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Return of Chloroquine Antimalarial Efficacy in Malawi

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ABSTRACT

BACKGROUND

In 1993, Malawi became the first country in Africa to replace chloroquine with the combination of sulfadoxine and pyrimethamine for the treatment of malaria. At that time, the clinical efficacy of chloroquine was less than 50%. The molecular marker of chloroquine-resistant *falciparum* malaria subsequently declined in prevalence and was undetectable by 2001, suggesting that chloroquine might once again be effective in Malawi.

METHODS

We conducted a randomized clinical trial involving 210 children with uncomplicated *Plasmodium falciparum* malaria in Blantyre, Malawi. The children were treated with either chloroquine or sulfadoxine–pyrimethamine and followed for 28 days to assess the antimalarial efficacy of the drug.

RESULTS

In analyses conducted according to the study protocol, treatment failure occurred in 1 of 80 participants assigned to chloroquine, as compared with 71 of 87 participants assigned to sulfadoxine–pyrimethamine. The cumulative efficacy of chloroquine was 99% (95% confidence interval [CI], 93 to 100), and the efficacy of sulfadoxine–pyrimethamine was 21% (95% CI, 13 to 30). Among children treated with chloroquine, the mean time to parasite clearance was 2.6 days (95% CI, 2.5 to 2.8) and the mean time to the resolution of fever was 10.3 hours (95% CI, 8.1 to 12.6). No unexpected adverse events related to the study drugs occurred.

CONCLUSIONS

Chloroquine is again an efficacious treatment for malaria, 12 years after it was withdrawn from use in Malawi. (ClinicalTrials.gov number, NCT00125489.)

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MALARIA CONTINUES TO BE A LEADING killer of the world's poorest children. Six decades after chloroquine was widely deployed in a global program to eradicate malaria, *Plasmodium falciparum* continues to plague most of sub-Saharan Africa.¹ Chloroquine resistance first emerged in Southeast Asia and South America in the late 1950s, and by the late 1970s, it had made its way to the African continent, where it contributed to increased transmission of malaria and deaths.²

In 1993, in response to high rates of treatment failure with chloroquine, Malawi became the first country in sub-Saharan Africa to replace chloroquine with the combination of sulfadoxine and pyrimethamine for the treatment of falciparum malaria.³ Since then, sulfadoxine–pyrimethamine has been the only available treatment for uncomplicated malaria in government health facilities. Although chloroquine remained available on a limited basis, a national information campaign was largely successful in persuading health practitioners and the public to replace it with sulfadoxine–pyrimethamine as first-line treatment for malaria.

It has been suggested that reducing the use of chloroquine in a region could result in the reemergence of chloroquine-sensitive *P. falciparum*, thus permitting the reintroduction of this safe and affordable drug. Decreases in the effective concentrations of chloroquine required to inhibit the growth of the parasite in vitro have been reported after the use of chloroquine was reduced in Asia and West Africa, but a return of its clinical efficacy for the treatment of falciparum malaria has not been demonstrated.⁴⁻⁷

A point mutation in the *P. falciparum* chloroquine-resistance transporter gene (*PfCRT*) is associated with chloroquine-resistant falciparum malaria.⁸ We previously measured the prevalence of this molecular marker of chloroquine resistance in Blantyre, Malawi, before, during, and after the withdrawal of chloroquine from use. From 1992 to 2000, the prevalence of the marker gradually decreased, disappearing completely by 2001.⁹ In contrast, in neighboring countries where chloroquine continued to be used, more than 90% of *P. falciparum* infections were caused by chloroquine-resistant parasites.^{9,10}

Small, uncontrolled studies found that in Malawi, low-level, asymptomatic *P. falciparum* infections cleared after treatment with chloroquine.^{9,11}

These studies did not assess the efficacy of chloroquine in patients with clinical malaria, and some of these asymptomatic infections might have cleared without treatment. To determine whether chloroquine could again be used to treat Malawian children for uncomplicated falciparum malaria safely and effectively, we conducted a clinical trial of chloroquine, as compared with standard antimalarial therapy.

