

# A Randomized Controlled Trial of Extended Intermittent Preventive Antimalarial Treatment in Infants

Robin Kobbe,<sup>1</sup> Christina Kreuzberg,<sup>1</sup> Samuel Adjei,<sup>1,3</sup> Benedicta Thompson,<sup>3</sup> Iris Langefeld,<sup>1</sup> Peter Apia Thompson,<sup>4</sup> Harry Hoffman Abruquah,<sup>4</sup> Benno Kreuels,<sup>1</sup> Matilda Ayim,<sup>4</sup> Wibke Busch,<sup>1</sup> Florian Marks,<sup>1,5</sup> Kwado Amoah,<sup>4</sup> Ernest Opoku,<sup>4</sup> Christian G. Meyer,<sup>2</sup> Ohene Adjei,<sup>4</sup> and Jürgen May<sup>1</sup>

<sup>1</sup>Infectious Disease Epidemiology Group and <sup>2</sup>Department of Molecular Medicine, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany; <sup>3</sup>Ministry of Health/Ghana Health Service, District Health Directorate, Agona, Ashanti Region, and <sup>4</sup>Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana; and <sup>5</sup>International Vaccine Institute, Seoul, South Korea

(See the editorial commentary by Greenwood on pages 26–8)

**Background.** Intermittent preventive antimalarial treatment in infants (IPTi) with sulfadoxine-pyrimethamine reduces falciparum malaria and anemia but has not been evaluated in areas with intense perennial malaria transmission. It is unknown whether an additional treatment in the second year of life prolongs protection.

**Methods.** A randomized, double-blinded, placebo-controlled trial with administration of sulfadoxine-pyrimethamine therapy at 3, 9, and 15 months of age was conducted with 1070 children in an area in Ghana where malaria is holoendemic. Participants were monitored for 21 months after recruitment through active follow-up visits and passive case detection. The primary end point was malaria incidence, and additional outcome measures were anemia, outpatient visits, hospital admissions, and mortality. Stratified analyses for 6-month periods after each treatment were performed.

**Results.** Protective efficacy against malaria episodes was 20% (95% confidence interval [CI], 11%–29%). The frequency of malaria episodes was reduced after the first 2 sulfadoxine-pyrimethamine applications (protective efficacy, 23% [95% CI, 6%–36%] after the first dose and 17% [95% CI, 1%–30%] after the second dose). After the third treatment at month 15, however, no protection was achieved. Protection against the first or single anemia episode was only significant after the first IPTi dose (protective efficacy, 30%; 95% CI, 5%–49%). The number of anemia episodes increased after the last IPTi dose (protective efficacy, –24%; 95% CI, –50% to –2%).

**Conclusion.** In an area of intense perennial malaria transmission, sulfadoxine-pyrimethamine–based IPTi conferred considerably lower protection than reported in areas where the disease is moderately or seasonally endemic. Protective efficacy is age-dependent, and extension of IPTi into the second year of life does not provide any benefit.

The causative agent of malignant malaria, *Plasmodium falciparum*, kills >1 million children aged <5 years in sub-Saharan Africa every year [1, 2]. Intermittent preventive treatment in infants (IPTi) with sulfadoxine-pyrimethamine (SP) was evaluated as a malaria control measure, combining therapeutic and prophylactic effects of

antimalarial drugs administered in intervals short enough to prevent disease and long enough to allow for the development of immunity [3, 4]. However, the exact mechanisms of protection are yet to be elucidated. Combined with vaccinations provided in the frame of the World Health Organization Expanded Program on Immunization (EPI) that treats infants aged 2, 3, and 9 months, IPTi reduced incidences of malaria and anemia in an area with moderate transmission by >50% [5]. Protective efficacy was lower in 2 additional trials from areas with moderate and seasonal transmission [6, 7]. Impairment of immune responses after routine vaccinations or clinical rebound effects known to occur after continuous chemoprophylaxis have not been reported

Received 15 December 2006; accepted 15 February 2007; electronically published 29 May 2007.

Reprints or correspondence: Dr. Jürgen May, Infectious Disease Epidemiology Group, Bernhard Nocht Institute for Tropical Medicine, Bernhard Nocht Str. 74, D-20359 Hamburg, Germany (may@bni-hamburg.de).

**Clinical Infectious Diseases** 2007;45:16–25

© 2007 by the Infectious Diseases Society of America. All rights reserved.  
1058-4838/2007/4501-0005\$15.00  
DOI: 10.1086/518575

[5, 6, 8–10], but concern exists about possible rebound in the incidence of malaria with high parasite densities in 1 of the studies [7].

To date, IPTi with SP has not been evaluated in areas with intense perennial transmission, where the malaria burden is highest [11, 12]. We hypothesized that an extended IPTi schedule with an additional drug application at month 15 might prolong protection. Therefore, we used a randomized, double-blind, placebo-controlled trial to investigate the efficacy and safety of SP administered at 3, 9, and 15 months of age in an area where malaria is highly endemic.

## METHODS

**Study area.** The study was conducted in the rural Afigya Sekyere district in the Ashanti Region in Ghana (estimated HIV prevalence, <3%) [13]. The area is holoendemic for falciparum malaria, with intense perennial transmission and seasonal peaks. Entomological evaluation during the study period indicated ~400 infective bites per person-year (unpublished data). Subsidized insecticide-treated bed nets were available, and their use was encouraged.

**Inclusion criteria and study procedures.** Infants aged 3 months (4-weeks tolerance accepted) with permanent residence in the study area were recruited from January 2003 through January 2004 at regular EPI sessions that took place at health centers in 9 neighboring villages. The trial was completed on 22 September 2005. A study team of 2 doctors, a nurse, a technician, and a field worker, all blinded to group assignment, was responsible for recruitment, treatment, and subsequent visits.

After enrollment, infants were assigned by the coordinating physician to the lowest identification number available, and photo identity cards were issued. The investigational products contained either a combination of 250 mg of sulfadoxine and 12.5 mg of pyrimethamine or placebo (La Roche) and were blister-packed and precoded after block-randomization (blocks of 10). Tablets were crushed, mixed with water, and administered independent of body weight; the first dose (IPTi-1) was administered to infants aged 3 months. The second (IPTi-2) and third (IPTi-3) doses were administered to infants aged 9 and 15 months, respectively. Treatment was repeated within 30 min if vomiting occurred. The sealed, computer-generated allocation list was locked until completion of the follow-up period. After cessation of the trial, the content and quality of the investigational product was verified.

