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Assessing the Efficacy of Sulfadoxine-Pyrimethamine, Sulfadoxine-Pyrimethamine + Artesunate, and Artemether-Lumefantrine for treatment of uncomplicated malaria in Tanzanian Children

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Abstract

Assessing the Efficacy of Sulfadoxine-Pyrimethamine, Sulfadoxine-Pyrimethamine + Artesunate, and Artemether-Lumefantrine for treatment of uncomplicated malaria in Tanzanian Children

By Julie Gutman, M.D.

In 2001 the Tanzanian government adopted sulfadoxine-pyrimethamine (SP) as the first-line antimalarial treatment. Continuous monitoring of antimalarial efficacy is crucial in light of increasing parasite resistance to antimalarials. We measured *in vivo* efficacy of SP alone versus SP-artesunate (SPAS) or artemether-lumefantrine (AL), three and five years after SP introduction and prior to widespread deployment of AL. Patients <5 years old with uncomplicated *P. falciparum* mono-infection were enrolled and randomized to receive either SP, SPAS, or AL using the standard WHO 28-day protocol. PCR genotyping was used to distinguish recrudescence from re-infection and characterize known molecular markers of antimalarial drug resistance. In 2004 we enrolled 425 patients; 143 each in the SP and SPAS arms and 139 in the AL arm, while in 2006 we enrolled 361 patients: 121 in the SP arm, 122 in the SPAS arm, and 118 in the AL arm. The 2006 uncorrected cure rates were 39%, 56%, and 77% in the SP, SPAS, and AL groups, respectively. This represents a significant decrease in efficacy for SP and SPAS since 2004, when respective uncorrected cure rates were 58%, 78%, and 80%, respectively. The PCR corrected cure rates in 2004 were 71%, 91%, and 94%, respectively; PCR corrected cure rates for 2006 are pending. Using treatment with SP as the baseline, the hazard ratio (95% CI) for infection was 0.26 (0.14-0.48) for SPAS and 0.14 (0.06-0.31) for Coartem; both of these were highly significant ($p<0.0001$). In comparison to AL, treatment with both SP and SPAS resulted in a significant increase in the hazard ratio for infection (3.6, 95% CI: 2.3-5.6 for SP and 2.2, 95% CI: 1.4- 3.5 for SPAS). Both SPAS and AL were significantly more efficacious for treatment of uncomplicated malaria than is SP. However, the efficacy of SPAS is rapidly decreasing. SP should no longer be used for treatment of malaria illness in Tanzania, either as monotherapy or as part of artemisinin combination therapy.

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INTRODUCTION

Malaria is one of the most significant infectious diseases globally with an estimated 300- 500 million clinical cases and 1 million deaths occurring annually (1). Sub-Saharan Africa accounts for approximately 90% of the global burden (2). Children less than 5 years of age account for the majority of cases and deaths caused by malaria worldwide. Increasing resistance of the parasite to inexpensive antimalarials (chloroquine and sulfadoxine-pyrimethamine) results in increased morbidity and mortality as these antimalarials become ineffective.

The artemisinin derivatives have been developed as a new class of antimalarials in the last few decades. They are the most highly effective antimalarial drugs available, but, due to their short half-life, a long course of therapy is required if they are used as monotherapy (3). To allow administration of short courses of antimalarials (which have a higher adherence than longer courses) and to prevent the development of resistance to these valuable therapeutic agents, artemisinin-based combination therapies (ACTs) were developed, consisting of an artemisinin derivative (artesunate or artemether) in combination with a longer acting partner drug (4). ACTs are highly effective, but are significantly more expensive (>10 fold more expensive) than the antimalarials which had been in widespread use. Given the significant economic burden that a switch to these agents poses on a developing economy, the decision of when to switch the first line therapy to an ACT required balancing between the decreasing efficacy of the current regimen and the cost of these new regimens. In addition,

it was important to field test these agents prior to large scale implementation to be sure that the added cost was justified.

The World Health Organization (WHO) advocates monitoring drug resistance using *in vivo* efficacy testing. The recommended threshold at which therapy should be changed has been adjusted downward, as new drugs have been developed and made available at more affordable prices. At the time SP was introduced, the recommendation was to change the first line antimalarial therapy once the 14 day failure rate exceeded 25% (5). More recent guidelines recommend changing the first line antimalarial therapy once failure rates at 28 days exceed 10% (6). In order to track the development of resistance, and to determine the best replacement therapies, continuous monitoring of *in vivo* drug efficacy is essential.

We conducted an open-label randomized, controlled, clinical trial to measure the *in vivo* efficacy of sulfadoxine-pyrimethamine (SP), then the recommended first line treatment for uncomplicated malaria in Tanzania, versus sulfadoxine-pyrimethamine -artesunate (SPAS) or artemether-lumefantrine (AL), three and five years after the introduction of SP. We calculated survival using the Kaplan-Meier product limit estimator. We also used the Cox proportional hazards model to assess the effect of additional covariates, including age, gender, district, initial parasite density, and bednet ownership, on failure. Some have argued that patients presenting with a higher initial parasitemia may be more likely to have a recrudescence of their parasitemia, due to the fact that there would be more chance for a resistant parasite to develop (the higher number of parasites increases the chances of developing a random resistance mutation as well as increases the time until all of the parasites are cleared from the bloodstream) (7). This finding has been seen in some studies

but not in others (8-10). Insecticide treated bednets have been shown to decrease malaria related morbidity and mortality, therefore it is important to consider the effect that they may have on treatment outcomes (11). In addition, we looked at the effect of treatment on the prevalence of anemia, as increasing drug resistance is associated with higher prevalence of anemia (12).

BACKGROUND

Malaria is caused by parasites of the *Plasmodium* genus. Human infection results from inoculation of motile *Plasmodium* sporozoites by a malaria-infected female *Anopheles* mosquito during a blood meal. The sporozoites infect liver cells where they mature into schizonts (exo-erythrocytic schizogony); these develop into merozoites which are released into the bloodstream following rupture of the hepatocyte. In the case of *P. vivax* and *P. ovale* a dormant hypnozoite stage may form and can persist in the liver, causing relapses weeks or years later. The merozoites which are released into the bloodstream proceed to infect erythrocytes, where they undergo asexual replication (erythrocytic schizogony) and develop into ring stage trophozoites. The trophozoites mature into schizonts, which rupture releasing merozoites. These infect new erythrocytes, continuing the infection with an exponentially enlarging parasite biomass resulting in fever and pathological processes such as anemia and cerebral malaria. Some the parasites differentiate into gametocytes, the sexual stage of the parasite. Sexual replication occurs when male and female gametocytes, which are ingested by the mosquito during feeding, combine in the stomach of the mosquito, and subsequently replicate, releasing sporozoites to the mosquito's salivary gland.

The developing trophozoites consume the cytoplasm of the erythrocyte. In doing so, they also ingest large amounts of hemoglobin, the predominant cytosolic protein of the red blood cell (RBC). Most of the hemoglobin is degraded within digestive vacuoles, releasing heme and generating amino acids. Free micromolar heme can damage cellular metabolism by the inhibition of enzymes (13, 14) the peroxidization of membranes (15), and the production of

oxidative free radicals as ferrous heme iron is oxidized to the ferric (+3) state (16). Plasmodial species do not possess heme oxygenase that vertebrates use for heme catabolism, therefore, they render this toxic byproduct inert by transforming it to a chemically inert crystal known as the malaria pigment hemozoin that can be visualized as intra-erythrocytic pigment by light microscopy of thin blood smears (17, 18).

Malaria symptoms and disease are caused exclusively by parasites in the asexual blood stage. Symptoms of malaria may be non-specific. Essentially all patients will present with fever. Other symptoms may include arthralgias, myalgias, diarrhea, vomiting, and abdominal cramps. Findings on examination may include pallor, jaundice, or hepatosplenomegaly. Anemia is common. Other laboratory abnormalities may include hypoglycemia, thrombocytopenia, hyponatremia, and elevations in creatinine. Severe disease is characterized by hyperparasitemia (>5% infected red blood cells), severe anemia, renal insufficiency, acute respiratory distress syndrome (ARDS), or cerebral malaria. Cerebral malaria is often associated with seizures, and may be associated with long-term cognitive and language impairment.

Four species are responsible for the majority of human disease: *Plasmodium falciparum*, *P. vivax, P. malariae*, and *P. ovale*. Occasionally, humans may also be infected with simian malaria; recently, several fatal cases in Southeast Asia were attributed to infections with the simian parasite *P. knowlesi*. In general, however, *P. falciparum* causes the most severe disease and is responsible for the greatest number of cases and deaths of all the species globally. Development of resistance to antimalarials is also seen primarily in *P. falciparum* (19),

although resistance of *P. vivax* to CQ has been reported from Indonesia (20-22), India (23), Myanmar (24), and the Amazon (25-28).

For the past 30 years, chloroquine (CQ), which interferes with the crystallization of heme to hemozoin, forming toxic heme complexes (29), has been the drug of choice for the treatment of malaria throughout Africa, based upon its rapid action, safety and low cost relative to other antimalarials. However, widespread use has led to significant resistance to CQ, mediated by mutations in the *P. falciparum* chloroquine resistance transporter (PfCRT) gene (30), with failure rates as high as 72% in 1999 (31). Increasing parasite resistance has led to a significant increase in malaria-related mortality (32), and prompted a change in first line therapy to sulfadoxine-pyrimethamine (SP). The components of sulfadoxine-pyrimethamine inhibit the enzymes dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) which catalyze sequential steps of the folate pathway, thus blocking plasmodium nucleic acid synthesis. SP had the advantage of being inexpensive (less than US$0.20 per adult treatment course (33)) and requiring only one dose to provide complete treatment, due to it's long half-life (95.5 and 184 h for pyrimethamine and sulfadoxine, respectively (34)). However, the development of resistance to this combination was reported from multiple sites soon after its implementation in Africa, Asia, Indonesia, and South America (35-40), leading to a push to develop novel, efficacious, and, inexpensive anti-malarial therapies.

