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Artemisinin resistance in *Plasmodium falciparum*

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Artemisinin resistance in *Plasmodium falciparum* is observed in young (0-3 h) ring-stage parasites, which have a high parasite survival rate in the in-vitro ring-stage survival assay ($RSA^{0\text{-}3h}$), but then become susceptible as they mature to late rings in the $RSA^{9\text{-}12h}$ and early trophozoites in the $TSA^{18\text{-}21h}$. In the $RSA^{0\text{-}3h}$, young ring-stage parasites are exposed to a pharmacologically relevant dose of dihydroartemisinin for 6 h and parasite survival is quantified 66 h later. Using this assay, we recently reported that parasite survival rates differ significantly between fast- and slow-clearing parasite isolates from malaria patients treated with an artemisinin in western Cambodia (10.88% *vs* 0.23%, p=0.007, Mann-Whitney test).[1]

Of the 26 parasites tested, however, we found that 4 gave 'discordant' results (Fig 1). Of the 13 fast-clearing parasites, 3 had high survival rates (5.30, 19.32, and 51.39%) but a resistant stage-dependent pattern (Δ=1.2%, 17.3%, and 50.2%, respectively). We hypothesized that these 3 parasites were indeed artemisinin-resistant, but that their clearance was fast because circulating parasites seen in blood films were predominantly older, drug-sensitive rings at the time the patients received artemisinins. Of the 13 slow-clearing parasites, 1 had a paradoxically low survival rate (0.16%). We hypothesized that this parasite was in fact artemisinin-sensitive, but that its clearance was slow because the patient had low levels of parasite-clearing immunity[2] or a poor pharmacokinetics profile.

We recently reported K13-propeller polymorphism as a new molecular marker for artemisinin-resistant *P. falciparum* malaria,[3] and hypothesized that K13-propeller genotypes

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would definitively resolve the 4 discordant results. To test this possibility we genotyped all 26 parasites and found that the 3 parasites with discordantly high survival rates each carry a different mutant K13-propeller allele (Y493H, C580Y, and R539H), and that the parasite with a discordantly low survival rate carries a wild-type allele (Fig 1). Furthermore, all the fast- and slow-clearing parasites with concordant survival rates carry wild-type or mutant alleles (Y493H and C580Y), respectively.

These data suggest that parasite survival rates in the RSA^{0-3h} are more relevant than parasite clearance half-lives in identifying artemisinin-resistant *P. falciparum*, and further validate K13-propeller polymorphism as a molecular marker of artemisinin resistance in vitro and in vivo.

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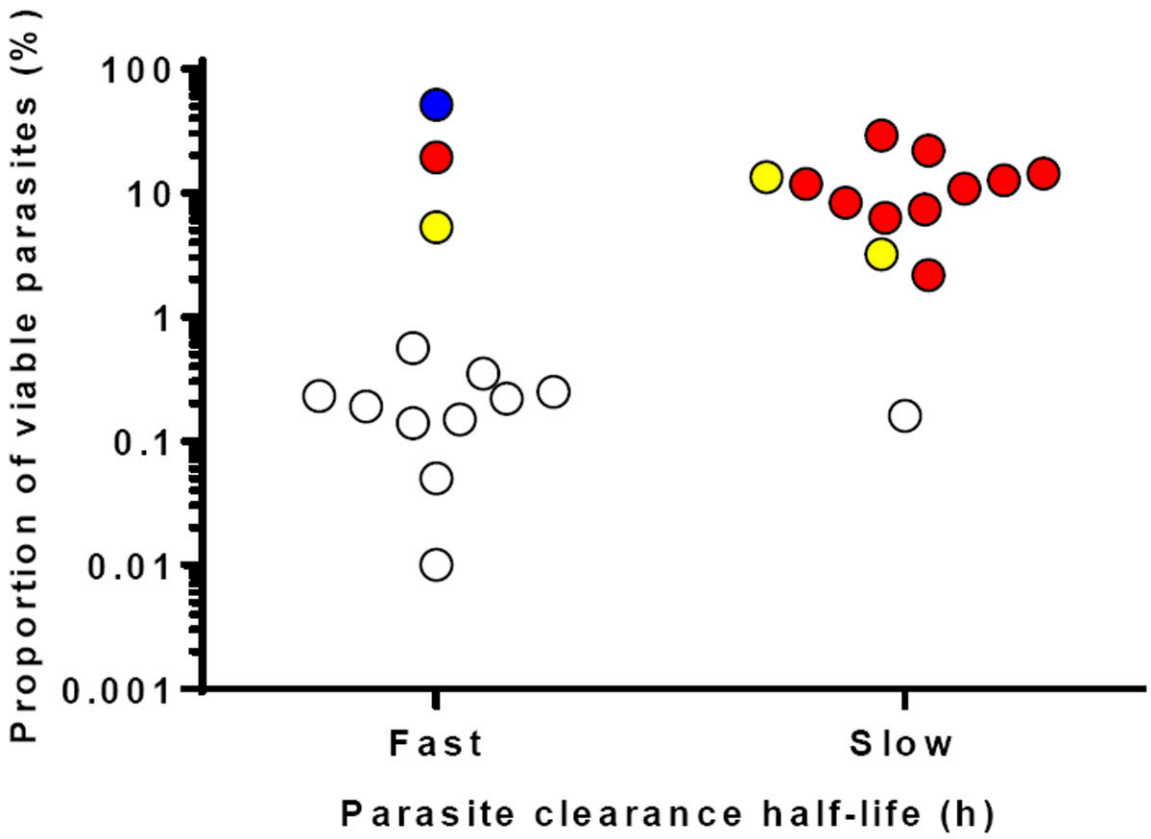

Figure 1.

Associations between parasite survival rates in the in-vitro RSA$^{0\text{-}3h}$, parasite clearance half-lives in patients treated with artemisinin-based combination therapy, and K13-propeller alleles: wild-type (clear), R539T (blue), C580Y (red), and Y493H (yellow).