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# Antidogmatic approaches to artemisinin resistance: reappraisal as treatment failure with artemisinin combination therapy

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**The definition of artemisinin resistance is becoming one of a prolonged parasite clearance phenotype, although this variable is a complex function of both host and parasite characteristics. We discuss some of the limitations of this definition of artemisinin resistance, particularly because of its potential global impact. This opinion article reviews the mechanisms underlying parasite clearance after artemisinin treatment and how these might relate to *in vitro* methods to assay for resistance. It revisits criteria for defining artemisinin resistance that are not currently being applied and suggests the term 'treatment failure of artemisinin combination therapy' (TFACT) as a more accurate description of most cases of 'artemisinin resistance'.**

## Classical antimalarial resistance

Artemisinins (artesunate, dihydroartemisin, or artemether) are partnered with unrelated antimalarials to treat most cases of malaria worldwide. Although resistance has already emerged to most drug classes that are used in combination with artemisinins, until recently resistance to artemisinins themselves was not considered to be clinically important. However, if artemisinin resistance has emerged, this will imperil the efficacy of the antimalarial combinations currently in use [1]. The impact of artemisinin resistance is therefore potentially large because hundreds of millions of doses of artemisinin combination treatments are dispensed every year [2]. Descriptions of artemisinin resistance in Southeast Asia [3–6] invite critical review as they do not always fulfil classical criteria for antimalarial resistance. Promulgation of the view that artemisinin resistance has emerged and therefore requires containment has widespread ramifications for global efforts to contain malaria and needs constant reappraisal [7]. This opinion article highlights some of the limitations of clinical definitions of artemisinin resistance and also suggests directions for further research that may increase our understanding of this important topic. Recent discussions emphasising different aspects of 'artemisinin resistance' have been reviewed [8] and debated [7,9,10], with an appropriate note of caution already sounded ('Defining artemisinin resistance is a work in progress and currently no consensus exists on the standard definition; thus claims of artemisinin resistance should be considered with caution' [8]).

In considering artemisinin resistance (Table 1), it may be useful to exemplify resistance to atovaquone, which has a mode of action that is well understood. Atovaquone inhibits the $bc_1$ electron transport complex of the parasite's mitochondrion. It can be docked into this target with molecular modelling approaches [11], and elegant studies that substitute for mitochondrial function using cytosolic yeast dihydroorotate dehydrogenase transfected into parasites achieve a predicted large increase in the resistance of parasites to atovaquone [12]. In patients, a relatively high frequency of mutations in certain residues (e.g., Y268C/S/N) that interact with atovaquone are associated with treatment failure as well as large (>3 log orders) increases in measures of *in vitro* resistance [13]. Several criteria are met for defining atovaquone resistance, including treatment failures with adequate doses of drug, and mutations in the target are associated with unequivocal resistance *in vitro* that is explicable in molecular terms. There remain some cases without mutations in the target that fail treatment for pharmacokinetic reasons or as yet undefined mechanisms, which clearly merit further investigations [14]. The possibility that resistance to atovaquone may arise through more than one mechanism may also be relevant to the artemisinins. Atovaquone by itself does not achieve high cure rates and is used in combination with proguanil, an antimalarial of a different class with which it synergises [12], to minimise emergence of resistant clones and maintain efficacy rates.

## Treatment failure does not necessarily mean resistance to artemisinins

Treatment failure with 3-day courses of artemisinins is comparable in frequency to that seen with atovaquone

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**Table 1. Features of resistance to artemisinins**

| Feature | How studied? |
|---|---|
| *In vitro* correlate | Conventional methods with parasite cultures |
| Plausible mechanism(s) | Monitoring changes in target and/or transporter(s) |
| Appropriate dosing | Pharmacokinetic and pharmacodynamic assessments with some estimate of immunological status |
| Confounding by partner drug minimised | Artemisinin monotherapy studies |
| Prolonged parasite clearance | Parasitaemia estimations with correlations to treatment failures |
| Reinfections excluded | Conventional methods using PCR |

monotherapy, although with artesunate, treatment failures do not necessarily arise because there is artesunate resistance. In Gabon, a 3-day course of artesunate (4 mg/kg/day) given to children with malaria in whom there was some presumed antimalarial immunity only cured approximately 70% after 1 month [15]. At 2 weeks after treatment, 'cure rates' were >90%, suggesting that artesunate treatment failure is best assessed later, and is not associated with prolongation of parasite clearance times because all patients cleared parasites by 72 h after treatment began.