METHODS

STUDY POPULATION

The research clinic of the Blantyre Malaria Project is adjacent to the Ndirande District Health Centre, which serves a population of 200,000 in a township on the outskirts of Blantyre, Malawi. Children brought to the health center for an illness suggestive of malaria and whose blood smears were positive for malaria parasites were screened for eligibility. Children 6 months to 12 years of age with symptoms of malaria were eligible for the study if they had *P. falciparum* mono-infection with a parasite level in the peripheral blood of 2000 to 200,000 per cubic millimeter. Children were excluded if they had signs of severe malaria, had an allergy to a study drug, had an acute nonmalarial infection, or were receiving daily trimethoprim–sulfamethoxazole.

The research and ethics committee of the University of Malawi College of Medicine was the primary review board that approved the study. The institutional review boards of the University of Maryland School of Medicine and Michigan State University also reviewed and approved the study. Written informed consent was obtained from parents or guardians and assent was obtained from children 6 years of age or older before the initiation of study procedures.

INITIAL EVALUATION

A full physical examination was performed to rule out concurrent bacterial infections that would interfere with the assessment of antimalarial efficacy of the drug. Reports of prior use of antimalarial medications were recorded but were not an exclusion criterion. Blood smears for malaria were made and hemoglobin levels were measured from finger-prick blood samples. Blood smears for malaria were read separately by two microscopists and adjudicated by a third expert reader if the second count differed from the first by more than

25% or if the readings led to a conflict in eligibility or the classification of the treatment outcome. Thin smears of peripheral blood obtained on enrollment were reviewed to confirm the presence of *P. falciparum* mono-infection.

RANDOMIZATION

Treatment was randomly assigned at enrollment according to study identification numbers, and sealed envelopes containing treatment-allocation cards were opened immediately before the administration of the study drug. The study was not conducted in a blinded fashion.

STUDY INTERVENTION

Two hundred ten children were assigned to receive either chloroquine sulfate (Nivaquine syrup, Aventis Pharma, at a dose of 10 mg of chloroquine base per kilogram of body weight on days 0 and 1, and 5 mg per kilogram on day 2) or the combination of sulfadoxine and pyrimethamine (Fansidar tablets, Roche, at a dose of 1.25 mg of sulfadoxine per kilogram and 25 mg of pyrimethamine per kilogram on day 0). Study drugs were purchased from commercial sources. The drugs were administered under direct observation by study nurses. If the participant vomited within 30 minutes after receiving the drug, the drug was administered again. Children who vomited the study medication twice were withdrawn from the study and transferred to Queen Elizabeth Central Hospital in Blantyre for further care.

FOLLOW-UP

Outcomes were measured with the use of a standard protocol for assessing antimalarial treatment.¹² The day of enrollment was considered study day 0, and follow-up visits were scheduled on study days 1, 2, 3, 7, 14, 21, and 28. Unscheduled follow-up visits were encouraged at any time between scheduled visits when symptoms developed. Blood was obtained for blood smears for malaria and hemoglobin measurements at each visit except that on day 1.

Study staff visited the homes of participants who missed scheduled visits to remind them or their parents or guardians that they should return for follow-up or to ascertain the reason for their withdrawal from the study. Participants who missed scheduled visits on day 1, 2, or 3, or who were not seen within 24 hours after the visit

scheduled for day 7, 14, 21, or 28, were considered lost to follow-up.

Because this was the first study of chloroquine efficacy in children with malarial illness in Malawi since the withdrawal from use of this drug in 1993 because of unacceptably poor efficacy, the first 60 participants enrolled (30 participants assigned to the chloroquine group and 30 to the sulfadoxine-pyrimethamine group) were admitted to the research clinic for continuous observation. Physical examinations were performed, blood smears were examined, and hemoglobin levels were determined every 12 hours and vital signs were recorded every 6 hours until two consecutive 12-hour blood smears for malaria were negative and the participant had been afebrile for 12 hours. After discharge from the observation unit, follow-up continued on an outpatient basis. A safety monitoring committee reviewed the results of treatment of the first 60 participants and judged that it was safe to continue the study with outpatient follow-up only.