The artemisinins, derived from the plant *Artemisia annua,* also known as sweet wormwood, have been used in China for the treatment of malaria for many years. With the increasing

interest in developing new antimalarials, these compounds were evaluated and found to rapidly reduce parasitemia. Artemisinin derivatives are currently the most highly effective antimalarial drugs in widespread use and are capable of producing a more rapid reduction in parasite biomass than any other current antimalarial (33). In addition, they are one of few anti-malarials with gametocidal activity, which may contribute to reducing transmission, especially in low transmission settings (41-43). Artemisinins have a very short half-life of about an hour (44). This may help to make them less susceptible to development of resistance, but it means that a minimum of a seven day course is required to achieve acceptable cure rates when used alone (3). In order to be effective as part of a short course of therapy, a second, longer acting antimalarial must be given in combination with the artemisinin (41).

Since April 2001, WHO has recommended the use of artemisinin-based combination therapies (ACTs) in all areas with chloroquine resistant malaria. Artemisinin-based combination therapies pair an artemisinin derivative with a longer acting partner drug, such as lumefantrine or amodiaquine. The rationale behind the use of combination therapy is that the administration of two drugs with different mechanisms of action will delay the development of resistance to either drug, as resistance will require not one, but at least two mutations. This same rationale has been proven effective in treatment strategies for other infectious diseases, including tuberculosis and HIV. Although the discrepant half-lives of the two drugs mean that the partner drug is left with an unexposed tail, because the artemisinin component rapidly reduces parasite density, only a small fraction of parasites are still present, thus the likelihood of developing resistance is greatly decreased.

Artemisinins are considerably more expensive than either CQ or SP monotherapy, and at the time when the recommendation to use ACTs was put forward by WHO, their availability was greatly limited (45). Deciding when to switch the antimalarial policy of a country relied on balancing the need for an effective therapy with the need to delay as long as possible, to prevent unnecessarily burdening the country financially. Furthermore, in order to justify the added expenditure related to ACTs, it was important to field test these new drugs prior to large scale implementation, to ensure that the benefits would justify the increased costs.

Once a new therapy has been implemented, it is crucial to continue to monitor the efficacy of both currently recommended and alternative therapies to continue to provide effective treatment. WHO recommends monitoring of efficacy using *in vivo* testing, in which a drug is administered to children age 6- 59 months with uncomplicated *P. falciparum* malaria who are then followed for a minimum of 28 days to document cure rates (6, 46, 47). Especially in areas of high transmission, clinical failures may be due to drug resistance in the original infection or an entirely new infection that emerges after complete treatment of the original infection. In evaluating drug efficacy, it is important to differentiate failures due to new infections from those due to recrudescence of parasites that were inadequately treated. In order to differentiate recrudescence from new infections, WHO recommends molecular genotyping. The most commonly used molecular markers are Merozoite Surface Peptide (msp)-1, msp-2, and glutamate-rich protein (glurp) (48). A 'new infection' is defined as a parasitemia in which all the alleles in parasites from the post-treatment sample are different from those in the admission sample, for one or more loci tested, while a 'recrudescence' is

defined as a parasitemia in which at least one allele at each locus is common to both the admission and relapse samples.

In Tanzania, at the time the government changed its first line malaria treatment from chloroquine to SP in 2001, SP failure rates were already as high as 25- 35% in parts of the country (8, 49, 50). Several studies in Southeast Asia in areas with significant mefloquine resistance had shown that the addition of artesunate to mefloquine resulted in high cure rates, even when mefloquine failure rates were approximately 25% (5, 51, 52). This had been shown to be true for the addition of artesunate to SP as well (10). Therefore, it was thought that the addition of artesunate to SP might prolong the useful lifespan of SP, thus providing a lower cost alternative to the other ACTs. This *in vivo* study was conducted at the time when SP was still the first line therapy in Tanzania, to explore whether the combination of SP+ artesunate would improve treatment efficacy versus SP alone. Given the possibility that the efficacy of SP+AS might be sub-optimal, the efficacy of artemether-lumefantrine (AL) was explored as another possible option.

METHODS

Hypothesis. The null hypothesis was that there is no difference in the therapeutic efficacy of SP, SPAS, or AL for treatment of uncomplicated *Plasmodium falciparum* infections among children less than 5 years of age based on clinical, parasitologic, and hematologic parameters.

Study Design. This was a prospective, open-label randomized, controlled clinical trial.

Study Area. This study was conducted at the Kibiti Health Center in Rufiji district and the Lupiro Health Center in Ulanga district of Tanzania. Malaria transmission is holoendemic, with year round transmission and seasonal peaks following the rainy seasons in November - December and March-May in both districts.

Rufiji and Ulanga are adjacent districts, divided by the Selous Game Reserve and form part of a contiguous ecosystem, the Greater Rufiji River Basin. The geography ranges from highland to forest, but most of the population is settled along broad flood plains where rice cultivation is possible. Rufiji is home to an estimated 202,001 people (32,661 less than 5 years of age), while Ulanga has a population of 193,280 people (31,553 less than 5 years) (53). Most are subsistence farmers and fishermen.

Participants. Patients 6 months to 59 months of age were eligible for enrollment if they presented to one of the participating health centers with *P. falciparum* mono-infection with a parasite density 2,000- 250,000 asexual parasites/mm^3 blood and a documented fever

(axillary temperature ≥37.5°C) or history of fever in the last 48 hours, with no obvious causes of fever other than malaria. Children were excluded if they had evidence of danger signs (unable to drink or breastfeed, profuse vomiting, convulsions, lethargy, altered mental status) or severe malaria (cerebral malaria, prostration, Hb < 5 g/dL, etc.) requiring hospitalization, a history of allergy to any antimalarial, other serious or chronic medical condition (heart failure, sickle cell disease), or weight < 5 kg. Written informed consent of the parent or guardian was required for participation.

Procedures. At enrollment, parents were questioned about the child's symptoms and any medications given. Axillary temperature and weight were measured, and a physical examination was performed. Parents were asked to bring their children for follow-up assessments on Days 1, 2, 3, 7, 14, 21, 28, and at any other time the child appeared ill. At each follow-up visit, a physical exam was performed, and the parents were questioned regarding possible adverse effects; any medications which the child received in addition to the study medication were documented.

Evaluation of in vivo *efficacy.* Blood was obtained by finger prick for thick blood smears and storage on filter paper at enrollment and on all follow-up days except day 1. Hemoglobin was measured at enrollment and day 14; in 2006 hemoglobin was also measured at day 28. Thick blood smears were prepared with 2% Giemsa stain for a minimum of 10 minutes and the number of parasites per 200 WBC counted. Parasite density was estimated assuming an average WBC count of 6,000/mm^3. A second microscopist, who was unaware of the results of the first reading, reread all slides. A third microscopist unaware of the first two readings

resolved discrepant slides as needed. Gametocytemia was also assessed from thick blood smears. Hemoglobin measurements were made using a portable spectrophotometer (HemoCue, Angelholm, Sweden http://www.hemocue.com).

Antimalarial Therapy. Patients were randomized to receive SP, SPAS, or AL using a random number table. All drugs were administered orally as follows: SP (500mg sulfadoxine/ 25mg pyrimethamine tablets, Fansidar, Roche, Basel, Switzerland) as a single dose of 25 mg/kg of the sulfadoxine component, up to a maximum of 3 tablets, on Day 0; SPAS (500mg sulfadoxine/25mg pyrimethamine tablets and 50 mg artesunate sodium tablets, Arsumax, Sanofi-Aventis, Gentilly, France) as a single dose of SP (25mg/kg sulfadoxine) on Day 0 plus 4 mg/kg artesunate once daily for 3 days, starting on Day 0; AL (Coartem, Novartis, 20 mg artemether/120 mg lumefantrine tablets, Coartem, Novartis, Basel, Switzerland), administered according to weight as one (5–14 kg), two (15–24 kg), three (25–34 kg), or four (35 kg) tablets given twice daily for 3 days. The first dose each day was administered under supervision by the clinical staff. Patients were observed for 60 minutes following administration of treatment for adverse reactions or vomiting. Patients who vomited their medication within the first 30 minutes following administration received a repeat full dose, those vomiting between 30-60 minutes following administration received an additional half dose. For artemether-lumefantrine, which is given twice daily, the parent was given the evening dose each morning to administer at home.

Outcome variables. The primary outcome was clinical and parasitological response, assessed as the number and percent of patients who respond by day 3 (temperature < 37.5°C on Day 3)

and remain malaria free at day 28. PCR corrected survival rates (using PCR to discriminate recrudescence from new infections) are also reported. Hematologic response (mean change in hemoglobin concentration from Day 0 to Day 14 and Day 0 to Day 28, measured by HemoCue in mg/dl) was assessed as a secondary outcome variable.

Predictor Variables. The primary predictor variable was treatment arm. Other predictor variables examined were age, gender, and initial parasite density (measured by counting the number of parasites/ 200 WBC).

Sample size. Sample size was determined using the single population proportion sampling technique to show the single point estimate of the performance of each drug. Assuming a worst-case scenario failure rate of 80% for SP based on previous studies, a power of 80%, a 5% level of significance, and precision of 10%, 62 subjects needed to be enrolled in each arm of the trial. Allowing for 15% attrition during a 28-day study, a total of 72 subjects per treatment arm per health center/ year were enrolled.