The observation period of 21 months started at recruitment (i.e., at receipt of IPTi-1). A malaria episode was defined as fever (temperature  $\geq 38.0^\circ\text{C}$  or fever during the preceding 48 h reported by mothers without being asked), accompanied by asexual *P. falciparum* parasitemia of  $>500$  parasites/ $\mu\text{L}$ . According to the algorithm proposed by Smith et al. [14], this

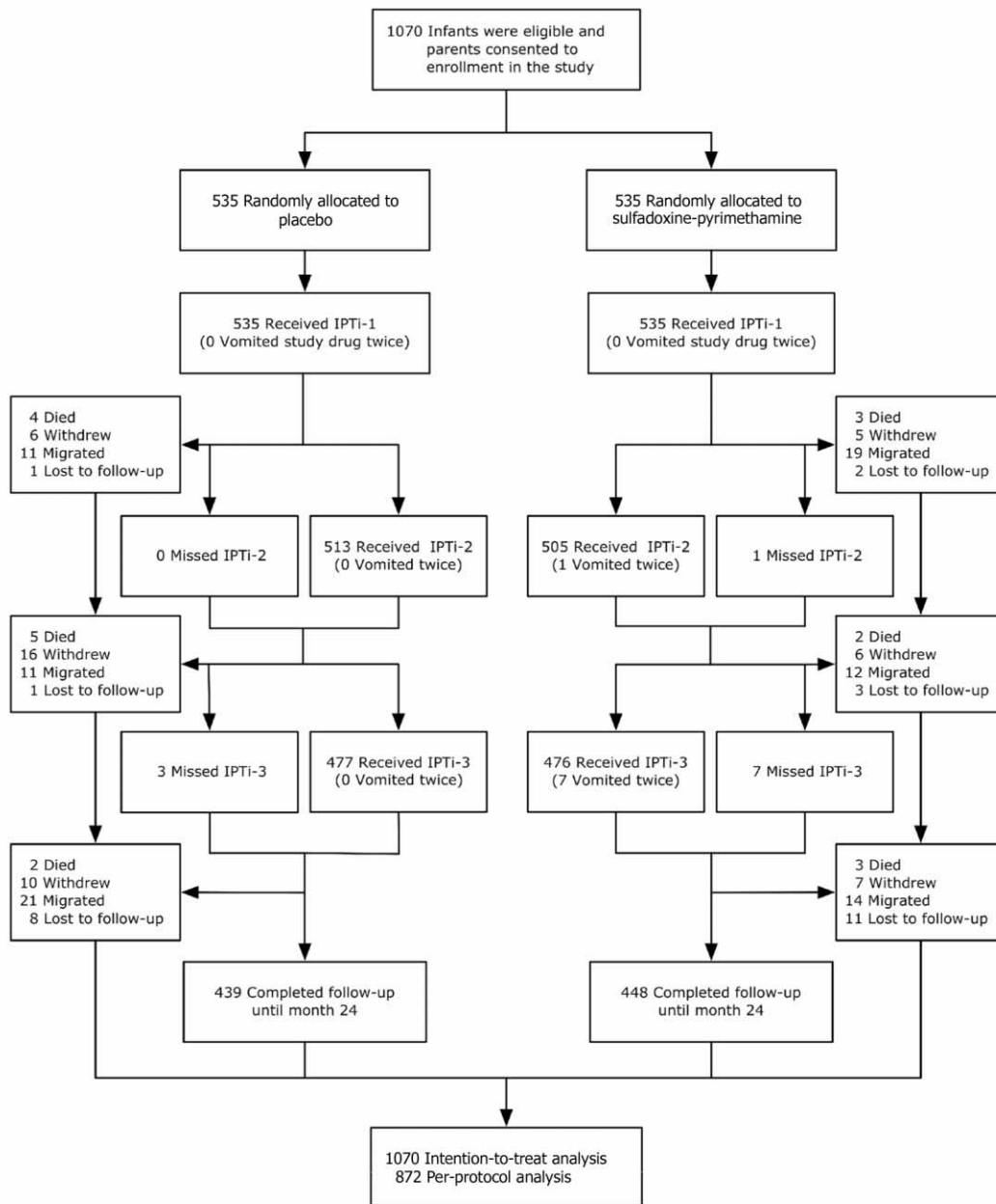
case definition yielded the highest sensitivity and specificity ( $>90\%$ ) for attribution of fever episodes to malaria among all events of fever occurring in the placebo group. Anemia and severe anemia were defined as hemoglobin levels  $<7.5$  g/dL and  $<5.0$  g/dL, respectively.

Episodes of clinical malaria, anemia, and adverse events were monitored monthly through active follow-up visits (20,733 visits in total). Blood films were obtained and hemoglobin levels were measured at each scheduled contact (active visit) or when patients presented independently from regular visits (passive case detection). Relevant events occurring between active visits and hospital attendances were documented on weighing charts. In addition to follow-up visits, trained field workers conducted house visits to improve compliance, foster information exchange, and encourage self-reporting of medical conditions. Adverse events were graded mild, moderate, severe, or serious. Serious adverse events were life-threatening or caused hospitalization, enduring disability, or death.

**Laboratory and field procedures.** Documentation of medical histories, as well as physical examinations, blood sampling, and laboratory techniques, were performed according to standard operating procedures. Blood films were air-dried, Giemsa-stained, and read independently by 2 microscopists. In case of discordances in parasite positivity or negativity, density (ratio,  $>3$ ), or species, reading of blood films was repeated by a senior laboratory technician. Parasite densities were estimated by counting the number of asexual parasites per 200 leukocytes (counts of  $<10$  parasites were related to 500 leukocytes). A leukocyte count of 8000 cells/ $\mu\text{L}$  was adopted to calculate densities. In case of 2 negative slide readings, final results were classified as negative, and in case of 2 positive slide readings, parasitemia was given as the mean of densities.

