

# Combination treatments for uncomplicated falciparum malaria in Kampala, Uganda: randomised clinical trial

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See Comment page 1922

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## Summary

**Background** *Plasmodium falciparum* resistance has rendered chloroquine monotherapy ineffective in much of Africa, but data on alternative regimens are limited. We compared chloroquine+sulfadoxine-pyrimethamine, amodiaquine+sulfadoxine-pyrimethamine, and amodiaquine+artesunate for treatment of uncomplicated malaria in Kampala, Uganda.

**Methods** Of 1017 consecutive patients aged 6 months to 10 years with uncomplicated malaria who were screened, 418 were randomised to receive: chloroquine (25 mg/kg over 3 days) and sulfadoxine-pyrimethamine (25 mg/kg sulfadoxine, 1.25 mg/kg pyrimethamine, single dose); amodiaquine (25 mg/kg over 3 days) and sulfadoxine-pyrimethamine; or amodiaquine and artesunate (4 mg/kg daily for 3 days). Primary efficacy outcomes were 28-day clinical failure risks, adjusted and unadjusted by genotyping to distinguish new infection and recrudescence. The primary safety endpoint was incidence of serious adverse events during follow-up. Analysis was intention to treat and per protocol.

**Findings** 18 patients were excluded before enrolment. Of those enrolled, 384 of 400 (96%) were assigned an efficacy outcome and 396 (99%) were assessed for safety. Risk of 28-day clinical treatment failure was significantly higher with chloroquine+sulfadoxine-pyrimethamine (44/125 [35%]) than with amodiaquine+sulfadoxine-pyrimethamine (12/129 [9%]; risk difference 26% [95% CI 16–36];  $p < 0.0001$ ) or amodiaquine+artesunate (3/130 [2%]; 33% [24–42];  $p < 0.0001$ ). The greater risk of clinical treatment failure with amodiaquine+sulfadoxine-pyrimethamine was balanced by a lower risk of new infection, resulting in a similar need for retreatment over 28 days for amodiaquine+sulfadoxine-pyrimethamine (17/129 [13%]) and amodiaquine+artesunate (16/130 [12%];  $p = 0.854$ ). Serious adverse events were uncommon with all regimens.

**Interpretation** Risk of treatment failure with chloroquine+sulfadoxine-pyrimethamine was unacceptably high. Combinations of amodiaquine and sulfadoxine-pyrimethamine or artesunate were significantly more efficacious, and each regimen could be an appropriate alternative for treatment of uncomplicated malaria in Africa.

## Introduction

Antimalarial drug resistance is a serious problem in Africa.<sup>1</sup> Resistance to chloroquine, once the mainstay of antimalarial therapy, has spread across the continent and has been associated with increased malaria-associated morbidity and mortality.<sup>2</sup> Unacceptably high levels of resistance have forced many African countries, including Uganda, to abandon chloroquine as first-line treatment.<sup>3</sup> However, identification of appropriate replacement regimens has been a challenge.<sup>4</sup> Use of second-line drugs such as sulfadoxine-pyrimethamine and amodiaquine has been limited by emerging resistance and concerns about toxic effects (severe cutaneous reactions with sulfadoxine-pyrimethamine; blood dyscrasias and hepatotoxicity with amodiaquine),<sup>5</sup> leaving few effective, affordable options.

Combination antimalarial treatment could improve therapeutic effectiveness, reduce gametocyte carriage, and delay the spread of drug resistance.<sup>6</sup> Unlike other options, regimens including a 4-aminoquinoline (chloroquine or amodiaquine) and sulfadoxine-pyrimethamine combine currently available and affordable drugs.<sup>7</sup> In Uganda, chloroquine+sulfadoxine-pyrimethamine was chosen to replace chloroquine monotherapy as first-line treatment of

uncomplicated malaria in 2000,<sup>3</sup> although no local data on the efficacy of this combination were available to support this policy over other options. Chloroquine+sulfadoxine-pyrimethamine subsequently proved more efficacious than sulfadoxine-pyrimethamine monotherapy in Kampala.<sup>8</sup> However, follow-up in that study was restricted to 14 days, which probably underestimated the risk of treatment failure.<sup>9</sup> Amodiaquine+sulfadoxine-pyrimethamine has been highly effective in Kampala,<sup>8,10,11</sup> and elsewhere in Africa.<sup>12–14</sup> However, combinations of a 4-aminoquinoline and sulfadoxine-pyrimethamine will probably be increasingly limited by resistance to the individual drugs and, therefore, their useful therapeutic lifespan might be short.

