Dynamics of *Plasmodium falciparum* Selection After Artemether-Lumefantrine Treatment in Africa

To the Editor—In an article recently published in the *Journal of Infectious Diseases*, Baliraine and Rosenthal [1]

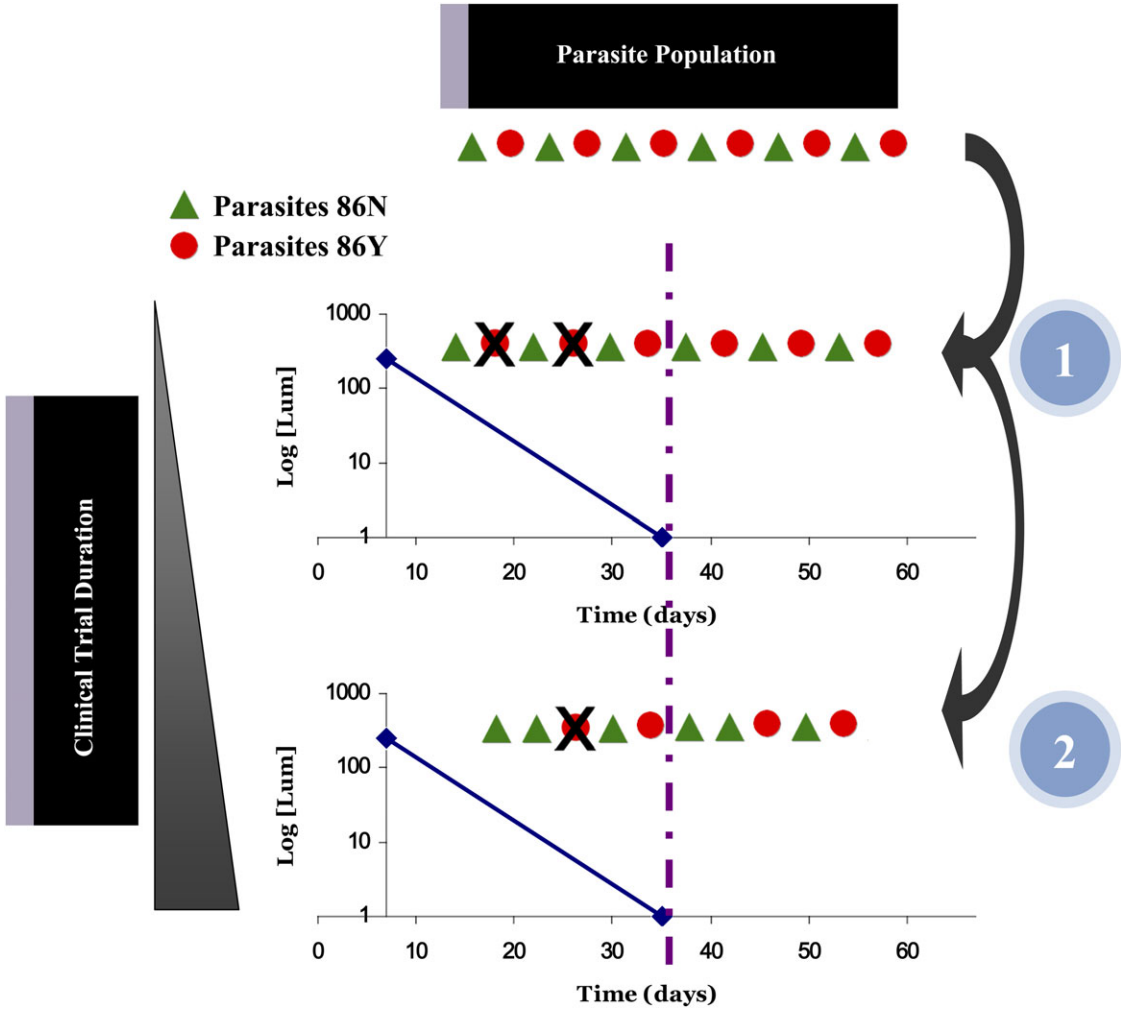

Figure 1. Dynamics of *pfmdr*1 86N allele selection following artemether-lumefantrine treatment. This scheme represents the selection of parasites carrying the 86N (lumefantrine-resistant) and 86Y (lumefantrine-sensitive) alleles (*green triangles and red circles, respectively*) over time. The model considers an initial population with an equal prevalence of 86N and 86Y alleles, an equal probability of 86N and 86Y alleles appearing through mutation, and an equal probability that either allele may be carried by parasites that cause reinfections. The selection process for the 86N allele is shown in a time-dependent manner; an initial selection phase is shown by population 1 (*blue circle 1*), which represents an intermediate stage in relation to the final selected population, shown in population 2 (*blue circle 2*).

