Evaluation of Recurrent Parasitemia after Artemether-Lumefantrine Treatment for Uncomplicated Malaria in Children in Western Kenya

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Abstract. From April 2005 to April 2006, a phase 2 malaria vaccine trial in Kenya enrolled 400 children aged 12–47 months. Each received mixed supervised and unsupervised artemether-lumefantrine for uncomplicated malaria, using a standard six-dose regimen, by weight. Children were followed for detection of parasitemia and clinical malaria. A median of two negative malaria blood films occurred during every recurrent parasitemia (RP) episode, suggesting reinfection over late recrudescence. Median time to RP after starting artemether-lumefantrine was 37 days (36–38). Of 2,020 evaluable artemether-lumefantrine treatments, there were no RPs in 99% by day 14, 71% by day 28, and 41% by day 42. By World Health Organization standards, 71% of treatment courses had adequate responses. Although recrudescence in some cannot be ruled out, our cohort had a shorter median time to RP compared with other artemether-lumefantrine treatment studies. This underscores patient counseling on completing all treatment doses for optimal protection from RP.

INTRODUCTION

The World Health Organization (WHO) estimates approximately 0.5 billion cases of malaria and over 1 million deaths from malaria around the world annually, with 90% of deaths in sub-Saharan Africa and most of these among children under 5 years old.¹ The fixed-dose combination, artemether-lumefantrine (AL), and the copackaged combination of amodiaquine and artesunate are currently the most widely used artemisinin-based combination therapies (ACTs) in sub-Saharan African countries for the treatment of uncomplicated malaria.² To date, 28 countries have adopted AL as first-line treatment of uncomplicated malaria.³ In 2004, the Kenyan Ministry of Health proposed that AL be given to all cases of uncomplicated malaria.⁴ Since implementation in 2006, this policy has had little evaluation of its effectiveness.

Recently, we prescribed AL as the first-line treatment of all clinical episodes of malaria to young children participating in a separately reported intervention trial.⁵ AL was administered in a manner consistent with public-health practices (i.e., the first dose was given in the clinic, and the remainder was given to the parent or guardian for administration at home). However, active and passive case detection for subsequent malaria was intense and prolonged. Mindful of the need for a thorough evaluation, we present data on the use of AL for treatment of *Plasmodium falciparum*, *P. ovale*, and *P. malariae* species and the species-specific risk of recurrent parasitemia (RP).

MATERIALS AND METHODS

Study site. Kombewa Division is a rural area on Lake Victoria about 40 km west of Kisumu in western Kenya. The majority of the population is subsistence farmers living in mud and clay homes with thatched roofs. Malaria is a significant cause of morbidity and mortality in most children in this area. It occurs throughout the year with two peak transmission seasons that coincide with a long and short rainy season. The burden of malaria particularly affects children under 5 years

of age, with *P. falciparum* accounting for over 95% of malaria infections and the documented average monthly parasite prevalence reaching up to 83%.⁶ In Kombewa, the total annual entomological inoculation rate (EIR) is as high as 22.0–31.1 infectious bites/person/year.⁷ The malaria burden is significant even into the surrounding highlands, with 10.6 infectious bites/person/year.⁸ In keeping with this high malaria transmission, there is a high prevalence of red blood cell polymorphisms associated with protection against malaria.^{9–13}

Study subjects. From April 2005 to April 2006, 400 children age 12–47 months were enrolled in a study at the Kenya Medical Research Institute (KEMRI)/US Army Medical Research Unit–Kenya (USAMRU-K) Clinical Research Center (CRC) in Kombewa Division, western Kenya. Inclusion criteria for enrollment into this vaccine trial included aged 12–47 months at the day of screening, written informed consent obtained from at least one parent or guardian before the start of the study, and available to participate for the duration of the study. Exclusion criteria included the following items: axillary temperature over 37.5°C, respiratory rate over 50 breaths per minute, serum alanine aminotransferase (ALT) over 50 IU/L, serum creatinine levels over 1.1 mg/dL, hemoglobin under 8.0 g/dL, platelets under 100,000 per mm³, and impaired immunity, active disease, or any other condition that may pose as a threat to the child. As a benefit of the study, participants received complete health care from the study clinic. This health care included AL treatment of any uncomplicated, microscopy-proven infection with malaria. Children had to have parasitemia with clinical symptoms to receive treatment of malaria. Asymptomatic, parasitemic patients were not treated with AL until they became symptomatic.