Monotherapy studies with artemisinins have already provided invaluable information on resistance to artesunate and continue to do so [16] if they are used in trials (as opposed to treatments for populations, Table 1) [17]. This is illustrated by a careful pharmacokinetic and dynamic analysis of different doses of oral artesunate used to treat uncomplicated malaria in western Cambodia. Despite there being almost a doubling in the proportion of patients with persistent parasitaemia at 72 h after treatment (from 31.7% to 57.9%; $P < 0.01$, Table 1) assessed in 2006 and then 2009, 7 days of artesunate treatment (4 mg/kg/day) maintained adequate clinical and parasitological responses for 94.7% of patients [18]. In an even more recent artesunate monotherapy study [16], 26.9% (14/52 evaluable participants) had persistent parasitaemia at 72 h, yet the cure rate at 28 days (after appropriate PCR correction) was 100%. This study also highlights the importance of correcting appropriately for reinfections or for *Plasmodium vivax* which frequently emerges after treatment of *Plasmodium falciparum*.

These observations clearly dissociate a delayed parasite clearance phenotype (parasitaemia present at 72 h after artesunate treatment) from a definition of resistance that includes treatment failure at 28 days follow up. The validity of parasitaemia persisting at 72 h as a (sole) marker of artemisinin resistance becomes harder to sustain in the face of these results.

Most resistance, if it fulfils criteria mentioned later, will be due to a combination of an artemisinin with another drug against which there is usually a high background of resistance already, or a high chance for selection of resistance to the partner [9,19,20]. This is illustrated by treatment failure rates (at day 42, after PCR adjustment) of <15% with mefloquine artesunate combination therapy in 2009 and 2010, when the proportion of individuals who were positive for parasites at 72 h was ~15%. One year later (2011) efficacy of this combination fell to <50% with an insignificant rise in the proportion of patients (~27%) who were parasitaemic at 72 h (see Figure 7 in [21]), once again separating a delayed parasite clearance phenotype from the risk of treatment failure. The 72-h parasitaemia positive rates were similar to those noted in the monotherapy study mentioned above [16] in which there was complete efficacy of artesunate monotherapy. There were no *in vitro* correlates of treatment failures, although notably there was also no reporting of increased *pfmdr1* copy number despite this being the best predictor of treatment failures with mefloquine artesunate treatment in the region [19]. The development of reliable molecular markers that predict failure of other combination partners (such as piperaquine) is also a priority, as well as the need to improve our understanding of the mechanisms of action and resistance to artemisinins [22].

Contending that there is artemisinin resistance when cure of patients relies on the partner drug of an artemisinin is difficult to substantiate without additional studies. It is more appropriate to describe the lack of observed efficacy as resistance to an 'artemisinin combination therapy' rather than as being 'artemisinin resistance'.

## Parasite clearance kinetics is a complex phenotype

The decreased speed at which parasites are cleared after being treated with artemisinins is now being used as a marker of artemisinin resistance in western Cambodia and neighbouring regions [3]. This measure may be entering dogma, although it relies not only on parasite factors that modulate sensitivity to antimalarials but also on host factors such as contributions from splenic clearance mechanisms [23,24]. The importance of the latter is illustrated by persistence of parasitaemia for several weeks, on occasion, in patients who have been treated with artesunate and are clinically well [25]. The spleen is also important in clearance of parasites after quinine treatment, although the pitting of parasites after artesunate may make splenic contributions to parasite clearance even more relevant [23]. According to the criteria for drug resistance by the World Health Organisation (WHO), splenectomised patients with slowly clearing parasites would be classified as having high grade artemisinin resistance [8]. It follows from these observations that it is insufficient to monitor parasite clearance kinetics as the only indicator of drug resistance, particularly in the light of monotherapy studies with artesunate that do not show treatment failure despite parasitaemia being detectable at 72 h in a proportion of patients (see above). We need to know much more about the state of parasites that are being cleared relatively slowly, bearing in mind that exposure to artesunate itself reduces the deformability of infected red cells [26]. If parasite clearance is prolonged, is this because parasites are alive and capable of recrudescing, or because they are already incapable of replication but are being cleared in a different way from more rapidly cleared populations? How much of a function of host factors is delayed parasite clearance in an individual patient? Host factors may include the genotype of host red cells such as in haemoglobinopathies, red cell transporter variants, or enzymopathies [4,8]. Genotypic factors influence host immune