Analysis

Efficacy. Treatment outcomes were classified according to 2005 WHO guidelines as early treatment failure (ETF; danger signs or complicated malaria or failure to adequately respond to therapy days 0–3); late clinical failure (LCF; danger signs or complicated malaria or fever and parasitemia on days 4–28 without previously meeting criteria for ETF or LPF); late parasitological failure (LPF; asymptomatic parasitemia days 7–28 without previously meeting

criteria for ETF or LCF); or adequate clinical and parasitological response (ACPR, absence of parasitemia on day 28 without previously meeting criteria for ETF, LCF, or LPF) (46). Patients who failed therapy were treated with amodiaquine (10 mg/kg/day for Days 1 and 2 and 5 mg/kg for Day 3). Patients were excluded from further follow-up after enrollment if any of the following occurred: (1) use of antimalarial drugs outside of the study protocol; (2) withdrawal of consent; (3) loss to follow-up; (4) protocol violation; or (5) death due to a non-malarial illness.

The primary efficacy outcomes were the 28-day risk of early treatment failure or recurrent parasitemia (LCF or LPF), unadjusted and adjusted by genotyping. Secondary efficacy outcomes included prevalence of fever and parasitemia during the first 3 d of follow-up, change in mean hemoglobin from day 0 to day 14, and prevalence of gametocytemia (presence of gametocytes on thick smears) during follow-up in participants lacking gametocytes at enrollment. Molecular genotyping techniques were used to distinguish recrudescent from new infections for all patients with LCF or LPF response. Briefly, parasite DNA was isolated from filter paper blood samples collected at enrollment and on the day of recurrent parasitemia. PCR genotyping for allelic variation of merozoite surface peptides (msp) 1 and msp 2 and glutamate rich protein (glurp) was used to distinguish recrudescence from re-infection (54).

Safety. Safety outcomes included risks of serious adverse events and common adverse events of any severity. An adverse event was defined as any untoward medical occurrence,

irrespective of its suspected relationship to the study medications [18]. At each follow-up visit, patients were assessed for any new or worsening event.

Statistical Methods. Data were entered and verified using FoxPro version 3.0 (Microsoft, Redmond, WA) and analyzed using SAS Version 9.1.3 (SAS Institute, Cary, NC). Efficacy and safety data were evaluated using a modified intention-to-treat analysis which included all patients who were enrolled and received at least one dose of study medication. The risk of recurrent parasitemia at Day 28 (adjusted and unadjusted by genotyping) was estimated using the Kaplan-Meier product limit formula. Data were censored for patients who did not complete follow-up and for new infections when estimating outcomes adjusted by genotyping. The log-rank test was used to compare the difference in outcomes between different arms. Three pair-wise log-rank tests were used to compare the efficacy of each drug to the other two. A Cox proportional hazard model was used to further explore the effect of various covariates, including age, gender, weight, fever at enrollment, initial parasite density, district, treatment group, and bednet ownership. Age was modeled as both a continuous and categorical variable with 3 groups: 6- 12 months (reference group), 13-23 months, and 24-59 months. Initial parasitemia was modeled as both a continuous variable and a categorical variable ($<$10,000/ μl (reference group) and $\geq$10,000/ μl). SP was the reference group for treatment and Ulanga was the reference district. The final model for 2004 included treatment, district, bednet ownership, age as a categorical variable, and a treatment- district interaction, expressed as follows:

$$Log\ [HR(\ infection)] = \beta_0 + \beta_1(SPAS) + \beta_2(AL) + \beta_3(district) + \beta_4(bednet\ ownership) + \beta_5(age\ category\ 13\text{-}23\ months) + \beta_6(age\ category\ 24\text{-}\ 59\ months) + \beta_7(SPAS\text{-}district\ interaction) + \beta_8(AL\text{-}district\ interaction).$$

The final model for 2006 included treatment, district, bednet ownership, and age as a categorical variable and parasitemia as a categorical variable (hyperparasitemia). Treatment-district interaction was explored, but was found to be non-significant. The final model was:

$$Log\ [(HR\ infection)] = \beta_0 + \beta_1(SPAS) + \beta_2(AL) + \beta_3(district) + \beta_4(bednet\ ownership) + \beta_5(age\ category\ 13\text{-}23\ months) + \beta_6(age\ category\ 24\text{-}\ 59\ months) + \beta_7(hyperparasitemia)$$

Categorical variables were compared using Chi-squared or Fisher exact test and continuous variables were compared using the independent samples t-test for variables with a normal distribution and the Wilcoxon test for variables with a non- normal distribution. All reported p-values are two sided; p-values were adjusted with the Bonferroni correction as needed for multiple testing and were considered statistically significant if below 0.05.

Ethics. Both verbal and written informed consent was obtained from parents/ guardians in their native language (Swahili) at the time of screening and again before randomization to one of the study treatment arms. The study was approved by the Institutional Review Boards of The Centers for Disease Control and Prevention (CDC) and The Ifakara Health Research and Development Center. The Tanzanian Ministry of Health also gave approval for the conduct of this study under the authority of the National Medical Research Coordinating Committee.

RESULTS

In 2004, 425 patients were enrolled; 143 each in the SP and SPAS arms and 139 in the AL arm, while in 2006, 361 patients were enrolled: 121 in the SP arm, 122 in the SPAS arm, and 118 in the AL arm. In 2004, patients were enrolled between June and October; in 2006, enrollment began in June and was completed in January, 2007. There were no significant differences in gender, age, temperature, parasite density, hemoglobin, or bednet ownership between the three treatment groups in either year (Table 1), however, there was a significant increase in bednet ownership and a significant decrease in the percent of children with gametocytemia from 2004 to 2006 (Table 2). There was also a significant increase in the age of enrolled children, with a concurrent increase in the mean weight and decrease in mean respiratory rate. In addition to a significant increase in bednet ownership in both districts from 2004 to 2006, there was a significant difference in the ownership of bednets between the 2 districts. In 2004, only 29% of patients in Rufiji reported owning a bednet, while in Ulanga 80% of patients owned a bednet ($P<0.0001$). In 2006, bednet ownership in Rufiji jumped to 94%, while in Ulanga it increased much more modestly to 85% ($p= 0.0057$). Only a small percentage of children had used other drugs with potential antimalarial activity. One child in 2004 reported use of SP and one child in 2006 reported use of amodiaquine. In 2004 7-8% of children in each treatment arm reported using cotrimoxazole. There were no reports of cotrimoxazole use in 2006 nor of any other antimalarials or agents with potential antimalarial activity (i.e. clindamycin). A high percentage of children reported use of antipyretics. This was more marked in 2006 (80%) than in 2004 (60%). Antibiotics were also used frequently; 54% and 46% of children received antibiotics other than cotrimoxazole

in 2004 and 2006, respectively (p=0.03). There was not a significant difference in the proportion of children using antibiotics by treatment arm in either year.

Outcomes

Primary efficacy outcomes. There were six (4%) early treatment failures in 2004, all in the SP group. Recurrent parasitemia was first detected on day 14 in the SPAS arm and day 21 in the AL arm (Figure 1; Table 3). The uncorrected 14 day cure rates were 90%, 99%, and 100% for SP, SPAS, and AL, respectively. The uncorrected 28 day cure rate was significantly lower in the SP arm than in either the SPAS or AL arm with cure rates of 58%, 76%, and 81%, respectively (p- value <0.0001). There was a significant difference between SP and each of the other drugs (p-value <0.0001 for both). There was not a statistically significant difference between SPAS and AL (p-value= 0.33). Similar trends were seen when results were corrected by genotyping, with cure rates of 71%, 91%, and 94%, respectively. PCR data was missing for a total of 7 participants, 4 (2.8%) in the SP arm, 2 (1.4%) in the SPAS arm, and 1 (0.7%) in the AL arm. The rate of re-infection was similar across all treatment groups (12- 14%, p=0.83).

In 2006, there were 10 early treatment failures - 9 (7% of total subjects) in the SP group and 1 (1%) in the AL group. Recurrent parasitemia was first detected in the SPAS group at day 7. Following a single early treatment failure in the AL group at day 3, no additional failures occurred until day 12. The uncorrected 14 day cure rates were 70%, 89%, and 96% for SP, SPAS, and AL, respectively. By day 28, the uncorrected cure rates had dropped to 51%, 64%, and 86% in the SP, SPAS, and AL groups, respectively (p- value <0.0001) (Figure 2;

Table 3). There was a significant difference (p-value <0.0001) in the cure rate at 28 days between AL and SP and between AL and SPAS. The difference in cure rate between SP and SPAS at 28 days was of borderline significance following Bonferroni correction (p-value = 0.016). This represents a potentially significant decrease in efficacy for SPAS since 2004. PCR corrected cure rates for 2006 are pending.

Parasite Density & Gametocytes

The geometric mean parasite density was higher for the SP group than for either of the other groups at every follow-up day in both years (Table 4, Figures 3 & 4). Gametocytes were present in 10%, 1.5%, and 0% of patients at day 14 (p<0.0001) and in 5%, 1.6%, and 0% (p=0.004) of patients at day 28 in the SP, SPAS, and AL groups in 2004. In 2006, gametocytes were present on day 14 in 6.6%, 0%, and 0% (p=0.0001) and on day 28 in 2.5%, 0%, and 0% (p=0.037) in the SP, SPAS, and AL groups, respectively.

Hemoglobin.

In both 2004 and 2006, patients treated with SP had a smaller mean rise in hemoglobin concentration from day 0 to day 14 than did the other arms (0.5 mg/dl, 0.8 mg/dl, and 0.8 mg/dl for SP, SPAS, and AL in 2004 and 0.3 mg/dl, 0.5 mg/dl, and 0.7 mg/dl, respectively, in 2006), as well as a higher percentage of patients remaining anemic on day 14 (64% in the SP arm versus 52% in the SPAS arm and 55% in the AL arm in 2004 and 73%, 64%, and 64%, respectively in 2006). However, these differences were not statistically significant (Table 5).