Laboratory evaluation. At the time of screening, venous whole blood was drawn for assay to detect sickle-cell disease or trait, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and alpha-thalassemia.

To obtain hemoglobin typing by high performance liquid chromatography (HPLC), blood samples for preparation of hemolysates were collected into K₂ ethylenediaminetetraacetic acid (EDTA) anticoagulated tubes (Becton Dickinson Vacutainer, Franklin Lakes, NJ). All specimens were analyzed on the Bio-Rad Variant I cationic-exchange HPLC system using Variant I β-Thalassemia Short Program as recommended by the manufacturer (Bio-Rad Laboratories, Hercules, CA). The DNA was isolated from EDTA blood using QIAamp

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DNA Blood Mini Kits (QIAGEN Inc., Valencia, CA) and stored at -20°C until required for G6PD genotyping.

To detect the $-\alpha^{3,7}$ deletions and the $\alpha\alpha\alpha^{\text{anti}3,7}$ triplication for alpha-thalassemia status determination, a method described by Liu and others¹⁴ was adopted with modification.¹⁵

Study design. The CRC is located in the center of Kombewa Division of Kisumu West District. The study area has field stations, and children were drawn from within a 1-mile radius of each station for easy and rapid access. These 13 field stations were within 10 miles of the CRC throughout the division and were staffed 24 hours/day throughout the study to facilitate medical care.

Children were given the first dose of AL with milk under directly observed treatment. Parents of the children were instructed on how to give the remaining five doses at 8, 24, 36, 48, and 60 hours after the initial dose. Parents were encouraged to administer AL only after a meal. AL was dispensed as a fixed-dose combination tablet (20 mg artemether and 120 mg lumefantrine). Dose was calculated for body weight. One tablet per dose was given for children weighing 5.0–14.9 kg, those children weighing 15.0–24.9 kg received two tablets, and three tablets were given to those children weighing 25.0–35.0 kg. Parents of the children were instructed to return to the CRC for a review visit and repeat malaria blood film (MBF) at 7 days after starting AL treatment or anytime they noted that the children were not improving or had any other abnormality. There was no blood film taken before day 7 unless the child was symptomatic and presented to the clinic. During review visits at 7 days after starting AL treatment, compliance with AL dosing, concomitant drug use, persisting symptoms, and possible adverse side effects were assessed. Rescue treatment with atovaquone-proguanil (AP) was provided if early treatment failure from AL was suspected or confirmed.

After the day 7 follow-up, children were followed with both passive and active detection of parasites during study scheduled home and clinic visits. Because children received all health care from the study clinic, an MBF microscopy could be performed at every presentation (passive detection). Additionally, children received MBFs every 1 month regardless of symptoms (active detection) as per the scheduled study activity. History of fever during any clinic visit or field follow-up led to clinical evaluation and documentation of fever. Transportation to the CRC was provided for all children and their caretakers, for both acute care and scheduled follow-ups, to ensure that they could present to the clinic for all care. Field workers were used to notify children of upcoming visits and coordinate transportation. Follow-up rates for the study were $>90\%$.

For asymptomatic children getting MBFs as part of active detection, MBFs were air dried overnight and individually stained (no dip wells were used) with a 3% Giemsa solution. MBFs from symptomatic patients were rapidly stained with 10% Giemsa solution so that the clinician could make clinical decisions. Another slide was made with slow drying, and 3% Giemsa was used in confirmation. Each MBF was read by two independent microscopists, each of whom examined 100 oil-immersion fields of the thick film before declaring a slide to be negative. Discrepancies between the two reads (parasite detection, speciation, or parasite density) were finalized by a third expert microscopist. If a child missed a scheduled MBF but was MBF negative at the next blood draw, he or she was considered negative for the entire period for the purposes of this secondary data analysis.