Resistance to artemisinin antimalarials has, up to now, not been identified, but the efficacy of this class of drugs is limited by late reappearance of parasites after monotherapy.<sup>15</sup> In southeast Asia, use of artesunate+mefloquine improved efficacy, reduced gametocyte carriage, and was associated with a fall in *Plasmodium falciparum* transmission.<sup>16</sup> In view of this success, combination therapy including an artemisinin has been widely advocated for Africa.<sup>17</sup> However, the epidemiology of malaria in this continent is vastly different from that in

southeast Asia, and little data on the efficacy and safety of artemisinins in Africa are available.<sup>17</sup> In available African studies, the efficacy of regimens pairing artesunate with sulfadoxine-pyrimethamine or amodiaquine has varied, probably owing to the level of resistance to the companion drug.<sup>11,14,18,19</sup> To further investigate combination anti-malarial regimens in Africa, we compared the efficacy and safety of chloroquine+sulfadoxine-pyrimethamine, amodiaquine+sulfadoxine-pyrimethamine, and amodiaquine+artesunate for the treatment of uncomplicated falciparum malaria.

## Methods

### Study site

The study was undertaken between August, 2002, and July, 2003, at Mulago Hospital, Kampala, Uganda. Kampala is an urban centre where malaria is mesoendemic (25% palpable spleen rate, 25% parasitaemia rate), arising perennially with peaks during the two rainy seasons (Talisuna A, Ugandan Ministry of Health, unpublished data, 1994). In drug-efficacy studies done between 1998 and 2002 at our research site, risk of 14-day clinical treatment failure was 49% with chloroquine, 14% with sulfadoxine-pyrimethamine, 7% with amodiaquine, 8% with chloroquine+sulfadoxine-pyrimethamine, and 2% with amodiaquine+sulfadoxine-pyrimethamine.<sup>8,10,11,20,21</sup> The study was approved by the international clinical studies review committee at the US National Institutes of Health (NIH), the Ugandan National Council for Science and Technology, and the institutional review boards of the University of California, San Francisco, and Makerere University, Kampala, and was overseen by a data and safety monitoring board.

### Patients

Children residing in Kampala who presented to the outpatient department of Mulago Hospital with symptoms suggestive of malaria and a positive screening thick blood smear were referred to the study clinic for further assessment. We screened consecutive patients for the following selection criteria: age 6 months to 10 years; temperature 38.0°C or higher (tympanic) or febrile symptoms in the previous 48 h; willingness to participate in follow-up for the next 28 days; written informed consent provided by parent or guardian; no history of serious side-effects to study treatments, including sulfa drugs; absence of severe malaria, including severe anaemia (haemoglobin <50 g/L), or danger signs such as inability to stand or drink, lethargy, recent convulsions, or persistent vomiting;<sup>22</sup> absence of alternative diagnosis for febrile illness; not previously enrolled in the study; and no history of antifolate use in the previous 4 weeks. We assessed the final selection criteria after enrolment: successful phlebotomy; *P falciparum* mono-infection; parasite density 500–200 000 parasites per  $\mu$ L; and haemoglobin 50 g/L or greater.

Because laboratory results were generally not available until the following day, a patient could be excluded after randomisation. Laboratory personnel not involved in treatment allocation or outcome classification determined such exclusions. The original selection criterion for parasite density was without an upper limit, but, after 57 (14%) children were enrolled, an upper limit of 200 000 parasites per  $\mu$ L was incorporated to follow updated WHO guidelines.<sup>22</sup>

### Procedures

At enrolment, we asked children and their parents or guardians about previous antimalarial treatment, use of other drugs, and common symptoms. We measured temperature (tympanic) and weight and did a physical examination. A brief neurological assessment, consisting of tests for coordination (heel-toe ataxia), fine finger dexterity (ability to pick up a small object), hearing, nystagmus, and balance (standing with feet together, Romberg test), was undertaken in children of appropriate age (tablet test,  $\geq 9$  months; walking in a straight line,  $\geq 2$  years; heel-toe walking and Romberg test,  $\geq 4$  years). We obtained blood by venepuncture for thick and thin blood smears, complete blood count (white-blood cells, differential, haemoglobin, and platelets), creatinine and alanine transaminase concentrations, and to store on filter paper for molecular analysis.

On day 0, we randomly assigned patients to receive one of three oral treatments: chloroquine+sulfadoxine-pyrimethamine, amodiaquine+sulfadoxine-pyrimethamine, or amodiaquine+artesunate. We administered drugs as follows: chloroquine (250 mg tablets, 25 mg/kg per treatment) 10 mg/kg on days 0 and 1, and 5 mg/kg on day 2; sulfadoxine-pyrimethamine (500 mg/25 mg tablets, 25 mg/kg sulfadoxine and 1.25 mg/kg pyrimethamine per treatment) as one dose; amodiaquine (200 mg tablets, 25 mg/kg per treatment) 10 mg/kg on days 0 and 1, and 5 mg/kg on day 2; and artesunate (50 mg tablets, 12 mg/kg per treatment) 4 mg/kg once daily for 3 days.

An off-site investigator generated randomisation codes with a computer for two age-groups (6–59 months and 5–10 years), using variable blocking. Sequentially numbered sealed envelopes containing the treatment group assignments were prepared from the randomisation lists. The study nurse assigned treatment numbers sequentially and allocated treatment by opening the envelope corresponding to the treatment number. All other study personnel, including the doctors, were unaware of treatment assignments. The nurse administered study drugs according to weight-based guidelines for administration of fractions of tablets modified from WHO recommendations.<sup>23</sup> The study drugs were not identical in appearance or taste, and no placebos were used. Before administration, all medications were crushed and mixed with chocolate syrup to mask the patients to the colour and taste of the tablets. All treatment was directly observed.

