.19 to .53)                                | .35            |

Abbreviations: AMA1, apical membrane antigen 1; CI, confidence interval; CSP, circumsporozoite protein; EBA175RII, erythrocyte-binding antigen 175, region 3-5; MSP, merozoite surface protein; \( \text{PC}_{50} \), parasite clearance half-life; Rh2, reticulocyte-binding ligand homologue 2; VSA3D7, variant surface antigen, 3D7 strain

*Linear regression analysis adjusted for age and treatment arm. Analysis did not adjust for kelch13 genotype because only 3 individuals had \textit{Plasmodium falciparum} infections with \textit{kelch13} mutations associated with resistance. Sensitivity analysis indicated their inclusion had no effect on the association between antibody positivity and \( \text{PC}_{50} \).*

*In patients with a mean age of 4.6 years receiving 4 mg/kg/d of artesunate for 3 days (base values for linear regression model).*
artesunate [5]). Differences in innate immune mechanisms of parasite removal in children relative to adults [44, 45] may also play a role in the faster parasite clearance observed in Kinshasa compared with Asia. Further investigation is required to elucidate the factors underpinning differences in PC½ between populations, but our findings suggest that naturally acquired antibodies to the *P. falciparum* antigens investigated here do not contribute to differences in PC½ between patients from the Kinshasa, DRC, and Asian study sites.

Since the TRAC study was completed in 2013, artemisinin resistance has continued to spread throughout the Greater Mekong subregion [9, 10], west to West Bengal in India [11] and resistance to piperaquine and other partner drugs has emerged and spread in the Greater Mekong subregion [9]. Nonsynonymous mutations in *kelch13* have been identified in isolates from west, central and east Africa [13] and de novo emergence of *kelch13* mutations conferring slow-clearance resistance phenotypes have been identified in Rwandan (RS61H) [14, 15] and Ugandan patients (A675V or C469Y) [16]. In populations living in highly endemic areas in Africa, with naturally acquired immunity, the current PC½ cutoff for defining resistance (≥5 hours) may be too strict [16]. As the artemisinin resistance evolves in Africa, it will be important to define how to quantitate the contribution of immunity to parasite clearance and its confounding effects on measures of parasite clearance in children, who form the sentinel surveillance populations for artemisinin therapeutic efficacy studies. Current threshold measures of resistance, including day 3 parasite positivity and PC½ of ≥5 hours, need to be revised to facilitate monitoring of the emergence of artemisinin resistance across Africa.

### Supplementary Data

**Supplementary materials** are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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### References

1. World Health Organization. World malaria report 2021. Geneva, Switzerland: World Health Organization, 2021.
2. Bhatt S, Weiss DJ, Cameron E, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature 2015; 526:207–11.
3. World Health Organization. World malaria report 2020: 20 years of global progress and challenges. Geneva, Switzerland: World Health Organization, 2020.
4. Aruye F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature 2014; 505:50–5.
5. Ashley EA, Dhorda M, Fairhurst RM, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 2014; 371:411–23.
6. World Health Organization. Artemisinin and artemisinin-based combination therapy resistance: status report. Geneva, Switzerland: World Health Organization, 2018.
7. Donkor AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 2009; 361:455–67.
8. Takala-Harrison S, Jacob CG, Arze C, et al. Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. J Infect Dis 2015; 211:670–9.
9. van der Pluijm RW, Imwong M, Chau NH, et al. Determinants of dihydroartemisinin-piperacillin treatment failure in *Plasmodium falciparum* malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. *Lancet Infect Dis* 2019; 19:952–61.

10. van der Pluijm RW, Tripura R, Hoglund RM, et al. Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated *Plasmodium falciparum* malaria: a multicentre, open-label, randomised clinical trial. *Lancet* 2020; 395:1345–60.

11. Das S, Saha B, Hati AK, et al. Evidence of artemisinin-resistant *Plasmodium falciparum* malaria in eastern India. *N Engl J Med* 2018; 379:1962–4.

12. MalariaGEN Plasmodium falciparum Community Project. Genomic epidemiology of artemisinin resistant malaria. *eLife* 2016; 5:e08714.

13. Kayiba NK, Yobi DM, Tshibangu-Kabamba E, et al. Spatial and molecular mapping of PfKelch13 gene polymorphism in Africa in the era of emerging *Plasmodium falciparum* resistance to artemisinin: a systematic review. *Lancet Infect Dis* 2021; 21:e82–92.

14. Uwimana A, Legrand E, Stokes BH, et al. Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. *Nat Med* 2020; 26:1602–8.

15. Uwimana A, Umulisa N, Venkatesan M, et al. Association of *Plasmodium falciparum* kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. *Lancet Infect Dis* 2021; 21:1120–8.

16. Balikagala B, Fukuda N, Ikeda M, et al. Evidence of artemisinin-resistant malaria in Africa. *N Engl J Med* 2021; 385:1163–71.

17. Slater HC, Griffin JT, Ghani AC, et al. Assessing the potential impact of artemisinin and partner drug resistance in sub-Saharan Africa. *Malar J* 2016; 15:10.