All children with microscopy-proven *Plasmodium* malaria treated with AL were included in the current analysis to evaluate the effectiveness of this ACT. Any scheduled or unscheduled visit for routine follow-up or symptomatic care provided data points for this analysis. The time to RP (or the risk of RP) was defined as the number of days between taking the first dose of AL and the day of microscopically detecting malaria parasites in the blood film (above 0 per μL) after having at least one negative MBF recorded. The time at risk ended whenever one of the following conditions occurred: RP, loss to follow-up, withdrawal, or end of follow-up period. To eliminate possible drug interaction between antimalarial treatments, time intervals between AL treatments that included any non-AL antimalarial treatment ($N = 33$) were excluded from analysis (Figure 1).

Data analysis. WHO guidelines for assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria were used to evaluate the time to RP after starting AL.¹⁶ Each new treatment was considered a new case for analysis purposes; therefore, one patient could afford multiple cases for analysis (Figure 2). Early treatment failure (ETF) was defined as danger signs (i.e., need for hospitalization, inability to tolerate the drug regimen, or other serious adverse events), complicated malaria, or failure to adequately respond to therapy on days 0–3. Late clinical failure (LCF) consisted of danger signs or complicated malaria or fever and parasitemia on days 4–28 without previously meeting criteria for ETF. Late parasitological failure (LPF) was defined as the presence of parasitemia on day 28 and an axillary temperature of $<37.5^{\circ}\text{C}$ without previously meeting any of the criteria for ETF or LCF. Adequate clinical and parasitological response (ACPR) was defined as the absence of parasitemia on day 28 irrespective of axillary temperature without previously meeting any of the criteria for ETF, LCF, or LPF.¹⁶

Data were entered and analyzed using STATA version 10 software (StataCorp LP, College Station, TX). Cumulative survival, median time to RP, and proportion with RP at 7, 14, 21, 28, and 42 days of follow-up were estimated using the Kaplan–Meier method (Figure 1). Survival curves were estimated for the entire sample and subgroups defined by the presence or absence of sickle-cell trait, alpha-thalassemia, and G6PD deficiency. The significance of the associations between these potential risk factors and the risk of RP was assessed using Cox proportional hazards regression models. The Anderson–Gill counting method was used to account for multiple RP per patient.¹⁷ All reported P values are two-sided without adjustment for multiple testing and were considered statistically significant if below 0.05.

RESULTS

Study flow. Of the 400 enrollees, 383 completed their entire 12 months. The median number of infections per volunteer was 5.5 symptomatic parasitemias over 12 months. Twenty-three percent of the children had seven or more symptomatic parasitemias in 1 year, with some children (2%) being treated with AL every 1 month for 12 consecutive months (Figure 3).

Overall effect of RP. During the 12-month study period, there were 2,209 AL treatments for uncomplicated malaria among 383 of 400 participants. These treatments were defined as individual cases (Figure 2). Three of three hundred

FIGURE 1. Study algorithm ($N = 400$).

eighty-three participants had no recordable time to RP for any AL treatment; 365 of 400 participants (91%) completed the 12-month study. Overall, 99% of all episodes had no detectable parasites up to day 14, 71% up to day 28, and 41% up to day 42 after the start of AL treatment. Fifteen episodes (0.7%) had positive MBFs on day 7 follow-up after completing AL treatment. After performing extensive chart review, seven of these were determined to be non-compliant with the regimen. The remaining eight episodes experienced by eight different children met the WHO definition of LCF, and the children were started on rescue AP therapy for AL treatment failure.¹⁶

FIGURE 2. Timeline of study. This timeline illustrates that multiple MBFs were collected from participants before and after AL treatment. The time to RP (or the risk of RP) was defined as the number of days between taking the first dose of AL and the day of microscopically detecting malaria parasites in the blood film (above 0 per μL) after having at least one negative MBF recorded. The subsequent start day of AL begins the day 0 for the next time to RP interval.