OUTCOME MEASURES

Outcomes were assessed according to the recommendations of the World Health Organization.¹² The primary end point of the study was an adequate clinical and parasitologic response, defined as the absence of parasites on day 28 in participants in whom treatment failure had not occurred. Treatment failure was classified as early treatment failure, late clinical failure, or late parasitologic failure. Early treatment failure was defined by the occurrence of one of the following: signs of severe disease or danger signs (obtundation, seizures, respiratory distress, prostration, bleeding, inability to drink, or persistent vomiting) on day 1, 2, or 3 with parasitemia; a level of parasitemia on day 2 that exceeded the level on day 0; an axillary temperature of 37.5°C or higher on day 3 in the presence of parasitemia; or a level of parasitemia on day 3 that was at least 25% of the level at the time of enrollment. Among participants who did not have early treatment failure, late clinical failure was defined by the occurrence of one of the following during days 4 to 28: danger signs, severe malaria, or an axillary temperature of 37.5°C or higher in the presence of parasitemia. Among participants who had parasitemia at day 28 but never had signs of severe disease or fever, the outcome was classified as late parasitologic

failure. Participants who had no response to treatment received rescue therapy with halofantrine (Halfan, GlaxoSmithKline) and were followed to ascertain that the parasitemia resolved. The microscopists who read the blood smears were unaware of the participants' treatment assignments.

MOLECULAR METHODS

Drops of blood were collected on filter papers each time blood was obtained for blood smears.¹³ After DNA was extracted from dried filter papers, we used a nested polymerase-chain-reaction (PCR) assay followed by mutation-specific restriction-endonuclease digestion to detect the molecular marker for chloroquine resistance, which is a single-nucleotide mutation encoding a change from lysine to threonine at codon 76 in *PfCRT*.⁸ Selected samples were subjected to DNA sequencing of the region of *PfCRT* surrounding codon 76. Samples were coded before sequencing so that laboratory personnel were unaware of the clinical outcomes (described in detail in the Supplementary Appendix, available with the full text of this article at www.nejm.org).

STATISTICAL ANALYSIS

The study was designed to have a statistical power of 90% to detect a difference in efficacy of at least 20% between the chloroquine group and the sulfadoxine-pyrimethamine group that was significant at the 5% level. The data were analyzed with the use of Stata software (version 8.0). Cumulative efficacy at 28 days was calculated with the use of data on all participants until they exited the study, including those in whom treatment failed or who were later lost to follow-up. Rates of cumulative efficacy were compared with the use of the proportional-hazards model. Parasite-clearance time

was defined as the time to the first blood smear negative for malaria. The time to the resolution of fever was defined as the time to the first continuous 24-hour period in which a child's temperature was less than 37.5°C and was determined only for those who had a fever within the first 24 hours among the 60 participants under continuous observation. The incidence of adverse events in the two groups was compared by Fisher's exact test.

RESULTS

From May through November 2005, 3406 children were screened for malaria, of whom 324 were found to have parasitemia within the eligible range and 215 were evaluated for eligibility for the study. Of these 215 children, 210 were enrolled in the study. Follow-up continued through December 2005. On enrollment, the characteristics of the participants in the two study groups were similar (Table 1). Eighty participants in the chloroquine group and 87 in the sulfadoxine-pyrimethamine group completed the study (Fig. 1).

