

## RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF MALARONE FOR MALARIA PROPHYLAXIS IN NON-IMMUNE COLOMBIAN SOLDIERS

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**Abstract.** Malarone was compared with placebo in a double-blind, randomized, placebo-controlled trial of prophylaxis of malaria in predominately *Plasmodium vivax* areas of Colombia. The study population consisted of 180 completely non-immune Colombian soldiers, male, average age 19 years, and average weight 63 kg. Twenty-four subjects were considered unevaluable because of compliance issues, including one Malarone subject (with no detectable drug levels) who became infected with *P. vivax*. Of the 97 evaluable subjects who received Malarone (250 mg atovaquone plus 100 mg proguanil hydrochloride) daily from 1 day before entering the endemic area to 7 days after leaving the endemic area, none became parasitemic. Of the 46 evaluable placebo subjects, 11 became infected with *P. vivax* and 2 became infected with *Plasmodium falciparum*. The protective efficacy of Malarone for all malaria and for *P. vivax* malaria was 100% (LL 95% CI = 63%) and 100% (LL 95% CI = 58%), respectively, and was 96% if the one case with undetectable blood levels was included. Malarone has high protective efficacy for *P. vivax* in Colombia.

### INTRODUCTION

Malaria remains one of the greatest contributors to morbidity and mortality for residents of tropical and subtropical areas and for travelers to these regions. Prior infection offers significant immunity to both *Plasmodium falciparum* and *Plasmodium vivax*.<sup>1</sup> Thus, significant disease primarily occurs in endemic areas, in children < 5 years of age, before their acquisition of semi-immunity, and in non-immune persons who travel to malarious regions. Intermittent presumptive treatment (IPT) for early gravid pregnancies<sup>2</sup> and for infants<sup>3</sup> in *P. falciparum*-endemic Africa is now recommended. Prophylaxis has long been recommended for non-immune travelers who enter an endemic region.

Malarone (atovaquone + proguanil) (GlaxoSmith Kline Co., Greenford, UK) is an anti-malarial that has been extensively evaluated for treatment and prophylaxis of *P. falciparum* malaria. Malarone cured 98.5% of 471 *P. falciparum* patients in eight treatment studies.<sup>4,5</sup> Six Malarone prophylaxis studies have been performed for *P. falciparum*. For three placebo-controlled trials in semi-immune African subjects, 2 Malarone subjects compared with 92 placebo subjects developed parasitemia.<sup>5</sup> For two active-comparator trials in non-immune travelers, no Malarone subjects, no mefloquine subjects, and three chloroquine-plus-proguanil subjects developed parasitemia.<sup>5–7</sup> In one active-comparator trial in purely pediatric travelers, no subjects developed malaria.<sup>8</sup> One placebo-controlled trial in generally non-immune subjects has been conducted. The trial was conducted in Indonesians, of whom ~40% had never had malaria and 25% who had had malaria only once and who then transmigrated into a malaria-endemic region. One Malarone subject compared with 23 placebo subjects showed *P. falciparum* parasitemia (protective efficacy = 96%).<sup>9</sup>

Although *P. vivax* causes 56% of non-African malaria infections,<sup>10</sup> there is relatively little information on the efficacy of Malarone against *P. vivax*. In one study in Thailand,<sup>11</sup> each of 46 Thai subjects treated for 3 days with Malarone and then

for 14 days with primaquine cleared their parasitemia. In the trans-migrant Indonesian trial, there were 3 cases of *P. vivax* malaria in the Malarone group versus 16 cases in the placebo group (protective efficacy = 84%).<sup>9</sup>

This study was designed to address questions not answered by previous studies: the protective efficacy of Malarone against *P. vivax* in an endemic region in South America and in totally non-immune subjects. The study was performed in regions in Colombia where *P. vivax* historically predominated and in soldiers who had never been in a malarious region.

### MATERIALS AND METHODS

**Study design.** This was a phase IV, randomized, double-blind, placebo-controlled single center trial to compare the effectiveness of Malarone versus placebo for the prophylaxis of *P. vivax* and *P. falciparum* malaria.