In 2004, there are insufficient Day 28 hemoglobin values to perform statistical analysis. In 2006, there was a significant increase in hemoglobin from Day 14 to Day 28 for all three treatment arms. The group treated with AL had a greater increase in mean hemoglobin concentration (1.5 mg/dl) than either the group treated with SP (1 mg/dl) or SPAS (0.85 mg/dl); however, this difference was statistically significant only for the difference between SPAS and AL (p= 0.016). By day 28, the percent of children remaining anemic in the AL group was significantly less than in either other group (41% in AL group versus 62% in SP and 68% in SPAS groups, p=0.001) (Table 5).

Univariate and Multivariate Modeling

The effects of various covariates were explored using a Cox Proportional Hazard Model. In univariate modeling, the effects of both SPAS and AL compared to SP alone were significant in both 2004 and 2006. The effect of bednet ownership was non-significant in both univariate and multivariate analysis in all years. There was no interaction of bednet and district (data not shown). The effect of initial parasitemia was significant in 2006 but not in 2004. The effect of gender, weight, temperature at enrollment, and use of cotrimoxazole were all found to be non-significant in univariate analysis (Table 6).

In the univariate analysis of 2004 data, compared to SP, treatment with SPAS resulted in a hazard ratio of 0.45 (95% CI = 0.30- 0.69, p = 0.0002) for infection during the 28 day follow-up period. Treatment with AL resulted in a hazard ratio of 0.36 (95% CI= 0.23- 0.56, p <0.0001) compared to treatment with SP. Treatment with AL was not significantly different from treatment with SPAS. In 2006, using treatment with SP as the baseline, the

hazard ratio (95% CI) for infection was 0.64 (0.43- 0.94) for SPAS and 0.22 (0.12- 0.38) for AL; both of these were highly significant ($p= 0.02$ and $p<0.0001$, respectively). In comparison to AL, treatment with SPAS resulted in a significant increase in the hazard ratio for infection (2.9, 95% CI: 1.7- 5.2).

In multivariate analysis of 2004 data (Table 7), AL showed a significant benefit over SP in both districts, with a hazard ratio of 0.3 (0.15- 0.62, $p= 0.001$) and 0.5 (0.28- 0.995, $p=0.048$) for Ulanga and Rufiji, respectively. SPAS, however, provided a greater benefit than SP in Ulanga only, with a hazard ratio of 0.19 (0.08- 0.44, $p<0.0001$). In 2006 (Table 8), there was no interaction of district and treatment. Treatment with both SPAS and AL showed a significant benefit over treatment with SP, HR= 0.55 (0.37- 0.82, $p=0.003$) and HR= 0.22 (0.13- 0.39, $p <0.0001$), respectively.

Bednets

We evaluated the utility of bednets for preventing infection. As stated above, bednet ownership was not associated with a decreased risk of uncorrected treatment failure in either univariate or multivariate analysis. Bednet ownership was, however, associated with a decreased risk of re-infection during the follow-up period, although this was not statistically significant except for SPAS (Table 9).

Resolution of symptoms

In 2004, the percent of patients reporting symptoms on day 2 was significantly higher in the SP group than in the other groups: fever was reported in 51% of the SP group versus 5%

and 9% in the SPAS and AL arms, respectively ($p<0.0001$). Weakness was reported in 18% of the SP group versus 2% and 3% in the SPAS and AL arms, respectively, $p<0.0001$, and vomiting was reported in 12% of the SP group versus 1% and 4% of the SPAS and AL respectively, $p<0.001$ (Table 10, Figure 5). No significant differences between treatment groups were observed for the proportion of patients reporting diarrhea and rash on any of the follow-up days. Six children in the SP group had seizures. Two occurred early, on days 2 and 3, and the remainder occurred late, at day 20, 26, and 27 (two). None of the children in the other groups had seizures.

Similar results were seen for 2006, with fever, weakness, and vomiting reported on Day 2 in 51%, 18%, and 13% of patients in the SP group compared to 5%, 2%, and 1% and 9%, 3%, and 1% in the SPAS and AL groups, respectively(p-value <0.0001) for all (Table 11, Figure 6). Diarrhea was seen more commonly in the AL group on the day of enrollment; subsequently, there was no difference in proportion of patients reporting diarrhea among the groups. There was no difference in the proportion of patients reporting rash at any follow-up day. One child in the SPAS group had a seizure on the day of enrollment. No other children reported seizures.

Safety concerns

There were no reports of any serious adverse events.

DISCUSSION

The results show that both SPAS and AL are significantly more efficacious than SP monotherapy at day 14, but by 2006, the 28 day efficacy of SPAS is declining significantly. Despite the declining efficacy of SPAS, the combination therapies resulted in a more rapid resolution of symptoms (fever, weakness, vomiting), clearance of parasitemia, and a decreased proportion of children with circulating gametocytes. There was a general trend towards higher mean hemoglobin values and a lower percentage of anemic children in the AL group. This was most marked in 2006 at the 28 day follow-up; however, a statistically significant difference was seen only between AL and SPAS in 2006, and not between AL and SP. This may be due to the relatively small sample size.

In Tanzania, the study site, the decision to switch the first line antimalarial therapy from CQ to SP was made in 1998, but due to lack of funds, was not able to be implemented until 2001. By that time, there was already a significant level of resistance to SP in some parts of the country (8, 49, 50). Within three years of its implementation as first line therapy, the efficacy of SP monotherapy was 71% at 28 days following correction for re-infections. This is well below the current standards which recommend use of antimalarials with at least 90% efficacy. However, the uncorrected day 14 failure rate was 10%, which was still within the acceptable range at that time (WHO recommendation was to change therapy once there was >25% resistance at 14 days (5)). There was only a minimal incremental drop in SP efficacy between 2004 and 2006, from 58 to 51% (uncorrected), likely due to the level of resistant alleles being near saturation in the population by this time. This rapid drop in efficacy was

observed following SP implementation in other countries (10, 55-58).

Although initially, addition of artesunate to SP resulted in a significantly higher likelihood of cure, by 2006 the uncorrected cure rate dropped to 89% at 14 days and only 64% by 28 days. Assuming that the true re-infection rate was close to those observed in 2004, one would expect that the corrected cure rate would rise to 76-78%, still well below the 90% cure rate currently recommended by WHO. Furthermore, one might expect that the rates of re-infection in 2006 would be lower than in 2004, as the rate of bednet ownership had increased, and the prevalence of parasitemia in the area had decreased from about 30% to about 10% (59). The addition of artesunate to SP did not have the hoped for effect of prolonging the useful lifespan of SP. The low efficacy of SPAS can likely be explained by the existing high level resistance to SP. Dorsey *et al.* showed a similar picture of relatively high cure rates with SPAS at 14 days, with a significant decline in efficacy by 28 days in an area with high level resistance to SP (10).

While 14 day cure rate with AL in 2006 remained excellent at 96%, the 28 day cure rate were less than desired at 86%. However, the uncorrected 2006 cure rate actually increased from that seen in 2004 (81%). Assuming a roughly 10-12% rate of re-infection (similar to what was seen in 2004), we suspect that the corrected cure rates would be closer to 96- 98%, however, as mentioned previously, it is likely that there will be lower rates of re-infection in 2006 than 2004, resulting in a lower cure rate for AL, but it is unlikely that the corrected cure rate will be <90%. In making this judgment, however, it is important to recognize the significant differences between 2004 and 2006. As the prevalence of parasitemia in the

community has rapidly decreased, children at risk for infection in 2006 are different to those in 2004. They likely are of lower socioeconomic status, and have decreased access to insecticide treated nets (ITNs) or health services in general. The fact that they are the few that are infected in the community may mean that they have increased risk for any number of reasons; therefore, their re-infection rates may be higher than expected based on 2004 data.

Modeling was used to explore the effects of various covariates on the rate of recrudescence. Only treatment arm, district, age, and initial parasitemia were shown to have any effect on failure rates. In theory, initial parasite density may be related to treatment outcome, as a higher parasite load corresponds both with more severe disease, and also theoretically, provides a larger pool from which resistance could develop. There was no significant association between the initial parasite density and outcome in 2004, in keeping with the results of some previous studies (8, 9), but a very significant association with initial parasite density of greater than 10,000 parasites/ μl in 2006, as has previously been reported by Dorsey *et al* (10). This finding is likely related to the fact that the proportion of children with high level parasitemia in 2004 was much greater than that in 2006. In 2004, 58% of children had initial parasitemia greater than 10,000 parasites/ μl; 30% of these children failed therapy, with no difference by treatment group. In 2006, only 5% of children had parasitemia greater than 10,000 parasites/ μl, but all of them failed therapy.

One of the most interesting findings of this study was the differential benefit of the antimalarials by district in 2004. AL was slightly more beneficial in Ulanga than in Rufiji;

however, SPAS was more efficacious than SP only in Ulanga. One possible explanation for the lower efficacy of SPAS in Rufiji might be increased resistance following the introduction of SPAS as first line therapy as a pilot project in Rufiji in 2003. Although bednet ownership was significantly different between patients enrolled from the two districts at that time, owning a bednet does not appear to be responsible for this difference. However, the community-wide prevalence of bednets might have contributed to these differences. Community-wide bednet ownership among people of all ages was 85.6% in communities near the Ulanga site in 2004, and increased to 92.4% in 2006, while in villages near the Rufiji enrollment site, coverage of bednets was only 51.3% in 2004, increasing to 63.4% in 2006 (unpublished data, S. Patrick Kachur). Insecticide treated bednets have been shown to decrease malaria transmission rates (60-63), with a higher level of coverage resulting in more substantial decreases. Use of bednets has been shown to decrease the mean parasite density of infection and the multiplicity of infection (the number of different infecting strains) (64); both of these factors are associated with the development of resistance (65, 66). It is possible that the higher bednet coverage in Ulanga helped to decrease transmission and thus also impacted the prevalence of resistant parasites. Mathematical modeling suggests that decreasing transmission results in a decreased rate of acquisition of resistance, at least up to a point, and therefore, advocates the use of bednets to help delay the spread of resistance (65, 66). In a study by Alifrangis *et al.*, bednet usage was associated with a decrease in the prevalence of resistant alleles of *dhfr* (conferring resistance to pyridoxine), but no difference in the prevalence of resistant alleles to *dhps* (conferring resistance to sulfadoxine) (64). As resistance to *dhfr* develops more quickly than that to *dhps*, the authors speculate that loss of resistance may occur in the same order and that with a

more prolonged observation period, susceptibility to SP may occur. This effect would occur at the community level rather than at the level of the individual, possibly partially explaining the significant effect of district and not of bednets observed in our study.