Determination of molecular markers of SP resistance of parasite isolates with a parasite load  $>500$  parasites/ $\mu\text{L}$  were performed by PCR, as described elsewhere [15]. Hemoglobin levels were measured photometrically (HemoCue). Rectal temperature was assessed until 12 months of age, and electronic ear thermometers (ThermoScan plus IRT 3520, Braun) were used thereafter. All episodes of clinical malaria were treated for 3 days (with 4 mg/kg per day of artesunate plus 10 mg/kg per day of amodiaquine). Children with severe malaria, according to World Health Organization criteria, were admitted to the hospital and treated according to hospital guidelines [16]. Children with hemoglobin levels  $<8$  g/dL received oral supplementation of ferrous-sulfate and folic acid (folic acid was not applied for 7 days after study drug administration because of possible inhibition of SP) [17]. Other diseases were treated according to national guidelines.

**Ethical considerations.** Before enrollment of participants, the aims and procedures of the study were explained to parents



**Figure 1.** Trial schema for a study of intermittent preventive antimalarial treatment in infants (IPTi). IPTi-1, IPTi dose 1; IPTi-2, IPTi dose 2; IPTi-3, IPTi dose 3.

or guardians. Their understanding was confirmed before written or thumb-printed (in the presence of an unbiased witness) consent was obtained. All clinical investigations were conducted in accordance with the principles of the Helsinki Declaration, and the protocol was approved by the Ethics Committee of the Kwame Nkrumah University of Science and Technology (Kumasi, Ghana).

**Sample size calculation and data analysis.** A sample size of 1070 infants was estimated to provide 80% power for detecting a 20% reduction of hazards of developing malaria in

the SP group, compared with the placebo group. The recruitment period was 12 months, and follow-up visits were conducted for 21 months; the expected drop-out rate was 20% (2-sided  $\alpha$  .05).

Data were collected until September 2005 and were entered in a database with predefined variable ranges and internal consistency controls before files were locked. According to a predefined analysis plan, the results presented here refer to the primary analysis, which was based on intention-to-treat, including all participants who received at least 1 dose of SP. A

**Table 1. Characteristics of study participants at recruitment.**

Characteristic	Placebo group (n = 535)	SP group (n = 535)	P <sup>a</sup>
<b>Sex</b>			
Male	272 (50.8)	264 (49.3)	.63
Female	263 (49.2)	271 (50.7)	
Age at IPTi dose 1, <sup>b</sup> mean weeks ± SD	12.3 ± 1.6	12.3 ± 1.7	.85
Z score, <sup>c</sup> mean value ± SD	0.31 ± 1.1	0.43 ± 1.2	.13
<b>Use of bednets<sup>d</sup></b>			
Yes	201 (37.6)	210 (39.3)	
No	263 (49.2)	261 (48.8)	.75
Missing data	71 (13.3)	64 (12.0)	
<b>Distance to study site<sup>e</sup></b>			
<0.5 km	135 (25.2)	146 (27.3)	
0.5–1 km	218 (40.8)	213 (39.8)	.89
>1 km	174 (32.5)	169 (31.6)	
Missing data	8 (1.5)	7 (1.3)	
<b>Parasitemia</b>			
Positive	86 (16.1)	72 (13.5)	
Negative	448 (83.8)	463 (86.5)	.29
Missing data	1 (0.2)	0 (0)	
<b>Clinical malaria<sup>f</sup></b>			
Yes	10 (1.9)	8 (1.5)	
No	524 (97.9)	527 (98.5)	.54
Missing data	1 (0.2)	0 (0)	
<b>Hemoglobin level, mean g/dL ± SD</b>			
At time of recruitment	10.3 ± 1.3	10.4 ± 1.4	.14
At start of potential rebound period	9.9 ± 1.4	10.1 ± 1.4	.19
<b>Hemoglobin genotype<sup>g</sup></b>			
HbAA	394 (73.6)	380 (71.0)	
HbAS	54 (10.1)	56 (10.5)	
HbSS	4 (0.8)	2 (0.4)	
HbAC	47 (8.8)	59 (11.0)	.85
HbCC	1 (0.2)	1 (0.2)	
HbCS	5 (0.9)	7 (1.3)	
Missing data	30 (5.6)	30 (5.6)	

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. IPTi, intermittent preventive antimalarial treatment in infants; SP, sulfadoxine-pyrimethamine.

<sup>a</sup> Determined using Wilcoxon test or  $\chi^2$  test. None of the parameters in both study arms was significantly different.

<sup>b</sup> Age at IPTi dose 1 is equal to age at time of recruitment.

<sup>c</sup> According to US Centers for Disease Control growth charts for 2000.

<sup>d</sup> Information obtained by interview of participants' mothers (use of insecticide-treated or untreated bed nets was not distinguished).

<sup>e</sup> Distance assessed by use of Global Position System.

<sup>f</sup> Clinical malaria was defined as fever (temperature  $\geq 38.0^\circ\text{C}$  or fever during the preceding 48 h reported by mothers without being asked) accompanied by asexual *Plasmodium falciparum* parasite load  $>500$  parasites/ $\mu\text{L}$ .

<sup>g</sup>  $\beta$ -globin genotype determined by PCR.

secondary analysis included only infants who received all study drugs within 4 weeks before or after the scheduled age. The primary end point was malaria incidence. Secondary end points were malaria episodes with high-density parasitemia (fever or reported fever during the preceding 48 h and parasite load  $>5000$  parasites/ $\mu\text{L}$ ), anemia, severe anemia, outpatient atten-

dance, hospital admissions, and death. Safety was determined by the number, severity, and assumed causality of adverse events.