There were 2,020 (91%) evaluable periods for the time to RP. Each interval encompassed when AL treatment was started, a negative MBF was then recorded, and the interval was completed by a positive MBF (Figure 2). These episodes were spread among 380 participants, all of which had at least one time to RP episode; 28 of these 380 participants had only one time to RP (Figure 3). For all 2,020 calculable times to RP intervals, the median time to RP was 37 days (36–38 days) (Figure 4). A median of two negative MBFs existed during every RP episode.

Effect of blood dyscrasias on RP. After adjusting for multiple events per volunteer, study children with sickle-cell trait showed a tendency to a lower risk of RP (10% lower) compared with children who were sickle-cell negative ($P = 0.095$). Overall, episodes involving children with sickle-cell trait ($N = 350$) had a median time to RP of 42 days (40–45 days) compared with 36 days (35–37 days) for those episodes involving children who were sickle-cell negative ($N = 1,653$). There was no difference in RP between the groups through day 14. As of day 28, 80% of episodes involving children with sickle-cell trait were negative compared with 69% of episodes involving children who were sickle-cell negative. By day 42, 50% of episodes with children with sickle-cell trait were negative compared with 39% of episodes involving children who were sickle-cell negative.

There was no difference in median time to RP for episodes with G6PD-deficient ($N = 239$) children versus episodes with non-G6PD ($N = 1,781$) children (36 versus 37 days, respectively). Similarly, episodes with heterozygous and homozygous alpha-thalassemia children showed no effect on median

FIGURE 3. Distribution of RP episodes.

time to RP compared with episodes with children with normal alpha-hemoglobin chains ($P = 0.947$ and $P = 0.465$, respectively). Gender status had no effect on time to RP ($P = 0.475$). The age of participants had a statistically significant effect on the median time to RP ($P = 0.010$), with episodes involving 12- to 24-month-old children having a 15% higher risk of RP compared with the episodes involving 36- to 48-month-old children ($P = 0.041$).

Episodes involving children who had malaria at the time of initial screening ($N = 320$) had an 18% increased overall risk of RP compared with those episodes involving children who did not have malaria at the time of initial screening ($N = 1,700$; $P = 0.015$). There was no difference in the median time to RP between the groups. At the time of screening, episodes involving children with G6PD or sickle-cell trait had no difference in the risk of RP compared with their counterparts ($P = 0.623$ and $P = 0.380$, respectively). Episodes involving children with heterozygous alpha-thalassemia at the time of screening had significant difference in the risk of RP compared with those episodes involving children with normal alpha-hemoglobin chains ($P = 0.031$). Episodes involving homozygous alpha-thalassemia children had no difference in the risk

of RP at the time of screening compared with those episodes involving children with normal alpha-hemoglobin chains ($P = 0.942$).

Examining the intervals where a time to RP was calculable ($N = 2,020$), of the 311 total episodes with no reported MBF recorded between days 1 and 10, 95% ($N = 296$) had at least one subsequent negative MBF between days 11 and 42, and 30% ($N = 90$) of these had two or more negative MBFs between day 11 and 42. Comparatively, of the 1,709 total episodes where there was at least one recorded MBF between days 1 and 10, 61% ($N = 1,035$) had at least one subsequent negative MBF between days 11 and 42, and 26% ($N = 272$) of these had two or more negative MBFs between day 11 and 42.

Effect of *P. ovale* and *Plasmodium malariae* mono-infections on RP. Although confounded by surrounding *P. falciparum*, there were episodes when AL was given as an off-label use to treat *P. ovale* or *P. malariae* mono-infections. One hundred percent of AL treatments given for mono-infections with either *P. ovale* ($N = 21$) or *P. malariae* ($N = 20$) had negative MBFs as of day 14. Twenty different children had one *P. malariae* mono-infection, and *P. malariae* was present as a mixed infection at the time of retreatment during one episode. Conversely, 3 of 18 different children with *P. ovale* mono-infections had two *P. ovale* mono-infections throughout the study. There were six episodes when *P. ovale* was present at the time of retreatment. Compared with *P. falciparum* mixed and mono-infections ($N = 1,979$), the median time to RP was 40 days (29–48 days) for *P. ovale* ($P = 0.74$) and 52 days (33–139 days) for *P. malariae* ($P = 0.017$) (Figure 5).