After treatment, children were observed for 30 min and the dose was re-administered if vomiting happened. Those who vomited persistently were referred for treatment with parenteral quinine. We provided all patients with a 3-day supply of paracetamol for treatment of febrile symptoms. Those with a concentration of haemoglobin of less than 100 g/L were treated according to Integrated Management of Childhood Illness guidelines with ferrous sulphate for 14 days and given anthelmintic treatment if they were older than 1 year and had not been treated in the previous 6 months.

Follow-up appointments were scheduled for days 1, 2, 3, 7, 14, 21, and 28 and consisted of completion of a standardised history form and physical examination. Neurological assessment was repeated on days 7, 14, and 28. We obtained blood (by fingerprick on days 1, 2, 3, 14, 21, and any unscheduled day that fever was reported and by venepuncture on days 7 and 28) to repeat thick blood smears and for storage on filter paper. Laboratory testing was repeated on days 7 and 28. We encouraged patients to return to the clinic at any time if they felt ill. If they did not return for follow-up, we visited them at home.

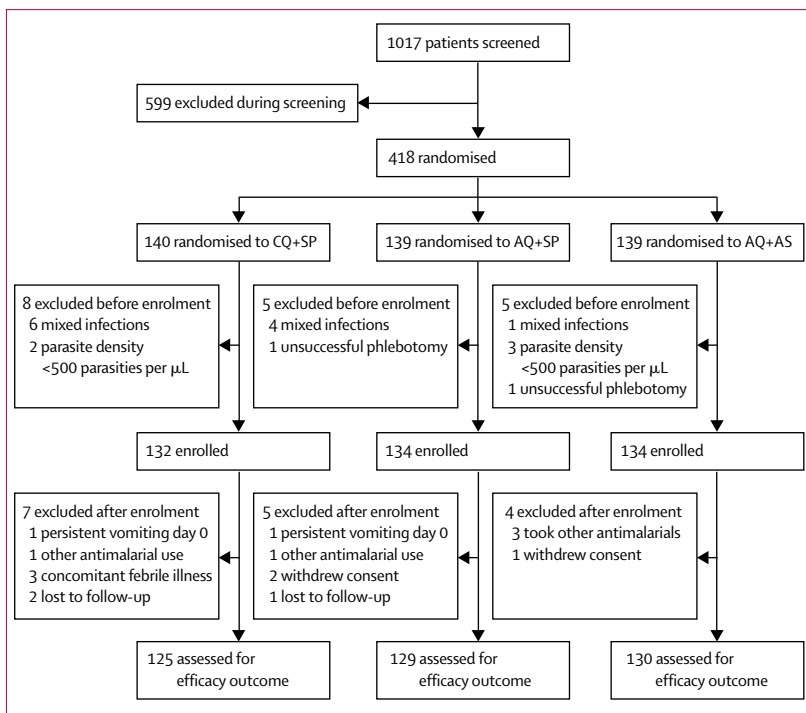
We stained thick and thin blood smears with 2% Giemsa for 30 min. We determined parasite density from thick blood smears by counting the number of asexual parasites per 200 white-blood cells and by calculating parasites per  $\mu\text{L}$ , assuming a white-blood-cell count of 8000 cells per  $\mu\text{L}$ . A smear was judged to be negative if no parasites were seen after review of 100 high-powered

fields. We also assessed gametocytaemia from thick blood smears. Thin blood smears were reviewed for non-falciparum infections. A second microscopist, who was unaware of the results of the first reading, reread all slides; a third reviewer screened and resolved discrepant results.

At every follow-up visit, we assessed patients for any new or worsening event and assessed laboratory results. We defined an adverse event as any untoward medical occurrence irrespective of its suspected relation to the study drugs, as per International Conference of Harmonization guidelines. We graded all events by severity (none, mild, moderate, severe, life-threatening) and relation to study treatment (none, unlikely, possible, probable, definite), using guidelines from WHO (toxicity grading scale for determining the severity of adverse events) and the NIH (paediatric toxicity tables). A serious adverse event was defined as any event that resulted in death, life-threatening experience, admission, persistent or clinically significant disability or incapacity, or specific medical or surgical intervention to prevent serious outcome.

