Title: Efficacy, Safety and Tolerability of *Pyronaridine-artesunate* in Asymptomatic Malaria-infected Individuals: a Randomized Controlled Trial

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Background. A major challenge for malaria elimination is transmission from asymptomatic malaria-infected individuals carrying low-density infections. Interventions targeting the human transmission reservoir, such as mass drug administration (MDA), can reduce malaria prevalence and transmission. Effective MDA requires high coverage, good adherence to an efficacious, well-tolerated and affordable treatment for this purpose. *Pyronaridine-artesunate (PA)* is a fixed-dose artemisinin based combination therapy (ACT) shown to be highly efficacious and well tolerated for the treatment of uncomplicated *plasmodium falciparum* malaria. A study was done to evaluate PA efficacy, safety, and tolerability in individuals with asymptomatic *Plasmodium falciparum* infection.

Objectives. To assess the efficacy of *Pyronaridine-artesunate* administered for three, two and one day by measuring polymerase chain reaction (PCR)-adjusted adequate parasitological response (APR) at Day 28 in asymptomatic *P. falciparum* carriers

Methodology. This was a phase II, multi-center, open label, randomized clinical trial conducted in The Gambia and Zambia (Nchelenge) between October 2018 and May 2019. Male and female asymptomatic participants aged above 5 years and weighing above 20 kg were -pre-screened with

a hyper-sensitive Rapid Diagnostic Test (HS-RDT) for detection of *P. falciparum malaria* after signing a pre-screening informed consent/assent. Individuals with a positive pre-screening HS-RDT and confirmatory blood smear had the study further explained and those interested in study participation were required to sign a Study Specific Informed Consent / Assent detailing all study procedures. Those who met the inclusion criteria and none of the exclusion criteria were enrolled and randomised 1:1:1 to receive treatment with Pyramax orally for 3 days, 2 days or 1 day (Treatment Arms A, B and C, respectively) and followed up for 63 days. Screen failures were offered local standard of care treatment. Blood samples were collected on Days 0, 1, 2, 3, 7, 14, 21, 28, 35, 42 and 63 for malaria diagnostics, assessment of parasite density and qPCR analysis. The primary efficacy outcome was PCR-adjusted APR at day 28 in the per-protocol population.

Results. 303 participants were randomized. Day 28 PCR-adjusted APR was 100% for both the 3-day (98/98) and 2-day regimens (96/96), and 96.8% (89/94) for the 1-day regimen. Efficacy was maintained at 100% until day 63 for the 3-day and 2-day regimens but declined to 94.4% (84/89) with the 1-day regimen. Adverse event frequency was similar between the 3-day (51.5% [52/101]), 2-day (52.5% [52/99]), and 1-day (54.4% [56/103]) regimens; the majority of adverse events were of grade 1 or 2 severity (85% [136/160]). Asymptomatic, transient increases in ALT/AST (>3 ULN) were observed for 6/301 (2.0%) participants.

Conclusions. Pyramax administered over 1, 2 or 3 days was well tolerated, with no new safety concerns. It was highly efficacious in clearing initial parasitemia in carriers and in preventing new infections up to 63 days post first dose. Overall, in this population the efficacy 2-day treatment regimen with pyramax was similar to that of standard treatment of 3-days. However, the efficacy for the 1-day treatment regimen was less than that of the standard 3-day dosing regimen. These data are very informative for MDA strategies which Zambia is considering in pursuit of malaria elimination.