**Study subjects.** All subjects were male members of the Colombian armed forces. None had ever been in a malarious region. Each was without concomitant medical problems history, physical examination, and baseline laboratory tests (hematology: hemoglobin, platelet count, white blood count (WBC); clinical chemistry profile: alanine aminotransferase (ALT), total bilirubin, serum creatinine). The protocol was approved by The Ethics Committee, Ministry of National Defense (Central Military Hospital) and by the Bioethics Committee, Santafe de Bogota, DC. All subjects gave written informed consent.

The sample size was calculated using the Blackwelder likelihood scores method. Sample sizes were calculated assuming a 2:1 randomization (Malarone: placebo). With 20% of placebo subjects expected to develop *P. falciparum* malaria and Malarone to have a protective efficacy of 95%, a sample size of 144 (96:48) gives an 80% probability (power) that the lower limit of the 90% confidence interval (CI) for protective efficacy is > 60%. Also, with 20% of placebo subjects expected to develop *P. vivax* malaria and Malarone to have a protective efficacy of 90%, a sample size of 156 (104:52) gives an 80% probability that the lower limit of the 90% CI for protective efficacy is > 50%. To allow for dropouts, two cohorts of subjects totaling 180 subjects were ultimately recruited: soldiers

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deployed to Granada in June–July 2000 (61 Malarone and 31 placebo subjects) and soldiers deployed to Uraba in July 2000 (59 Malarone and 29 placebo subjects). Some of the soldiers who originally deployed to Granada later redeployed to Uraba.

The intent-to-treat population (ITT) consisted of all randomized subjects (120 of whom received Malarone, 60 of whom received placebo). The first per protocol population (PP1) consisted of randomized subjects who were compliant with study criteria including taking study drug and weekly blood smears to detect parasitemia. A subject was considered non-compliant with study drug administration if consent was withdrawn, if he missed three consecutive doses of study drug, or if he was lost to follow-up before the last scheduled visit during prophylaxis.

After completion of the study, review of drug concentration data revealed that 13 subjects randomized to Malarone had no component of the drug detectable in their serum in one of two plasma samples. This included one subject who failed prophylaxis with Malarone. In addition, 11 placebo subjects had atovaquone, proguanil, and/or cycloguanil detected in their plasma at one or more time-points. Most of these soldiers were subjects who redeployed from Granada to Uraba under conditions where compliance could not be assured, and it is believed that some soldiers missed some doses and/or shared study medication. A second per protocol population (PP2) was therefore defined as those subjects who were compliant with study criteria as verified by drug concentration data.

**Study drug.** Subjects were assigned to Malarone (250 mg atovaquone and 100 mg proguanil hydrochloride) or matching placebo in 2:1 allocation. For both Malarone and placebo groups, the dose regimen was one tablet daily with breakfast, from 1 day before entering the malaria endemic areas through the 10–16 weeks of residence in the area and for 7 days after leaving the endemic areas. Study drug was administered under the supervision of the combat health technician or designee. An exception to such supervision occurred for Granada subjects during their redeployment to Uraba. As an additional verification of compliance, a plasma sample was collected between weeks 5 and 7 and weeks 10 and 12, and if the subject exhibited malaria, for determination of atovaquone, proguanil, and cycloguanil concentrations.

**Study procedures.** Blood smears and body temperatures were obtained weekly during the chemoprophylaxis period (1 day before until 7 days after being in the endemic region) and for a further 4 weeks of follow-up. Smears were also obtained at any time malaria was suspected. Parasite counts were quantified per 200 WBC and, assuming a WBC count of 8,000/ $\mu$ L, expressed as the number of parasites per microliter. A positive result had to be confirmed by two technologists. A slide was not considered negative until examination of 200 oil immersion fields revealed no parasites.

**Tolerance.** Tolerance to study medications was determined by history and physical examination at weekly intervals and repetition of baseline laboratory tests at week 8 of dosing.

**Endpoints and statistical evaluation.** The primary efficacy endpoint was protective efficacy based on the proportion of subjects who experienced parasitemia and therefore failed prophylaxis.

Proportion who failed prophylaxis = number of subjects who failed/number of subjects treated.

