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Efficacies of artemether–lumefantrine, artesunate–amodiaquine, dihydroartemisinin–piperaquine, and artesunate–pyronaridine for the treatment of uncomplicated *Plasmodium falciparum* malaria in children aged 6 months to 10 years in Uganda: a randomised, open-label, phase 4 clinical trial

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MRK, JIN, CE, BA, JO, GS, SLN, LFM, DR, EM, BB, and AY conceived the idea for the study. MRK, CE, VA, SLN, PJR, LFM, and AY wrote the protocol. CE, MRK, DK, and AY were responsible for management of the study participants. VA, MK, SO, MO, ST, BA, SLN, and PJR contributed to laboratory studies. JIN, SB, MDC, and LFM performed the statistical analysis with inputs from MRK, PMC, and GA. MRK, JIN, SB, PJR, and LFM wrote the first draft of the manuscript. SB, JIN, and LFM had access to raw data; MDC, PJR, and MRK verified the data. All authors interpreted the data and critically reviewed the manuscript. MRK had full access to all data and final responsibility to submit for publication.

For the Swahili translation of the abstract see **Online** for appendix 1

See **Online** for appendix 2

Declaration of interests

We declare no competing interests. The findings and conclusions in this Article are those of the authors and do not necessarily represent the official position of the US Agency for International Development, the US Centers for Disease Control and Prevention, the US Department of Health and Human Services, or the US Government.

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Summary

Background: Anti-malarial artemisinin-based combination therapies (ACTs) might be losing efficacy in east Africa, with the spread of artemisinin partial resistance and reduced partner drug activity. Our trial aimed to measure the efficacies of artemether–lumefantrine, artesunate–amodiaquine, dihydroartemisinin–piperaquine, and artesunate–pyronaridine in three sites in Uganda.

Methods: This randomised, open-label, phase 4 clinical trial was carried out at three sites in the Agago, Arua, and Busia districts of Uganda. Children aged 6 months to 10 years with uncomplicated *Plasmodium falciparum* malaria were randomly assigned to receive either artemether–lumefantrine (20 mg artemether; 120 mg lumefantrine; twice a day for 3 days) in all sites or dihydroartemisinin–piperaquine (40 mg dihydroartemisinin and 320 mg piperaquine, once a day for 3 days) in Agago, artesunate–amodiaquine (25 mg artesunate and 67·5 mg amodiaquine for children <9 kg or 50 mg artesunate and 135 mg amodiaquine for children ≥9 kg, once a day for 3 days) in Busia; and artesunate–pyronaridine (60 mg artesunate and 180 mg pyronaridine for children >15 kg or 20 mg artesunate and 60 mg pyronaridine for children <15 kg, once a day for 3 days) in Arua, with follow-up to 42 days. Participants were not blinded to group assignments; however, investigators and those assessing outcome were masked. The primary outcome was parasitaemia, assessed by microscopy, either uncorrected or PCR-corrected to distinguish recrudescence from new infection. All participants who received the treatment per protocol and were not lost to follow-up were included in the primary outcome. All participants who were randomly allocated to treatment groups were included in the safety analyses. This study is registered with the Pan African Clinical Trials Registry, number PACTR202301796134887, and is complete.

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Findings: Between Nov 7, 2022, and March 24, 2023, 808 participants (437 [54%] female) were enrolled and assigned to treatment groups; 15 (2%) were lost to follow-up and 793 (98%) completed follow-up. The uncorrected adequate clinical and parasitological response for artemether–lumefantrine was 87 (51·8%; 95% CI 44·0–59·5) of 168 participants in Arua, 88 (51·8%; 44·0–59·4) of 170 and Busia, and 131 (79·4%; 72·3–85·1) of 165 in Agago. This response for artemether–lumefantrine was lower than that of the other ACTs at all sites: 97 (98·0%; 92·2–99·6) of 99 for dihydroartemisinin–piperaquine in Agago, 95 (99·0%; 93·5–99·9) of 96 for artesunate–amodiaquine in Busia, and 73 (73·7%; 63·8–81·8) of 99 for artesunate–pyronaridine in Arua. PCR-corrected 28-day efficacies were 88 (81·5%; 72·6–88·1) of 108 for artemether–lumefantrine and 95 (100%; 95·2–100·0) of 95 for artesunate–amodiaquine in Busia; 131 (97·0%; 92·1–99·0) of 135 for artemether–lumefantrine and 97 (100%; 95·3–100·0) of 97 for dihydroartemisinin–piperaquine in Agago; and 87 (82·1%; 73·2–88·6) of 106 for artemether–lumefantrine and 73 (92·4%; 83·6–96·9) of 79 for artesunate–pyronaridine in Arua. All regimens were well tolerated. The most common adverse events were upper respiratory tract infection, diarrhoea, and anaemia. None of the reported adverse events were attributed to the study drugs. There were two serious adverse events, both cases of severe malaria in Arua, one in each of the treatment groups. Parasite clearance half-lives were prolonged with parasites carrying the PfK13 Cys469Tyr (median 4·2 h; IQR 3·4–4·9) and Ala675Val (4·9 h; 3·4–5·7) mutations compared with wild-type parasites (2·8 h; 2·3–3·6; p<0·0001).

Interpretation: Artemether–lumefantrine was associated with a higher risk of recurrent malaria than other antimalarial combinations tested, and K13 mutations were associated with delayed parasite clearance. Changes in first-line therapy for uncomplicated malaria must be considered in response to suboptimal efficacy of artemether–lumefantrine.

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Introduction

Prompt diagnosis and effective treatment of malaria with artemisinin-based combination therapies (ACTs) is the foundation of malaria management.[1] The principal ACTs recommended for the treatment of uncomplicated malaria in Africa are artemether–lumefantrine, the first-line therapy in most countries, including Uganda, and the most widely procured, artesunate–amodiaquine, dihydroartemisinin–piperaquine, and artesunate–pyronaridine.[1] Artemisinin partial resistance (ART-R) is defined as delayed parasite clearance after treatment of malaria with an artemisinin-based regimen and is mediated by any of about 20 mutations in the *Plasmodium falciparum* kelch protein (PfK13); the effects of ART-R on the efficacies of ACTs in Africa are uncertain. ART-R has emerged in eastern Africa, including in Uganda, Rwanda, Tanzania, and Eritrea;[2–4] however, different PfK13 mutations have been observed in different countries, indicative of multiple independent emergences of resistant parasites. In southeast Asia, ART-R has been accompanied by resistance to key ACT partner drugs, leading to high rates of ACT treatment failure, particularly for dihydroartemisinin–piperaquine.[5] However, the effects of ART-R in southeast Asia have been limited by a relatively low malaria burden. The consequences of

