INTRODUCTION

Malaria remains a major public health problem with estimates of 198 million (124–283 million) clinical cases and 367,000–755,000 deaths reported worldwide in 2013, 90% of which occurred in sub-Saharan Africa.1 In Mali, of the 39,283 deaths due to malaria, 28,859 were children under 5 years of age in 2010.2 Artemisinin-based combination therapies (ACTs) are the first-line treatments of uncomplicated malaria in endemic countries. Artemisinins are a powerful class of antimalarial drugs that are able to rapidly reduce malaria parasite load and morbidity.10,11 These drugs along with quinine are the most efficacious antimalarial drugs currently used to control malaria. Unfortunately, artemisinin resistance, defined as delayed parasite clearance, has emerged in southeastern Asia.2,6,7 Mutations in the kelch 13 propeller domain have been associated with artemisinin resistance in vitro and in vivo in southeast Asia.9,10 To date, artemisinin resistance has not been reported in Africa and ACTs remained highly efficacious.10,14 However, sub-Saharan Africa remains at risk because of drug pressure from wide use of ACTs and intercontinental movement of people. Furthermore, circulation of substandard or counterfeit drugs and nonadherence to treatment course are factors that contribute to rapidly selecting resistant parasites.14–16 Since 2006, Mali adopted artether–lumefantrine (AL) and artesunate–amodiaquine as first-line treatments for uncomplicated malaria management. Large quantities of artesunate + sulfadoxine–pyrimethamine (AS+SP) were donated to Mali by the Chinese cooperation in 2010. China is a major partner of Mali, supporting its health system since the 1960s. This cooperation allowed the training of many Malian health professionals and the building of three hospitals. When thousands of doses of AS+SP were donated, it was critical to assess the efficacy of this combination and compare it to one of the standard first-line ACT, that is, AL.

MATERIALS AND METHODS

Study sites. The study was conducted in three clinical trial sites of the Malaria Research and Training Center, namely Banambani, Sotuba, and Kolle.

Banambani village is located 30 km north of Bamako, the capital city of Mali, in the Savanna area. The population is estimated to be 1,400 inhabitants. Malaria transmission is seasonal (June–November). Malaria incidence in children under 5 years of age is 2.1 episodes per child per year. The entomological inoculation rate is 137–167 infective bites per person per year.17 Sotuba is a peri-urban area of Bamako with 6,472 inhabitants. The entomological inoculation rate is 4–12 infective bites per person per year.17 Kolle is a village of 2,500 inhabitants located 57 km southwest of Bamako in the Sudan savanna area and 9 km away from the Niger River. The entomological inoculation rate is nearly identical to the level of Banambani.17

Study volunteers were recruited between October 2010 and January 2011 in Sotuba and Kolle and between January 2013 and January 2014 in Banambani.

Anopheles gambiae s.l. and Anopheles funestus s.l. are the two Anopheles complex species responsible for malaria transmission in all three study sites.

Sample size calculation and sampling. The sample size was calculated using a non-inferiority hypothesis of AS+SP versus AL, which had an efficacy rate of 95%.18 A non-inferiority range of 5%, an alpha risk of 5%, a power of 80%, and lost to follow-up of 10% were assumed to yield a total sample size of 503 subjects. Volunteers were recruited by applying a
simple random sampling among patients visiting our medical teams during the study periods. Volunteers were then assigned to either AS+SP or AL through a computer-generated random list (Excel; Microsoft Corporation, Redmond, WA).

A nested parasite clearance evaluation sub-study was conducted on the cohort of volunteers recruited between January 2013 and January 2014. Among the volunteers recruited during that period, a systematic sampling was used to draw the volunteers included into the nested sub-study. The first volunteer was randomly determined and then every 6th volunteer was selected until reaching the 45 patients of the sub-study.