Protective efficacy =  $1 - (\text{proportion of Malarone failures} / \text{proportion of placebo failures})$ .

## RESULTS

**Subject entrance characteristics.** There were 120 Malarone subjects and 60 placebo subjects. For all 180 subjects, the age [median (range)] was 19 (17–27) and the weight was 63 (48–81) kg, without statistical difference between the groups. In terms of race, 75% of the subjects were Hispanic and 25% were black in both groups.

**Compliance.** Eight Malarone subjects and 3 placebo subjects withdrew their consent (Table 1). Two Malarone subjects and no placebo subjects missed three or more consecutive doses of medication. The PP1 population was therefore 110 Malarone subjects and 57 placebo subjects.

After completion of the study, review of plasma drug concentration data revealed that 13 subjects randomized to Malarone had no component of the drug detectable in their serum in one of two plasma samples. In addition, 11 placebo subjects had atovaquone, proguanil, and/or cycloguanil detected in their plasma at one or more time-points. The PP2 population consists of the PP1 population minus these 13 Malarone and 11 placebo subjects: 97 Malarone subjects and 46 placebo subjects.

**Efficacy.** For the ITT population of all Malarone and placebo subjects, the prophylactic efficacy was 87%. Of the nine Malarone failures included in the ITT analysis, however, eight withdrew consent and were not administered any drug.

A more meaningful efficacy analysis derives from the subjects who were administered the drug. For the subjects deployed to Granada, there were no prophylactic failures in the Malarone group and nine failures (eight caused by *P. vivax*) in the placebo group. For the subjects deployed to Uraba, no Malarone subjects failed and four placebo subjects failed (three caused by *P. vivax*). There was also one Malarone failure caused by *P. vivax* and four placebo failures caused by *P. vivax* in subjects whose blood levels did not correspond to the assigned study medication.

The first *P. vivax* failure occurred at week 4, the last on week 12, and most failures (12) occurred between weeks 7 and 11. Parasitemias for *P. vivax* had mean (SD) values of 8,725 (6,589) parasites/ $\mu$ L and were between 1,176 and 19,800 parasites/ $\mu$ L. For the two *P. falciparum* cases, parasitemias were 6,400 and 1,862/ $\mu$ L. Of the total of 18 patients who had parasitemia, 4 failures were detected on routine weekly examination, and 14 failures were detected mid-week in patients who requested medical attention.

The failure rates for Malarone were 0% for the Granada and Uraba subjects in the PP2 population who demonstrably took drug. The failure rates for placebo were 47% and 15% for the PP2 population. The protective efficacy for Malarone for all malaria and for *P. vivax* malaria was 100%, with lower limits (LL) of the 95% CI being 63% and 58%, respectively.

If a conservative approach to the subjects with aberrant pharmacokinetics (PK) data is taken, and the one Malarone failure is included but the four placebo failures are omitted, the total Malarone failure rate becomes 1 per 110 subjects and the total placebo failure rate becomes 13 per 57 subjects overall and 11 per 51 subjects for *P. vivax*. With this conservative approach, the protective efficacy for all malaria and for *P. vivax* malaria was 96%.

TABLE 1  
Compliance and efficacy

Group	Malarone	Placebo
Randomized [ITT] population	120	60
Prophylactic failure	9	19
Prophylactic efficacy all malaria	87%	
Withdrew consent or missed $\geq 3$ doses	10	3
PP1 population	110	57
Prophylactic failure	0 <i>P. falciparum</i> 1 <i>P. vivax</i>	2 <i>P. falciparum</i> 15 <i>P. vivax</i>
Protective efficacy all malaria	97% (LL 95% CI = 74%)	
Protective efficacy <i>P. vivax</i> malaria*	97% (LL 95% CI = 69%)	
PP2 population†	97	46
Granada deployment		
Total number	47	19
Prophylactic failures	0 <i>P. falciparum</i> 0 <i>P. vivax</i>	1 <i>P. falciparum</i> 8 <i>P. vivax</i>
Failure rate	0/47 = 0%	9/19 = 47%
Uraba Deployment		
Total number	50	27
Prophylactic failures	0 <i>P. falciparum</i> 0 <i>P. vivax</i>	1 <i>P. falciparum</i> 3 <i>P. vivax</i>
Failure rate	0/50 = 0%	4/27 = 15%
Total failure rate	0/97 = 0%	13/46 = 28%
Protective efficacy all malaria	100% (LL 95% CI = 63%)	
Protective efficacy <i>P. vivax</i> malaria*	100% (LL 95% CI = 58%)	

\* Protective efficacy for *P. vivax* malaria is based on the number of exposed individuals minus the subjects who were infected with *P. falciparum* and therefore not available to be infected with *P. vivax*.