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ART-R will probably be much worse in sub-Saharan Africa, where around 95% of malaria cases and deaths occur, but such consequences have not yet been well characterised. In a few studies in Rwanda, Eritrea, and Tanzania, validated ART-R PfK13 mutations were associated with delayed parasite clearance or enhanced parasite survival in the ring survival assay, an in-vitro correlate of ART-R, but not with adverse treatment outcomes based on standard WHO criteria.[3]

Malaria treatment strategies should be guided by therapeutic efficacy studies, following standard protocols. According to WHO, a change in the national malaria treatment policy should be initiated if PCR-corrected treatment failure rate exceeds 10%.[6] However, molecular genotyping to assign treatment outcomes is imprecise, especially in areas with high levels of malaria transmission, such as much of Africa. Historically, artemether–lumefantrine and other ACTs had high efficacy in east Africa, but with the emergence of ART-R in the past decade, treatment efficacies below the WHO threshold of 90% corrected efficacy have been observed in several countries.[7–9] In Uganda, the corrected efficacy of artemether–lumefantrine in 2019 was 92% in Arua and 87% in Busia, with uncorrected efficacies ranging from 63% to 88%.[7] Children bear the highest burden of malaria morbidity and mortality and are particularly vulnerable due to their low immunity, making treatment efficacy in this group especially important. The aim of our study was to examine treatment efficacies of leading ACTs for uncomplicated malaria in children to inform policy makers regarding best antimalarial treatment practices in Uganda; this included assessments of parasite clearance rates, lumefantrine blood concentrations, ex-vivo drug susceptibilities, and safety.

Methods

Study design

We conducted a randomised, open-label, phase 4 clinical trial at Patongo Health Centre III (outpatient care only), in the Agago District in north Uganda (high prevalence of molecular markers associated with ART-R); Arua Regional Referral Hospital (inpatient and outpatient care), in the Arua District in northwest Uganda (borderline to low efficacy of artemether–lumefantrine); and Masafu General Hospital (inpatient and outpatient care), in the Busia District in east Uganda (borderline to low efficacy of artemether–lumefantrine). All sites have moderate (ie, 10–50% parasite prevalence) or high (ie, >50% parasite prevalence) malaria transmission intensity, according to standard classifications by WHO and the Malaris Atlas Project (ie, Agago 37·5%, Arua 53·9%, and Busia 34·4%). This study is registered with the Pan African Clinical Trials Registry, number PACTR202301796134887.

The study protocol was approved by the Makerere University School of Biomedical Sciences Research and Ethics Committee, the Uganda National Council for Science and Technology, and the Uganda National Drug Authority; it was also approved as a non-research programme evaluation by the Office of the Associate Director for Science, Global Health Center, US Centers for Disease Control and Prevention (0900f3eb82311595). The study protocol is available in appendix 2.

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Participants

Children aged 6 months to 10 years diagnosed with uncomplicated *P falciparum* malaria were recruited from participating health facilities; during the study period, all outpatients presenting to the participating health facilities were screened for eligibility. Inclusion criteria were *P falciparum* mono-infection with microscopy measured parasite density of 2000–200 000 parasites per μL; axillary temperature of 37·5°C or higher or history of fever in the preceding 24 h; haemoglobin concentration higher than 8·0 g/dL; ability to swallow oral medication; informed consent from a parent or guardian; and agreement for follow-up for 42 days. Exclusion criteria were evidence of severe malnutrition, defined as very low weight for height (ie, a z-score <–3), severe wasting, symmetric oedema, or in children aged 6–60 months, mid-upper arm circumference lower than 115 mm; weight of 5 kg or lower; history of serious adverse effects or hypersensitivity to study medications; regular use of medications that could interact with study medications; evidence of severe malaria or danger signs; and evidence of concomitant febrile illness or other underlying chronic or severe diseases.[6] Data on sex were collected by self-report with the provided options male or female. Written informed consent was obtained from parents or guardians, and assent was sought from children aged 8–10 years, in accordance with Uganda's ethical guidelines.

Randomisation

In Agago, patients were randomly assigned to receive artemether–lumefantrine or dihydroartemisinin–piperaquine; in Busia, artemether–lumefantrine or artesunate–amodiaquine; and in Arua, artemether–lumefantrine or artesunate–pyronaridine. Randomisation at each site was performed using a computer-generated list by an individual not involved in the study. Sequentially numbered, sealed, opaque envelopes containing the treatment assignment were secured in a locked cabinet accessible to an unblinded study nurse responsible for dispensing the medications. Only the investigators and those assessing outcomes were masked to group assignment. Participants, study nurses administering the interventions, and those analysing the data were not masked. To minimise bias, outcomes were assessed using standardised criteria, and data analysis followed a pre-specified plan.

Procedures

Enrolled children were admitted to hospital for at least 3 days, and all doses of study medication were orally administered as directly observed treatment by study nurses. Artemether–lumefantrine (Coartem [Novartis], Basel, Switzerland; 20 mg artemether and 120 mg lumefantrine) was administered twice a day for 3 days following the consumption of milk. The other ACTs were administered once a day for 3 days. These ACTs were: artesunate–amodiaquine (Winthrop [Sanofi Aventis], France; 25 mg artesunate and 67·5 mg amodiaquine for children <9 kg or 50 mg artesunate and 135 mg amodiaquine for children ≥9 kg); dihydroartemisinin–piperaquine (D-Artepp [Forsun], Shanghai, China; 40 mg dihydroartemisinin and 320 mg piperaquine); and artesunate–pyronaridine (Pyramax [Shin Poong Pharmaceutical], South Korea) tablets for children >15 kg (60 mg artesunate and 180 mg pyronaridine), or granules for children <15 kg (20 mg artesunate and 60 mg pyronaridine). If a child vomited within 30 min, the medication was readministered. Children with fever were given paracetamol and those with haemoglobin concentrations

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lower than 10 g/dL were treated with ferrous sulphate and anthelminthics per the Integrated Management of Childhood Illness guidelines.[10]

Thick blood smears were collected every 6 h after initiation of treatment until two consecutive negative parasitaemia results, and then daily for the first 3 days and weekly thereafter for 28 days (artemether–lumefantrine and artesunate–amodiaquine groups) or 42 days (dihydroartemisinin–piperaquine and artesunate–pyronaridine groups). Participants were encouraged to visit the study clinic on any day they felt unwell. At each follow-up visit, response to treatment was monitored through a standardised history and physical examination and examination of thick and thin blood films. Haemoglobin was measured using a portable spectrophotometer (Hemocue; Ängelholm, Sweden) on days 0, 21, and 28 for all groups and also on days 35 and 42 for the dihydroartemisinin–piperaquine and artesunate–pyronaridine groups. A dried blood spot was collected on the day of enrolment and follow-up days for molecular studies.

