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A randomised trial to assess the safety and efficacy of artemether—lumefantrine (Coartem®) for the treatment of uncomplicated *Plasmodium falciparum* malaria in Rwanda

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Summary Coartem® is a fixed-dose combination of artemether—lumefantrine that, given in six doses, provides effective treatment for children with uncomplicated *Plasmodium falciparum* infection in areas with highly endemic and multidrug-resistant malaria. In Rwanda since 2001, amodiaquine + sulfadoxine—pyrimethamine (AQ + SP) has been the first-line treatment, but resistance to this combination has rapidly emerged and spread. Coartem was considered as a possible alternative, and a randomised, open-label, clinical trial to test its safety, tolerability and efficacy was carried out in 2004—2005. Five hundred children aged 12—59 months with uncomplicated *P. falciparum* malaria were randomly allocated to AQ + SP or Coartem. Patients were followed up until day 28 after treatment. Adverse events and clinical and parasitological outcomes were recorded. Adequate clinical and parasitological response (ACPR) was significantly higher in children treated with Coartem than in those treated with AQ + SP: the PCR-adjusted 28-day ACPR was 96.68% for Coartem and 79.35% for AQ + SP. Both treatments rapidly cleared parasitaemia and fever, although parasite clearance was significantly faster in children treated with Coartem. Mean packed cell volume increased in all patients, with no significant differences between treatments. Coartem proved to be more efficacious than AQ + SP, with a good safety and tolerability profile.

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1. Introduction

Coartem® (ALN) is a fixed-dose combination of artemether—lumefantrine (20 mg artemether and 120 mg lumefantrine). When given in six doses it provides effective treatment in children with uncomplicated *Plasmodium falciparum* infection in areas with highly endemic and multidrug-resistant malaria. Lumefantrine conforms structurally, physico-chemically and in its mode of action to the aryl amino alcohol group of antimalarials, including quinine, mefloquine and halofantrine. It has a half-life of 3 to 6 d. Artemether is effective in the treatment of malaria, although it shows high recrudescence rates and poor oral bioavailability when given as monotherapy (Haynes, 2001); its half-life is 2—4 h. ALN is a synergistic combination in which the artemether component gives a rapid reduction of the parasite biomass and consequent rapid resolution of symptoms, and the lumefantrine component eliminates any residual parasites. The lumefantrine component prevents the high level of recrudescence of the artemether used alone and it also protects against resistance, because the parasite is never exposed to artemether alone. Lumefantrine exerts blood schizontocidal activity in *P. falciparum*, *P. vivax* and many other plasmodia. It lacks gametocytocidal and tissue schizontocidal activity. Artemether inhibits or disrupts blood schizogony and gametocytogony (Wernsdorfer, 2004), thus reducing the chance of transmission of the infection. However, there is no effect on the liver stage of the parasite, so the drug does not provide a radical cure. Both drugs are characterised by poor oral bioavailability. The absorption varies considerably between individual doses and between patients and is reduced significantly in the acute phase of malaria (Ezzet et al., 1998, 2000; White et al., 1999). Lumefantrine is highly lipophilic, and its bioavailability increases substantially if the drug is administered after a meal rich in fat. Although the pharmacokinetic properties of lumefantrine are similar to those of halofantrine, an antimalarial known to have adverse cardiac effects, no such effect has been reported with ALN (Bakshi et al., 2000; Bindschedler et al., 2002). However, as a result of work carried out with animal models, concern has arisen over the neurotoxic potential of the principal metabolite (DHA) of the artemisinin derivatives (Haynes, 2001).

A recent addition to the Essential Drugs List, ALN is being promoted in Africa as a replacement for chloroquine, and WHO has commended the manufacturer, Novartis, for providing the drug to malaria-endemic countries at discounted prices (see www.who.int/malaria/cmc_upload/0/000/016/747/who_coartem_review_summary_report.pdf).