We classified treatment outcomes according to WHO guidelines for areas of intense transmission, as adequate clinical and parasitological response (ACPR), early treatment failure (ETF), late clinical failure (LCF), and late parasitological failure (LPF).<sup>22</sup> The WHO system was adapted to 28-day follow-up and we modified classification of LCF to include history of fever within the previous 24 h on days 4–28 with parasitaemia.<sup>22</sup> We stratified clinical outcome into success (ACPR/LPF) or failure (ETF/LCF) and parasitological outcome into success (ACPR) or failure (ETF/LCF/LPF). Some patients were not assigned an efficacy outcome classification for the following reasons: persistent vomiting of study drugs on day 0; receipt of antimalarial drugs outside of the study protocol; emergence of any concomitant febrile illness that would interfere with outcome classification; withdrawal of consent; and loss to follow-up. Participants meeting criteria for clinical treatment failure or LPF were treated with quinine (10 mg/kg three times daily for 7 days). To distinguish recrudescence and new infections, we adjusted day 28 treatment outcomes using molecular genotyping, as previously described.<sup>24</sup>

Briefly, we amplified the block 3 region of the merozoite surface protein 2 gene by nested PCR and characterised this gene on the basis of sequence and size polymorphisms identified by restriction endonuclease digestion and gel electrophoresis. Genotyping patterns on the day of repeat therapy were compared with those at treatment initiation, using GelCompar II software (Applied Maths, Austin, TX, USA). We defined an outcome as recrudescence if a sample at the time of repeat therapy contained identical alleles or a subset of alleles that were present at the time of treatment initiation. A new infection outcome was identified if a sample at the time of repeat therapy contained only new alleles. If, at repeat therapy, a sample contained alleles



**Figure 1: Trial profile**

CQ+SP=chloroquine+sulfadoxine-pyrimethamine. AQ+SP=amodiaquine+sulfadoxine-pyrimethamine. AQ+AS=amodiaquine+artesunate.

	Chloroquine+sulfadoxine-pyrimethamine (n=125)	Amodiaquine+sulfadoxine-pyrimethamine (n=129)	Amodiaquine+artesunate (n=130)
<b>Demographics</b>			
Female sex	64 (51%)	68 (53%)	73 (56%)
Median (IQR) age (years)	4.3 (3.0–7.0)	4.5 (2.2–6.0)	4.0 (2.1–7.0)
Age <5 years	67 (54%)	69 (54%)	73 (56%)
<b>Clinical characteristics</b>			
Antimalarial use in past 2 weeks	49 (39%)	49 (38%)	59 (45%)
Initial temperature (°C)	38.0 (1.1)	37.9 (1.0)	37.9 (1.1)
Geometric mean (range) parasite density (per $\mu\text{L}$ )	25 097 (585–951 360)	33 286 (1120–320 880)	22 483 (560–626 400)
Parasite density >200 000 parasites per $\mu\text{L}$ (%)	3 (2)	4 (3)	2 (2)
<b>Blood measures</b>			
White-blood-cell count ( $\times 10^9/\text{L}$ )	7.76 (3.02)	7.90 (3.17)	8.54 (3.98)
Neutrophil count ( $\times 10^9/\text{L}$ )	4.00 (2.44)	3.96 (2.36)	4.14 (2.77)
Haemoglobin (g/L)	102 (18)	105 (19)	100 (18)
Platelet count ( $\times 10^9/\text{L}$ )	176.01 (88–18)	187.85 (96–27)	199.13 (98–27)
Creatinine ( $\mu\text{mol/L}$ )	39.78 (19–45)	39.78 (15–91)	40.66 (14–14)
Alanine transaminase (IU/L)	20 (14)	25 (74)	18 (11)

Data are mean (SD) or number of patients (%) unless otherwise indicated.

**Table 1: Baseline characteristics of patients with efficacy outcomes**

present at the time of treatment initiation and new alleles, we classified the outcome as recrudescence if more than half the alleles were identical and new infection otherwise. Findings of a sensitivity analysis showed this method to be the best one for classification of genotyping outcomes in our population.<sup>24</sup>

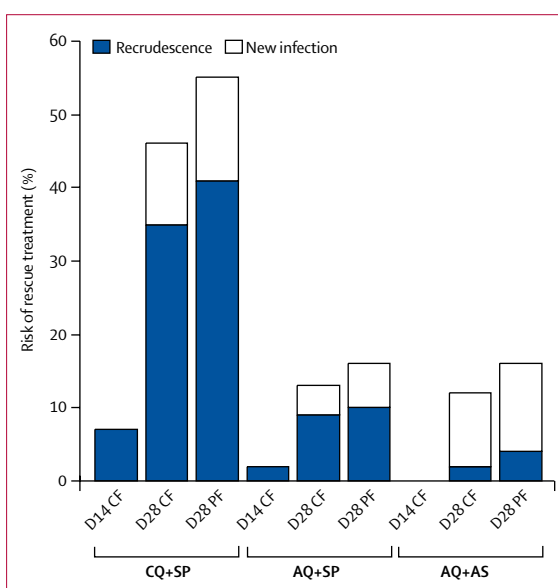
Primary efficacy outcomes were 28-day clinical failure risks, adjusted and unadjusted by genotyping. We defined rescue therapy as quinine treatment given to a patient when treatment failure was identified on the basis of results unadjusted by genotyping, and included all early and late treatment failures (LCF for clinical outcomes and LCF/LPF for parasitological outcomes). Clinical and parasitological treatment failure included all early and late failures due to recrudescence after adjustment by genotyping (excluding new infections). The primary safety endpoint was incidence of serious adverse events during follow-up. Secondary efficacy endpoints included clinical outcome at day 14, parasitological outcome at day 28, resolution of fever and febrile symptoms, parasite clearance, change in haemoglobin from day 0 to day 28, and presence of gametocytes during follow-up.