P falciparum infection was assessed by trained microscopists using thick and thin blood smears. A thick smear stained with 10% Giemsa for 10 min was used for screening. Smears stained with 2% Giemsa for 30 min were used to count the number of asexual parasites per 200 leukocytes in thick films and detect different parasite species in thin films. Parasite density was calculated by multiplying the number of parasites by 40, assuming 8000 leukocytes per μL, until more than 500 parasites were identified, or a smear was declared negative when no parasites were detected following examination of 100 high-power fields. Gametocytes were recorded when observed. Each smear was read by two blinded microscopists; any disagreements, defined as differences in species diagnosis, parasite density over 50%, or the presence of parasites, were settled by a third microscopist, with final parasite density calculated as the mean between the two closest readings.

To differentiate between patients with recrudescence and recurrent infections, genotyping was performed based on the sizes of fragments amplified from polymorphic loci of merozoite surface protein 1 (*msp1*; block-6 for the K1, MAD20, and RO33 alleles), *msp2* (the central polymorphic region for the 3D7/IC and FC27 alleles), and the polyadenosine (*poly*-α) microsatellite locus, using primers and PCR protocols as previously described[11] on samples from day 0 and day of recurrence. Genomic DNA was extracted from a dried blood spot using Chelex-100 resin (Bio-Rad Laboratories, Hercules, CA, USA).[12] Amplicon sizes were resolved through capillary electrophoresis using the QIAxcel Advanced system (Qiagen, Hilden, Germany).[13] Results were categorised using the WHO 3/3 match algorithm; an outcome was considered recrudescence when day 0 and day of recurrence samples shared at least one allele for *msp1*, *msp2*, and *poly*-α.[14]

To identify markers of ART-R and partner drug resistance, sequencing of the PfK13 propeller domain was performed on all day 0 isolates. Primers targeting the PfK13 β-propeller domain (amino acids 350–726) and PCR conditions have been reported elsewhere.[15] Amplicons were dideoxy sequenced on an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using POP-7 polymer with BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA, USA). Raw sequence data were analysed in Codon Code aligner, version v10.0.2, with 3D7 as the reference sequence. *P falciparum*

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plasmepsin 2/3 copy number was determined in samples from the dihydroartemisinin–piperaquine group using an Agilent Mx3005 real-time PCR machine (Agilent Technologies, CA, USA), using a protocol described elsewhere.[16] Multiplicity of infection was determined as the highest number of unique alleles among *msp1*, *msp2*, or *poly*-α on day 0.

For ex-vivo growth inhibition assays, 50% inhibitory concentrations (IC_{50}) were determined as described elsewhere.[17–19] Briefly, drug stocks were prepared and diluted in 96-well microplates to capture dose–response curves. *P falciparum* cultures were maintained under standard conditions[19] for 72 h, and parasite growth was quantified based on SYBR Green fluorescence (Thermo Fisher Scientific, Waltham, MA, USA). IC_{50} values were derived through non-linear regression with the four-parameter Hill equation.

Dihydroartemisinin susceptibility was measured using the ring survival assay, as described elsewhere.[19,20] Cultures at parasitaemia 0·2–1·0%, were incubated with 700 nM dihydroartemisinin or, for controls, 0·1% dimethyl sulfoxide, for 6 h under standard culture conditions, washed to remove dihydroartemisinin, and continued. At 72 h after culture initiation, Giemsa-stained thin smears were read to assess parasitaemia. Cultures were appropriate for assessment if control parasitaemia was 0·2% or higher and 25% or higher than the starting parasitaemia. Ring survival assay survival was expressed as the proportion of viable parasites in the dihydroartemisinin-treated cultures relative to controls.

Plasma was collected on day 7 from all participants in artemether–lumefantrine treatment groups. Lumefantrine concentrations were determined using liquid chromatography with tandem mass spectrometry, modified to a calibration range of 2·5–1000 ng/mL, with 5 μL plasma volume.[21]

Treatment failures as defined by WHO[6] were treated with quinine tablets (10 mg/kg; Agog Pharma, Vasai, India) every 8 h for 5 days, following Uganda Clinical Guidelines. Patients with evidence of severe malaria or danger signs including haemoglobin concentration lower than 5 g/dL, convulsions, lethargy, inability to drink or breastfeed, repeated vomiting, or inability to sit or stand because of weakness were referred for treatment with intravenous artesunate (Sanjar Pharmaceuticals, Sabarkantha, India). Participants who took antimalarial medications outside the study, had adverse events requiring a change in treatment, withdrew informed consent, or were lost to follow-up were excluded at the time of these events.

Outcomes

The primary outcome was parasitaemia, assessed by microscopy, either uncorrected or PCR-corrected to distinguish recrudescence from new infection. The categories for parasitaemia are as follows: early treatment failure was defined as the occurrence of danger signs or severe malaria on day 1 to day 3 in the presence of parasitaemia; parasitaemia on day 2 higher than day 0, irrespective of axillary temperature; parasitaemia on day 3 with axillary temperature of 37·5°C or higher; or parasitaemia on day 3 at or above 25% of the count on day 0. Late clinical failure was defined as the occurrence of danger signs or severe malaria in the presence of parasitaemia between day 4 and day 28 (or day 42) in participants who did not previously meet early treatment failure criteria; or parasitaemia with axillary temperature of 37·5°C or higher during the same period in patients without previous early treatment

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failure. Late parasitological failure was defined as the presence of parasitaemia with axillary temperature lower than 37·5°C between day 7 and day 28 (or day 42) in patients who did not previously meet early treatment failure or late clinical failure. Adequate clinical and parasitological response was defined as the absence of parasitaemia on day 28 (or day 42), irrespective of axillary temperature, in patients without previous early treatment failure, late clinical failure, or late parasitological failure.[6]

Secondary outcomes were prevalence of fever and parasitaemia on days 1–3, prevalence of gametocytaemia, risk of adverse events, presence of genetic polymorphisms associated with ART-R, and haemoglobin concentrations for day 28 or 42. Adverse events were evaluated at each study visit, graded according to WHO and US National Institute of Allergy and Infectious Diseases scales, and defined based on International Conference on Harmonization guidelines as untoward medical occurrences. Serious adverse events included death, life-threatening experience, hospitalisation, incapacity, or events that required medical or surgical intervention to prevent serious outcomes.

Statistical analysis

A sample size for each of the artemether–lumefantrine groups of 139 participants was calculated based on an estimated PCR-corrected treatment failure rate of 10%, with a 5% margin of error and a 95% CI. To account for an expected high rate of new infections during follow-up, the sample size was increased by 20%, resulting in 170 participants per site. A sample size of 73 participants was calculated for the alternate treatment group at each site based on an estimated failure rate of 5%, with a 5% margin of error and a 95% CI; this was increased by 35% to account for new infections and loss to follow-up, leading to 100 participants per site.[22,23]

All patients who received the treatment per protocol and were not lost to follow-up by day 28 or day 42 depending on treatment group were included in the primary outcome. All participants randomly allocated to treatment were included in the safety analyses. Study data were collected using standardised case report forms and entered into a secure database with double entry. Baseline characteristics and primary efficacy outcomes were summarised as means or medians for continuous variables and as frequencies and percentages for categorical variables. Kaplan–Meier survival analysis was used to estimate cumulative probabilities of uncorrected and corrected treatment failures. Efficacies were calculated for single-arm studies stratified per site per WHO standards using per protocol study populations. Comparisons between sites for PfK13 mutations and lumefantrine concentrations were performed using the Mann–Whitney U test for continuous variables and Pearson’s chi-square or Fisher’s exact test for categorical data. Statistical significance was set at $p<0·05$. Analyses were performed using STATA (version 18) and R (version 4.4.1).