To estimate protective efficacy, the period from recruitment until 3 months after IPTi-3 was considered to be the intervention period. The 8-month period starting from 5 weeks after

**Table 2. Frequency of sulfadoxine-pyrimethamine drug-resistance markers at time of intermittent preventive antimalarial treatment in infants (IPTi) applications.**

Characteristic	Placebo group	SP group	<i>P</i> <sup>a</sup>
No. (%) of persons with >3 mutations in <i>pfdhfr</i> and/or <i>pfdhps</i> genes <sup>b</sup>			
IPTi dose 1	24/38 (63.2)	18/30 (60.0)	.79
IPTi dose 2	45/59 (76.3)	40/58 (69.0)	.34
IPTi dose 3	45/57 (79.0)	49/53 (79.3)	.97

**NOTE.** SP, sulfadoxine-pyrimethamine.

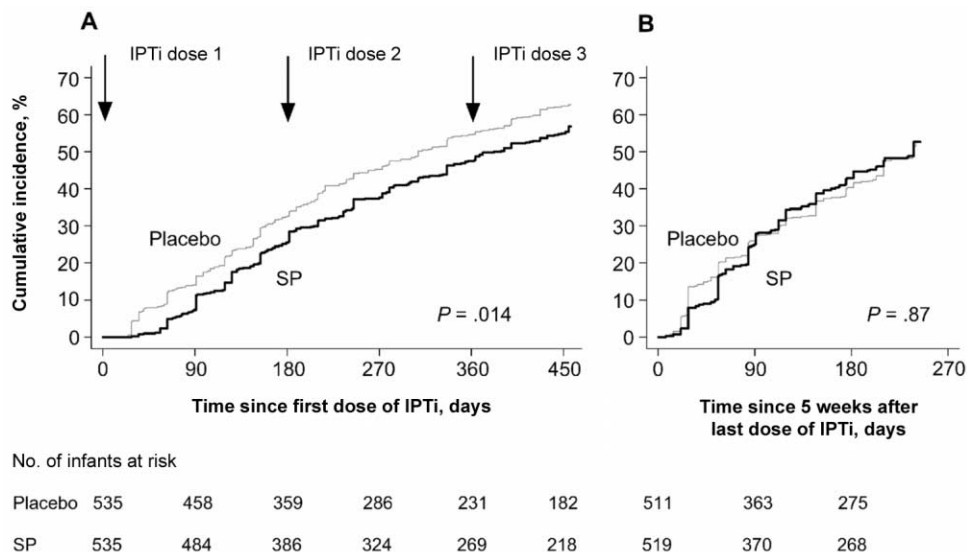
<sup>a</sup> Determined using  $\chi^2$  test.

<sup>b</sup> Molecular marker of sulfadoxine-pyrimethamine-resistant parasite isolates. The denominator is the number of isolates with >500 parasites/ $\mu$ L that are PCR-positive for a *pfdhfr* fragment (Ser108Asn, Asn51Ile, and Cys59Arg) and *pfdhps* fragment (Ala437Gly and Lys540Glu) in the respective groups. In total, *pfdhfr* and/or *pfdhps* sequences could not be PCR-amplified in 135 DNA samples of infections with >500 parasites/ $\mu$ L (31, 39, and 65 at IPTi-1, IPTi-2, and IPTi-3, respectively).

IPTi-3 was considered to be the period of potential rebound (a period of 5 weeks was chosen, because the residual sulfadoxine plasma level after this time is <5%, consistent with negligible inhibitory activity). Children were not considered to be at risk for malaria for 21 days after confirmed malaria episodes or after presumptive antimalarial therapy; children were not considered to be at risk for anemia for 21 days after a preceding episode of anemia, iron administration, or antimalarial therapy.

Characteristics of both study arms were compared using contingency ( $\chi^2$ ) and nonparametric (Wilcoxon) tests;  $P < .05$  was considered to be statistically significant. Cox regression was

used to calculate protective efficacy (defined as 1 minus the hazard ratio) against first or single malaria or anemia episodes and death. Poisson regression was used to assess protective efficacy (defined as 1 minus the rate ratio) against multiple events. Confounding was determined as a relative difference of >15% between crude hazard ratios and hazard ratios adjusted for predefined covariates without signs of effect modification. Proportional hazards assumptions were displayed in log-minus-log plots for categories of nominal covariates versus log (analysis time) and were formally tested by Schoenfeld residuals. A multivariate Poisson regression model was generated with time-dependent covariates defined as 6-month periods after each



**Figure 2.** Cumulative incidence of a first or single episode of malaria. *A*, Cumulative incidence of a first or single episode of malaria in infants during the intervention period. Arrows indicate times of application of intermittent preventive antimalarial treatment in infants (IPTi). The difference between the treatment group and the placebo group was significant ( $P = .01$ , by Breslow test). *B*, Cumulative incidence in children during the 8-month potential rebound period starting 5 weeks after the last administration of sulfadoxine-pyrimethamine (SP) or placebo. No significant difference between the groups was observed.

**Table 3. Incidence rates and protective efficacies during the first 2 years of life.**

Period, event	Placebo group			SP group			Protective efficacy, % (95% CI)	P <sup>a</sup>
	No. of events	PYAR	Rate of events per year	No. of events	PYAR	Rate of events per year		
<b>Intervention period<sup>b</sup></b>								
First or single event								
Malaria	310	388.4	0.80	278	426.0	0.65	18.4 (4.0–30.6)	.01
Anemia <sup>c</sup>	221	440.1	0.50	203	462.6	0.44	12.8 (–5.4 to 28.0)	.16
Severe anemia <sup>d</sup>	14	527.3	0.03	14	534.8	0.03	0.0 (–109.7 to 52.3)	1.00
All-cause mortality	8	630.2	0.01	5	628.0	0.01	37.2 (–92.1 to 79.4)	.42
Multiple events								
Malaria								
>500 parasites/ $\mu$ L	657	548.9	1.20	530	555.4	0.95	20.3 (10.6–28.9)	<.001
>5000 parasites/ $\mu$ L	360	551.5	0.65	296	557.0	0.53	18.6 (5.0–30.2)	.009
Anemia <sup>c</sup>	356	530.7	0.67	335	538.1	0.62	7.2 (–7.7 to 20.1)	.33
Severe anemia <sup>d</sup>	14	531.9	0.03	17	539.5	0.03	–19.7 (–242.9 to 41.0)	.62
Outpatient visits	1575	630.2	2.50	1526	628.0	2.43	2.8 (–4.3 to 9.4)	.43
Hospital admissions	89	630.2	0.14	81	628.0	0.13	8.7 (–23.4 to 32.4)	.56
<b>Potential rebound period<sup>e</sup></b>								
First or single event								
Malaria	232	204.5	1.13	239	207.3	1.15	–1.5 (–21.6 to 15.3)	.87
Anemia <sup>c</sup>	144	218.7	0.66	154	219.9	0.70	–6.2 (–33.3 to 15.4)	.60
Severe anemia <sup>d</sup>	4	248.1	0.02	5	252.4	0.02	–22.5 (–356.6 to 67.1)	.76
All-cause mortality	8	299.8	0.03	7	306.3	0.02	14.8 (–134.9 to 69.1)	.76
Multiple events								
Malaria								
>500 parasites/ $\mu$ L	365	258.3	1.41	396	262.8	1.51	–6.6 (–22.9 to 7.5)	.38
>5000 parasites/ $\mu$ L	223	259.1	0.86	223	264.1	0.84	1.9 (–18.1 to 18.5)	.84
Anemia <sup>c</sup>	186	247.4	0.75	235	252.9	0.93	–23.6 (–49.8 to –2.0)	.03
Severe anemia <sup>d</sup>	4	248.3	0.02	6	253.5	0.02	–46.9 (–420.4 to 58.6)	.55
Outpatient visits	708	299.9	2.36	759	306.4	2.48	–4.9 (–16.2 to 5.3)	.36
Hospital admissions	35	299.8	0.12	42	306.3	0.14	–17.4 (–83.9 to 25)	.48