18. White NJ. Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. *Antimicrob Agents Chemother* 1997; 41:1413–22.

19. Flegg JA, Guerin PJ, White NJ, et al. Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator. *Malar J* 2011; 10:339.

20. WWARN Parasite Clearance Study Group. Baseline data of parasite clearance in patients with falciparum malaria treated with an artemisinin derivative: an individual patient data meta-analysis. *Malar J* 2015; 14:359.

21. Intharabut B, Kingston HW, Srinamon K, et al. Artemisinin resistance and stage dependency of parasite clearance in falciparum malaria. *J Infect Dis* 2019; 219:1483–9.

22. Doolan DL, Dobaño C, Baird JK. Acquired immunity to malaria. *Clin Microbiol Rev* 2009; 22:13–36.

23. Marsh K. Kinyanjui S. Immune effector mechanisms in malaria. *Parasite Immunol* 2006; 28:51–60.

24. Griffin JT, Hollingsworth TD, Reyburn H, Drakeley CJ, Riley EM, Ghani AC. Gradual acquisition of immunity to severe malaria with increasing exposure. *Proc Biol Sci* 2015; 282:20142657.

25. Healer J, Chiu CY, Hansen DS. Mechanisms of naturally acquired immunity to *P. falciparum* and approaches to identify merozoite antigen targets. *Parasitology* 2018; 145:839–47.

26. Ataide R, Ashley EA, Powell R, et al. Host immunity to *Plasmodium falciparum* and the assessment of emerging artemisinin resistance in a multinational cohort. *Proc Natl Acad Sci U S A* 2017; 114:3515–20.

27. O’Flaherty K, Ataide R, Zaloumis SG, et al. Contribution of functional antimalarial immunity to measures of parasite clearance in therapeutic efficacy studies of artemisinin derivatives. *J Infect Dis* 2019; 220:1178–87.

28. Ataide R, Powell R, Moore K, et al. Declining transmission and immunity to malaria and emerging artemisinin resistance in Thailand: a longitudinal study. *J Infect Dis* 2017; 216:723–31.

29. Richards JS, Arumugam TU, Reiling L, et al. Identification and prioritization of merozoite antigens as targets of protective human immunity to *Plasmodium falciparum* malaria for vaccine and biomarker development. *J Immunol.* 2013; 191:795–809.

30. Kurtovic L, Behet MC, Feng G, et al. Human antibodies activate complement against *Plasmodium falciparum* sporozoites, and are associated with protection against malaria in children. *BMC Med* 2018; 16:61.

31. Chan JA, Howell KB, Reiling L, et al. Targets of antibodies against *Plasmodium falciparum*-infected erythrocytes in malaria immunity. *J Clin Invest* 2012; 122:3227–38.

32. Amaratunga C, Andrianaranjaka VH, Ashley E, et al. Association of mutations in the *Plasmodium falciparum* Kelch13 gene (PF3D7_1343700) with parasite clearance rates after artemisinin-based treatments—a WWARN individual patient data meta-analysis. *BMC Med* 2019; 17:1.

33. Cui L, Yan G, Sattabongkot J, et al. The central role of the spleen in malaria parasite clearance. *J Infect Dis* 2002; 185:1538–41.
36. Buffet PA, Safeukui I, Deplaine G, et al. The pathogenesis of *Plasmodium falciparum* malaria in humans: insights from splenic physiology. Blood 2011; 117:381–92.

37. Angus BJ, Chotivanich K, Udomsangpetch R, White NJ. In vivo removal of malaria parasites from red blood cells without their destruction in acute falciparum malaria. Blood 1997; 90:2037–40.

38. Newton PN, Chotivanich K, Chierakul W, et al. A comparison of the in vivo kinetics of *Plasmodium falciparum* ring-infected erythrocyte surface antigen-positive and -negative erythrocytes. Blood 2001; 98:450–7.

39. Ndour PA, Lopera-Mesa TM, Diakite SA, et al. *Plasmodium falciparum* clearance is rapid and pitting independent in immune Malian children treated with artesunate for malaria. J Infect Dis 2015; 211:290–7.

40. Dogovski C, Xie SC, Burgio G, et al. Targeting the cell stress response of *Plasmodium falciparum* to overcome artemisinin resistance. PLoS Biol 2015; 13:e1002132.

41. Boyle MJ, Reiling L, Feng G, et al. Human antibodies fix complement to inhibit *Plasmodium falciparum* invasion of erythrocytes and are associated with protection against malaria. Immunity 2015; 42:580–90.

42. Dutta S, Haynes JD, Barbosa A, et al. Mode of action of invasion-inhibitory antibodies directed against apical membrane antigen 1 of *Plasmodium falciparum*. Infect Immun 2005; 73:2116–22.

43. Lopera-Mesa TM, Doumbia S, Chiang S, et al. *Plasmodium falciparum* clearance rates in response to artesunate in Malian children with malaria: effect of acquired immunity. J Infect Dis 2013; 207:1655–63.

44. Dobbs KR, Crabtree JN, Dent AE. Innate immunity to malaria—the role of monocytes. Immunol. Rev 2020; 293:8–24.

45. Chua CLL, Ng IMJ, Yap BJM, Teo A. Factors influencing phagocytosis of malaria parasites: the story so far. Malar J 2021; 20:319.