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

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Results

Between Nov 7, 2022, and March 24, 2023, 932 patients with uncomplicated malaria were screened for eligibility to participate. Of these 932 participants, 124 (13%) were excluded at screening; 808 (87%) were enrolled (437 [54%] female) and randomised to treatment groups. 509 participants were assigned to receive artemether–lumefantrine, 168 in Agago, 170 in Arua, and 171 in Busia; 100 to receive dihydroartemisinin–piperaquine in Agago; 99 to receive artesunate–amodiaquine in Busia, and 100 to receive artesunate–pyronaridine in Arua. 15 participants were lost to follow-up, leaving 793 (98%) of 808 participants who completed follow-up and were included in primary outcome assessments (figure 1). Baseline characteristics of all study participants are shown in table 1.

Primary efficacy outcomes are summarised in table 2 and appendix 2 (pp 4–6). No episodes of early treatment failure were observed. The uncorrected adequate clinical and parasitological response for artemether–lumefantrine was 87 (51·8%; 95% CI 44·0–59·5) of 168 participants in Arua, 88 (51·8%; 44·0–59·4) of 170 and Busia, and 131 (79·**4%**; 72·3–85·1) of 165 in Agago. This response for artemether–lumefantrine was lower than that of the other ACTs at all sites: 97 (98·0%; 92·2–99·6) of 99 for dihydroartemisinin–piperaquine in Agago, 95 (99·0%; 93·5–99·9) of 96 for artesunate–amodiaquine in Busia, and 73 (73·7%; 63·8–81·8) of 99 for artesunate–pyronaridine in Arua (table 2).

PCR-corrected 28-day efficacies were 88 (81·5%; 95% CI 72·6–88·1) of 108 for artemether–lumefantrine and 95 (100%; 95·2–100·0) of 95 for artesunate–amodiaquine in Busia; 131 (97·0%; 92·1–99·0) of 135 for artemether–lumefantrine and 97 (100%; 95·3–100·0) of 97 for dihydroartemisinin–piperaquine in Agago; and 87 (82·1%; 73·2–88·6) of 106 for artemether–lumefantrine and 73 (92·4%; 83·6–96·9) of 79 for artesunate–pyronaridine in Arua (table 2). Corrected outcomes showed efficacy below the WHO recommended cutoff for artemether–lumefantrine at Arua and Busia, but for no other study regimens. The multiplicity of infection was similar across sites: 7·7 (3·7) in Agago, 8·4 (3·8) in Arua, and 8·9 (4·0) in Busia.

For the sensitivity analysis, the risk of recurrent parasitaemia was similar between children younger than and older than 5 years (p=0·98; appendix 2 p 2). Determination of corrected 28-day and 42-day efficacies using the 2/3 match algorithm were reduced compared with those calculated with the 3/3 match algorithm (appendix 2 p 3).

Parasitaemia persisted to day 3 in less than 10% of participants in all treatment groups (table 3). The parasite clearance half-life was under 5 h at all sites, with slower parasite clearance in Agago, the site with high prevalence of ART-R mutations (figure 2).[2]

The most common adverse events were upper respiratory tract infection, diarrhoea, and anaemia. None of the reported adverse events were attributed to the study drugs. There were two serious adverse events, both instances of severe malaria in Arua, one in each of the treatment groups (table 4).

Of the 754 isolates collected at enrolment, three validated markers of ART-R were identified (appendix 2 p 1). One of the trial sites, Agago, has a known high prevalence of two

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PfK13 mutations, Cys469Tyr and Ala675Val, which are validated markers of ART-R.[2] The prevalences of these mutations were higher in Agago (combined prevalence 34·6%) than the other two study sites (combined prevalence 2·7% in Busia and 7·7% in Arua; appendix 2 p 1). Parasite clearance half-lives were prolonged in participants infected with parasites carrying the Cys469Tyr mutation (median 4·2 h; IQR 3·4–4·9) and the Ala675Val mutation (median 4·9 h; 3·4–5·7) compared with those with wild-type parasites (median 2·8 h; 2·3–3·6; p<0·0001 for both comparisons; appendix 2 p 1). Clearance half-lives exceeding 5·5 h were observed in 16 (20%) of 79 infections with Cys469Tyr and nine (28%) of 32 infections with Ala675Val, compared with 33 (5%) of 637 infections with PfK13 wild-type infections (p=0·0001 for both comparisons). Day-3 parasitaemia was more frequent in participants infected with parasites harbouring Cys469Tyr (seven [9%] of 79; p=0·018) and Ala675Val (7 [21%] of 33; p=0·0001) mutations than in those with wild-type (19 [3%] of 638; appendix 2 p 1). Increased plasmepsin 2/3 copy number, linked to piperaquine resistance in southeast Asia, was not observed in any of 139 isolates studied.

Ex-vivo drug susceptibility testing was performed on 27 isolates from Agago and 69 isolates from Busia (appendix 2 p 1). Susceptibilities were assessed for dihydroartemisinin and the ACT partner drugs lumefantrine, monodesethyloamodiaquine (the active metabolite of amodiaquine), piperaquine, and pyronaridine. Results were similar for the two sites and similar to results reported for other samples collected in these regions of Uganda, without evidence of ex-vivo resistance to any study drug.[17,19]

The median day-7 lumefantrine concentration across the study population was 383·5 ng/mL, with 96 (20%) of 478 participants having concentrations below a previously assigned therapeutic threshold of 200·0 ng/mL (appendix 2 p 2).[24] The proportion with concentrations below 200 ng/mL did not differ significantly between participants with recurrent malaria (35 [19%] of 188) or adequate clinical and parasitological response (61 [21%] of 290; p=0·52).

Discussion

In this trial, we evaluated the efficacy of artemether–lumefantrine, the standard-of-care treatment for uncomplicated malaria in Uganda, and three alternate ACTs. We assessed parasite clearance, markers of drug resistance, ex-vivo drug susceptibilities, and lumefantrine blood concentrations. Both uncorrected 28-day clinical outcomes (considering all episodes of recurrent malaria infection) and PCR-corrected 28-day efficacy outcomes (indicating infections classified as recrudescence) showed that artemether–lumefantrine efficacy was less effective than the other ACTs. All identified adverse events were deemed unrelated to study drugs. As treatment of uncomplicated malaria with artemether–lumefantrine was followed by frequent episodes of recurrent malaria and, after PCR-correction, true treatment failures were seen in over 10% of participants at two of the three study sites, a change in malaria treatment recommendations should be considered.

