The title of the article, “Parasite viability as a superior measure of antimalarial drug activity in humans” [1], suggests a significant advance, but it is not clear why or how it would be used to assess antimalarial drugs. It is stated that “the use of parasite clearance to measure drug activity and to inform decisions about drug development should be reconsidered in view of these new insights.” It is unclear what these insights are and whether these difficult and laborious serial in vivo studies would offer any advantage over the currently used, simple ring-stage in vitro tests [4, 6], which identify the loss of ring-stage activity in artemisinin-resistant parasites very well.

The meaning and predictive value of the estimated half-life from the viability studies are also unclear. The observed log-linear decline in parasite densities in blood after artemisinin treatment provides a clearance half-life of about 3.5 hours, which, if continued, would result in an approximately 16 000-fold decrease per life-cycle. This predicts that ≥5 days of artemisinin monotherapy (regardless of dosing frequency) are needed to clear an infection with a biomass of $10^{12}$ parasites. This matches clinical observations [9]. But what is the meaning or utility of the half-life estimated from the viability study? Interpreted literally, a continued half-life of 0.75 hours would kill all the infecting malaria parasites within a day, which clearly does not match clinical observations.

As for dose finding, the results presented in [1] fig 3 suggest that the fits to the serial viability log-linear declines are poor and, thus, the derived viability half-lives are imprecise in comparison with the parasite clearance profiles. Indeed, it is unclear whether declines are exponential and, therefore, whether the model is appropriate. This does not give confidence that a concentration-effect (dose-response) estimate derived from these
viability data will be more informative than one derived from parasite clearance profiles. Serial circulating malaria parasite viability estimations are certainly unsuited for field assessments and, importantly, they are not relevant for the majority of current antimalarial drugs, which have little or no effects on ring-stage parasites.

Notes

Financial support. This work was supported by the Wellcome Trust.

Potential conflicts of interest. Both authors: No reported conflicts. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Nicholas J. White and James A. Watson
Mahidol Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

References

1. Rebelo M, Pawliw R, Gower J, et al. Parasite viability as a superior measure of antimalarial drug activity in humans. J Infect Dis 2021;223:2154–63.

2. Watts RE, Oledra A, Marquart L, et al. Safety and parasite clearance of artemisinin-resistant Plasmodium falciparum infection: a pilot and a randomised volunteer infection study in Australia. PLoS Med 2020;17:e1003203.

3. Witkowski B, Khim N, Chim P, et al. Reduced artemisinin susceptibility of Plasmodium falciparum ring stages in western Cambodia. Antimicrob Agents Chemother 2013;57:914–23.

4. Witkowski B, Amaratunga C, Khim N, et al. Novel phenotypic assays for the detection of artemisinin-resistant Plasmodium falciparum malaria in Cambodia: in-vitro and ex-vivo drug-response studies. Lancet Infect Dis 2013;13:1043–9.

5. Klonis N, Xie SC, McCaw JM, et al. Altered temporal response of malaria parasites determines differential sensitivity to artesinin. Proc Natl Acad Sci U S A 2013;110:5157–62.

6. Chotivanich K, Tripura R, Das D, et al. Laboratory detection of artemisinin-resistant Plasmodium falciparum. Antimicrob Agents Chemother 2014;58:3157–61.

7. Flegg JA, Guerin PJ, White NJ, Stepniewska K. Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator. Malar J 2011;10:339.

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

9. White NJ, Watson J, Ashley EA. Split dosing of artemisinins does not improve antimalarial therapeutic efficacy. Sci Rep 2017;7:12132.

Reply to White and Watson

To the Editor—In their letter White and Watson raised questions about findings presented in our article [1, 2]. They contend that the ex vivo viability approach used in the context of a volunteer infection study could be considered as “a laborious in vivo ring-stage survival assay (RSA).” The RSA methodology is not used to assess parasite viability after in vivo drug exposure. Instead, it assesses the response of clinical isolates collected before drug treatment to in vitro drug exposure [3]. Our ex vivo assay provides information on the speed of drug activity in vivo, by measuring the number of parasites that remain viable in an individual at any sampled time point [2]. Furthermore, the ex vivo RSA is not technically feasible in volunteer infection studies, where the parasitemia of volunteers is very low and below the level of microscopic quantitation. The main metric used to assess in vivo drug activity is the parasite clearance curve, which does not distinguish viable from nonviable parasites. The major insight gained from our serial assessment of parasite viability after in vivo drug exposure is that artesunate activity is more rapid than is evidenced by parasite clearance.

White and Watson also suggest that the parasite half-life estimated from measurements of parasite viability does not match clinical observations [1]. They observe that if parasites are killed continuously for 24 hours with our estimated 0.75-hour half-life, then complete clearance of parasite biomass should occur within a day, which is inconsistent with clinical observations. However, artesunate and dihydroartemisinin (DHA, the active metabolite) are not thought to act continuously for 24 hours and their very short half-lives indicate that the direct drug effect should not last for more than 6–8 hours after administration [4]. Therefore, if one assumes that parasite killing occurs at a 0.75-hour half-life for 6 hours after each dose, more than 5 doses of artesunate would be required to clear an infection of 10^{12} parasites, consistent with clinical observations. Furthermore, the decline in viable parasite numbers coincides with the short time that DHA is detectable in plasma, and when DHA is no longer detectable viable parasite numbers increase