In 2001, as an interim strategy, Rwanda chose amodiaquine + sulfadoxine—pyrimethamine (AQ + SP) as the first-line antimalaria treatment. Although the clinical response to this combination was relatively good in 2001, since then its efficacy has steadily declined: in 2002 the proportion of successful treatment (recorded at 28 days and PCR-unadjusted) was 83% (Rwagacondo et al., 2003), and in 2003 it was 74% (Karema et al., 2006). Different artemisinin-based combination treatments (ACTs), such as amodiaquine + artesunate (AQ + AS) and dihydroartemisinin—piperaquine (DHAPPQ) have been tested in the past few years as possible alternatives to the current policy (Karema et al., 2006). In this study the authors show that both DHAPPQ and AQ + AS were efficacious for the treatment of uncomplicated *P. falciparum* malaria, with a significantly higher prevalence of successful treatment in children compared with the first-line AQ + SP (95.2, 92.0 and 84.7%, respectively).

In the present clinical trial we present the results of a study evaluating the safety, tolerability and efficacy of the six-dose regimen of ALN compared to AQ + SP at two Rwandan sites.

2. Materials and methods

2.1. Study design, sites and patient treatment

This study was a randomised open trial carried out at two sites in Rwanda: Mashesha and Rukara. Both sites are rural health centres: the first located near the eastern border with Uganda and the second near the border with the Democratic Republic of Congo, at 900 m above sea level and surrounded by rice fields. Malaria transmission in Rwanda varies widely, being stable with seasonal peaks in the valleys and unstable in the hills, with some epidemic-prone districts. At Mashesha and Rukara malaria transmission lasts from February to November. The major vectors are *Anopheles gambiae* and *A. funestus*. Malaria incidence seems to have increased in recent years, possibly due to environmental changes, demographic pressure, population movements due to political instability in the Great Lakes region, increasing mean temperatures and loss of efficacy of commonly used antimalarial drugs, especially in the Eastern part of the country where Rukara is located.

Patients attending the two health centres with suspected clinical malaria were screened and enrolled if they met the following inclusion criteria: age 12—59 months; weight $\geq$10 kg; infection with *P. falciparum* and no other *Plasmodium* species; parasite density between 2000 and 200 000/μl; fever (axillary body temperature $\geq$37.5 °C) or history of fever in the preceding 24 h; and packed cell volume (PCV) >15%. Patients with severe malaria, mixed malaria infection, any other concomitant illness or underlying disease, known allergy to the study drugs being used in this trial or a clear history of adequate antimalarial treatment in the previous 72 h were excluded. Cases of severe malaria were referred to the nearest hospital for treatment with intravenous quinine and other supportive therapy, according to Rwandan national treatment guidelines. Patients with mixed infections were treated according to national guidelines. Patients were randomly allocated to receive ALN or AQ + SP in blocks of 20. Patients allocated to AQ + SP received the treatment according to body weight: AQ 10 mg/kg/d for 3 days and SP 25 mg/kg of sulfadoxine and 1.25 mg/kg of pyrimethamine the first day; ALN was dispensed as a fixed-dose combination tablet (20 mg artemether and 120 mg lumefantrine). The number of tablets was given according to body weight. The minimum dosage for patients weighing less than 15 kg was one tablet per dose, and those between 15 and 24 kg received two tablets; ALN was administered twice daily (at 0 and 8 h) for 3 days. Children were hospitalised and therefore received regular meals during the 3 days. However, no fatty meal was given at the time of drug administration.

After drug administration, patients were observed for 1 h, and the dose was repeated in full if vomiting occurred within 30 min and halved if vomiting occurred between 30 min and 1 h after dosing. If vomiting persisted, a full course of quinine was administered as rescue treatment. A case-record form was completed for each patient documenting all symptoms before clinic attendance, concomitant illness and drug history.

All subjects enrolled in the study were given a unique code and the corresponding treatment according to a randomisation list prepared in Belgium. Allocation of treatment was concealed from both the doctor and the patient, until final recruitment of the patient.

The primary outcome was the incidence of microscopically and genotypically confirmed recrudescent infections in the different treatment groups by day 28. Secondary outcomes were the immediate treatment responses: parasite and fever clearance and occurrence of adverse events.

2.2. Follow-up

Children were hospitalised until day 3 after treatment. Parents/guardians of children were asked to return with the children for the scheduled tests on days 7, 14, 21 and 28. If a patient did not attend the scheduled visits, every effort was made to locate him/her at home. Parents/guardians were encouraged to take the child to the hospital whenever the child was sick. At each visit, the history, clinical signs and symptoms, body temperature and a blood sample for parasitaemia were collected. A blood spot on filter paper for molecular analysis was also collected at each visit. If malaria was diagnosed, a full course of quinine was administered.