report the selection of *pfmdr1* 86N, 184F, and 1246D alleles in a parasite population under long-term artemether-lumefantrine treatment. They suggest that "decreased drug sensitivity can appear long after predicted exposure to antimalarial drugs." This study supports earlier reports describing antimalarial drug–mediated selection of *pfmdr1* alleles in parasites reinfecting successfully treated malaria patients [2, 3].

The pharmacokinetics of artemether-lumefantrine are such that, on average, lumefantrine is completely cleared from the body at around day 35 posttreatment [4], which correlates with the reported selective window for *pfmdr1*. Thus, an explanation for the observed selection of resistance-associated alleles outside this window is required.

It is possible that the dynamics of antimalarial resistance selection can explain the increase of associated alleles outside the selective window. To demonstrate this, a model (Figure 1), which was derived from previous reports [2–4], illustrates the increase in prevalence of this allele beyond the 35-day selective window. This model considers an initial population with an equal prevalence of 86N and 86Y alleles (green triangles and red circles, respectively, in Figure 1), an equal probability of 86N and 86Y alleles appearing through mutation, and an equal probability that either allele may be carried by parasites that cause reinfections. Two parasite populations at different time points during the selection process are represented at points 1 and 2 (blue circles).

According to this model, the 86N allele frequency increases to 0.7 during

the latter part of the clinical trial (represented by population 2 in Figure 1). The 86N allele frequency in *reinfections* is 0.75 in patients treated <35 days prior to reinfection and 0.6 in those treated >35 days prior to reinfection. To explain the apparent increase of the resistant allele outside the 35-day selective window, an intermediate state in the selection process is shown by population 1 (Figure 1). At the early stage of the hypothetical clinical trial, frequency of the 86N allele is measured at 0.75 within the 35-day lumefantrine selection window, whereas no selection is observed (compared with the initial population) outside this window (ie, allele frequency is 0.5). With the increase of 86N in the general population, further reinfections occurring at >35 posttreatment in "population 2" will carry allele frequencies reflecting the parasite population following selection of "population 1."

Baliraine and Rosenthal's [1] results raise important questions regarding how clinical trials of antimalarial efficacy should be analyzed in terms of drug resistance selection at the parasite population level. It is becoming increasingly clear that posttreatment reinfections act as a strong mechanism driving selection of antimalarial resistance in *Plasmodium falciparum*. This fact suggests that the design of clinical trials of antimalarial drug efficacy and their molecular analysis should be carefully considered. Procedures concerning follow-up periods and duration/season of clinical trials need to be taken into consideration, as fluctuations in transmission intensity will have a direct effect on the rate of reinfection.

The selection of drug resistance within a parasite population is a dynamic process that results in the accumulation of adaptive characteristics in a temporal manner. Commonly, large antimalarial efficacy trials are performed over a period of several months. During the latter periods of such trials, the allele frequencies of genes linked to drug resistance within the parasite populations may already be altered due to the drug pressure that results from the trial. In practical terms, this becomes important when the allele frequencies of the parasite population sampled from patients at the beginning of the trial are used as the "baseline" for all comparisons, regardless of the time point of the secondary sampling. In summary, there is a cumulative effect of selection of alleles associated with drug resistance, which, in the case of analyses of reinfections following antimalarial treatment, may lead to an overestimation of the selection pressure. The same principle is applicable to the (non–drug resistance-related) genetic background of parasites that cause reinfections at different points during the clinical trial.

We believe that there is a need to establish standard operational procedures for the performance of future clinical efficacy trials of artemisinin combination therapy if they are to assess selection of resistant alleles. This is particularly relevant for trials conducted in Africa, where malaria transmission intensity plays an important etiologic role.

Notes

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