† The PP2 population excludes the 13 Malarone subjects and the 11 placebo subjects in whom blood analysis showed that correct treatment was not taken.

**Adverse events.** There were no serious adverse events in this study, and no subject discontinued study medication because of adverse events. In total, 47 of 120 (39%) subjects in the Malarone group and 24 of 60 (40%) subjects in the placebo group reported one or more adverse events. The most common events in the Malarone and placebo groups were Tinea infection (18% and 28%, respectively), parasitic gastrointestinal infection (7% and 5%, respectively), headache (7% and 3%, respectively), and fever (5% and 0%, respectively).

## DISCUSSION

At the time of this study, the Colombian military afforded a unique situation for the study of anti-malarial prophylaxis. Some units consisted only of personnel who had lived their whole lives at high altitudes where malaria does not exist and who were completely malaria naïve. Anti-malarial prophylaxis was not routinely given to soldiers (according to Colombian military procedures), even though these units could be deployed to operations in highly malaria endemic terrain.

In this study, malaria-naïve Colombian soldiers were randomly assigned to receive either Malarone prophylaxis or placebo when they engaged in military operations in the primarily *P. vivax* regions of Granada and Uraba. Placebo attack rates were substantial (> 25%). However, study conditions could not be completely maintained for soldiers who deployed to Granada and then redeployed to Uraba.

The data from the subjects in whom pharmacokinetic analysis indicated that the assigned medication was taken correctly form the basis of this report. None of 97 Malarone subjects were prophylactic failures. Thirteen of 46 placebo subjects (28%) were prophylactic failures. As expected from the choice of study region, most (11) of the failures were caused by *P. vivax* infection. The protective efficacy for all malaria and for *P. vivax* malaria was 100%. If a conservative

adjustment is made such that we include the *P. vivax* infection in a Malarone subject whose PK data were aberrant, the protective efficacy for all malaria and for *P. vivax* malaria remains high at 96%.

Two randomized, double-blind, placebo-controlled trials of malaria chemoprophylaxis have previously been conducted in the Colombian soldier population. In a 1997 primaquine study, the protective efficacy based on incidence density was 89% for all malaria (94% for *P. falciparum* and 85% for *P. vivax*).<sup>12</sup> In a follow-up study in 1998, chloroquine plus primaquine was used in an attempt to improve on primaquine use alone. However, the protective efficacy of the chloroquine plus primaquine group remained at 88% for all malaria (89% for *P. falciparum* and 88% for *P. vivax*).<sup>13</sup>

It is difficult to compare results between different study situations and different decades. Nevertheless, the > 95% protective efficacy for Malarone against *P. vivax* in this study represents a higher value than was found for Malarone in the Indonesian study or for primaquine in the Colombian soldier population.

Travelers to malaria-endemic regions other than Africa require prophylaxis not only against *P. falciparum* but also against *P. vivax*. The data of this report suggest that, in addition to the recognized potency of Malarone for *P. falciparum*, at least in Colombia, Malarone has high potency against *P. vivax*.

Received February 13, 2006. Accepted for publication April 13, 2006.

**Acknowledgment:** The authors thank the efforts and participation of the commanders and soldiers from Brigada XIV in Uraba and Batallón 21 Vargas in Granada.

**Disclosure:** Stephane Duparc is an employee of GlaxoSmithKline and is identified as such on the authorship page. This statement is made in the interest of full disclosure and not because the author considers this to be a conflict of interest.

Financial support: This study was supported by a grant from Glaxo Smith Kline to Dr. Soto.

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