2.3. Laboratory methods

Thick blood films were stained with Giemsa. Parasite density was determined on the basis of the number of parasites per 200 white blood cells (WBC) on a thick film, assuming a total WBC count of 8000/μl. If gametocytes were seen, the gametocyte count was extended to 1000 WBC. Laboratory technicians reading malaria slides did not know the treatment received by individual patients. Quality control was performed monthly by a qualified technician from the official reference national laboratory (Kigali) on all positive slides and 10% of the negative slides. PCV (measured by microhaematocrit centrifugation) and total and differential WBC counts were assessed at days 0 and 14. If the child had a second episode of parasitemia within 28 days, blood samples on filter paper from the first and second episodes were used to type parasite strains, to distinguish between new infections and recrudescence. For this, DNA was purified (Irion et al., 1998) and three polymorphic markers of *P. falciparum* (MSP1, MSP2 and GLURP) were analysed (Cattamanchi et al., 2003). A recrudescent infection was defined as one that matched in size at least one allele of the MSP1, MSP2 and GLURP loci between the first and second samples. If any clone of a polyclonal primary infection was detected during a second episode, this was considered a recrudescence.

2.4. Outcome measurements

Treatment outcome was established according to standard WHO classification (WHO, 2003): early treatment failure (ETF) was defined as: (1) danger signs of severe illnesses or severe malaria on days 1, 2 or 3 with parasitaemia (defined according to WHO, 2003); (2) parasite density at day 2 greater than at day 0; (3) parasitaemia on day 3 with axillary temperature $\geq$37.5 °C; and (4) parasite density at day 3 equal to or greater than 25% of that at day 0. Late clinical failure (LCF) was defined as danger signs or severe malaria or parasitaemia with axillary temperature $\geq$37.5 °C between day 4 and day 28, without having been previously classified as ETF. Late parasitological failure (LPF) was defined as the reappearance of parasitaemia between day 4 and day 28 without fever and without previously meeting any of the criteria for ETF or LCF. An adequate clinical and parasitological response (ACPR) was defined as the absence of parasitaemia by day 28 without previously meeting any of the criteria for ETF, LCF and LPF. The number of cases of total treatment failure (TTF) was computed as ETF + LCF + LPF. Late clinical and parasitological failures were confirmed by PCR.

All adverse events (AEs) were recorded on the clinical record form (CRF) and a causality assessment of the AEs was made according to the guidelines of the WHO—Uppsala Monitoring Centre (see http://www.who-umc.org/).

2.5. Statistical analysis

For the sample size calculation, we assumed a parasitological failure of 5% for ALN at both sites; a failure to AQ + SP of 20% in Rukara, and 16% in Mashesha, and about 10% of patients lost to follow-up. Therefore we estimated that 100 patients per arm in Rukara and 150 in Mashesha would have been able to detect a significant difference at the 5% level and with 80% power.

Data were double-entered and validated using EpiInfo 6.4b (CDC, Atlanta, GA, USA). Descriptive statistics were used to summarise baseline values and demography data. For the per protocol analysis, the χ^2 test was used to compare proportions. The odds ratio for failure was calculated with 95% CI with a two-sided Fisher test. Mantel—Haenszel was used to adjust for sites. For the intention to treat analysis, the log-rank test adjusted by site was used. Analysis of variance (ANOVA) was used for normally distributed continuous data. The non-parametric Kruskal—Wallis test was used to analyse continuous data with a skewed distribution. All analyses were performed using STATA statistical analysis software package version 8 (Stata Corp., College Station, TX, USA).

2.6. Ethical approval

The study was reviewed and approved by the Ministry of Health of Rwanda and by the Ethical Committee of the Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium. An informed written consent was provided by the parents/guardians of all patients before they were included in the study.