While bednets were not shown to affect the crude failure rates, they did decrease the rate of re-infection, as has been shown previously (67). Although one would not necessarily expect sleeping under a bednet to decrease recrudescence rates, some argue that sleeping under a bednet reduces multiplicity of infection (64), which may then impact recrudescence rates. However, this was not observed in our study.

A significant proportion of the treatment failures in the SPAS group occurred after 14 days of follow-up. By this time, there is no circulating artesunate, and the concentration of SP has dropped to a level below the treatment threshold. Exposure of parasites to these low levels of drug are felt to select for resistance (68). This suggests that SPAS is not an appropriate new regimen for treatment of uncomplicated malaria in regions, such as Tanzania, with substantial resistance to SP.

The late 1990s were a time of much confusion and debate over malaria treatment policy throughout Africa. By 2001, there was a growing consensus that artemisinin-containing combination therapies were likely to be more efficacious than traditional monotherapies almost everywhere they were examined (69). Whether the combinations would lower malaria transmission pressure or forestall the development and spread of drug resistance—which had been claimed to have occurred in southeast Asia—became less important as the

number of efficacious alternative antimalarial monotherapies quickly diminished. In 2006, following the results of this study, the Tanzanian Ministry of Health made the decision to change the first line therapy to artemether-lumefantrine, based on the poor efficacy of SP and only slight advantage of SPAS. Following this change, continued monitoring of the efficacy of AL will be essential to detect resistance before it reaches the levels seen with SP. As newer antimalarials are developed, they will require continued evaluation to determine the most cost-effective strategy for treatment of uncomplicated malaria.

CONCLUSION

Both SPAS and AL were significantly more efficacious for treatment of uncomplicated malaria than SP. However, the efficacy of SPAS is rapidly decreasing. SP should no longer be used for treatment of malaria illness in Tanzania, either as monotherapy or as part of artemisinin combination therapy.

TABLES

Table 1. Characteristics of enrolled patients in 2004 and 2006.

	2004			2006		
	SP	**SPAS**	**AL**	**SP**	**SPAS**	**AL**
N	**143**	**143**	**139**	**121**	**122**	**118**
Resident of Rufiji District (%)	52%	52%	54%	49%	50%	52%
Bednet use (%)	53%	51%	55%	93%	85%	91%
Male (%)	52%	50%	46%	48%	42%	53%
Mean age (months)	25	27.6	24.8	31.7	29	30.4
Mean weight (kg)	10.8	11	10.7	11.8	11.3	11.6
Fever at enrollment (%)	66%	58%	63%	64%	66%	63%
Mean axillary temperature (˚C)	38.7	38.7	38.6	38.4	38.5	38.5
Geometric mean parasite density (parasites/ ul)	54,719	49,137	44,737	41,963	49,269	40,705
Gametocytes (%)	2.9%	2.8%	5.6%	1.65%	0.82%	0%
Mean hemoglobin (mg/dl)	9.1	9.4	8.9	9.1	8.9	9
Mean respiratory Rate (bpm)	43	44	43	37	38	38
Antipyretic use	62%	57%	60%	78%	80%	78%
Sulfa drug (cotrimoxazole)	8%	8%	7%	0	0	0

Table 2. Differences between enrolled patients in 2004 and 2006.

	2004	**2006**	p-value
N	425	361	
District (Rufiji)	53%	50%	0.43
Bednet (%)	**53%**	**89%**	**<0.0001**
Male (%)	49%	48%	0.65
Mean age (months)	**25.8**	**30.3**	**<0.0001**
Mean weight (kg)	10.7	11.6	<0.0001
Fever at enrollment (%)	62%	64%	0.64
Mean temperature (˚C)	36.9	36.9	0.69
Geometric mean parasite density (parasites/ ul)	49,576	44,021	0.25
Gametocytes (%)	**3.8%**	**0.83%**	**0.01**
Mean hemoglobin (mg/dl)	9.1	9	0.38
Mean respiratory Rate (bpm)	33	29	<0.0001
Antipyretic use	60%	79%	<0.0001
Use of sulfa drugs (cotrimoxazole)	8%	0%	<0.0001

SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine

Table 3. Uncorrected cure rates and failure rates by type of failure for each treatment arm

	2004			2006		
	SP	**SPAS**	**AL**	**SP**	**SPAS**	**AL**
N	143	143	139	121	122	118
ETF	6 (4%)	0	0	9 (7%)	0	1 (0.85%)
LCF	26 (18%)	13 (9%)	9 (6%)	20 (17%)	11 (9%)	3 (3%)
LPF	28 (20%)	21 (15%)	18 (13%)	30 (25%)	33 (27%)	12 (10%)
ACPR*	83 (58%)	109 (76%)	112 (81%)	62 (51%)	78 (64%)	102 (86%)

The rate of Adequate clinical and parasitologic cure was significantly different among the treatment groups in both 2004 and 2006 ($p<0.0001$).

SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine
ETF= Early treatment failure
LCF= Late clinical failure
LPF= Late parasitologic failure
ACPR= Adequate clinical and parasitologic response

Table 4. Geometric Mean Parasite Density (95% Confidence Interval) by Follow-up Day and Treatment Group

	2004						
Follow-up		SP		SPAS		AL	
Day	N	Geo Mean	N	Geo Mean	N	GeoMean	p-value
0	143	10.39 (10.16- 10.6)	143	10.35 (10.15- 10.6)	139	10.29 (10.09- 10.5)	0.7659
2	140	2.2 (1.60- 2.80)	140	0.18 (0.02- 0.33)	139	0.25 (0.05- 0.46)	<0.0001
3	136	1.54(0.97- 2.11)	141	0.03 (0- 0.10)	138	0.15 (0- 0.33)	<0.0001
7	126	0.36 (0.07- 0.66)	140	0	137	0	0.0013
14	123	0.93 (0.45- 1.41)	138	0.11 (0- 0.27)	135	0	<0.0001
21	106	1.67 (0.99- 2.36)	129	0.57 (0.199- 0.94)	135	0.85 (0.39- 1.31)	0.0109
28	96	2.99 (2.15- 3.84)	124	1.82 (1.15- 2.49)	125	1.4 (0.80- 2.00)	0.0048

	2006						
Follow-up		SP		SPAS		AL	
Day	N	Geo Mean	N	Geo Mean	N	GeoMean	p-value
0	121	9.06 (8.68- 9.44)	122	9.24 (8.85- 9.63)	118	8.89 (8.47- 9.30)	0.4291
2	119	3.06 (2.44- 3.68)	120	0.51 (0.25- 0.76)	115	0.44 (0.18- 0.70)	<0.0001
3	115	2.04 (1.44-2.64)	119	0.17 (0- 0.35)	114	0.19 (0.02- 0.35)	<0.0001
7	106	0.72 (0.30- 1.13)	117	0.13 (0- 0.25)	113	0.13 (0- 0.28)	0.0077
12	18	4.72 (2.70- 6.75)	10	7.92 (5- 10.83)	3	1.12 (0-5.95)	0.0882
14	97	1.13 (0.60- 1.66)	115	0.34 (0.06- 0.63)	109	0.12 (0- 0.26)	0.0002
21	88	1.55 (0.94- 2.17)	105	1.36 (0.77- 1.95)	108	0.11 (0- 0.28)	<0.0001
28	79	2.56 (1.75- 3.37)	95	1.48 (0.81- 2.14)	104	0.49 (0.15- 0.83)	<0.0001

P-values were calculated using the Wilcoxon Rank Sum test.
GeoMean= geometric mean parasite density.
SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine

Table 5. Mean hemoglobin level, mean rise in hemoglobin, and percent of anemic children by treatment group

2004

		N	Mean Hemoglobin	Mean rise in Hemoglobin	Anemic
Day 14	**SP**	121	9.7 (9.42- 9.98)	0.51 (0.15- 0.87)	64% (56- 72%)
	SPAS	136	10.08 (9.83- 10.34)	0.83 (0.55- 1.11)	52% (44-61%)
	AL	136	9.73 (9.46- 9.99)	0.85 (0.57- 1.12)	55% (47- 64%)
	p-value		*0.0808*	*0.2362*	*0.107*
Day 28	**SP**	16	10.06 (9.05- 11.07)	0.81 (-0.05- 1.66)	38% (11-64%)
	SPAS	4	10.75 (8.64- 12.86)	1.33 (-1.32- 3.97)	25% (-55-105%)
	AL	22	9.99 (9.28- 10.69)	0.74 (-0.29- 1.76)	41% (19-63%)
	p-value		*0.7077*	*0.8657*	*0.842*

2006

		N	Mean Hemoglobin	Mean rise in Hemoglobin	Anemic
Day 14	**SP**	94	9.42 (9.10- 9.74)	0.34 (0.02- 0.66)	73% (65- 81%)
	SPAS	114	9.43 (9.18- 9.68)	0.48 (0.18- 0.77)	64% (55- 73%)
	AL	108	9.65 (9.39- 9.91)	0.7 (0.44- 0.97)	64% (55- 72%)
	p-value		*0.4229*	*0.228*	*0.233*
Day 28	**SP**	80	10.14 (9.81- 10.47)	1.08 (0.65- 1.50)	62% (53- 71%)
	SPAS	92	9.77 (9.49- 10.05)	0.85 (0.48- 1.22)	68% (60- 76%)
	AL	104	10.5 (10.25- 10.76)	1.54 (1.25- 1.84)	41% (32- 50%)
	p-value		***0.001****	***0.0172***†	***<0.0001***≠

This shows the mean hemoglobin and 95% confidence interval for each treatment group as well as the mean rise in hemoglobin and 95% confidence interval from day 0 until day 14 and day 28 for each treatment group. Children were considered anemic if their hemoglobin was <10 mg/dl. The p-value is the groupwise p-value.