### Statistical analysis

Sample size was calculated to test the hypothesis that treatment with either of the amodiaquine-containing regimens would reduce the risk of rescue therapy by day 28 compared with treatment with chloroquine+sulfadoxine-pyrimethamine in all age-groups. Based on previous studies in Kampala, using data unadjusted by genotyping, we estimated the risk of clinical failure for amodiaquine+sulfadoxine-pyrimethamine at 13%,<sup>11</sup> and for chloroquine+sulfadoxine-pyrimethamine at 30%. With these estimates, a study with 121 patients in each arm (134 to allow for 10% loss to follow-up) would obtain 80% power with a two-sided significance of 5%.

We assessed efficacy data by intention-to-treat (including all enrolled patients) and per-protocol (including those assigned efficacy outcomes) analyses. Participants who completed study follow-up and were assessed on day 28, but who had missing data points during follow-up (n=6), were assigned treatment outcomes based on the available data. An age-stratified subgroup analysis was planned before the study started.

Data were double-entered (EpiInfo 6.04, Centers for Disease Control and Prevention, Atlanta, GA, USA), verified, and analysed with SPSS version 10.0 (SPSS, Chicago, IL, USA) and Stata version 7.0 (Stata, College



**Figure 2: Risk of rescue treatment**

D14 CF=day 14 clinical failure. D28 CF=day 28 parasitological failure. CQ+SP=chloroquine+sulfadoxine-pyrimethamine. AQ+SP=amodiaquine+sulfadoxine-pyrimethamine. AQ+AS=amodiaquine+artesunate.

	Failure risk comparisons	Risk difference (95% CI)	p
<b>Risk of rescue therapy</b>			
CQ+SP vs AQ+SP	46% vs 13%	33% (23 to 44)	<0.0001
CQ+SP vs AQ+AS	46% vs 12%	34% (24 to 45)	<0.0001
AQ+SP vs AQ+AS	13% vs 12%	1% (-7 to 9)	0.854
<b>Risk of clinical treatment failure</b>			
CQ+SP vs AQ+SP	35% vs 9%	26% (16 to 36)	<0.0001
CQ+SP vs AQ+AS	35% vs 2%	33% (24 to 42)	<0.0001
AQ+SP vs AQ+AS	9% vs 2%	7% (1 to 13)	0.018

CQ+SP=chloroquine+sulfadoxine-pyrimethamine. AQ+SP=amodiaquine+sulfadoxine-pyrimethamine.  
AQ+AS=amodiaquine+artesunate.

**Table 2: Clinical treatment outcome at day 28 (per-protocol analysis)**

Station, TX, USA). Parasite densities were log-transformed. We compared categorical variables with Fisher's exact test and continuous variables with an independent samples *t* test. We judged  $p < 0.05$  (two-tailed) significant. Risk differences, 95% CIs, and exact *p* values were calculated with the Stata statistical analysis software package. We prepared an interim report for a data and safety monitoring board at about the midpoint of the trial, which included analyses of safety and efficacy.

#### Role of the funding source

The NIH was involved in protocol development, reporting of serious adverse events, and trial monitoring but had no role in data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

#### Results

Of 1017 patients referred for assessment, 599 were excluded during screening and 18 after randomisation (figure 1). Of the 400 patients enrolled, 384 (96%) were assigned efficacy outcomes. Table 1 shows baseline characteristics for the three treatment groups.

All 384 patients with efficacy outcomes were included in the per-protocol analysis. The risk of rescue therapy with quinine was low in all treatment groups at day 14, but increased substantially by day 28 (figure 2). At day 28, chloroquine+sulfadoxine-pyrimethamine was

significantly inferior to the other treatment groups, for either overall need for rescue therapy or risk of clinical treatment failure (table 2). Comparing the amodiaquine-containing regimens, risk of clinical treatment failure was higher for amodiaquine+sulfadoxine-pyrimethamine. However, risk of new infection was greater with amodiaquine+artesunate, resulting in a similar need for rescue therapy for the two groups. No patient treated with amodiaquine+artesunate progressed to severe malaria. When results were stratified by age (children <5 years vs those aged 5–10 years), outcomes with chloroquine+sulfadoxine-pyrimethamine or amodiaquine+artesunate were similar for the two age-groups (data not shown). For amodiaquine+sulfadoxine-pyrimethamine, clinical treatment failure was higher in younger children than in those aged 5–10 years (16% vs 2%; risk difference 14% [95% CI 5–24];  $p=0.006$ ). The intention-to-treat results were similar to those of the per-protocol analysis (table 3).