Since 2013, there has been increasing concern regarding the efficacy of artemether–lumefantrine, the most widely used antimalarial drug in Africa, and the first-line therapy in Uganda.[9] Although most recent trials have shown adequate efficacy of artemether–lumefantrine for the treatment of uncomplicated malaria, some have shown efficacies less

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than 90%, including trials in Angola, the Democratic Republic of Congo, Burkina Faso, and Tanzania, as well as a trial conducted in Uganda in 2018–19, which reported a 28-day PCR-corrected artemether–lumefantrine efficacy of 87·2% in Busia.[7,9] These studies have been reported in the context of the emergence and spread of ART-R in eastern Africa,[3] including in Uganda,[2,25] and evidence of decreased activity of lumefantrine in Uganda.[18] However, all trials reporting suboptimal artemether–lumefantrine efficacy were at sites with low or no prevalence of ART-R K13 mutations,[3] including Busia at the time of the Ugandan study noted previously. The results of suboptimal artemether–lumefantrine efficacy are therefore not clearly explained by the recent emergence of ART-R in Africa.

In our new trial, considering uncorrected outcomes, recurrences were remarkably common after treatment with artemether–lumefantrine, as noted previously in Uganda. In locations with very high malaria transmission, recurrent infections within 28 days of follow-up were observed in about 50% of children treated with artemether–lumefantrine in studies at multiple sites around Uganda in 2004–05[22] and 2013.[23] In our new study, recurrences were less common in Agago despite its higher prevalence of ART-R mutations. This result could be explained by higher partner drug efficacy or lower transmission intensity, and thus lower risk of recurrent infections at Agago. Lumefantrine has the shortest half-life (~4 days) of the ACT partner drugs, probably explaining the higher rates of recurrence with lumefantrine compared with other partner drugs. All comparators had much lower rates of recurrence (ie, late clinical failure and late parasitological failure combined) after therapy at 28 days, varying from 26 (26%) of 99 for artesunate–pyronaridine (pyronaridine half-life ~8 days), to two (2%) of 99 for dihydroartemisinin–piperaquine (piperaquine half-life ~28 days), to one (1%) of 96 for artesunate–amodiaquine (amodiaquine half-life ~14 days). The results for artesunate–amodiaquine, showing very low rates of recurrence, were surprising, as this regimen had much higher levels of recurrence (15–66%) in previous trials.[22,23] The improved efficacy of artesunate–amodiaquine is probably due to the loss of resistance to amodiaquine, with parasites reverting to wild type PfCRT Lys76Thr and PfMDR1 Asn86Tyr sequences[26] and good ex-vivo activity of monodesethyloamodiaquine[17,19] in the last decade in Uganda. These changes could also partly explain decreased artemether–lumefantrine efficacy, as the wild-type PfMDR1 Asn86Tyr sequence has been associated with reduced lumefantrine susceptibility.[27]

With high malaria transmission intensity, the precision of genotyping to define treatment outcomes is challenged, as with increasing parasite diversity, the likelihood of independent clones identified before and after therapy being characterised as identical increases. The WHO 3/3 match algorithm (requiring a match at all three alleles to designate a recrudescence) to interpret genotype data is less likely than the 2/3 match algorithm to define late treatment failures as instances of recrudescence. As expected, the 2/3 match algorithm yielded lower estimates of corrected artemether–lumefantrine treatment efficacy at all study sites (appendix 2, p 3). There remains a risk of misclassification, often due to missing alleles in the data in samples with multiplicity of infection higher than 1. Improved genotyping methods, including amplicon deep sequencing to improve discrimination of clones, and improved analytical methods to decrease the likelihood of mischaracterisation,[28] are under study. Although assigning precise treatment outcomes is difficult, it is noteworthy that corrected efficacies for artemether–lumefantrine, using similar

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methods over time, appear to have decreased,[23,29] and the identified suboptimal efficacies of artemether–lumefantrine at two sites, following similar results in Uganda and elsewhere in recent years, are of great concern. Additionally, reports of travellers from Uganda who failed artemether–lumefantrine treatment in non-endemic countries adds to concern about the efficacy of the drug.[30]

We measured ex-vivo drug susceptibilities of isolates collected at enrolment from a subset of participants. Susceptibilities to all study drugs were similar in Arua and Busia (appendix 2 p 1). Thus, differences in artemether–lumefantrine efficacies between study sites were not easily explained by differences in drug susceptibilities. Ring survival assay percent survivals were higher at both sites than described in studies from southeast Asia, as we have observed previously with the ex-vivo ring survival assay and Ugandan isolates.[17]

To approximate relative drug exposure in study participants, we measured plasma lumefantrine concentrations at day 7 after treatment. Plasma drug concentrations were adequate at all sites and not significantly different in patients with different study outcomes.[24] Thus, differences in outcomes after treatment with artemether–lumefantrine could not be attributed to inadequate drug exposure.

One of the trial sites, Agago, has known high prevalence of two validated PfK13 markers, Cys469Tyr and Ala675Val. As expected, the prevalences of these mutations were higher (combined prevalence 35% in Agago) than at the other two study sites (combined prevalence 4% in Busia and 9% in Arua). Consistent with this finding, the parasite clearance half-life for both treatment groups was higher in Agago than the other two sites, although half-lives were below the generally accepted resistance cutoff of 5·5 h. As noted earlier, the modest decrease in clearance at Agago was not accompanied by decreased artemether–lumefantrine efficacy. However, participants with PfK13 mutations exhibited a higher prevalence of day 3 parasitaemia and a longer parasite clearance half-life, suggesting that if resistance to partner drugs also develops, ACTs could frequently fail.