CLINICAL OUTCOMES

Only one treatment failure occurred in the chloroquine group, in a participant whose parasitemia level 2 days after enrollment was higher than at enrollment (8880 vs. 6480 parasites per cubic millimeter). In the sulfadoxine-pyrimethamine group, treatment failure occurred in 71 participants: 21 episodes of early treatment failure, 32 episodes of late clinical failure, and 18 episodes of late parasitologic failure. All cases of treatment failure resolved after the administration of rescue therapy. The 28-day cumulative efficacy of chloroquine was 99% (95% confidence interval [CI], 93 to 100), as compared with 21% (95% CI, 13 to 30) for sul-

Table 1. Characteristics of the 210 Participants at Enrollment.*

Characteristic	Chloroquine Group (N=105)	Sulfadoxine-Pyrimethamine Group (N=105)
Age — yr	2.6±2.2	2.9±2.2
Female sex — no. (%)	57 (54)	51 (49)
Mean hemoglobin — g/dl	9.5±1.9	9.2±1.9
Geometric mean parasitemia — no. of parasites/mm ³ (95% CI)	19,379 (15,163–24,768)	18,856 (12,245–23,322)
Reported use of antimalarial drug during preceding week — no. (%)	13 (12)	13 (12)

* Plus-minus values are means ±SD.

fadoxine–pyrimethamine ($P < 0.001$) (Fig. 2A). As part of a sensitivity analysis, we reclassified all unknown outcomes as treatment failure. According to this worst-case scenario, the 28-day cumulative efficacy of chloroquine was 80% (95% CI, 70 to 86).

The mean time to parasite clearance in the chloroquine group was 2.6 days (95% CI, 2.5 to 2.8) (Fig. 2B), and the mean time to the resolution of fever was 10.3 hours (95% CI, 8.1 to 12.6) (Fig. 2C). The mean times to parasite clearance and defervescence could not be calculated accurately for sulfadoxine–pyrimethamine because of the high rates of early withdrawal in this group to receive rescue therapy.

ADVERSE EVENTS

With regard to serious adverse events, four participants required hospitalization during the study: two children vomited after two attempts to administer chloroquine and required parenteral quinine; cervical adenitis developed in one child who had received chloroquine and was treated with intravenous antibiotics and surgical drainage; and one child who had received sulfadoxine–pyrimethamine had a febrile seizure on study day 1. The condition of another child who received sulfadoxine–pyrimethamine met the criteria for severe malaria (hemoglobin level, < 5 g per deciliter), but the child was in clinically stable condition and did not require hospitalization. All five participants made complete and uneventful recoveries. Table 2 summarizes the adverse events.

MOLECULAR MARKER FOR CHLOROQUINE RESISTANCE

Assays for the T76 molecular marker for chloroquine resistance in *PfCRT* were successfully performed on 199 of the 210 filter-paper samples obtained from participants at enrollment. All samples, including those from the one participant who had treatment failure 2 days after chloroquine treatment was started and the two participants who were admitted to the hospital after vomiting chloroquine, had the wild-type K76 *PfCRT* genotype associated with chloroquine susceptibility. DNA sequencing confirmed that the T76 mutation in *PfCRT* was absent from samples obtained before and after treatment from the one participant who had chloroquine treatment failure and in four other random samples obtained before treatment.