Effect of vaccination status on RP. One possible limitation of this study was whether the 50% of volunteers who were vaccinated with the trial vaccine might have a longer time to RP after AL was used compared with control volunteers who did not receive the trial vaccine. Those who received the trial vaccine had a median time to RP of 37 days (35–39 days) compared with 37 days (35–39 days) for those who did not receive the trial vaccine ($P = 0.192$). There was no difference in the median time to RP between those who received vaccine and those who did not receive trial vaccine. In addition, the trial vaccine did not reduce the overall incidence of clinical malaria episodes or malaria infections.⁵

FIGURE 4. Time to RP after initiation of AL treatment ($N = 2,020$). Figure shows that the median time to RP is 37 days. FIGURE 5. Time to RP by *Plasmodium* species after initiation of AL treatment.

DISCUSSION

This paper presents an evaluation of a mixed supervised and unsupervised treatment (although largely the latter) course of AL in microscopy proven, symptomatic, uncomplicated malaria in 1- to 4-year-old children from a high-transmission area of western Kenya. The large number of treatments (400 patients with 2,209 treatments) and the intensity of follow-up make these field-effectiveness data in western Kenya the first study of its kind in East Africa to evaluate the important question of time to RP after repeated treatment with AL, and this has great programmatic impact for national malaria-control programs. With multiple (median = 5.5) AL treatment episodes/child/year documenting the time to RP for each participant, confounders such as rainfall or other seasonality changes are less likely to affect the current study's time to RP findings compared with other reported time to RP malaria studies that measure the first and only time to RP at a certain time of year.

In this group, 71% and 41% of children after treatment with AL had no detectable parasites by days 28 and 42, respectively, and this evidence corroborates findings from a study from the neighboring state of Uganda.¹⁸ The Ugandan study confirms that AL does not confer post-treatment prophylaxis,¹⁸ and this is related to the shorter half-life of lumefantrine.^{19,27} Gene mapping was not performed in the current study to distinguish recrudescence from reinfection; however, by the WHO criteria for assessing and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria,¹⁶ this study shows a 71% and 41% programmatic ACPR rate by days 28 and 42, respectively. This shows that, in areas of high transmission, about 30-60% will require a repeat treatment with AL within 4-6 weeks of initial treatment, and this should be factored in drug supplies.

In other similar, tightly controlled efficacy studies of AL using genotyping, at least 96% of the RP after day 28 were caused by new infections. In a study by Bukirwa and others²⁰ comparing artesunate plus amodiaquine (AS + AQ) and AL in a hyperendemic malaria region of Uganda with an EIR of 591, 51% of those treated with AL (compared with 29% in our study) and 66% of those treated with amodiaquine plus artesunate had RP within 28 days. Interestingly, genotyping revealed that 99% of episodes of RP at day 28 were caused by new infections.²⁰ In a trial conducted in a holoendemic region of Congo that compared artesunate plus sulfadoxine-pyrimethamine (AS + SP), AS + AQ, and AL, the 28-day polymerase chain reaction (PCR)-corrected cure rate was 90% for AS + SP, 98.5% for AS + AQ, and 100% (95.8-100%) for AL.²¹ In a holoendemic region of Burkina Faso, Zongo and others²² showed that amodiaquine plus sulfadoxine-pyrimethamine prevented RP and recurrent symptomatic malaria better than AL ($P = 0.0001$ and $P = 0.0002$, respectively). Nested PCR and restriction-enzyme digestion analysis revealed that 98% of recurrences were caused by new infections for this study.²² All of these studies have shown that ACTs effectively treat uncomplicated malaria, and the time to RP after starting AL is generally comparable with other ACT therapies.