* The SPAS and AL groups are significantly different from one another, p- value= 0.0007. None of the other pairwise comparisons reveal statistically significant differences between the groups.

† The SPAS and AL groups are significantly different from one another, p- value= 0.016. None of the other pairwise comparisons reveal statistically significant differences between the groups.

≠ The AL group is significantly different from both the SP and the SPAS groups, p- value= 0.002 and <0.0001, respectively.

SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine

Table 6. Results of Univariate Analysis using a Cox Proportional Hazards Model

2004	HR	95% Hazard Ratio CL		p-value
SPAS	**0.45**	**0.30**	**0.69**	**0.000**
AL	**0.36**	**0.23**	**0.56**	**<0.001**
District (Rufiji vs. Ulanga)	**1.76**	**1.22**	**2.55**	**0.003**
Bednet ownership	0.76	0.53	1.09	0.138
Age- continuous (months)	1.01	0.99	1.02	0.477
Age (categorical)- 12-23 mo	**2.07**	**1.14**	**3.75**	**0.016**
Age (categorical)- 24-59 mo	**1.86**	**1.06**	**3.25**	**0.031**
Weight (kg)	1.02	0.95	1.10	0.579
Gender (female vs. male)	1.12	0.78	1.60	0.538
Temperature	1.08	0.89	1.32	0.440
Parasite density (continuous)	1.06	0.91	1.23	0.488
Parasite density (categorical)	1.17	0.81	1.68	0.408
Use of cotrimoxazole	0.94	0.48	1.86	0.867

2006	HR	95% Hazard Ratio CL		p-value
SPAS	**0.64**	**0.43**	**0.94**	**0.024**
AL	**0.22**	**0.12**	**0.38**	**<0.001**
District (Rufiji vs. Ulanga)	1.39	0.97	2.00	0.076
Bednet ownership	0.79	0.46	1.36	0.400
Age- continuous (months)	1.01	1.00	1.02	0.080
Age (categorical)- 12-23 mo	1.77	0.83	3.76	0.137
Age (categorical)- 24-59 mo	**2.12**	**1.10**	**4.08**	**0.024**
Weight (kg)	1.04	0.97	1.11	0.263
Gender (female vs. male)	1.05	0.73	1.50	0.806
Temperature	0.96	0.74	1.25	0.747
Parasite density (continuous)	1.03	0.96	1.10	0.466
Parasite density (categorical)	**6.47**	**3.95**	**10.6**	**<0.0001**
Use of cotrimoxazole	**-**	**-**	**-**	

SPAS= Sulfadoxine- Pyrimethamine + Artesunate, compared to the reference, SP
AL= Artemether- Lumefantrine, compared to the reference, SP
Parasite density as a categorical variable was divided into 2 groups: those with initial parasitemia < 10,000 parasites/ μl, and those with ≥ 10,000 parasites/ μl.

Table 7. Results of Multivariate Analysis using a Cox Proportional Hazards Model for 2004, showing the effect of treatment- district interaction.

	HR	95% Hazard Ratio CL		p-value
SPAS, Ulanga	**0.19**	**0.08**	**0.44**	**<.0001**
SPAS, Rufiji	0.85	0.47	1.52	0.579
AL, Ulanga	**0.30**	**0.15**	**0.62**	**0.001**
AL, Rufiji	**0.53**	**0.28**	**0.995**	**0.048**
Bednet	1.12	0.74	1.72	0.587
Age (13-23 mo vs 6-12mo)	**2.04**	**1.12**	**3.71**	**0.019**
Age (24-59 mo vs 6-12mo)	**1.92**	**1.09**	**3.38**	**0.023**

Table 8. Results of Multivariate Analysis for 2006 using a Cox Proportional Hazards Model.

	HR	95% Hazard Ratio CL		p-value
SPAS vs SP	**0.55**	**0.37**	**0.82**	**0.003**
AL vs SP	**0.22**	**0.13**	**0.39**	**<.0001**
District (Rufiji vs Kilombero)	**2.37**	**1.55**	**3.64**	**<.0001**
Bednet ownership	0.82	0.47	1.45	0.500
Age (13-23 mo vs 6-12mo)	1.75	0.82	3.76	0.148
Age (24-59 mo vs 6-12mo)	**2.32**	**1.20**	**4.49**	**0.012**
Hyperparasitemia (>10,000/ µl)	**9.30**	**5.15**	**16.80**	**<.0001**

SPAS= Sulfadoxine- Pyrimethamine + Artesunate, compared to the reference, SP
AL= Artemether- Lumefantrine, compared to the reference, SP

Table 9. Percentage of patients who owned a bed-net among those with and without new infections during the course follow-up by treatment group (2004)

	SP	SPAS	AL
N	**139**	**141**	**138**
New Infection	**38%**	**26%**	**47%**
	6/ 16	5/ 19	9/ 19
Not infected	**56%**	**56%**	**56%**
	69/123	68/ 122	67/ 119
p- value	***0.16***	***0.017***	***0.47***

New infections during the course of follow-up were less likely among patients who owned a bednet than among those who did not, Mantel-Haenszel OR = 0.461 (0.255, 0.832). This effect did not differ by treatment arm (Breslow-Day Test p-value = 0.47).

SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine

Table 10. Side effects reported by treatment group in 2004

Fever	SP	SPAS	AL	Total	p- value
Day 0	78/ 78 (100%)	71/ 73 (97%)	72/ 72 (100%)	221/ 223	0.209*
Day 1	44/ 142 (31%)	50/ 142 (35%)	49/ 139 (35%)	143/ 423	0.684
Day 2	49/ 140 (35%)	3/ 140 (2%)	5/ 139 (4%)	57/ 419	<.0001
Day 3	7/ 136 (5%)	0/ 141	3/ 138 (2%)	10/ 415	0.020
Day 7	4/ 126 (3%)	3/ 140 (2%)	1/ 137 (0.7%)	8/ 403	0.360
Day 14	8/ 123 (7%)	2/ 138 (1.5%)	3/ 135 (2%)	13/ 396	0.051
Day 21	5/ 106 (5%)	6/ 129 (5%)	9/ 135 (7%)	20/ 370	0.718
Day 28	9/ 96 9%)	9/ 124 (7%)	12/ 125 (10%)	30/ 345	0.776
Total	215/ 961 (22%)	153/ 1040 (15%)	159/ 1028 (15%)	527/ 3029	<0.0001
Vomiting					
Day 0	70/ 143 (49%)	70/143 (49%)	64/ 139 (46%)	204/425	0.854
Day 1	11/ 142 (8%)	12/ 142 (8%)	7/139 (5%)	30/ 423	0.501
Day 2	17/ 140 (12%)	2/ 140 (1%)	5/ 139 (4%)	24/ 419	<0.001
Day 3	3/ 136 (2%)	0/ 141 (0%)	0/ 138 (0%)	3/ 415	0.035*
Day 7	0/126 (0%)	2/ 140 (1.4%)	0/137 (0%)	2/ 403	0.151
Day 14	1/ 123 (0.8%)	1/ 138 (0.7%)	1/ 135 (0.7%)	3/ 396	0.996
Day 21	2/ 106 (2%)	2/ 129 (1.6%)	2/ 135 (1.5%)	6/ 370	0.967
Day 28	2/ 96 (2%)	3/ 124 (2%)	2/ 125 (1.6%)	7/ 345	0.899
Total	106/ 1012 (10%)	92/ 1097 (8%)	81/ 1087 (7%)	279/ 3196	0.044
Diarrhea					
Day 0	16/143 (11%)	(11/143 (8%)	13/ 139 (9%)	40/ 425	0.599
Day 1	4/ 142 (3%)	5/ 142 (4%)	5/ 139 (4%)	14/ 423	0.945*
Day 2	6/ 140 (4%)	2/ 140 (1%)	2/ 139 (1%)	10/ 419	0.319*
Day 3	4/ 136 (3%)	0/ 141	1/ 138 (1%)	5/ 415	0.031*
Day 7	1/ 126 (0.8%)	3/ 140 (2%)	2/ 137 (1.5%)	6/ 403	0.875*
Day 14	2/123 (2%)	0/ 138	5/ 135 (4%)	7/ 396	0.043*
Day 21	0/ 106 (0%)	0/ 129 (0%)	2/ 135 (1.5%)	2/ 370	0.335*
Day 28	1/ 96 (1%)	0/ 124 (0%)	2/ 125 (1.6%)	3/ 345	0.499*
Total	34/ 1012 (3%)	21/ 1097 (2%)	32/ 1087 (3%)	87/ 3196	0.108
Rash					
Day 0	3/ 143 (2%)	1/ 143 (1%)	2/ 139 (1%)	6/ 425	0.704*
Day 1	2/ 142 (1%)	0/ 142 (0%)	1/139 (1%)	3/ 423	0.549*
Day 2	0/ 140 (0%)	1/ 140 (1%)	1/139 (1%)	2/ 419	0.776*
Day 3	0/ 136 (0%)	1/ 141 (1%)	1/ 138 (1%)	2/ 415	1*
Day 7	0/ 126 (0%)	2/ 140 (1.4%)	2/ 137 (1.5%)	4/ 403	0.554*
Day 14	1/ 123 (0.8%)	0/ 138 (0%)	0/ 135 (0%)	1/ 396	0.311*
Day 21	1/ 106 (0.9%)	2/ 129 (1.6%)	1/ 135 (0.7%)	4/ 370	0.839*
Day 28	0/ 96 (0%)	0/ 124 (0%)	0/ 125 (0%)	0/ 345	
Total	7/ 1012 (0.7%)	7/ 1097 (0.6%)	8/ 1087 (0.7%)	22/ 3196	0.962