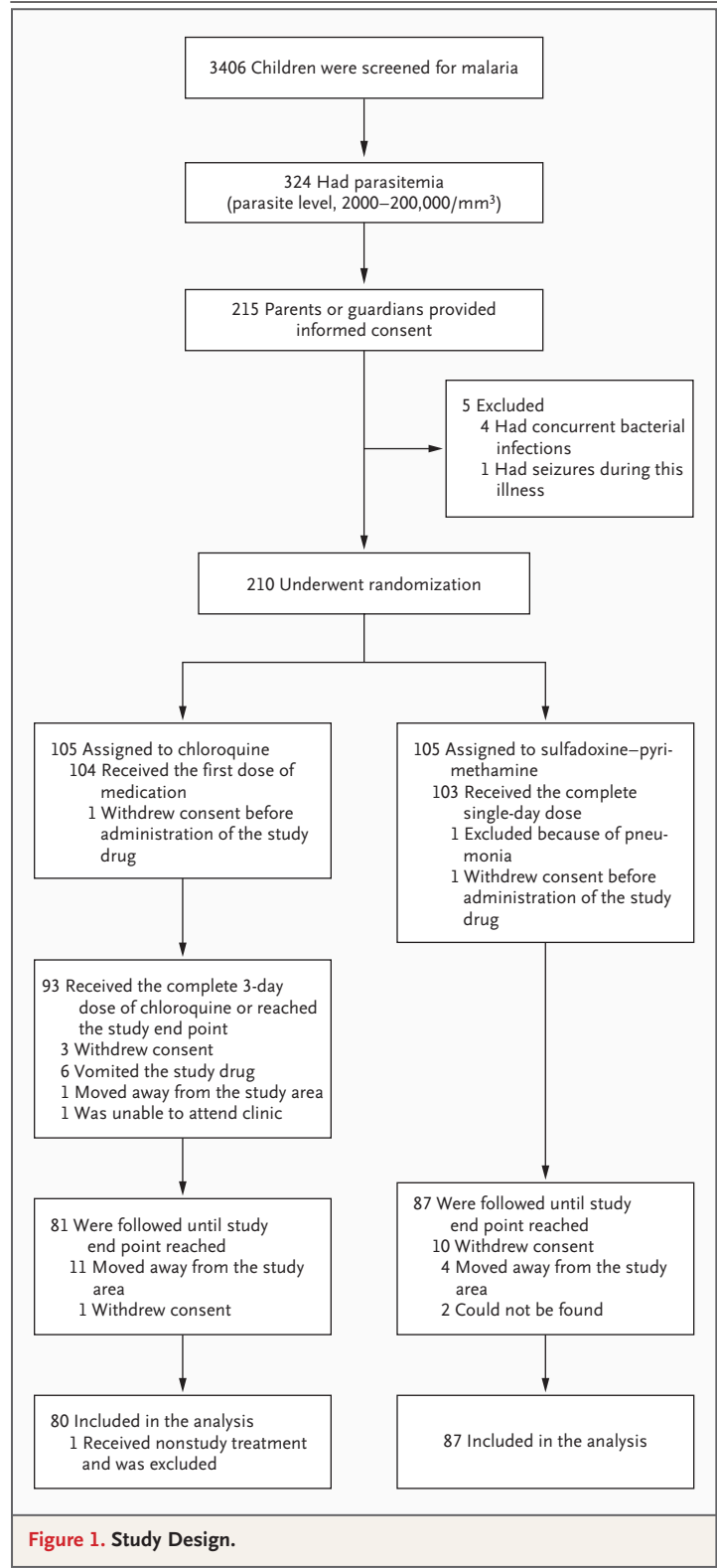
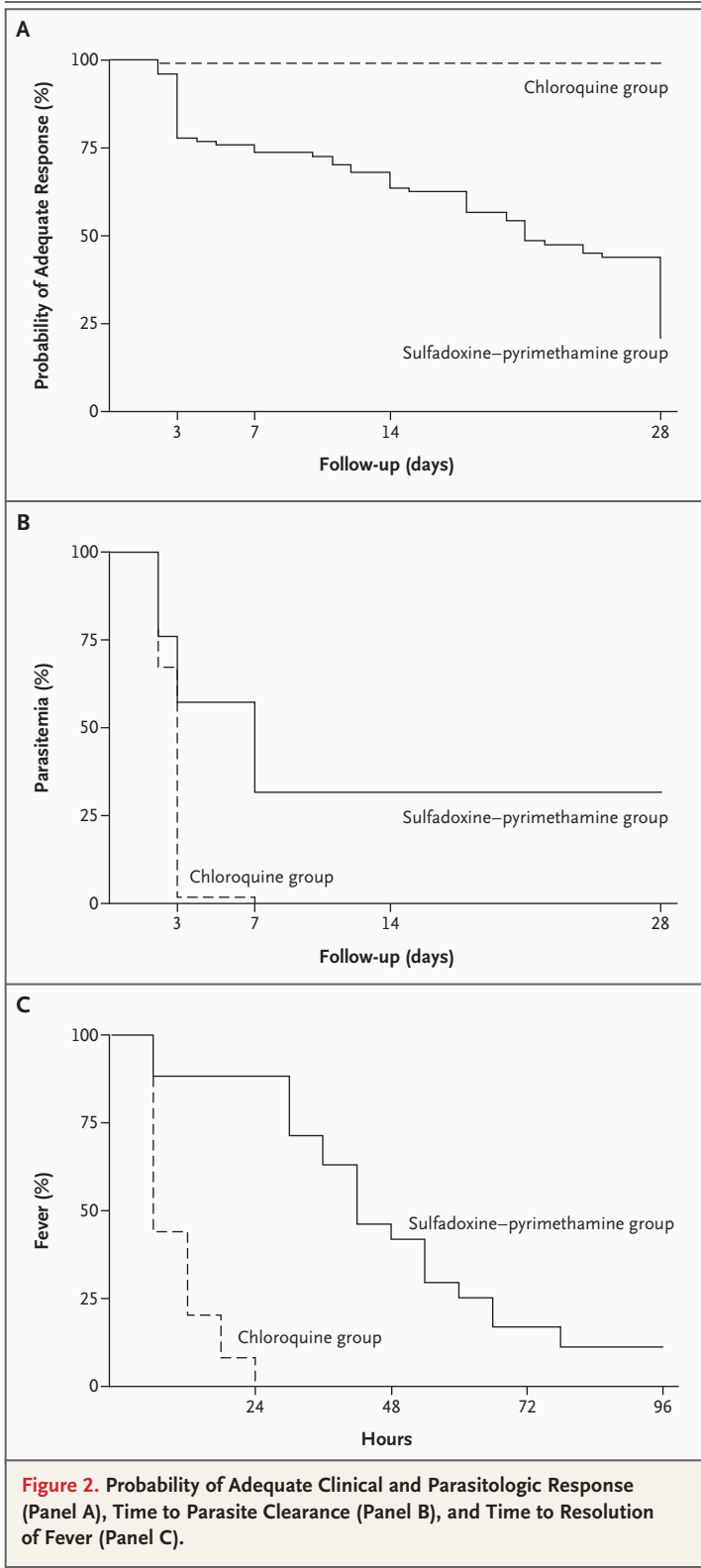