**NOTE.** All infants who received at least 1 dose of sulfadoxine-pyrimethamine or placebo were included. Protective efficacy is defined as 1 minus the hazard ratio after Cox regression analysis for single events and 1 minus the rate ratio after Poisson regression for multiple events. PYAR, person-years at risk. SP, Sulfadoxine-pyrimethamine.

<sup>a</sup> Determined using Breslow test.

<sup>b</sup> Months 3–18 of life.

<sup>c</sup> Hemoglobin level <7.5 g/dL.

<sup>d</sup> Hemoglobin level <5.0 g/dL.

<sup>e</sup> Eight-months observation time, starting 5 weeks after the last dose of intermittent preventive antimalarial treatment.

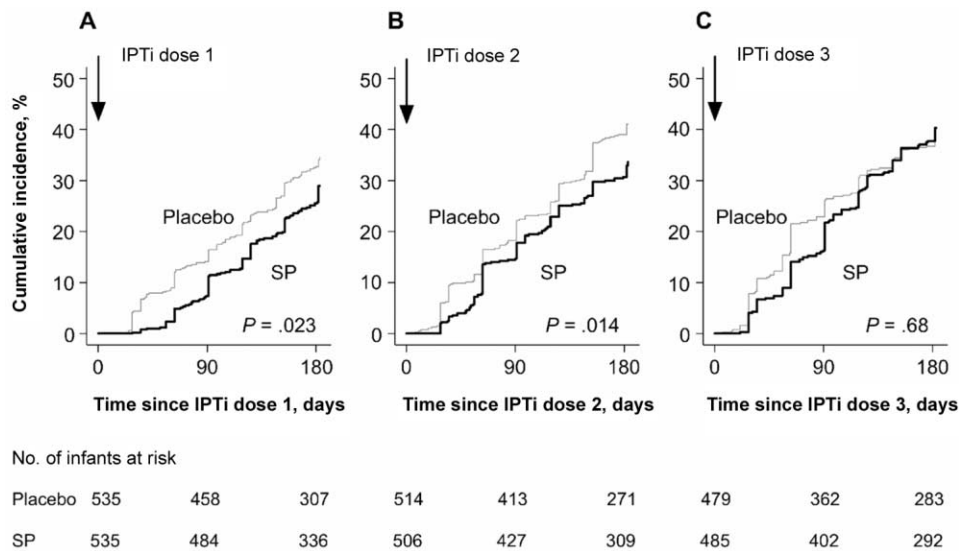
IPTi dose. Effect modification of efficacy with time strata was evaluated by log-likelihood tests. The study is registered at <http://www.ClinicalTrials.gov> (NCT00206739).

## RESULTS

**Trial schema, characteristics of participants, and SP resistance.** One thousand seventy infants were randomly assigned to receive either SP or placebo. The number of participants receiving all 3 doses was similar in both groups (figure 1). Demographic and laboratory characteristics (table 1) of participants and the proportions of isolates carrying markers for SP resistance (table 2) did not differ significantly between groups.

**Protective efficacy: intervention and potential rebound period.** Efficacy of the extended IPTi schedule against first or single malaria episodes until 15 months after receipt of IPTi-1 was 18% (95% CI, 4%–31%;  $P = .01$ ) (figure 2A). Protective efficacy against cumulative episodes was 20% (95% CI, 11%–29%;  $P < .001$ ), and protective efficacy against cumulative episodes with high-density parasitemia was 19% (95% CI, 5%–30%;  $P = .009$ ) (table 3). Efficacy against first or single and all episodes of anemia was 13% (95% CI, –5% to 28%;  $P = .16$ ) and 7% (95% CI, –8% to 20%;  $P = .33$ ), respectively (table 3).

During the potential rebound period, no differences in the incidence of malaria episodes were observed between the



**Figure 3.** Cumulative incidence of a first or single episode of malaria in infants during the 6-month period after each treatment. Differences between treatment and placebo groups were significant after the first dose (A;  $P = .02$ ) and second dose (B;  $P = .01$ ) of intermittent preventive antimalarial treatment in infants (IPTi) but not after the third dose (C;  $P = .68$ ).  $P$  values were determined using the Breslow test.

groups. Notably, the frequency of anemia increased by 24% after preceding SP treatment (protective efficacy,  $-24\%$ ; 95% CI,  $-50\%$  to  $-2\%$ ;  $P = .03$ ) (table 3). Although anemia rebound could be reproduced with various lengths of time-at-risk, the effect was strongly dependent on the predefined hemoglobin cutoff level for the definition of anemia (hemoglobin level,  $<7.5$  g/dL).