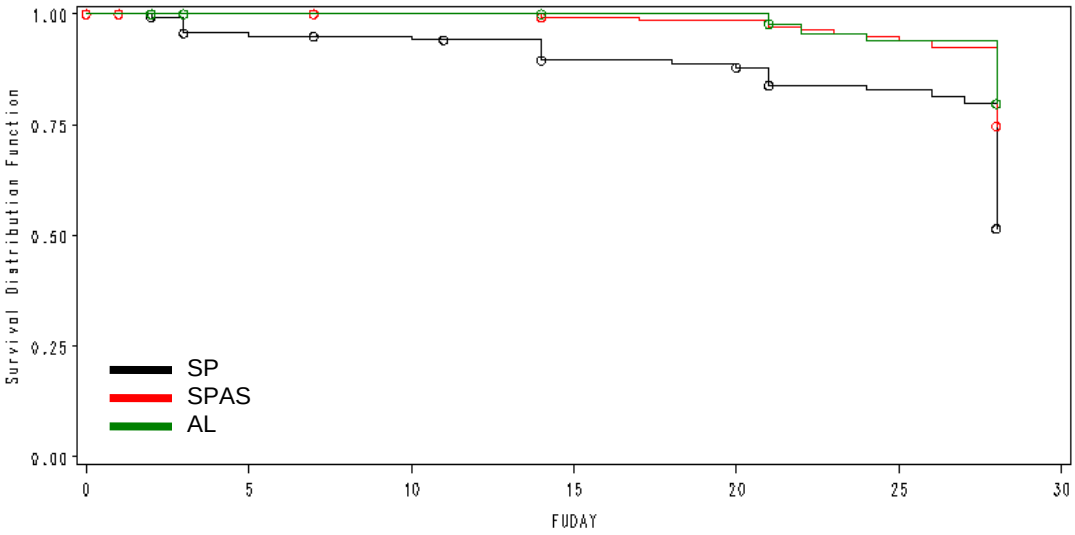
* Fisher exact test was used to calculate the p-value
SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine

Table 11. Side effects reported by treatment group in 2006

Fever	SP	SPAS	AL	Total	p- value
Day 0	121/121 (100%)	120/ 121 (99%)	117/ 118 (99%)	358/ 360	0.7734*
Day 1	52/ 119 (44%)	48/119 (40%)	58/ 117 (50%)	158/ 355	0.3525
Day 2	61/ 120 (51%)	6/ 120 (5%)	10/ 115 (9%)	77/ 355	<0.0001
Day 3	16/ 115 (14%)	2/ 119 (2%)	4/ 114 (4%)	22/ 348	<0.0001
Day 7	9/ 106 (8%)	5/ 117 (4%)	3/ 113 (3%)	17/ 336	0.128
Day 14	27/ 113 (24%)	12/ 121 (10%)	10/ 115 (9%)	49/ 349	0.001
Day 21	10/ 88 (11%)	10/ 105 (10%)	6/ 108 (6%)	26/ 301	0.3274
Day 28	12/ 80 (15%)	12/96 (13%)	5/ 104 (5%)	29/ 280	0.0555
Total	308/ 862 (36%)	215/ 918 (23%)	213/ 904 (24%)	736/ 2684	<0.0001
Vomiting					
Day 0	42/ 119 (35%)	34/ 121 (28%)	34/ 118 (29%)	248/ 358	0.4144
Day 1	11/ 118 (9%)	10/ 120 (8%)	6/ 117 (5%)	27/ 355	0.4478
Day 2	15/ 120 (13%)	1/ 120 (1%)	1/ 115 (1%)	17/ 355	<.0001
Day 3	4/ 111 (3.5%)	1/ 119 (1%)	1/ 114 (1%)	6/ 348	0.2102
Day 7	0/ 105	0/ 117	1/ 113 (1%)	1/ 335	0.3733
Day 14	8/ 112 (7%)	2/ 122 (2%)	2/ 116 (2%)	12/ 350	0.004*
Day 21	3/ 90 (3%)	3/ 105 (3%)	0/ 109	6/ 304	0.1757
Day 28	3/ 80 (4%)	2/ 96 (2%)	0/ 104	5/ 280	0.1573
Total	86/ 859 (10%)	53/ 920 (6%)	45/ 906 (5%)	184/ 2685	<.0001
Diarrhea					
Day 0	5/ 120 (4%)	6/ 121 (5%)	14/ 118 (12%)	25/ 359	0.0374
Day 1	1/ 119 (0.8%)	1/ 120 (0.8%)	1/ 117 (0.9%)	3/ 356	0.9998
Day 2	1/ 120 (0.8%)	0/ 120	2/ 115 (2%)	3/ 355	0.3465
Day 3	1/ 114 (0.9%)	0/ 119	1/ 114 (0.9%)	2/ 348	0.5929
Day 7	1/ 106 (0.9%)	2/ 117 (2%)	1/ 113 (0.9%)	4/ 336	0.8136
Day 14	2/ 113 (2%)	2/ 121 (2%)	1/ 112 (0.9%)	5/ 346	0.8352
Day 21	1/ 89 (1%)	2/ 105 (2%)	0/ 108	3/ 302	0.3705
Day 28	1/ 80 (1%)	1/ 95 (1%)	0/ 105	2/ 280	0.54
Total	13/ 862 (2%)	14/ 918 (2%)	20/ 902 (2%)	47/ 2682	0.426
Rash					
Day 0	0/ 120	3/ 121 (2%)	1/ 118 (0.9%)	4/ 359	0.176
Day 1	0/ 119	2/ 120 (2%)	1/ 117 (0.9%)	3/ 356	0.3704
Day 2	0/ 120	2/ 120 (2%)	0/ 115	2/ 355	0.1395
Day 3	0/ 115	2/ 119 (2%)	0/ 114	2/ 348	0.1444
Day 7	0/ 107	0/ 117	0/ 113	0/ 337	
Day 14	0/ 113	1/ 121 (0.8%)	0/ 112	1/ 346	0.3936
Day 21	0/ 88	0/ 105	0/ 108	0/ 301	
Day 28	0/ 80	0/ 96	1/ 105 (1%)	1/ 281	0.4312
Total	0/ 862	10/ 919 (1%)	3/ 902 (0.3%)	13/ 2683	0.0031

* Fisher exact test was used to calculate the p-value
SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine

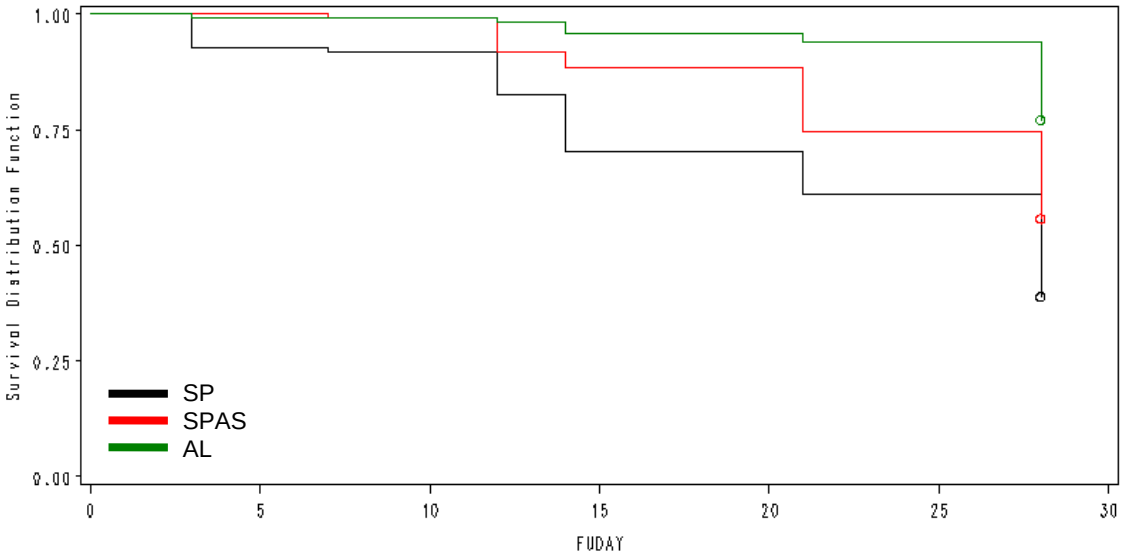
Figure 1. 2004 Uncorrected failure rates stratified by treatment group



This figure shows uncorrected survival curves for the three different treatment arms in 2004. The uncorrected cure rate was significantly lower in the SP arm than in either the SPAS or AL arm, with cure rates of 58%, 76%, and 81% (Log Rank test p- value <0.0001). There was a significant difference between SP and each of the other drugs (p-value <0.0001 for both). There was not a statistically significant difference between SPAS and AL (p-value= 0.33). Similar trends were seen when results were corrected by genotyping, with cure rates of 71%, 91%, and 94%, respectively (Log Rank test p- value <0.0001, data not shown).

SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine

Figure 2. 2006 Uncorrected failure rates stratified by treatment group



This figure shows uncorrected survival curves for the three different treatment arms in 2006. The uncorrected cure rates were 51%, 64%, and 86% in the SP, SPAS, and AL arms, respectively (Log Rank test p- value <0.0001). There was a significant difference (p-value <0.0001) between AL and SP and between AL and SPAS. The difference between SP and SPAS was of borderline significance following Bonferroni correction (p-value = 0.016).

SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine

Figure 3. Parasite clearance by treatment arm in 2004

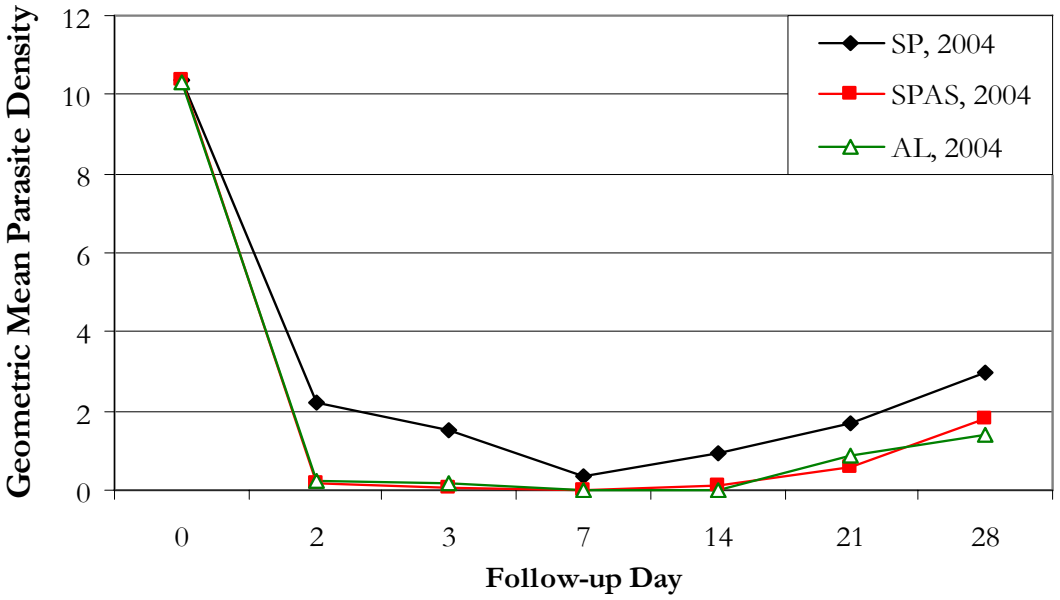
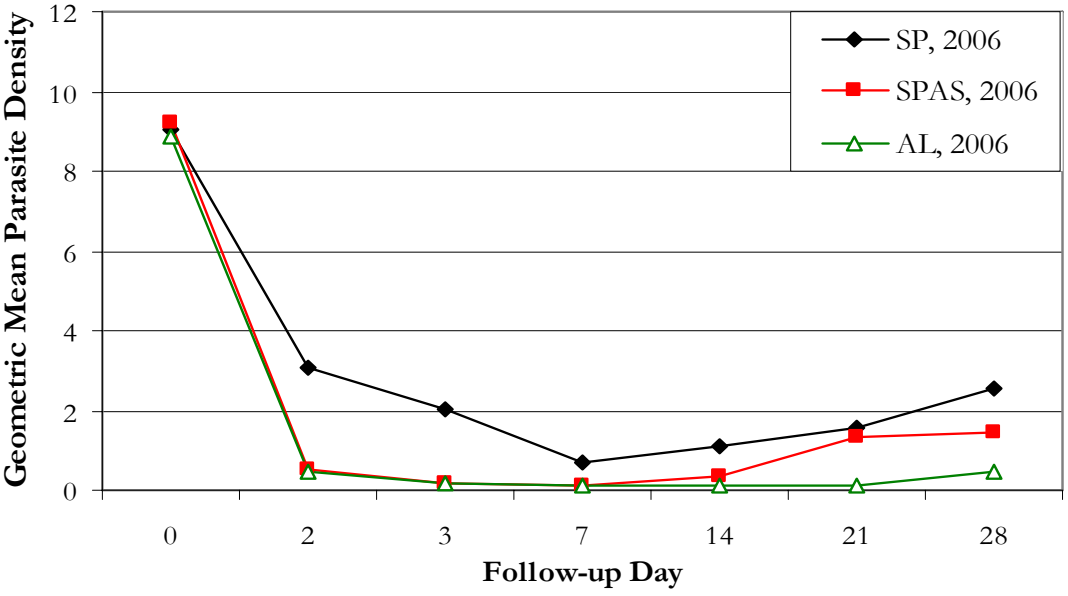


Figure 4. Parasite clearance by treatment arm in 2006



SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine

Figure 5. Fever clearance by treatment arm, 2004

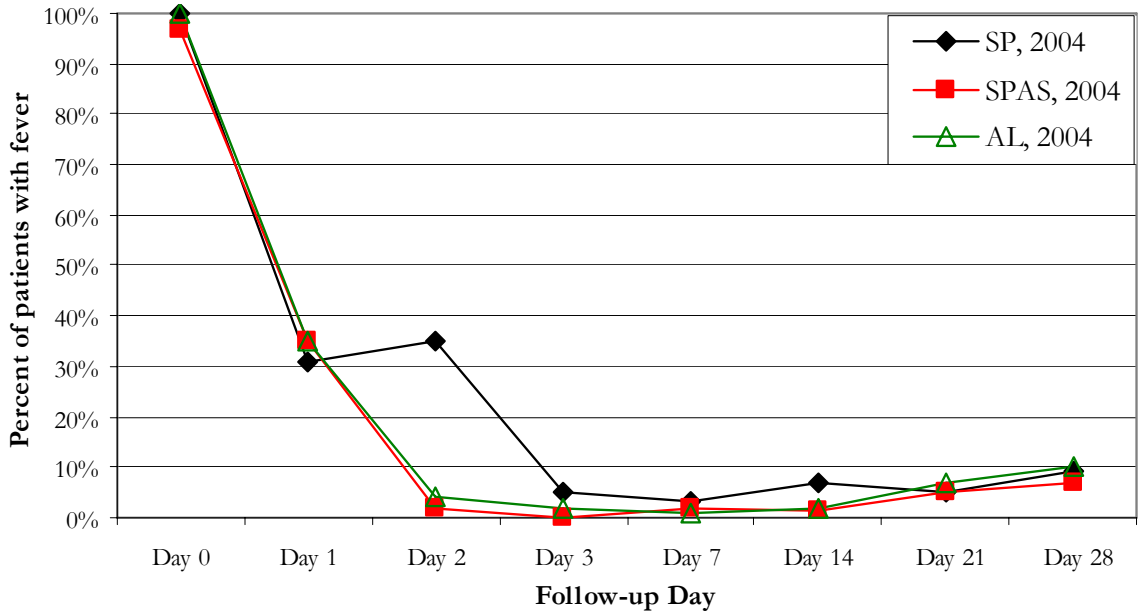
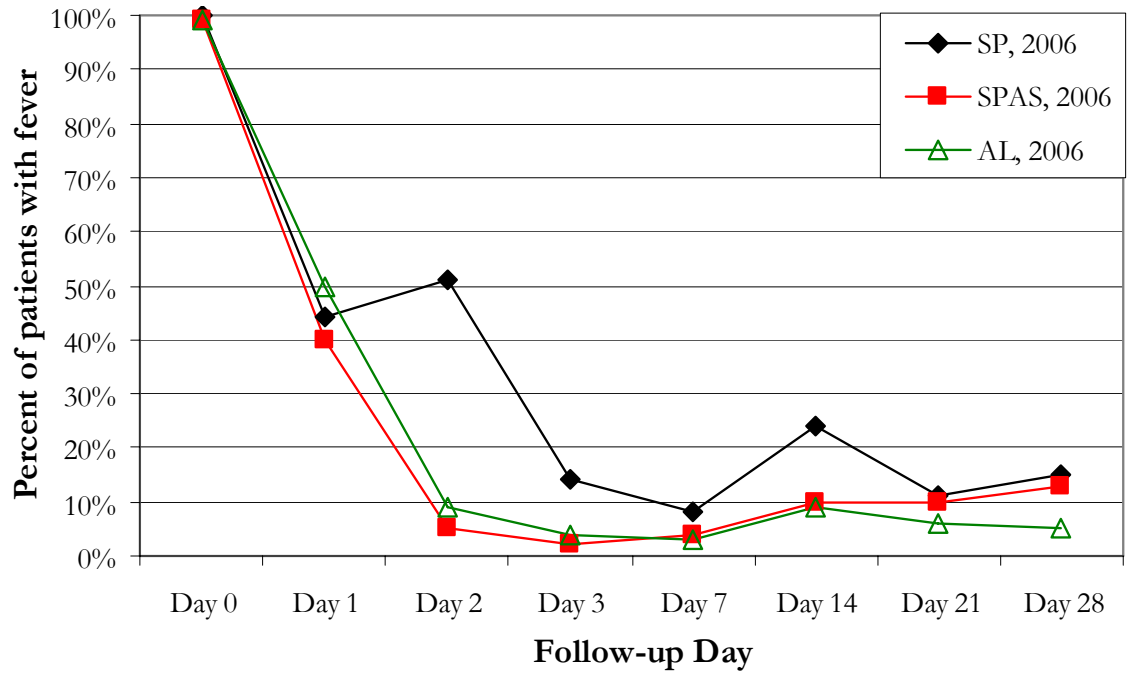


Figure 6. Fever clearance by treatment arm, 2006



SP= Sulfadoxine- Pyrimethamine
SPAS= Sulfadoxine- Pyrimethamine + Artesunate
AL= Artemether- Lumefantrine

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