Study design. We used the standard World Health Organization (WHO) 28-day in vivo antimalarial efficacy protocol. Patients aged 6 months and older suffering from uncomplicated falciparum malaria were recruited. Cases of coinfection with other malaria parasite species were excluded. Inclusion parasitemia ranges were 1,000–200,000 trophozoites/μL. Pregnant women, breast-feeding mothers, and patients allergic to any of the study drugs were systematically excluded. Exclusion criteria also included general signs of danger in children under 5 years of age, severe falciparum malaria and severe illness according to WHO 2003 protocol. Patients previously treated by any antimalarial drug in the preceding 14 days before inclusion were excluded. During scheduled visits at days 0, 1, 2, 3, 7, 14, 21, and 28, each enrolled patient was subjected to full clinical examination, collection of dry blood spots on 3 MM filter papers (Whatman®, Maidstone, United Kingdom) hemoglobin measurements and parasitemia assessments. Drugs were administered to volunteers over the first 3 days by the research teams. Doses were repeated whenever there was immediate vomiting. In case of repeated vomiting, patients were excluded and treated by parenteral route. A presence of other Plasmodium species or a parasitemia outside the range of 1,000–200,000/μL, as confirmed by the third reader, was excluded.

Follow-up ended when the following criteria were met: 1) lost to follow-up, 2) refusal, 3) occurrence of any sign of severe malaria, and 4) any treatment failure. Volunteers selected for parasite clearance evaluation were checked for parasitemia at hours 0 (before first drug administration), 8, 16, 24, 36, 48, and 60. After hour 60, those individuals continued to be followed as part of the main study until day 28 or when one of the above study termination criteria was met.

Study drugs. The drugs tested included fixed dose tablets of AL (20/120 mg, Coartem® Novartis, Basel, Switzerland) and co-blistered tablets of AS+SP (Artespers®, Guilin Pharmaceutical Co. Ltd., Guilin, China) in three different packages (3 × 50 mg + 1 × 500/25 mg, 6 × 50 mg + 2 × 500/25 mg, and 6 × 100 mg + 3 × 500/25 mg). For AL, the standard dosing was used, that is, one tablet for patients weighing 5–14 kg, two tablets for 15–24 kg, three tablets for 25–34 kg, and four tablets for ≥ 35 kg taken at hours 0, 8, 24, 36, 48, and 60. For AS+SP, tablets of SP were given at a unique dose of 25 mg/kg of sulfadoxine and 1.25 mg/kg of pyrimethamine on day 0 along with artesunate at 4 mg/kg on days 1 and 2.

Quality control of thick and thin blood smears. Previously described methods were used to perform quality control of all slides. Parasitemia was estimated with Giemsa-stained thick smears by counting the number of asexual stages per 300 white blood cells (WBCs) or more. Indeed, the quotient from the division of the asexual stage counts by ≥ 300 WBCs was multiplied by 7,500 to estimate parasitemia per microliter of blood. Thin smears were used to search the presence of other Plasmodium species before inclusion on day 0 or to check the appearance of other malaria parasite species during follow-up. All thick/thin smears were read a second time by an independent qualified microscopist. A third read was done if the first two readers had significant qualitative or quantitative discrepancies. Qualitative discrepancy was deemed met when the second reader observed another malaria parasite species. Quantitative discrepancy was estimated by dividing the difference of the two counts by their mean. Third read was warranted for discrepancy ≥ 25% (significant) when parasitemia was comprised in 1–999/μL and > 50% (significant) for parasitemia ≥ 1,000/μL. The mean value of the two counts was used in the absence of significant discrepancy between the two first readers. However, in case of significant discrepancy the mean value of the third read and the closest read was retained.

Molecular correction. Msp2 gene and two microsatellites (Cal and Ta99) were genotyped by nested polymerase chain reaction (PCR) to discriminate recrudescent parasites from new infections as previously described. Three qualified investigators assessed gel pictures. Outcomes were classified: 1) recrudescence, if the alleles on day 0 and days of treatment failure were the same for msp2, Cal, and Ta99 or if similar alleles were found on day 0 and days of treatment failure for all the three markers in the presence of additional bands; 2) reinfection, if the alleles on day 0 and days of treatment failure were distinct for any of the three markers; and 3) indeterminate, if either or both the day 0 and days of treatment samples could not be amplified.

Data analysis. Data collected in case report forms were entered in Microsoft Excel (Microsoft® Corporation). Efficacy data were analyzed using GraphPad Prism 6 (http://www.graphpad.com/scientific-software/prism/). Parasite clearance was analyzed with the Worldwide Antimalarial Resistance Network parasite clearance estimator (www.wwarn.org) and the WHO online parasite clearance estimator (http://www.who.int/malaria/areas/treatment/drug_efficacy/en/). This software provided individual estimation of parasite clearance half-life, which were used to perform different comparisons using GraphPad Prism 6. Pearson’s χ² test and Fisher’s exact test were used for comparisons of efficacy data whereas Mann–Whitney test was used for parasite clearance data. A P value ≤ 0.05 was considered statistically significant.