**Stratified protective efficacy.** Different tests of the proportional hazards assumption suggested time-dependency of protective efficacy. Parallel curves for the treatment and placebo groups on a log-minus-log survival plot were ambiguous, but vertical differences of curves tended to be higher during the first half of the observation period. Kaplan Meier survival plots of observed data and predicted Cox curves deviated slightly for

**Table 4. Protective efficacies after each dose of sulfadoxine-pyrimethamine or placebo.**

Event	IPTi-1		IPTi-2		IPTi-3	
	Protective efficacy, % (95% CI)	$P$	Protective efficacy, % (95% CI)	$P$	Protective efficacy, % (95% CI)	$P$
<b>First or single events</b>						
Malaria	22.8 (3.5–38.2)	.02	23.4 (5.2–38.0)	.01	4.2 (–17.6 to 22.0)	.68
Anemia <sup>a</sup>	30.3 (5.1–48.8)	.02	–2.2 (–32.3 to 21.0)	.87	–4.4 (–34.5 to 18.9)	.74
Severe anemia <sup>b</sup>	34.1 (–133.5 to 81.4)	.52	–34.4 (–287.4 to 53.4)	.58	–141.1 (–1143.1 to 53.2)	.29
All-cause mortality	–1.0 (–616.7 to 85.8)	.99	49.9 (–173.7 to 90.8)	.43	51.3 (–436.5 to 95.6)	.56
<b>Multiple events</b>						
Malaria						
>500 parasites/ $\mu$ L	22.5 (5.8–36.2)	.01	17.2 (1.2–30.7)	.04	–5.2 (–24.5 to 11.1)	.56
>5000 parasites/ $\mu$ L	16.6 (–10.5 to 37.1)	.21	10.7 (–12.2 to 29.0)	.33	2.4 (–21.3 to 21.5)	.83
Anemia <sup>a</sup>	18.4 (–7.2 to 37.9)	.14	–3.4 (–29.5 to 17.5)	.77	–9.9 (–37.0 to 11.9)	.40
Severe anemia <sup>b</sup>	17.6 (–170.0 to 74.8)	.75	–30.5 (–276.2 to 54.7)	.62	–147.2 (–1147.1 to 52.0)	.28
Outpatient visits	3.9 (–6.9 to 13.6)	.46	1.8 (–10.0 to 12.3)	.76	–1.4 (–14.2 to 9.9)	.82
Hospital admissions	15.5 (–42.3 to 49.8)	.53	19.7 (–27.8 to 49.6)	.36	–58.6 (–163.7 to 4.6)	.08

**NOTE.** All infants who received at least 1 dose of sulfadoxine-pyrimethamine or placebo were included. The observation time of every child was stratified in 3 periods of 6 months after each dose of intermittent preventive antimalarial treatment in infants (IPTi). IPTi-1, 6 months after IPTi dose 1; IPTi-2, 6 months after IPTi dose 2; IPTi-3, 6 months after IPTi dose 3.

<sup>a</sup> Hemoglobin level  $<7.5$  g/dL.

<sup>b</sup> Hemoglobin level  $<5.0$  g/dL.

both groups during the first year. Schoenfeld residuals eventually provided evidence for violation of the proportional hazards assumption ( $\rho$ , 0.09;  $P = .02$ ).

In multivariate Poisson regression analysis, a model comprising interaction between efficacy against cumulative events and time of drug application fits better than a model neglecting interaction ( $P = .05$ , by log-likelihood ratio test). This effect was a result of a negative effect modification of the period after IPTi-3 ( $P = .02$ , by Wald test).

Efficacy against first or single malaria episodes during 6 months after IPTi-1 and IPTi-2 were each 23% (95% CI, 4%–38%;  $P = .02$ , and 95% CI, 5%–38%;  $P = .01$ , respectively). In contrast, IPTi-3 did not provide additional protection (protective efficacy, 4%; 95% CI, –18% to 22%;  $P = .68$ ) (figure 3 and table 3). A reduction in the total frequency of malaria episodes of 23% (95% CI, 6%–36%;  $P = .01$ ) was observed after IPTi-1, and a reduction of 17% was observed after IPTi-2 (95% CI, 1%–31%;  $P = .04$ ); a reduction was not observed after IPTi-3 (protective efficacy, –5%; 95% CI, –25% to 11%;  $P = .56$ ) (table 4).

Significant protection against first or single anemia episodes was conferred only after IPTi-1 (protective efficacy, 30%; 95% CI, 5%–49%;  $P = .02$ ), and during the 6-month periods after IPTi-2 and IPTi-3, no protection was provided (table 4). No reduction in the frequency of anemia episodes was observed during the 6-month periods after each drug administration.

No significant differences with regard to severe anemia, hospital admissions, outpatient attendance, and all-cause mortality were observed between the study groups (tables 3 and 4). Results of per-protocol and intention-to-treat analyses did not differ significantly.

**Adverse events.** Nineteen children died during the study period. Seven of the 11 deaths in the placebo group and 4 of the 8 deaths in the treatment group were most likely attributable to malaria. Two individuals in the SP group and 1 person in the placebo group developed Stevens-Johnson syndrome. Symptoms in both patients in the treatment group occurred within 14 days after IPTi-3, suggesting a causative relationship to SP (1 patient simultaneously received artesunate, amodiaquine, and paracetamol). Both children in the SP group recovered fully, and the patient in the placebo group, an HIV-positive infant, died at 5 months of age of multiple-organ failure. Five days before the onset of dermatological symptoms, this child had received antituberculous drugs and trimethoprim-sulfamethoxazole, which are agents that are also associated with Stevens-Johnson syndrome.

Vomiting was more frequent in the SP group (72 events among 1516 applications) than in the placebo group (32 events among 1525 applications; 4.7% vs. 2.1%; risk ratio, 2.26;  $P <$

.001). Other adverse events occurred at similar frequencies in both groups.

## DISCUSSION

The results of our study demonstrate that IPTi with SP was moderately efficacious in reducing the incidence of malaria and, to a certain extent, in preventing anemia episodes in an area with intense perennial malaria transmission. Notably, protection was age-dependent: IPTi administered to infants aged 3 or 9 months, together with the EPI routine vaccinations, conferred protection against malaria, and an extended IPTi dose administered to children aged 15 months did not provide additional benefit. This corresponds to results of studies on presumptive antimalarial treatment with SP for anemia control, which showed only marginal protection in older children [18–20]. When IPTi-3 was administered, 17.8% of children had a body weight  $>10$  kg, suggesting under-dosage of SP according to the most recent recommendations of the manufacturer. However, the level of efficacy did not change after excluding these children from analyses.

Efficacy could also have been diminished by preexisting drug resistance [3, 21] and by selection of resistant parasites, a phenomenon recently shown after single-dose SP treatment [22]. Indeed, there was a slight but nonsignificant increase in the proportion of parasites carrying mutations for SP resistance in both study arms when IPTi-2 and IPTi-3 were administered (table 2), which could partly explain the impaired protective efficacy of IPTi-2 and IPTi-3 against multiple adverse events. However, the equal distribution of molecular markers for SP resistance in both study groups at times of each IPTi administration argues against specific perpetual selection of resistant parasites.