Considering the suboptimal efficacy for artemether–lumefantrine reported in this and previous trials, should guidelines for the treatment of malaria be changed? Important goals are first, to provide the best possible treatment efficacy and second, to slow the spread of drug resistance. Our results identify three ACTs (artesunate–amodiaquine, dihydroartemisinin–piperaquine, and artesunate–pyronaridine) with improved efficacies compared with artemether–lumefantrine. The superiority of the other ACTs was mostly driven by improved prevention of new malaria infections after therapy, but corrected efficacies were also superior, probably due to declining activity of lumefantrine. Thus, consideration should be given to replacement of artemether–lumefantrine as the first-line antimalarial by other ACTs. Each potential choice has benefits and drawbacks. For artesunate–amodiaquine, efficacy was outstanding, but potential limitations are relatively poor perceived amodiaquine tolerability (although we did not observe increased adverse events in participants who received artesunate–amodiaquine) and the risk of rapid reselection of amodiaquine resistance. Isolates collected from refugees who recently departed South Sudan, where artesunate–amodiaquine is the first-line antimalarial, showed higher levels of amodiaquine resistance mediating mutations than isolates from Uganda.[31]

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For dihydroartemisinin–piperaquine, efficacy has generally been excellent in Africa, but in southeast Asia, combined resistance to artemisinins and piperaquine has led to poor treatment efficacy.[5] Artesunate–pyronaridine has, to date, been relatively little studied, and so the consequences of widespread use are not well appreciated. The cost of artesunate–amodiaquine is currently about equal to the cost of artemether–lumefantrine, whereas dihydroartemisinin–piperaquine and artesunate–pyronaridine are 2–3-times that of artemether–lumefantrine and artesunate–amodiaquine. Given resource constraints, it is improbable that efficacy and resistance data alone will be used to make case management decisions.

The use of multiple first-line therapies was recommended by WHO in 2021. All our tested ACTs might be included in a multiple first-line therapies programme, and consideration should be given to regimens that provide opposite resistance selection pressures, in particular artemether–lumefantrine and artesunate–amodiaquine. The triple ACT artemether–lumefantrine–amodiaquine also benefits from including drugs with opposite resistance pressures and are likely to be available soon, which also provides another reason to preserve artemether–lumefantrine efficacy through its discontinuation and implementation of multiple first-line therapies. Policy makers must carefully consider recent treatment efficacy and drug resistance data along with cost to optimise malaria treatment practices.

This study had limitations. First, genotyping to assign treatment outcomes used a WHO-recommended method, but newer methods would probably have improved the accuracy of our outcome determinations. Second, we were unable to assess the prevalence of gametocytaemia more than 3 days after therapy, limiting evaluation of its effect on malaria transmission. Third, we did not evaluate molecular markers of resistance to aminoquinolines, but prevalence of the PfCRT 76Thr and PfMDR1 86Tyr mutations associated with resistance to amodiaquine has been very low in Uganda.[17] Fourth, due to logistical challenges, few samples were assessed for ex-vivo drug susceptibilities. Fifth, pharmacological assessments were limited to day-7 lumefantrine concentrations, so insights from more detailed evaluation of exposure to lumefantrine and other ACT components are not available.

In summary, artemether–lumefantrine was associated with a higher risk of recurrent malaria infection after 28 days compared with dihydroartemisinin–piperaquine, artesunate–amodiaquine, or artesunate–pyronaridine, with corrected efficacy below 90% at two study sites. The prevalence of two ART-R-associated PfK13 mutations, 469Tyr and 675Val, was high in Agago, and the presence of these mutations was linked to delayed parasite clearance. Our study highlights the need for an immediate response to the declining efficacy of artemether–lumefantrine, such as implementing multiple first-line therapies, while awaiting the availability of new antimalarial drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data sharing

The deidentified individual participant data (including a data dictionary) used during the current study will be made available, along with related documents, such as the study protocol and statistical analysis plan, upon reasonable request. Requests will be considered on a case-by-case basis and must be approved by all investigators. Data will be shared with researchers whose proposed use is deemed methodologically sound and ethically appropriate, following the signing of a data access agreement. Investigator support will be provided if necessary to facilitate data use.

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Research in context

Evidence before this study

We reviewed the literature and found that WHO has confirmed artemisinin partial resistance (ART-R) in four African countries: Rwanda, Uganda, Eritrea, and Tanzania; with Uganda having the highest prevalence of WHO-validated and candidate markers of ART-R. Such resistance markers have also been reported in several other African countries. Trials in Angola, the Democratic Republic of Congo, Burkina Faso, and Tanzania have shown artemether–lumefantrine efficacies lower than 90% at some sites. In addition, a trial conducted in Uganda in 2018–19 showed 28-day genotype-corrected artemether–lumefantrine efficacy of 87·2% (95% CI 79·0– 95·0) in Busia. An ex-vivo ring survival assay (an in-vitro correlate of delayed artemisinin clearance) of *Plasmodium falciparum* isolates collected in northern and eastern Uganda in 2021 showed that those with the WHO-validated *kelch13* Cys469Tyr mutation had significantly prolonged parasite survival. Additional ex-vivo studies have shown reduced susceptibility to lumefantrine. Previous studies have shown that low antimalarial concentrations at day 7 after therapy are strongly associated with treatment failure, and detectable drug levels at the time of recurrent malaria have been associated with markers of decreased sensitivity to antimalarial drugs.

Added value of this study

This study took place during the emergence and spread of ART-R and the declining efficacy of artemether–lumefantrine in Africa. To the best of our knowledge, this trial is the first comprehensive assessment of the therapeutic efficacies of four artemisinin-based combination therapies (ACTs): artemether–lumefantrine, artesunate–amodiaquine, dihydroartemisinin–piperaquine, and artesunate–pyronaridine, all of which are recommended by WHO to treat uncomplicated malaria. In addition to evaluating the efficacies of these treatments, we also examined molecular markers associated with antimalarial resistance, standard growth inhibition assays, and day-7 plasma concentrations of lumefantrine. Both uncorrected 28-day clinical outcomes (considering all episodes of recurrent malaria infection) and PCR-corrected 28-day efficacy outcomes (indicating infections classified as recrudescence) showed that artemether–lumefantrine efficacy was less effective than the other ACTs. Parasite clearance half-lives were significantly prolonged in participants infected with parasites carrying the PfK13 Cys469Tyr mutation and the Ala675Val mutation, both of which have high prevalence in the Agago district compared with wild-type parasites.

Implications of all the available evidence

With ART-R emerging in Uganda and other regions of the world, using more effective non-artemether–lumefantrine ACTs must be considered to respond to spreading antimalarial drug resistance, and continued monitoring of the efficacy of antimalarials at multiple sites in Uganda and the region is a high priority. Our study highlights the need for an immediate response to the declining efficacy of artemether–lumefantrine, such as implementing the WHO-recommended multiple first-line therapy approach, beginning

with the districts where artemether–lumefantrine efficacy is lowest, while awaiting the availability of new compounds.