Ethical clearance. The study protocol obtained ethical clearance from WHO and the Ethics Committee of Faculty of Pharmacy and Faculty of Medicine and Odonto-stomatolgy, University of Science, Techniques and Technologies of Bamako, Mali. Community permission and individual informed consent and/or informed assent were obtained after explanation of benefits and probable risks related to the study. Data were anonymized to guarantee confidentiality of volunteers’ identities. All patients seen by our medical team received free medical care including treatments for concomitant diseases.

RESULTS

Of 3,000 patients screened during the study period, 503 volunteers were enrolled (Figure 1). After quality control of all
thick/thin smears, 479 volunteers were included in the final analyses. Between October 2010 and January 2011, 103 patients were enrolled in Kolle, 80 in Sotuba and between January 2013 and January 2014, 296 were enrolled in Banambani. Patients were randomized by treatment arm with 242 in AS+SP and 237 in AL arms. Because of refusal and travel 14 volunteers were lost to follow-up in AS+SP arm and nine in AL arm. Consequently, 228 volunteers were successfully followed up in each treatment arm.

Baseline characteristics of the patients are presented in Table 1. The two treatment arms were comparable for all assessed parameters. Patients under 5 years represented less than 28% in each group.

Before PCR correction, the rate of adequate clinical and parasitological response (ACPR) was significantly higher in the AS+SP group than in the AL group ($P = 0.016$; Pearson’s $\chi^2$ test) with 91.2% ($N = 228$) and 83.8% ($N = 228$), respectively (Table 2). The risk of occurrence of treatment failures in AL group was 9% higher than AS+SP group (relative risk [RR] = 1.09; 95% confidence interval [CI95] = 1.0, 1.2). When we compared the occurrence of treatment failures between groups of individuals under 5 years versus those $\geq 5$ years (Figure 2), we found no difference between the two groups (RR = 1.23; CI95 = 0.7, 2.1). However, when we performed this comparison between patients under 8 years versus those $\geq 8$ years (Figure 3), the risk of failure was significantly higher in AL group (RR = 1.94; CI95 = 1.2, 3.3).

Among the parasitological failures, five samples from AL arm and two from AS+SP arm failed to yield PCR results. In a per protocol analysis excluding these seven samples, the rates of ACPR for the two treatment arms were comparable ($P = 0.06$; Fisher’s exact test) with 100% for AS+SP and 98.2% for AL. The parasite reinfection rate for AS+SP was 8.0%. There was no significant difference with the reinfection rate of AL, which was 12.6% ($P = 0.11$; Pearson’s $\chi^2$ test).

From the 45 subjects followed for parasite clearance assessment, 10 did not meet the software requirements (too few data points; too low parasitemia; if the first zero parasitemia is replaced by software detection limit, which was 26 parasites/μL with the last positive parasitemia exceeding 1,000; and if the last positive parasitemia was $> 1,000$ with no zero parasitemia) and were therefore removed. After analysis, the medians of

### Table 1
Baseline characteristics of subjects by treatment arm

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AS+SP</th>
<th>AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (n)</td>
<td>242</td>
<td>237</td>
</tr>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>8.83 ± 7.18</td>
<td>8.98 ± 7.06</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>1.08–43.00</td>
<td>0.92–48.00</td>
</tr>
<tr>
<td>Age range (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 years</td>
<td>27.3</td>
<td>27.5</td>
</tr>
<tr>
<td>$\geq 5$ years</td>
<td>72.7</td>
<td>72.5</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>50.0</td>
<td>41.3</td>
</tr>
<tr>
<td>Male</td>
<td>50.0</td>
<td>58.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>22.32 ± 12.55</td>
<td>22.73 ± 11.65</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>7.00–67.00</td>
<td>7.00–62.00</td>
</tr>
<tr>
<td>Hemoglobin concentration (g/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>10.94 ± 2.00</td>
<td>10.89 ± 2.01</td>
</tr>
<tr>
<td>Minimum–maximum</td>
<td>5.20–17.90</td>
<td>5.50–17.70</td>
</tr>
</tbody>
</table>

AL = artemether + lumefantrine; AS+SP = artesunate + sulfadoxine–pyrimethamine.

