Artemisinin resistance (ART-R) in parasites in tropical settings is relevant to public health because it might play a yet-recognized role in larva migrans pathology, which can be severe. Increased contact between raccoons and humans also warrants further investigation to improve understanding and minimize zoonotic risk.

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With these threats, we must acknowledge the fact that the future of Plasmodium falciparum, one of the most dangerous parasites, is uncertain. The emergence of ART-R in parasites of vertebrates: archival volume. Wallingford (UK): CAB International; 2009. p. 463.

To the Editor: Artemisinin resistance (ART-R) in Plasmodium falciparum phenotypes may have evolved independently in various areas of the Greater Mekong Sub-region (1,2), prompting the World Health Organization to change its regional policy from containment to elimination (3). Risks associated with ART-R include compromised use of artemisinin combined therapy, partner drug resistance selection, total ART-R resistance, and geographic extension

Artemisinin-Resistant Plasmodium falciparum K13 Mutant Alleles, Thailand–Myanmar Border

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to other malaria-endemic regions (2,3). Characterization of ART-R in each setting and rapid update of listed phenotypes classified as in vitro resistant to this antimalarial drug are needed.

Detected in western Cambodia in 2008, ART-R has been observed in neighboring countries, notably at the Thailand–Myanmar border (4,5). Resistance is partial and manifests by an increased parasite clearance half-life (PCHL) of >5 hours in patients receiving artemisinin monotherapy or artemisinin combined therapy (6). In vitro, ART-R phenotype has been characterized by the ring-stage survival assay (RSA (7) (cutoff 1%) and mutations in the propeller domain of a kelch gene on chromosome 13 (K13) (8,9). However, tremendous K13 variability in different genetic backgrounds requires confirmation of specific alleles as ART-R markers (2,3); even statistically significant clinical associations are rarely unequivocal (5–9).

On the Thailand–Myanmar border where ART-R has been documented (4), we investigated K13 mutations in clinical and in vitro phenotypes. Patients with nonsevere \textit{P. falciparum} hyperparasitemia infections treated during 2011–2013 at the Shoklo Malaria Research Unit (Mae Sot, Thailand) were treated with artesunate, then combined artesunate/mefloquine (5). We retrospectively selected 33 case-patients on the basis of PCHL outcome to analyze a broad parasite clearance distribution with available cryopreserved isolates. Full written consent from all patients was obtained. PCHL was calculated on the basis of initial and repeated parasitemia measurement every 6 hours until undetectable asexual parasitemia (6) was achieved. Venous blood samples were cryopreserved before drug administration (day 0).

Short-term, culture–adapted parasites (3% hematocrit; RPMI-1640 supplemented with 10% human serum, 0.05 mg/mL hypoxanthine, 2 mg/mL sodium bicarbonate, 2 mg/mL glucose, 0.04 mg/mL gentamicin, 0.3 mg/mL L-glutamine in a 37°C candle-jar atmosphere) were split for blinded RSA (8,9) and K13 genotyping. We performed RSA in duplicate by selecting early rings (0–3 h) in a combination of percoll gradient and sorbitol lysis, followed by a 6-h exposure to 700 nmol/L dihydroartemisinin (7). RSA survival rate was measured microscopically 66 hours after drug removal and calculated as the quotient of parasite clearance half-life and RSA survival (artemisinin resistance >1%). K13 alleles with variable parasite clearance half-life and RSA survival rates above threshold. Bold text indicates K13 alleles with variable parasite clearance half-life and RSA associations. Horizontal bars represent median values for each K13 genotype. Mean survival rate of duplicate measures are showed for each isolate. Dashed line represents the cutoff value for parasite clearance half-life (artemisinin resistance >5 h) and RSA survival (artemisinin resistance >1%). K13 alleles N458Y and C580Y were consistently associated with PCHL >5h and RSA values >1%. The C580Y allele has been repeatedly confirmed as a molecular marker of ART-R (5,7–9). Previous reports have inconsistently associated the N458Y mutation with ART-R; 7 case-patients with PCHL >5 h were reported by Ashley et al. (5), and 1 artemisinin sensitive case was reported at the China–Myanmar border (10). Nevertheless, this mutation has not been confirmed in vitro (3). We confirmed the mutation in vitro, and in vivo, according to the World Health Organization definition (3), this K13 allele as a molecular marker of ART-R.

Conflicting data observed between PCHL and RSA values for 4 mutant alleles (E252Q, P441L, G538V, and R-ggatatgatgttcttct) before sequencing (8). The 33 monoclonal isolates yielded clear K13 gene sequences. All except 4 isolates from patients who had PCHL >5 h had a single K13 mutant allele (19/23), and all isolates except 1 (G538V) from case-patients who had PCHL <5 h carried the K13 3D7 wild-type allele (9/10). PCHL was significantly different between K13 wild-type (n = 13, median 4.3 h) and mutant (n = 20, median = 7.2 h) alleles (p<0.01 by Mann-Whitney U test). Among the 25 isolates successfully tested, RSA survival rates differed significantly between K13 wild-type (n = 10, median 0.5%) and mutant (n = 15, median 3.5%) alleles (p<0.001 by Mann-Whitney U test). When PCHL was present <5 h, RSA survival rates (n = 7, median 0.5%) were significantly lower than when PCHL was >5 hours (n = 18, median 3.1%) (p = 0.001 by Mann-Whitney U test).
A675V) require further targeted approaches to relate them to previous reports. In a study in which only PCHL were reported (5), the proportion of slowly clearing infections were 69%, 0%, 30%, and 61% for the P441L, E252Q, G538V, and A675V alleles, respectively. Discrepancies can result from confounding pharmacologic (drug level, partner drug), immunologic, and parasitologic (genetic background, parasitic stage at treatment initiation) factors.

RSA results and K13 genotypes were associated with delayed parasite clearance, emphasizing the pertinence of each method to define ART-R. In this area, N458Y is a marker of ART-R. To solve conflicts about specific mutations, more detailed characterization in vitro and in vivo is needed.

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M.B., B.W., F.N., and D.M. contributed to the study design. M.B., B.W., and V.D. performed the in vitro assays. T.A., S.N., M.M-W., and K.S. performed the genetic polymorphism analyses. A.P.P. and F.N. coordinated and supervised the clinical studies. M.B., B.W., F.N. and D.M. analyzed the data and wrote the first draft of the manuscript. All authors contributed to the writing of the manuscript.

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Meningococcal Group W Disease in Infants and Potential Prevention by Vaccination

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To the Editor: We recently reported that postvaccination serum samples from infants immunized with a novel, protein-based multicomponent meningococcal serogroup B (MenB) vaccine (Bexsero; GlaxoSmithKline Vaccines, Verona, Italy) have bactericidal activity against the hypervirulent meningococcal group W (MenW) strain belonging to the sequence type (ST) 11 clonal complex (1). Historically, MenW has been a rare cause of invasive meningococcal disease (IMD), accounting for <5% of confirmed cases in England and Wales (2). Since 2009, MenW cases caused by