Pooled non-comparative, multicenter studies of African children have shown the 28-day cure rate for AL effectiveness ranged from 86% to 89% uncorrected and 92% to 96% corrected by PCR for reinfection.^{23,24} Although the current study's

non-PCR corrected day 28 cure rate (71%) was not as effective as the previously mentioned non-comparative and comparative studies, it should be noted that the time to RP for all these previously mentioned studies was calculated as the first and only time to RP. The novelty of the current study is that multiple episodes of RP exist for the same participant, with a participant having a median of 5.5 episodes of evaluable RP in this high malaria-transmission area. This study adds to information in the growing body of knowledge on the effectiveness of AL and elucidates a means to account for multiple times to RP for the same participant through the Anderson-Gill counting method. The fact that there is no decrease in the time to subsequent RP shows that AL is effective in clearing parasitemias in RP infections, and a median of two negative MBFs during each time to RP supports that subsequent parasitemias were not caused by parasites resistant to AL but new infections. This may be an indicator that AL, because of the relatively shorter half-life of lumefantrine, is not likely to select resistant parasites rapidly.

This finding of high RP rate should be interpreted with caution in reference to choosing a first-line ACT. The short half-life is likely to confer a longer therapeutic life to AL in relation to ACTs with long-life second drug, such as dihydroartemisinin plus piperazine and artesunate plus mefloquine,^{18,25} in selection for resistance parasites, despite the immediate gain of post-treatment prophylaxis¹⁸ as was seen with SP.²⁶ In addition, this finding of high RP also underscores the need for patient counseling on completing all treatment doses for optimal protection from RP.

This study has also shown that AL is effective in the treatment of *P. malariae* in children. The recurrent *P. ovale* monoinfections raise questions as to whether AL can effectively treat *P. ovale*. Therefore, AL in combination with a drug like primaquine should be considered for the radical cure of two species of malaria.

Children who had malaria at the time of screening were much more likely to develop RP compared with those who did not have malaria at the time of screening. It could be postulated that these volunteers were from areas with higher transmission and received more infectious bites over the study period. However, there was a detectable difference in the log odds of infection across field stations ($P = 0.001$) and a range of median time to RP of 34-42 days. In areas with higher odds of infection, RP was probably from a new infectious bite. This sentiment of reinfection over recrudescence is furthered by the WHO guidelines that the longer the duration of post-treatment follow-up, the greater the chance that reappearance of parasites may be caused by reinfection rather than recrudescence.¹⁶

In this study, we have shown that sickle-cell trait tends to protect from RP after treatment with AL ($P = 0.095$), which is in keeping with that it confers protection against malaria and likely gives a false high-efficacy rate of an antimalarial.²⁶ The reduced risk of RP in older children in this study shows the relatively rapid acquisition of antiparasite immunity in an area of high transmission. This finding should be interpreted with caution in reference to choosing a first-line ACT. The short half-life is likely to confer a longer therapeutic life to AL in relation to ACTs with a long-life second drug, such as dihydroartemisinin/piperazine and artesunate plus mefloquine,^{18,25} in selection for resistance parasites, despite the immediate gain of post-treatment prophylaxis.¹⁸

CONCLUSION

AL is an effective treatment of uncomplicated malaria in 1- to 4-year-old children in the hyperendemic areas of western Kenya. In an area of high malaria transmission, parent-supervised AL protects patients for a median time of 37 days until possible RP. Further research should be dedicated to more closely examine the possible role that AL may play in preventing RP for *P. ovale* and *P. malariae*.

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Ethical review: Study subjects were recruited, screened, and enrolled under a phase II malaria vaccine protocol approved by the Kenyan Medical Research Institute, the Western Institutional Review Board, and the US Army Surgeon General's Institutional Review Board. The trial was registered as NCT 0023990. A subsequent amendment to provide additional data analysis was approved by the Walter Reed Army Institute of Research Human Use Review Committee and the Uniformed Services University of the Health Sciences Institutional Review Board.