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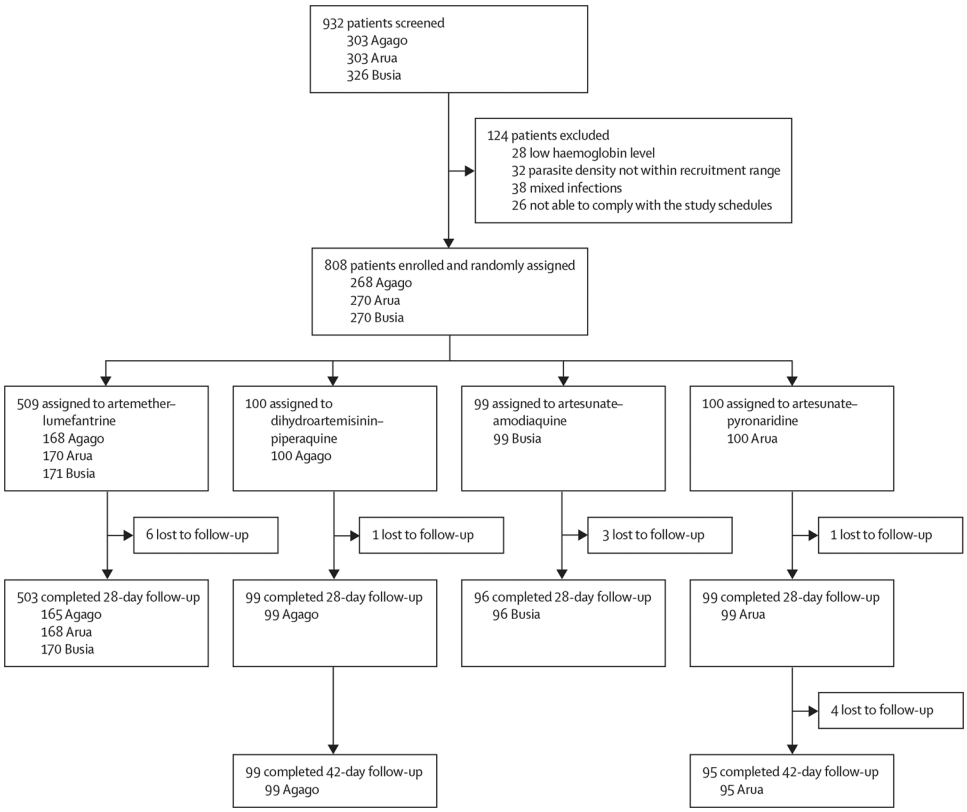


Figure 1:
Trial profile

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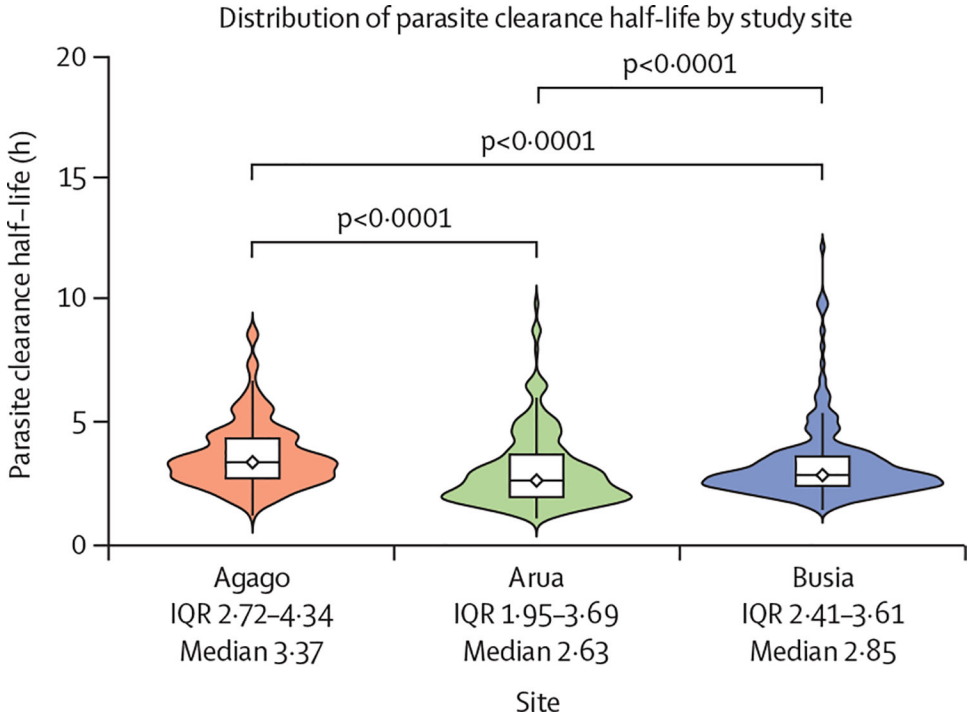


Figure 2:
Distribution of parasite clearance half-lives by site

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Table 1:

Baseline characteristics (N=808)

	Agago		Arua		Busia	
	Artemether–lumefantrine (n=168)	**Dihydroartemisinin–piperaquine (n=100)**	**Artemether–lumefantrine (n=170)**	**Artemether–lumefantrine (n=170)**	**Artemether–lumefantrine (n=170)**	**Artesunate–amodiaquine (n=99)**
Weight (kg)	17·8 (6·8)	17·8 (6·7)	15·1 (6·2)	15·6 (6·0)	16·2 (6·2)	15·8 (6·0)
Temperature (°C)	37·4 (0·9)	37·4 (0·9)	37·9 (0·9)	37·5 (1·0)	37·1 (3·4)	36·9 (3·9)
Parasite density per μL	27 090·0 (11 600·0–50 045·0)	29 860·0 (11 895·0–55 460·0)	42 460·0 (21 940·0–73 360·0)	39 750·0 (19 430·0–67 130·0)	30 420·0 (11 640·0–74 600·0)	21 380·0 (10 400·0–62 200·0)
Haemoglobin (g/dL)	10·1 (9·1–11·1)	10·5 (9·4–11·3)	10·3 (9·2–11·2)	10·5 (9·4–11·4)	10·2 (8·9–11·1)	9·7 (8·7–10·7)
Sex						
Male	69 (41·7%)	46 (46·0%)	85 (50·0%)	44 (44·0%)	82 (47·9%)	45 (45·5%)
Female	99 (58·9%)	54 (54·0%)	85 (50·0%)	56 (56·0%)	89 (52·0%)	54 (54·5%)
Age (years)	5·0 (2·0–7·0)	4·0 (2·0–7·0)	3·0 (2·0–6·0)	3·0 (2·0–6·0)	4·0 (2·0–6·0)	4·0 (2·0–6·0)
Prevalence of gametocytes	20 (11·9%)	11 (11·0%)	0	2 (2·0%)	18 (10·5%)	14 (14·1%)

Data are mean (SD), median (IQR), or n (%).