Patients treated with amodiaquine+artesunate had more rapid resolution of fever than did other participants, although this difference was not evident on day 1, when only 36 (29%) patients allocated chloroquine+sulfadoxine-pyrimethamine, 42 (33%) given amodiaquine+sulfadoxine-pyrimethamine, and 33 (25%) amodiaquine+artesunate did not have fever. On day 2, 101 (78%) patients given amodiaquine+artesunate were afebrile and did not have febrile symptoms compared with 69 (55%) allocated chloroquine+sulfadoxine-pyrimethamine ( $p=0.0002$ ) and 83 (64%) amodiaquine+sulfadoxine-pyrimethamine ( $p=0.02$ ). By day 3, fever had resolved in most patients, irrespective of treatment group. Amodiaquine+artesunate also accelerated clearance of parasitaemia compared with the other two treatments (figure 3). By day 2, nearly all patients treated with amodiaquine+artesunate had a negative blood smear.

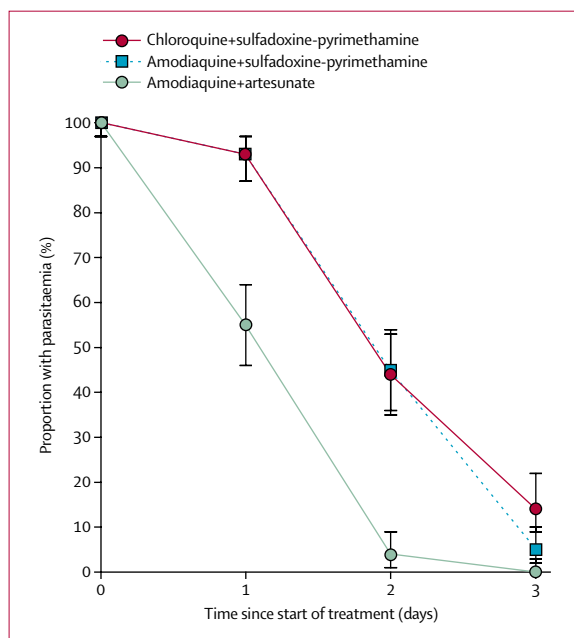
Anaemia (haemoglobin <100 g/L) was present in 45% (172/384) of patients at enrolment and fell in all treatment groups by day 28 (chloroquine+sulfadoxine-pyrimethamine 45% to 18%; amodiaquine+sulfadoxine-pyrimethamine 40% to 11%; amodiaquine+artesunate 49% to 5%). Patients allocated amodiaquine+artesunate showed the greatest increase in haemoglobin (amodiaquine+artesunate 19 g/L vs chloroquine+sulfadoxine-pyrimethamine 12 g/L,  $p=0.001$ ; and vs amodiaquine+sulfadoxine-pyrimethamine 13 g/L,  $p=0.004$ ).

Presence of gametocytes during follow-up was reduced in patients treated with an amodiaquine-containing regimen. Excluding those with gametocytes at enrolment, gametocytes were noted in nine of 125 (7%) patients allocated amodiaquine+sulfadoxine-pyrimethamine and six of 121 (5%) amodiaquine+artesunate patients, compared with 24 of 113 (21%) given chloroquine+sulfadoxine-pyrimethamine (amodiaquine+sulfadoxine-pyrimethamine vs chloroquine+sulfadoxine-pyrimethamine,  $p=0.002$ ; amodiaquine+artesunate vs chloroquine+sulfadoxine-pyrimethamine,  $p=0.0003$ ).

	Treatment failure risk comparisons	Risk difference (95% CI)	p
<b>Patients with no efficacy outcome classified as successes</b>			
CQ+SP vs AQ+SP	33% vs 9%	24% (15–34)	<0.0001
CQ+SP vs AQ+AS	33% vs 2%	31% (23–40)	<0.0001
AQ+SP vs AQ+AS	9% vs 2%	7% (1–12)	0.03
<b>Patients with no efficacy outcome classified as failures</b>			
CQ+SP vs AQ+SP	39% vs 13%	26% (16–36)	<0.0001
CQ+SP vs AQ+AS	39% vs 5%	33% (24–43)	<0.0001
AQ+SP vs AQ+AS	13% vs 5%	8% (1–14)	0.053

CQ+SP=chloroquine+sulfadoxine-pyrimethamine. AQ+SP=amodiaquine+sulfadoxine-pyrimethamine.  
AQ+AS=amodiaquine+artesunate.

**Table 3: Clinical treatment outcome at day 28 (intention-to-treat analysis)**



**Figure 3: Parasite clearance**

Error bars represent 95% CI. Significant pairwise comparisons include: days 1 and 2, amodiaquine+artesunate vs chloroquine+sulfadoxine-pyrimethamine and amodiaquine+sulfadoxine-pyrimethamine,  $p < 0.0001$ ; day 3, amodiaquine+artesunate vs amodiaquine+sulfadoxine-pyrimethamine,  $p = 0.014$ ; amodiaquine+artesunate vs chloroquine+sulfadoxine-pyrimethamine,  $p < 0.0001$ .