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REFERENCES

- World Health Organization, 2005. *World Malaria Report 2005*. Available at: <http://rbm.who.int/wmr2005/html/1-1.htm>. Accessed April 10, 2007.
- Chanda P, Masiye F, Chitah BM, Sipilanyambe N, Hawela M, Banda P, Okorosobo T, 2007. A cost-effectiveness analysis of artemether lumefantrine for the treatment of uncomplicated malaria in Zambia. *Malar J* 6: 21.
- Novartis, 2007. Patients benefit from artemether/lumefantrine community development. Proceedings of the American Society of Tropical Medicine and Hygiene 56th Annual Meeting, November 4–8, 2007, Philadelphia, PA.
- Kimani D, 2007. *New Drug Brings Down Cost of Treating Malaria*. Global Business Coalition on HIV/AIDS, Tuberculosis and Malaria. Available at: <http://www.businessfightsaids.org>. Accessed April 20, 2007.
- Ogutu BR, Apollo OJ, McKinney D, Okoth W, Siangla J, Dubovsky F, Tucker K, Waitumbi JN, Diggs C, Wittes J, Malkin E, Leach A, Soisson LA, Milman JB, Otieno L, Holland CA, Polhemus M, Remich SA, Ockenhouse CF, Cohen J, Ballou WR, Martin SK, Angov E, Stewart VA, Lyon JA, Heppner DG, Withers MR, MSP-1 Malaria Vaccine Working Group, 2009. Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers no protection to young children in Western Kenya. *PLoS One* 4: e4708.
- Bloland PB, Boriga DA, Ruebush TK, McCormick JB, Roberts JM, Oloo AJ, Hawley W, Lal A, Nahlen B, Campbell CC, 1999. Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission II. Descriptive epidemiology of malaria infection and disease among children. *Am J Trop Med Hyg* 60: 641–648.
- Ndenga B, Githeko A, Omukunda E, Munyekenye G, Atieli H, Wamai P, Mbogo C, Minakawa N, Zhou G, Yan G, 2006. Population dynamics of malaria vectors in western Kenya highlands. *J Med Entomol* 43: 200–206.
- Githeko AK, Ayisi JM, Odada PK, Atieli FK, Ndenga BA, Githure JJ, Yan G, 2006. Topography and malaria transmission heterogeneity in western Kenya highlands: prospects for focal vector control. *Malar J* 5: 107.
- Kifude CM, Polhemus ME, Heppner DG, Withers MR, Ogutu BR, Waitumbi JN, 2007. Hb Kenya among Luo adults and young children in malaria holoendemic western Kenya: screened by high performance liquid chromatography and confirmed by polymerase chain reaction. *Hemoglobin* 31: 401–408.
- May J, Evans JA, Timmann C, Ehmen C, Busch W, Thye T, Agbenyega T, Horstmann RD, 2007. Hemoglobin variants and disease manifestations in severe falciparum malaria. *J Am Med Assoc* 297: 2220–2226.
- Ruwende C, Khoo SC, Snow RW, Yates SN, Kwiatkowski D, Gupta S, Warn P, Allsopp CE, Gilbert SC, Peschu N, Newbold CI, Greenwood BM, Marsh K, Hill VS, 1995. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. *Nature* 376: 246–249.
- Wambua S, Mwangi TW, Kortok M, Uyoga SM, Macharia AW, Mwacharo JK, Weatherall DJ, Snow RW, Marsh K, Williams TN, 2006. The effect of alpha-thalassaemia on the incidence of malaria and other diseases in children living on the coast of Kenya. *PLoS Med* 3: e158.
- Williams TN, Wambua S, Uyoga S, Macharia A, Mwacharo JK, Newton CR, Maitland K, 2005. Both heterozygous and homozygous alpha+ thalassemias protect against severe and fatal *Plasmodium falciparum* malaria on the coast of Kenya. *Blood* 106: 368–371.
- Liu YT, Old JM, Miles K, Fisher CA, Weatherall DJ, Clegg JB, 2000. Rapid detection of alpha-thalassaemia deletions and alpha-globin gene triplication by multiplex polymerase chain reactions. *Br J Haematol* 108: 295–299.
- Waitumbi JN, Kifude CM, Polhemus ME, Heppner DG, Withers MR, Ogutu BR, 2007. Hb G-Philadelphia or Stanleyville II? When the phenotype and genotype do not agree. *Eur J Haematol* 79: 177–178.
- World Health Organization, 2003. *Assessment and Monitoring Antimalarial Drug Efficacy for the Treatment of Uncomplicated Falciparum Malaria*. Available at: <http://www.who.int/malaria/docs/ProtocolWHO.pdf>. Accessed April 10, 2007.
- Andersen P, Borgan O, Gill RD, Keiding N, 1993. *Statistical Models Based on Counting Processes*, 1st ed. New York, NY: Springer-Verlag.
- Kanya MR, Yeka A, Bukirwa H, Lugemwa M, Rwakimari JB, Staedke SG, Talisuna AO, Greenhouse B, Nosten F, Rosenthal PJ, Wabwire-Mangen F, Dorsey G, 2007. Artemether-lumefantrine versus dihydroartemisinin-piperaquine for treatment of malaria: a randomized trial. *PLoS Clin Trials* 18: e20.
- Kokwaro G, Mwai L, Nzila A, 2007. Artemether/lumefantrine in the treatment of uncomplicated falciparum malaria. *Expert Opin Pharmacother* 8: 75–94.
- Bukirwa H, Yeka A, Kanya MR, Talisuna A, Banek K, Bakyaita N, Rwakimari JB, Rosenthal PJ, Wabwire-Mangen F, Dorsey G, Staedke SG, 2006. Artemisinin combination therapies for treatment of uncomplicated malaria in Uganda. *PLoS Clin Trials* 1: e7.
- van den Broek I, Kitz C, Attas SA, Libama F, Balasegaram M, Guthmann JP, 2006. Efficacy of three artemisinin combination therapies for the treatment of uncomplicated *Plasmodium falciparum* malaria in the Republic of Congo. *Malar J* 5: 113.