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Table 2:

WHO primary efficacy outcomes

	Agago			Arua			Busia	
	Artemether–lumefantrine (28 days)	**Dihydroartemisinin–piperaquine (28 days)**	**Dihydroartemisinin–piperaquine (42 days)**	**Artemether–lumefantrine (28 days)**	**Artesunate–pyronaridine (28 days)**	**Artesunate–pyronaridine (42 days)**	**Artemether–lumefantrine (28 days)**	**Artesunate–amodiaquine (28 days)**
Uncorrected outcomes								
Early treatment failure[*]	0/165	0/99	0/99	0/168	0/99	0/95	0/170	0/96
Late clinical failure[†]	7/165 (4·2%)	1/99 (1·0%)	5/99 (5·1%)	19/168 (11·3%)	6/99 (6·1%)	21/95 (22·1%)	15/170 (8·8%)	1/96 (1·0%)
Late parasitological failure[‡]	27/165 (16·4%)	1/99 (1·0%)	14/99 (14·1%)	62/168 (36·9%)	20/99 (20·2%)	20/95 (21·1%)	67/170 (39·4%)	0/96
Adequate clinical and parasitological response[§]	131/165 (79·4%; 72·3–85·1)	97/99 (98·0%; 92·2–99·6)	80/99 (80·8%; 71·4–87·8)	87/168 (51·8%; 44·0–59·5)	73/99 (73·7%; 63·8–81·8)	54/95 (56·8%; 46·3–66·8)	88/170 (51·8%; 44·0–59·4)	95/96 (99·0%; 93·5–99·9)
Adequate clinical and parasitological response[§¶], % (95% CI)	**79·4% (73·5–85·8)**	**98·0% (95·2–100·0)**	**80·8% (73·4–89·0)**	**51·8% (44·8–59·9)**	**73·7% (65·6–82·9)**	**56·8% (47·7–67·7)**	**51·8% (44·8–59·8)**	**99·0% (96·9–100·0)**
Corrected outcomes								
PCR corrected efficacy	131/135 (97·0%; 92·1–99·0)	97/97 (100·0%; 95·3–100·0)	80/84 (95·2%; 87·6–98·5)	87/106 (82·1%; 73·2–88·6)	73/79 (92·4%; 83·6–96·9)	54/62 (87·1%; 75·6–93·9)	88/108 (81·5%; 72·6–88·1)	95/95 (100·0; 95·2–100·0)
PCR corrected efficacy[¶], % (95% CI)	97·0% (94·2–100·0)	100·0% (100·0–100·0)	95·2% (90·8–99·9)	82·1% (75·1–89·7)	92·4% (86·7–98·4)	87·1% (79·1–95·9)	81·5% (74·5–89·1)	100·0% (100·0–100·0)

Data are n/N (%) or n/N (%; 95% CI), unless otherwise indicated. In the uncorrected calculation, the denominator includes reinfections, whereas in the corrected calculation, reinfections are excluded.

[*] Occurrence of danger signs or severe malaria on day 1, 2, or 3 in the presence of parasitaemia; parasitaemia on day 2 higher than day 0, irrespective of axillary temperature; parasitaemia on day 3 with axillary temperature ≥37·5°C; or parasitaemia on day 3 ≥25% of the count on day 0.

[†] Danger signs or severe malaria in the presence of parasitaemia between day 4 and day 28 (or day 42) in patients who did not previously meet early treatment failure criteria; or parasitaemia with axillary temperature ≥37·5°C during the same period in patients without previous early treatment failure.

[‡] Presence of parasitaemia with axillary temperature <37·5°C between day 7 and day 28 (or day 42) in patients who did not previously meet early treatment failure or late clinical failure criteria.

[§] Absence of parasitaemia on day 28 (or day 42), irrespective of axillary temperature, in patients without previous early treatment failure, late clinical failure, or late parasitological failure.

[¶] Kaplan-Meier estimate.

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Table 3:

Secondary outcomes by site and treatment group

	Agago		Arua		Arua	
	Artemether–lumefantrine	**Dihydroartemisinin–piperaquine**	**Artemether–lumefantrine**	**Artesunate–pyronaridine**	**Artemether–lumefantrine**	**Artesunate–amodiaquine**
Prevalence of parasitaemia						
Day 1	147/168 (88%)	93/100 (93%)	145/170 (85%)	71/100 (71%)	144/171 (84%)	79/99 (80%)
Day 2	47/168 (28%)	28/100 (28%)	29/168 (17%)	11/98 (11%)	41/171 (24%)	20/99 (2%)
Day 3	4/168 (2%)	6/100 (6%)	6/169 (4%)	3/99 (3%)	14/171 (8%)	5/99 (5%)
Prevalence of gametocytes						
Day 1	12/168 (7%)	11/100 (11%)	0/169	1/99 (1%)	15/169 (9%)	13/96 (14%)
Day 2	9/168 (5%)	11/99 (11%)	0/153	1/92 (1%)	7/79 (9%)	6/39 (15%)
Day 3	4/166 (2%)	3/97 (3%)	0/154	0/89	1/22 (5%)	1/9 (11%)
Prevalence of fever						
Day 1	22/168 (13%)	7/100 (7%)	25/170 (15%)	8/100 (8%)	23/171 (13%)	4/99 (4%)
Day 2	1/168 (1%)	1/100 (1%)	5/170 (3%)	1/100 (1%)	10/171 (6%)	0/99
Day 3	0/168	1/100 (1%)	4/170 (2%)	0/100	1/171 (1%)	1/99 (1%)
Haemoglobin, g/dL (mean [SD])						
Day 28	11·3 (1·3)	11·6 (1·2)	10·8 (1·5)	11·3 (1·2)	10·9 (1·5)	11·4 (1·4)
Day 42	NA	11·5 (1·4)	NA	11·2 (1·4)	NA	NA
Parasite clearance half-life, h (median [IQR])	3·3 (2·6–4·4)	3·4 (2·8–4·3)	2·7 (2·0–3·7)	2·6 (2·0–3·4)	2·9 (2·4–3·5)	2·8 (2·4–3·7)

Data are n/N (%) unless otherwise indicated. NA=not applicable.

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Table 4:

Adverse events by site and treatment group

	Agago		Agago		Busia	
	Artemether–lumefantrine	**Dihydroartemisinin–piperaquine**	**Artemether–lumefantrine**	**Artesunate–pyronaridine**	**Artemether–lumefantrine**	**Artesunate–amodiaquine**
Individuals with any adverse event	87/168 (52%)	59/100 (59%)	153/170 (90%)	97/100 (97%)	127/171 (74%)	45/99 (45%)
Total number of adverse events reported	130/1343 (10%)	80/1000 (8%)	317/1381 (23%)	252/1008 (25%)	185/1388 (13%)	65/802 (8%)
Common adverse events						
Upper respiratory tract infection	38/86 (44%)	23/59 (39%)	100/153 (65%)	83/97 (86%)	34/126 (27%)	29/48 (60%)
Diarrhoea	8/86 (9%)	1/59 (2%)	32/153 (21%)	27/97 (28%)	7/126 (6%)	6/48 (13%)
Anaemia	16/86 (19%)	12/59 (20%)	27/153 (18%)	20/97 (21%)	54/126 (43%)	18/48 (38%)
Serious adverse events						
Severe malaria	0	0	1/170 (1%)	1/100 (1%)	0	0

Data are n/N (%) unless otherwise indicated.

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