Figure 1. Study Design.

DISCUSSION



Chloroquine, a safe and inexpensive treatment for malaria, is once again highly efficacious in Malawi, 12 years after it was withdrawn from use because of rates of treatment failure of more than 50%.³ In this study, treatment failure occurred in only 1 child among 105 participants assigned to receive chloroquine for uncomplicated falciparum malaria. Infection and fever cleared more quickly in the chloroquine group than in the sulfadoxine-pyrimethamine group. Vomiting was more frequent in the chloroquine group, but only two children ultimately required parenteral therapy. Malaria parasites that are clinically susceptible to chloroquine have returned to Malawi, as predicted by molecular surveys.^{9,11}

This rapid shift in the parasite population is most likely to be explained by a fitness cost incurred as a result of drug resistance: the survival advantage enjoyed by chloroquine-resistant parasites in the presence of the drug appears to be lost when chloroquine is absent.¹⁴⁻¹⁶ Chloroquine resistance did not disappear as rapidly or completely when its use was curtailed in Asia and South America.^{7,17-19} Greater success in reducing chloroquine use in Malawi may explain the more rapid decline in chloroquine resistance there than in Asia and South America. In a survey of more than 1000 children conducted in 2000 in the Malawian community where this study was conducted, information on chloroquine use was specifically sought, but no use was reported.²⁰ However, in early 2006, chloroquine could still be purchased in local pharmacies, where it is reportedly bought mainly by travelers for use as prophylaxis against malaria and by Malawian adults who use it to treat symptoms of malaria. Thus, the complete elimination of chloroquine use does not appear to be required for the number of chloroquine-resistant parasites to recede. With the introduction of combination anti-malarial therapies elsewhere in Africa, reductions in chloroquine use may be followed by a resurgence of chloroquine-susceptible falciparum malaria throughout the region.