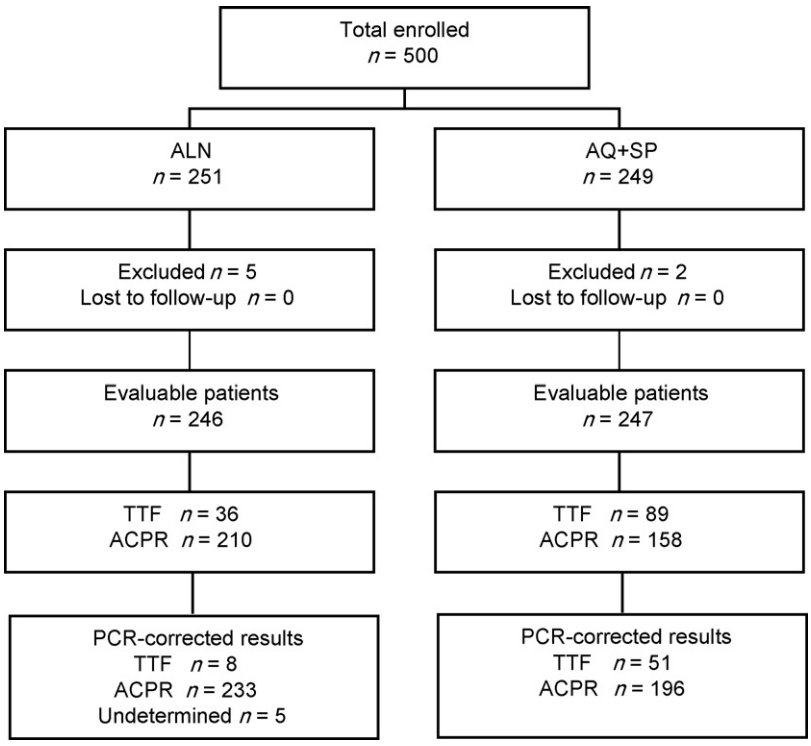

Figure 1 Flowchart of the study. ALN: artemether—lumefantrine (Coartem®); AQ + SP: amodiaquine + sulfadoxine—pyrimethamine; TTF: total treatment failures; ACPR: adequate clinical and parasitological response.

3. Results

Between July 2004 and December 2004, 500 patients were recruited in the two study sites (AQ + SP = 249 and ALN = 251) (Figure 1).

At enrolment the groups had similar demographic and clinical characteristics (Table 1). Seven patients were excluded: two were given an incorrect dose (one ALN, one AQ + SP); four were treated with erythromycin during the follow-up (three ALN; one AQ + SP); one developed severe malaria during the first day (ALN). By day 2 the proportion of patients still parasitaemic was significantly lower in the ALN group (2.80%; 7/250) than in the AQ + AS group (46.99%; 117/249) ($P < 0.0001$); and by day 3 there were no parasitaemic patients in the ALN group, whereas 6.43% (16/249) were still parasitaemic in the AQ + SP group ($P < 0.0001$). No differences were observed among groups in fever clearance on any day (Table 2).

One case of ETF was observed in the AQ + SP group. PCR genotyping was carried out on all late clinical and parasitological failures and showed for AQ + SP: 38 new infections and 50 recrudescences; for ALN: 23 new infections, eight recrudescent infections and five indeterminate results. Indeterminate results were excluded from the analysis. The PCR-adjusted ACPR at day 28 was lower in the AQ + SP group (79.35%; 196/247) than in the ALN (96.68%; 233/241) ($P < 0.0001$) with a marked difference between study sites (Table 3). Taking into account this difference, ALN was still significantly better than AQ + SP (MH-OR for failure 0.12; 95% CI 0.05—0.27) ($P < 0.001$). The log-rank

Table 1 Baseline characteristics of children at enrolment by treatment group

	ALN	AQ + SP
Total	251	249
Female:Male	112:139	123:126
Mean age in months (SD)	33.68 (13.39)	34.59 (13.53)
Weight in kg (SD)	12.08 (2.06)	12.46 (2.23)
Mean temperature in °C (SD)	38.77 (1.16)	38.68 (1.17)
Geometric mean asexual *P. falciparum*/μl (95% CI)	23 323.87 (20 277.87—26 827.42)	22 070.89 (19 221.93—25 342.10)
No. people carrying gametocytes (%)	10/251 (3.98)	14/249 (5.62)
Splenomegaly (%)	34/251 (13.55)	29/249 (11.65)
Hepatomegaly (%)	2/251 (0.80)	0/249 (0.00)
Packed cell volume (SD)	31.65 (4.39)	31.98 (4.63)

ALN: artemether—lumefantrine (Coartem®); AQ + SP: amodiaquine + sulfadoxine—pyrimethamine.