The absolute number of malaria episodes prevented by IPTi (0.25 cases per person-year at risk) was similar to that reported in Tanzania (0.26 cases per person-year at risk) [5]. Because, however, the malaria incidence rate in our study group (1.2 infections per person-year at risk) was higher than that in Tanzania (0.4 infections per person-year at risk), the protective efficacy that we observed was, in fact, lower. Varying degrees of protective efficacies might be explained by differing levels of SP resistance, differences in the use of insecticide-treated bednets, and differences in SP application schedules.

According to our protocol, the only SP application during the first 6 months of life was applied in infants aged 3 months. This contrasts to other IPTi studies, in which the first dose of SP was administered to infants aged 2 or 3 months and a second dose was administered 1 month after the first dose [5–7]. Our schedule was based on the assumption that, for a long-acting drug such as SP, monthly intervals might be too short to ensure postulated IPT characteristics (namely, complete elimination

and absence of the drug for a substantial period of time) [3]. Furthermore, infants aged 2 months are still protected from clinical malaria by maternal antibodies and fetal hemoglobin [23–25]. It is, however, also conceivable that 2 applications during the first 6 months of life might enhance protection by IPTi for unknown reasons.

The trial presented here is, to our knowledge, the first SP-based IPTi study involving active follow-up visits and allowing closer parasitological and hematological monitoring. Early treatment of malaria episodes through frequent patient contacts may have resulted in prevention of complications, thus producing an artificial healthy cohort. Accordingly, the number of hospital admissions was lower than in all previous trials that exclusively performed passive case detection [5–7]. Early treatment of mild malaria episodes, as performed in both study arms, inherently comprises the risk of equalization of study groups and, consequently, underestimation of intervention effects. This is of particular importance, because amodiaquine, part of the current first-line drug combination in Ghana and used for the treatment of uncomplicated malaria episodes in our study, has a long half-life and might exert effects that are similar to those associated with the study drug [26, 27].

It has been suggested that interventions, such as IPTi, could delay the manifestation of severe malaria to an age at which progression to severe malarial anemia becomes less likely and the risk of cerebral malaria has not yet increased [11]. We found that, in an area where the disease is holoendemic, the overall protective effect of IPTi against anemia in infancy is rather low and is statistically significant only after IPTi-1. Our study design, with active follow-up visits, enabled us to routinely assess events of moderate anemia. When applying analyses using our definition of anemia (hemoglobin level, <7.5 g/dL), the frequency of anemia episodes increased after suspending SP treatment. A limitation is that this effect was strongly dependent on the predefined hemoglobin cutoff level. Although unambiguous rebound of anemia has not been reported thus far, a similar trend was observed in northern Ghana [7]. Certainly, early prevention of anemia is of particular importance, because a considerable proportion of childhood deaths from malaria in areas where high transmission occurs are caused by anemia within the first year of life [28–30].

The frequency of adverse events was similar in both groups. However, vomiting was more frequent in children after they received SP therapy, as recently reported [31]. This may, indeed, modify EPI attendance and should be addressed in implementation studies. Serious adverse events were rare, and the 3 cases of Stevens-Johnson syndrome were not conclusively attributable to SP treatment [32, 33].

It has been recommended to link the IPTi schedule with EPI, because disease control in infants in most African countries is best established through this World Health Organization pro-

gram. Data from areas with seasonal transmission indicate, however, that the application schedule should be adapted to the local malaria transmission dynamics [34]. The moderate degree of protection that we observed was possibly caused by a suboptimal drug administration schedule. Protective efficacy is age-dependent, and it is conceivable that IPTi intervals should be shorter in areas with high incidence of malaria [4]. Extension of IPTi into the second year of life did not provide any benefit. These observations from an area where malaria is holoendemic should be considered for recommendations of implementing IPTi in Africa [35–37].

## Acknowledgments

We thank all participants and their parents and guardians, for participating in the study; the field workers, for dedicated assistance; the staff of the Kumasi Centre for Collaborative Research in Tropical Medicine; T. Kruppa; J. Evans (the local safety monitor); C. Bevilacqua, for external audit; all members of the Ghana Health Service in the Afigya Sekyere district; the School of Medical Science and the Department of Community Health; and the members of the IPTi Consortium, who gave expert advice.

**Financial support.** The Bundesministerium für Bildung und Forschung (grant 01KA0202) and the German Academic Exchange Service (to R.K., C.K., S.A., I.L., and H.H.A.). La Roche (Basel, Switzerland) manufactured study drugs free of charge, and Sanofi-Aventis donated Artesunate tablets for treatment of uncomplicated malaria episodes.

**Manuscript preparation.** All authors participated in design, implementation, field work, analysis, and interpretation of the study; J.M. designed the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; O.A. was the principle investigator; R.K., S.A., C.K., I.L., and H.H.A. were responsible for the field work, safety surveillance, and coordinating the passive case detection; additional acquisition of data was performed by B.T., P.A.T., B.K., M.A., F.M., K.A., and E.O.; analysis of data and writing of the manuscript was led by R.K., W.B., and J.M.; and C.G.M. critically reviewed the paper.

**Potential conflicts of interest.** All authors: no conflicts.

## References

1. Breman JG. The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am J Trop Med Hyg* **2001**; *64*:1–11.
2. Hay SI, Guerra CA, Tatem AJ, Noor AM, Snow RW. The global distribution and population at risk of malaria: past, present and future. *Lancet Infect Dis* **2004**; *4*:327–36.
3. White NJ. Intermittent presumptive treatment for malaria. *PLoS Med* **2005**; *2*:e3.
4. O'Meara WP, Breman JG, McKenzie FE. The promise and potential challenges of intermittent preventive treatment for malaria in infants (IPTi). *Malar J* **2005**; *4*:33.
5. Schellenberg D, Menendez C, Kahigwa E, et al. Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomised, placebo-controlled trial. *Lancet* **2001**; *357*:1471–7.
6. Macete E, Aide P, Aponte J, et al. Intermittent preventive treatment for malaria control administered at times of routine vaccinations in Mozambican infants: a randomized, placebo-controlled trial. *J Infect Dis* **2006**; *194*:276–85.
7. Chandramohan D, Owusu-Agyei S, Carneiro I, et al. Cluster randomised trial of intermittent preventive treatment for malaria in infants in area of high, seasonal transmission in Ghana. *BMJ* **2005**; *331*:727–33.
8. Rosen JB, Breman JG. Malaria intermittent preventive treatment in infants, chemoprophylaxis, and childhood vaccinations. *Lancet* **2004**; *363*:1386–8.