Of the 400 enrolled patients, 396 (99%) had at least one follow-up assessment and were included in the safety analysis. A more detailed account of these results will be reported separately. With respect to events of moderate or greater severity, patients treated with chloroquine+sulfadoxine-pyrimethamine were more likely to have fever and febrile symptoms, headache, and a fall in haemoglobin. Excluding those who needed rescue therapy, no significant difference was recorded in incidence of events of moderate or greater severity between the treatment groups (data not shown).

16 serious adverse events were reported in 12 patients (five chloroquine+sulfadoxine-pyrimethamine, six amodiaquine+sulfadoxine-pyrimethamine, and one artesunate+amodiaquine,  $p = 0.128$ ). No deaths or severe cutaneous reactions happened, and no important neurological events were seen. Most events were attributable to severe malaria, including convulsions (one chloroquine+sulfadoxine-pyrimethamine, three amodiaquine+sulfadoxine-pyrimethamine), severe anaemia (two chloroquine+sulfadoxine-pyrimethamine), vomiting (one amodiaquine+sulfadoxine-pyrimethamine), severe anaemia with vomiting (one chloroquine+sulfadoxine-pyrimethamine), and severe anaemia with altered mental status (one chloroquine+sulfadoxine-pyrimethamine). Two patients had serious events that were clearly related to other illnesses, including pyomyositis (amodiaquine+sulfadoxine-pyrimethamine) and measles (amodi-

aquine+artesunate). Thrombocytopenia, neutropenia, and anaemia were reported in one patient given amodiaquine+sulfadoxine-pyrimethamine who was successfully treated for malaria. Severe thrombocytopenia (33 000 cells per  $\mu\text{L}$ ) was present in this child at enrolment and persisted through study follow-up (32 000 cells per  $\mu\text{L}$  on day 7, 20 000 cells per  $\mu\text{L}$  on day 28), becoming life-threatening. Severe neutropenia and anaemia subsequently developed and the patient became transfusion-dependent. A bone-marrow biopsy revealed hypoplastic marrow of undetermined cause with all cell lines present.

Additional severe laboratory-related adverse events included anaemia (two chloroquine+sulfadoxine-pyrimethamine, one amodiaquine+artesunate), neutropenia (one amodiaquine+sulfadoxine-pyrimethamine), and an increase of alanine transaminase (amodiaquine+sulfadoxine-pyrimethamine). The patient with neutropenia was asymptomatic and the abnormality was transient (1170 cells per  $\mu\text{L}$  on day 0, 301 cells per  $\mu\text{L}$  on day 7, 1568 cells per  $\mu\text{L}$  on day 9, and 1224 cells per  $\mu\text{L}$  on day 28). The child with an increase in alanine transaminase (27 IU/L on day 0, 597 IU/L on day 7) developed clinical hepatitis with pruritus, rash, fever, anorexia, and vomiting (days 11–21). These symptoms resolved by day 28: alanine transaminase returned to normal (17 IU/L), HbsAg was non-reactive, and abdominal ultrasound showed typical findings.

## Discussion

In this randomised clinical trial, combinations of amodiaquine with sulfadoxine-pyrimethamine or artesunate were significantly more efficacious than chloroquine+sulfadoxine-pyrimethamine—the current first-line drug regimen—for treatment of uncomplicated malaria in Kampala, Uganda. In a comparison of the amodiaquine-containing regimens, risk of clinical treatment failure was higher with amodiaquine+sulfadoxine-pyrimethamine than with amodiaquine+artesunate. However, no difference was noted between the two regimens in the overall requirement for rescue therapy, since the lower treatment efficacy of amodiaquine+sulfadoxine-pyrimethamine was offset by a higher risk of new infection with amodiaquine+artesunate.

In this study and another from Kampala,<sup>8</sup> chloroquine+sulfadoxine-pyrimethamine had only a 7% risk of clinical failure at 14 days. However, after 28 days, failures with chloroquine+sulfadoxine-pyrimethamine increased greatly, reaching levels seen with sulfadoxine-pyrimethamine monotherapy.<sup>11</sup> Consistent with other reports,<sup>11,14</sup> these data emphasise the need for extended follow-up when assessing the efficacy of combination regimens. Furthermore, although the decision to change to chloroquine+sulfadoxine-pyrimethamine was only made in Uganda in 2000, risk of clinical treatment failure with this regimen (35%) is already unacceptably high in Kampala.

Assessment of antimalarial drug efficacy is generally based on risk of treatment failure. Use of genotyping to distinguish recrudescence from new infection allows for consideration of both the overall risk of rescue therapy (including early failures, recrudescence, and new infections) and the risk of treatment failure (excluding new infections). Although risk of treatment failure is an essential measure of efficacy, risk of rescue therapy provides a more meaningful public-health indicator, because the distinction between retreatment for recrudescence or reinfection is less clinically relevant. A relative efficacy benefit of amodiaquine+artesunate was offset by a relative benefit of amodiaquine+sulfadoxine-pyrimethamine in preventing new infections, such that use of the two regimens was followed by nearly identical risks of treatment for recurrent malaria over the ensuing 28 days.