22. Zongo I, Dorsey G, Rouamba N, Tinto H, Dokomajilar C, Guiguemde RT, Rosenthal PJ, Ouedraogo JB, 2007. Artemether-lumefantrine versus amodiaquine plus sulfadoxine-pyrimethamine for uncomplicated falciparum malaria in Burkina Faso: a randomized non-inferiority trial. *Lancet* 369: 491–498.
23. Falade CO, Makanga M, Premji Z, Ortmann CE, Stockmeyer M, de Palacios PI, 2005. Efficacy and safety of artemether-lumefantrine (Coartem) tablets (six-dose regimen) in African infants and children with acute, uncomplicated falciparum malaria. *Trans R Soc Trop Med Hyg* 99: 459–467.
24. Makanga M, Premji Z, Falade C, Karbwang J, Mueller EA, Andriano K, Hunt P, de Palacios PI, 2006. Efficacy and safety of the six-dose regimen of artemether-lumefantrine in pediatrics with uncomplicated *Plasmodium falciparum* malaria: a pooled analysis of individual patient data. *Am J Trop Med Hyg* 74: 991–998.
25. Bhatt KM, Samia BM, Bhatt SM, Wasunna KM, 2006. Efficacy and safety of an artesunate/mefloquine combination, (artequin) in the treatment of uncomplicated *P. falciparum* malaria in Kenya. *East Afr Med J* 83: 236–242.
26. Terlouw DJ, Aidoo MA, Udhayakumar V, Kolczak MS, Oloo AJ, Kager PA, Lal A, Nahlen B, ter Kuile FO, 2002. Increased efficacy of sulfadoxine-pyrimethamine in the treatment of uncomplicated falciparum malaria among children with sickle cell trait in western Kenya. *J Infect Dis* 186: 1661–1668.
27. Nzila AM, Nduati E, Mberu EK, Hopkins SC, Monks SA, Winstanley PA, Watkins WM, 2000. Molecular evidence of greater selective pressure for drug resistance exerted by the long-acting antifolate Pyrimethamine/Sulfadoxine compared with the shorter-acting chlorproguanil/dapsone on Kenyan *Plasmodium falciparum*. *J Infect Dis* 181: 2023–2028.