Table 2 Parasitaemia and fever clearance (%) day 0—day 3 by treatment group

Clearance	ALN	AQ + SP	*P*-value
Parasitaemia			
Day 2	7/249 (2.8)	117/247 (47.4)	<0.001
Day 3	0/249 (0)	16/248 (6.5)	<0.001
Fever			
Febrile[a] at baseline	209/248 (84.3)	215/246 (87.4)	0.319
Day 1	11/247 (4.5)	22/249 (8.8)	0.050
Day 2	5/249 (2.0)	4/248 (1.6)	0.741
Day 3	6/249 (2.4)	3/249 (1.2)	0.313

ALN: artemether—lumefantrine (Coartem®); AQ + SP: amodiaquine + sulfadoxine—pyrimethamine.
[a] Axillary temperature ≥37.5 °C.

test stratified by site gave comparable results: $\chi^2 = 36.03$; $P < 0.00001$.

Mean WBC count at day 14 was similar in both groups (data not shown). At recruitment, mean PCV was similar in the treatment groups (AQ + SP = 31.99, SD = 4.63; ALN = 31.68, SD = 4.38; $P = 0.45$) and by day 14 had increased in both groups (AQ + SP = 35.23, SD = 3.42; ALN = 34.70, SD = 3.67; $P = 0.1$), with no differences between treatments on any day.

At enrolment, no significant differences in gametocyte prevalence between the two treatment groups were found (Table 1). However, during the follow-up (at days 2, 3, 7, 14 and 21) the proportion of patients with gametocytes was significantly higher in the AQ + SP group compared with the ALN group (Figure 2). No patient had gametocytes at day 28.

Table 3 Efficacy results by site (%)

Outcome	ALN	AQ + SP	*P*-value
Mashesha			
Total	150	150	
Evaluable	146	148	
ETF	0	1	
LCF	3	18	
LPF	14	17	
ACPR	129 (88.4)	112 (75.7)	0.005
TTF	17 (11.6)	36 (24.3)	
ACPR PCR-adjusted	141 (98.6)	134 (90.5)	0.003
TTF PCR-adjusted	2 (1.4)	14 (9.5)	
Rukara			
Total	101	99	
Evaluable	100	99	
ETF	0	0	
LCF	5	15	
LPF	14	38	
ACPR	81 (81.0)	46 (46.5)	<0.001
TTF	19 (19.0)	53 (53.5)	
ACPR PCR-adjusted	92 (93.9)	62 (62.6)	<0.001
TTF PCR-adjusted	6 (6.1)	37 (37.4)	

ALN: artemether—lumefantrine (Coartem®); AQ + SP: amodiaquine + sulfadoxine—pyrimethamine; ETF: early treatment failure; LCF: late clinical failure; LPF: late parasitological failure; ACPR: adequate clinical and parasitological response; TTF: total treatment failure.

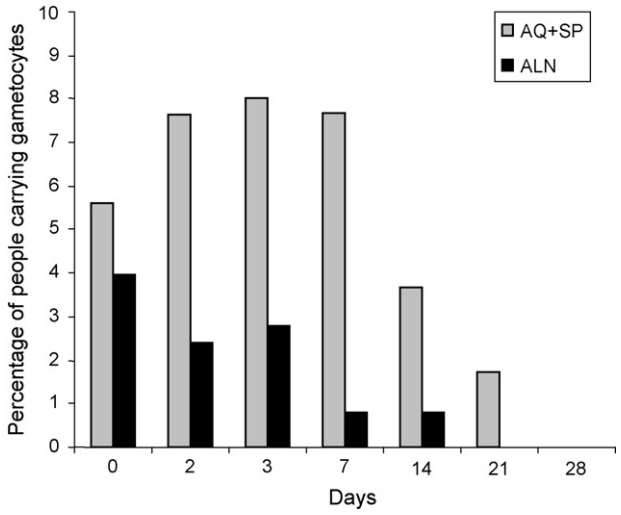

Figure 2 Percentage of people carrying gametocytes by treatment at days 0—28. ALN: artemether—lumefantrine (Coartem®); AQ + SP: amodiaquine + sulfadoxine—pyrimethamine.

Two hundred and fifty-one patients reported at least one AE concomitant with the administration of the study drug (125 of them had two or more) with no differences between the two groups: 130 (52.21%) patients in the AQ + SP group; 121 (48.21%) in the ALN group ($P = 0.37$).

The number of patients with an AE classified as possibly or probably related to the study drug was 35 for AQ + SP (22.73%) and 22 (14.47%) for ALN ($P = 0.06$). The most frequent AEs associated with AQ + SP treatment were fatigue, anorexia, vomiting and abdominal pain, and with ALN they were cough and diarrhoea.