9. Menendez C, Kahigwa E, Hirt R, et al. Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *Lancet* **1997**; 350:844–50.
10. Schellenberg D, Menendez C, Aponte JJ, et al. Intermittent preventive antimalarial treatment for Tanzanian infants: follow-up to age 2 years of a randomised, placebo-controlled trial. *Lancet* **2005**; 365:1481–3.
11. Reyburn H, Mbatia R, Drakeley C, et al. Association of transmission intensity and age with clinical manifestations and case fatality of severe *Plasmodium falciparum* malaria. *JAMA* **2005**; 293:1461–70.
12. Snow RW, Omumbo JA, Lowe B, et al. Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet* **1997**; 349:1650–4.
13. Mishra V, Vaessen M, Boerma JT, et al. HIV testing in national population-based surveys: experience from the Demographic and Health Surveys. *Bull World Health Organ* **2006**; 84:537–45.
14. Smith T, Schellenberg JA, Hayes R. Attributable fraction estimates and case definitions for malaria in endemic areas. *Stat Med* **1994**; 13: 2345–58.
15. Marks F, Evans J, Meyer CG, et al. High prevalence of markers for sulfadoxine and pyrimethamine resistance of *Plasmodium falciparum* in the absence of drug pressure in the Ashanti region of Ghana. *Antimicrob Agents Chemother* **2005**; 49:1101–5.
16. World Health Organization. Severe falciparum malaria. *Trans R Soc Trop Med Hyg* **2000**; 94:S1–19.
17. Carter JY, Loolpapit MP, Lema OE, et al. Reduction of the efficacy of antifolate antimalarial therapy by folic acid supplementation. *Am J Trop Med Hyg* **2005**; 73:166–70.
18. Tomashek KM, Woodruff BA, Gotway CA, Bloland P, Mbaruku G. Randomized intervention study comparing several regimens for the treatment of moderate anemia among refugee children in Kigoma Region, Tanzania. *Am J Trop Med Hyg* **2001**; 64:164–71.
19. Verhoef H, West CE, Kraaijenhagen R, et al. Intermittent administration of iron and sulfadoxine-pyrimethamine to control anaemia in Kenyan children: a randomized controlled trial. *Lancet* **2002**; 360: 908–14.
20. Desai MR, Mei JV, Kariuki SK, et al. Randomized, controlled trial of daily iron supplementation and intermittent sulfadoxine-pyrimethamine for the treatment of mild childhood anemia in western Kenya. *J Infect Dis* **2003**; 187:658–66.
21. Baird JK. Effectiveness of antimalarial drugs. *N Engl J Med* **2005**; 352: 1565–77.
22. Marks F, von Kalckreuth V, Kobbe R, et al. Parasitological rebound effect and emergence of pyrimethamine resistance in *Plasmodium falciparum* after single-dose sulfadoxine-pyrimethamine. *J Infect Dis* **2005**; 192:1962–5.
23. Hviid L, Staalsøe T. Malaria immunity in infants: a special case of a general phenomenon? *Trends Parasitol* **2004**; 20:66–72.
24. Pasvol G, Weatherall DJ, Wilson RJ, Smith DH, Gilles HM. Fetal haemoglobin and malaria. *Lancet* **1976**; 1:1269–72.
25. Snow RW, Nahlen B, Palmer A, et al. Risk of severe malaria among African infants: direct evidence of clinical protection during early infancy. *J Infect Dis* **1998**; 177:819–22.
26. Massaga JJ, Kitua AY, Lemnge MM, et al. Effect of intermittent treatment with amodiaquine on anaemia and malarial fevers in infants in Tanzania: a randomised placebo-controlled trial. *Lancet* **2003**; 361: 1853–60.
27. Olliaro P, Mussano P. Amodiaquine for treating malaria. *Cochrane Database Syst Rev* **2003**; 2:CD000016.
28. Murphy SC, Breman JG. Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. *Am J Trop Med Hyg* **2001**; 64(1–2 Suppl):S57–67.
29. Kitua AY, Smith TA, Alonso PL, et al. The role of low level *Plasmodium falciparum* parasitaemia in anaemia among infants living in an area of intense and perennial transmission. *Trop Med Int Health* **1997**; 2: 325–33.
30. Schellenberg D, Schellenberg JR, Mushi A, et al. The silent burden of anaemia in Tanzanian children: a community-based study. *Bull World Health Organ* **2003**; 81:581–90.
31. Cisse B, Sokhna C, Boulanger D, et al. Seasonal intermittent preventive treatment with artesunate and sulfadoxine-pyrimethamine for prevention of malaria in Senegalese children: a randomised, placebo-controlled, double-blind trial. *Lancet* **2006**; 367:659–67.
32. Sturchler D, Mittelholzer ML, Kerr L. How frequent are notified severe cutaneous adverse reactions to fansidar? *Drug Saf* **1993**; 8:160–8.
33. Forman R, Koren G, Shear NH. Erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis in children: a review of 10 years' experience. *Drug Saf* **2002**; 25:965–72.
34. Greenwood B. Review: intermittent preventive treatment—a new approach to the prevention of malaria in children in areas with seasonal malaria transmission. *Trop Med Int Health* **2006**; 11:983–91.
35. Meremikwu MM, Omari AA, Garner P. Chemoprophylaxis and intermittent treatment for preventing malaria in children. *Cochrane Database of Syst Rev* **2005**; 4:CD003756.
36. Egan A, Crawley J, Schellenberg D; IPTi Consortium. Intermittent preventive treatment for malaria control in infants: moving towards evidence-based policy and public health action. *Trop Med Int Health* **2005**; 10:815–7.
37. Crawley J. Reducing the burden of anemia in infants and young children in malaria-endemic countries of Africa: from evidence to action. *Am J Trop Med Hyg* **2004**; 71(2 Suppl):S25–34.