Rates of the decline of chloroquine resistance may also vary according to the intensity of malaria transmission. In parts of Africa where the rate of transmission is high, most infections are genetically mixed, allowing within-host competition be-

Table 2. Adverse Events.

Event	Chloroquine Group	Sulfadoxine–Pyrimethamine Group	P Value
	<i>no. of events</i>		
Moderate-to-severe anemia*	18	17	1.0
Pruritus†	13	1	0.001
Clinical treatment failure	1	53	<0.001
Severe adverse events‡	3	2	

* Moderate-to-severe anemia was defined as a hemoglobin level of less than 7.0 g per deciliter. No participant required transfusion.

† Self-limited, nonallergic pruritus is a common side effect of chloroquine.

‡ All participants who had severe adverse events recovered. Two participants in the chloroquine group were hospitalized for vomiting after receipt of drug, and one was hospitalized for cervical adenitis. One participant in the sulfadoxine–pyrimethamine group was hospitalized for febrile seizure, and one had severe malaria but was not hospitalized.

tween parasite clones with different degrees of fitness.²¹ In contrast, where the rate of transmission is low, such as much of Asia and the Amazon region, most malaria infections are characterized by a single clone, and distinctive clones or genotypes of *P. falciparum* may predominate over wide areas.^{22,23} Under these conditions, opportunities for direct competition between chloroquine-sensitive and chloroquine-resistant parasites may be limited, permitting resistant parasites to remain fixed in the population after the withdrawal of chloroquine.

Following Malawi's example, other countries in Africa switched from chloroquine to the combination of sulfadoxine and pyrimethamine. Predictably, resistance to sulfadoxine–pyrimethamine has compromised the efficacy of this single-dose, long-acting, inexpensive medication,²⁴ and most of these countries are now moving to artemisinin-based combination therapies for malaria, which are more expensive and require multiple doses. Malawi is currently seeking international donor support for a policy change from the combination of sulfadoxine and pyrimethamine to the combination of lumefantrine and artemether as first-line treatment for uncomplicated malaria (Malenga G: personal communication). The antifolate antibiotic combination of trimethoprim and sulfamethoxazole is cross-resistant with sulfadoxine–pyrimethamine, and its widespread use in Africa might maintain a selective pressure favoring malaria parasites resistant to sulfadoxine–pyrimethamine.²⁵ However, recent studies in Uganda and Mali found that trimethoprim–sulfamethoxazole prophylaxis did not increase rates of antifolate-resistant malaria,^{26,27} leaving open the possibility that reducing the use of sulfadoxine–pyrimeth-

amine could arrest and reverse resistance and that the use of this valuable drug might also be resurrected. The prevalence of molecular markers of malarial organisms resistant to both chloroquine and sulfadoxine–pyrimethamine should be monitored while these drugs are being replaced by combination therapies.

Despite its efficacy in this small study, chloroquine should not be used as monotherapy for malaria where chloroquine-sensitive *P. falciparum* has reemerged after its use was reduced. Although we did not find clinical or molecular evidence of chloroquine resistance in this study, chloroquine-resistant malaria remains common in neighboring countries, including Mozambique and Zambia, and would probably reemerge rapidly if chloroquine were to be reintroduced for the routine treatment of malaria.

Where its efficacy is impaired, chloroquine should be completely withdrawn from use and replaced by more effective drugs. If chloroquine-susceptible *falciparum* malaria returns across the region, it may become possible to reintroduce the drug in combination with other drugs to prevent the reemergence of resistance. Chloroquine has many desirable attributes as an antimalarial drug: it is inexpensive, rapid-acting, long-acting, and safe in all age groups and in pregnant women. It is an excellent drug for preventing malaria in travelers and may be an ideal candidate for intermittent preventive treatment, which has shown promise in studies involving pregnant women, infants, and children.²⁸⁻³¹ If chloroquine can be withdrawn from use throughout Africa as effectively as it was withdrawn in Malawi, it may be possible to include it as one component of a new generation of combination therapy in the not-too-distant future.

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