### Table 2
Treatment responses of study drugs before PCR correction

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AS+SP</th>
<th>AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment arm outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACPR</td>
<td>208 (91.2)</td>
<td>191 (83.8)</td>
</tr>
<tr>
<td>LPF</td>
<td>16 (7.0)</td>
<td>28 (12.3)</td>
</tr>
<tr>
<td>LCF</td>
<td>4 (1.8)</td>
<td>9 (4.0)</td>
</tr>
<tr>
<td>Size</td>
<td>228</td>
<td>228</td>
</tr>
</tbody>
</table>

ACPR = adequate clinical and parasitological response; AL = artemether + lumefantrine; AS+SP = artesunate + sulfadoxine–pyrimethamine; LCF = late clinical failure; LPF = late parasitological failure; PCR = polymerase chain reaction. AS+SP vs. AL regarding treatment efficacy (i.e., ACPR), $P = 0.016$ (Pearson’s $\chi^2$ test).
parasite clearance half-life (T_{1/2}) were 1.7 hours (interquartile range [IQR] = 1.3–2.2) for AS+SP and 1.9 hours (IQR 1.5–2.5) for AL (Table 3). The distribution of T_{1/2} by treatment arm and by age range (Figures 4 and 5) showed no significant difference between AS+SP and AL (P = 0.24, Mann–Whitney test) and in subjects <5 and ≥5 years (P = 0.70, Mann–Whitney test). Both parasite clearance estimation methods gave the same outcome and were highly correlated (r = 0.73; P < 0.001).

The proportions of negative parasitemia 24 hours after the treatment (or day 1) were 73.9% (N = 23) for AS+SP and 65.0% (N = 20) for AL (P = 0.53). Proportions of patients with positive smears at days 2 and 3 (48 hours) were 0% in both. All volunteers included in the parasite clearance assessment presented adequate clinical and parasitological response by day 28 of follow-up regardless of treatment arm (Table 3).

**DISCUSSION**

AL and AS+AQ have been used as first-line treatments for malaria in Mali since 2006, whereas AS+SP has been widely used in the main hospitals and many secondary healthcare centers of the country as part of Chinese and Malian governments’ efforts to control malaria. This study sought to provide evidence of the efficacy of the later regimen in the treatment of uncomplicated malaria as the partner drug is used in intermittent preventive treatment in pregnant women (IPTp) and seasonal malaria chemoprevention (SMC) in children in Africa.²³–²⁶ We evaluated efficacy of the combinations AL and AS+SP in children aged 6 months and older and adults living in a malaria-endemic area in Mali. We showed that AS+SP is as efficacious as the first-line treatment ACT with a high cure rate. In addition to high ACPR, other parameters such as parasite clearance time were comparable between the two regimens.

Our findings are in line with results from previous studies that showed that ACTs had a high cure rate and short parasite clearance time in malaria-endemic areas in Mali.²⁷–³² Although we did not observe a statistically significant difference in terms of reinfection rate between the two arms of treatment when the entire population was used, children under 8 years of age in the AL group were more likely to experience reinfection than children of the same age in the AS+SP group. This observation could be explained by the fact that volunteers were followed during 28 days instead of 42 days or it could be due to differences in the intensity of transmission between the study sites.

Parasitemia at day 3 was shown to be a good indicator of delayed parasite clearance.³³ Our results show that all patients cleared their parasite by day 2 in both treatment groups, indicative of a fast parasite clearance. These results are comparable to data reported from previous studies.²⁷–³²

SP is recommended in IPTp and has been used in other interventions such as SMC in Africa.²³–²⁶ There is a concern about the rapid emergence of SP resistance. One limitation of this study is the lack of genotyping data on molecular markers.
of SP resistance before and after treatment. However, our treatment failure rates for AS+SP arm were too low to provide adequate power to compare the rates of resistance-mediating polymorphisms in dihydropteroate synthase and dihydrofolate reductase before the treatment and after the follow-up. Although the addition of artesunate to SP may delay the emergence of SP resistance, the implementation of AS+SP at large scale in areas with high rate of SP resistance could be a threat to artemisinin derivatives. More importantly, the unavailability of this ACT as a fixed-dose combination could jeopardize patient adherence and increase the likelihood of using the artesunate tablets for monotherapy. This could add concerns to potential emergence of artemisinin resistance.

CONCLUSION

AS+SP and AL were highly efficacious in Mali 4–7 years after the implementation of the ACTs. This study provides baseline information on parasite clearance half-lives after ACT treatment, particularly for AS+SP, in Mali.

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