Amodiaquine+sulfadoxine-pyrimethamine and amodiaquine+artesunate have been regarded as alternatives to chloroquine+sulfadoxine-pyrimethamine for first-line antimalarial therapy in Uganda, and have been included in revised antimalarial drug policies in some other east African countries.<sup>14,25</sup> Each regimen has its potential advantages. Amodiaquine+sulfadoxine-pyrimethamine is inexpensive and both drugs are currently available. Amodiaquine+artesunate might provide the benefits expected from an artemisinin-containing regimen, including rapid action and added efficacy. In this study, the total number of rescue treatments needed with the two regimens was similar, suggesting that amodiaquine+sulfadoxine-pyrimethamine might currently be the more cost-effective regimen (approximate cost per adult treatment, US\$0.24 for amodiaquine+sulfadoxine-pyrimethamine, US\$1.30 for amodiaquine+artesunate).<sup>26,27</sup> However, the durability of amodiaquine+sulfadoxine-pyrimethamine should be considered. In this study, only 84% of children younger than 5 years of age treated with amodiaquine+sulfadoxine-pyrimethamine were clinical successes at day 28, compared with 96% in a previous study at the same site.<sup>28</sup> Although the study designs differed, these results suggest a decline in efficacy of this regimen. The lower risk of new infection seen with amodiaquine+sulfadoxine-pyrimethamine probably results from a post-treatment prophylactic effect of the two fairly long-acting drugs. Delayed drug clearance might prevent new infection with drug-sensitive parasites but is likely to contribute to the selection of drug resistance and could have an effect on the long-term effectiveness of amodiaquine+sulfadoxine-pyrimethamine.<sup>29</sup>

The safety profiles of new antimalarial regimens remain a concern. Both amodiaquine and sulfadoxine-pyrimethamine have been associated with serious toxic effects when used for long-term chemoprophylaxis,<sup>5</sup> but seem to be much safer in treatment regimens.<sup>30,31</sup> Artemisinin derivatives given at high doses produce selective neurotoxicity in laboratory animals, but this

effect has not been reported in people, and the drugs seem to be safe and well tolerated.<sup>32</sup> Evidence of reproductive toxic effects in animals treated with artemisinins in early pregnancy is perhaps of greater concern, and has led WHO to recommend avoidance of artemisinin compounds in the first trimester.<sup>33</sup> We monitored participants for severe toxic effects that have previously been associated with our study drugs.<sup>5,32</sup> Single episodes of transient neutropenia and hepatitis were seen with amodiaquine+sulfadoxine-pyrimethamine. The association between this combination and development of pancytopenia in one patient was unclear since the thrombocytopenia at enrolment could have been due to malaria or a pre-existing condition. Thus, our study did not have the power to compare incidence of rare severe adverse events but our results offer reassurance that all study regimens were well tolerated by nearly all participants.

African countries are faced with a challenge. Escalating drug resistance has rendered chloroquine ineffective, but the best replacement for first-line antimalarial therapy has been unclear. Artemisinin-containing combination therapy has been strongly advocated for use in Africa, but limited clinical experience and the high cost of these regimens are important obstacles. Despite these concerns, newly revised antimalarial drug policies in many African countries include artemisinin-containing combination regimens. In Uganda, the coformulation of artemether with lumefantrine was provisionally chosen to replace chloroquine+sulfadoxine-pyrimethamine in 2004, although comparative data on this combination were lacking. Artemether+lumefantrine is promising, but its complex dosing regimen raises concerns about adherence and it is currently expensive. Implementation of artemisinin-based combination therapy as first-line antimalarial treatment needs great logistic and economic commitment. The role of non-artemisinin-containing combination regimens is not clear. In Kampala, amodiaquine+sulfadoxine-pyrimethamine currently offers a readily available, efficacious, and economical alternative.

Although the lifespan of amodiaquine+sulfadoxine-pyrimethamine might be limited by resistance, this regimen could be appropriate for regions of Africa where resistance to the individual drugs remains low, as an interim policy pending introduction of artemisinin-containing combinations, or for presumptive treatment of fever outside of the formal health sector. Additional evaluation of new antimalarial regimens, particularly longitudinal studies to assess the clinical and economic effect of repetitive dosing, and further monitoring of drug safety, will be essential.

#### Contributors

S Staedke contributed to study design and coordination, supervised patients' enrolment and follow-up, analysed and interpreted data, and supervised preparation of the report. A Mpimbaza contributed to study

coordination, supervised patients' enrolment and follow-up, assisted with entry, verification, analysis, and interpretation of data, and participated in preparation of the report. B Nzarubara contributed to study design, patients' enrolment and follow-up, and participated in preparation of the report. M Kanya contributed to study design and coordination, supervised patients' enrolment and follow-up, and assisted with data interpretation and manuscript preparation. G Dorsey contributed to study design and coordination, supervised patients' enrolment and follow-up, participated with data analysis and interpretation, and assisted with preparation of the report. P Rosenthal contributed to study design and coordination, data interpretation, and preparation of the report.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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