4. Discussion

In the present study we evaluated the efficacy, safety and tolerability of the ACT artemether—lumefantrine compared to the recommended first-line treatment in Rwanda, AQ + SP. The results showed that ALN was significantly more efficacious than AQ + SP: the proportion of successful treatments recorded at 28 days and PCR-adjusted was 96.68% for ALN and 79.35% for AQ + SP. Moreover, a faster suppression of parasitaemia was observed with ALN. Some variation in the ACPR between study sites was observed, and this is consistent with previous studies (Karema et al., 2006), in which drug efficacy in Rukara was always lower than in other sites.

When implemented on a national scale, the effectiveness of ALN could be compromised by poor absorption as well as poor adherence and incorrect timing of doses (the drug needs to be administered twice a day for 3 days). In patients with acute malaria, who are reluctant to eat and often vomit, lumefantrine oral bioavailability is poor, but it improves markedly during recovery. Piola et al. (2005) evaluated the effectiveness of ALN when given supervised (all doses observed with fatty food intake) or unsupervised (first dose supervised followed by outpatient treatment with

nutritional advice) to patients of all ages (weight >10 kg); the proportion of successful treatments, recorded at day 28 and PCR-adjusted, was 97.7% in the supervised group and 98.0% in the unsupervised group, thus showing a high prevalence of successful treatment irrespective of food intake. Our study shows similar results despite the administration of ALN without a fatty meal. However, study children received three meals a day during their hospitalisation, and this might not be representative of the normal conditions at home.

Children treated with ALN were significantly less likely to be positive for gametocytes within the 4 weeks after treatment, implying a reduction of post-treatment transmission of *P. falciparum*, which conforms to previous studies (Sutherland et al., 2005; von Seidlein et al., 1998). In addition, ALN was well tolerated, and the proportion of observed AEs was similar between the two study groups.

Rwanda is now in the process of moving from the co-administered drug combination AQ + SP to ALN, which is the only co-formulated ACT available in Africa. An agreement with WHO makes Coartem available for public sector procurement at US$2.4/adult dose, with a final price in the pharmacies or local stores of probably around US$3.0, a subsidised price that would still be unaffordable by the majority of patients. A recent Cochrane review (Omari et al., 2005) evaluated all randomized controlled trials, comparing six doses of ALN administered orally with standard treatment regimens, single drug or combination (nine trials, 4547 participants). The conclusion of this review was that the six-dose regimen of ALN appears more effective than antimalarial regimens not containing artemisinin derivatives. Regarding the ACTs, ALN performed better than AQ + AS, worse than mefloquine + artesunate and did not differ from dihydroartemisinin—napthoquine—trimethoprim.

Clinical failure between 13 and 30% with ALN (see http://www.who.int/malaria/rbm/Attachment/20041108/Drugefficacybycountry.pdf) has already been observed in Cambodia. In Africa, Sisowath et al. (2005) reported a significant increase in the mutation *pfmdr1* 86N after exposure to ALN. The authors suggested that the 86N form leads to increased tolerance to the drug, rather than clinical resistance. This could be the first step of a multi-step process, as opposed to being a genetic background allowing the establishment of a single mutation that will lead to complete resistance (Gil et al., 2005).

As mentioned in the Introduction, two other ACTs have been tested in Rwanda. Of these, DHAPPQ, a relatively inexpensive drug (about US$1.5—3.0 for an adult treatment) with a simple dosage (once a day for 3 days), which favours good compliance, proved to be efficacious and well tolerated (Karema et al., 2006). Unfortunately, DHAPPQ is not yet produced according to good manufacturing practice (GMP) standards and no piperaquine-containing preparation is registered with the US Food and Drug Administration or similar international regulatory bodies (Davis et al., 2005). Therefore, though it is an extremely promising drug, DHAPPQ cannot be used as a first-line treatment yet.

In conclusion, ALN is efficacious for the treatment of *P. falciparum* infections and has a good safety and tolerability profile, thus supporting the choice of ALN as first-line treatment for malaria in Rwanda. The crucial factor at this point will be to monitor compliance to the complex treatment protocol and its price at community level.

Conflicts of interest

The authors have no conflicts of interest concerning the work reported in this paper.

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