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HIV-1 Protease Inhibitors and Clinical Malaria: a Secondary Analysis of the AIDS Clinical Trials Group A5208 Study

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HIV-1 protease inhibitors (PIs) have antimalarial activity *in vitro* and in murine models. The potential beneficial effect of HIV-1 PIs on malaria has not been studied in clinical settings. We used data from Adult AIDS Clinical Trials Group A5208 sites where malaria is endemic to compare the incidence of clinically diagnosed malaria among HIV-infected adult women randomized to either lopinavir/ritonavir (LPV/r)-based antiretroviral therapy (ART) or to nevirapine (NVP)-based ART. We calculated hazard ratios and 95% confidence intervals. We conducted a recurrent events analysis that included both first and second clinical malarial episodes and also conducted analyses to assess the sensitivity of results to outcome misclassification. Among the 445 women in this analysis, 137 (31%) received a clinical diagnosis of malaria at least once during follow-up. Of these 137, 72 (53%) were randomized to LPV/r-based ART. Assignment to the LPV/r treatment group ($n = 226$) was not consistent with a large decrease in the hazard of first clinical malarial episode (hazard ratio = 1.11 [0.79 to 1.56]). The results were similar in the recurrent events analysis. Sensitivity analyses indicated the results were robust to reasonable levels of outcome misclassification. In this study, the treatment with LPV/r compared to NVP had no apparent beneficial effect on the incidence of clinical malaria among HIV-infected adult women. Additional research concerning the effects of PI-based therapy on the incidence of malaria diagnosed by more specific criteria and among groups at a higher risk for severe disease is warranted.

HIV and malaria are highly coprevalent infections in several regions of the world, including sub-Saharan Africa. An antiretroviral treatment for HIV that is also effective in treating or preventing malaria would have important clinical implications to the millions of HIV-infected individuals residing in areas where malaria is endemic.

The effects of HIV infection on malaria and of malaria infection on HIV have been well documented, although the precise interactions between these two pathogens are incompletely understood. HIV infection increases the incidence of malaria (10, 27, 35); malaria increases HIV viral replication (18, 20) and decreases CD4 counts in individuals infected with HIV (22). Although drug interactions between antiretroviral therapies (ART) and antimalarials are only partially understood, there are examples of harmful effects for the coinfected patient (11) and alterations in the pharmacokinetics of several drugs that are used to treat HIV and malaria (12, 19, 32). An antiretroviral treatment regimen that not only inhibits HIV replication and improves immunologic competency but also has inhibitory activity against the *Plasmodium* parasite is highly desirable. There is emerging evidence that HIV protease inhibitors (PIs) may fill that role (3, 17, 26, 29, 28, 33).

In vitro studies have demonstrated that HIV PIs inhibit the growth of *Plasmodium falciparum* in both drug-sensitive and drug-resistant parasites (3, 33, 26). Parasites exposed to sera taken from HIV-infected subjects being treated with a coformulation of lopinavir and ritonavir (LPV/r) underwent a 59 to 95% reduction in growth compared to parasites exposed to sera from control subjects (29). *In vivo* evidence from murine models also supports the antimalarial effect of HIV PIs (3, 17). Nathoo et al. proposed that HIV PIs may have an impact on individuals with malaria that is independent of its antiparasitic effects after *in vitro* observations demonstrated that treating cells with HIV PIs resulted in a marked reduction in the expression of CD36, a human cell receptor associated with the sequestration of the malaria parasite, compared to control cells not treated with a PI (24).

PI-based ART is not currently a recommended first-line option in resource-limited settings (38). However, the recent development of a heat-stable LPV/r tablet, coupled with the broader degree of resistance found in patients failing first-line nonnucleoside reverse transcriptase inhibitor (NNRTI)-based ART

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(14) and the risk of NNRTI resistance in HIV-infected pregnant women who received single-dose NVP (sdNVP) for the prevention of mother-to-child transmission (PMTCT) (4), makes it likely that the use of PIs in the developing world will increase. If a direct antimalarial effect of HIV PIs is found to be clinically relevant in humans, their use in highly prevalent HIV regions where malaria is also prevalent would be even more valuable, particularly for patients coinfected with HIV and *P. falciparum* malaria, despite the increased expense relative to NVP.

The AIDS Clinical Trials Group (ACTG) recently completed two phase III randomized clinical trials (A5208) comparing the antiretroviral activity of LPV/r-based ART to NVP-based ART in ART-naive, HIV-infected women who had been exposed to sdNVP (trial 1) or who had no history of NVP use (trial 2). We conducted a secondary analysis using data derived from A5208 to measure the association between LPV/r and clinical malaria as defined by a compatible clinical syndrome.

MATERIALS AND METHODS

ACTG A5208. The study consisted of two open label trials that monitored subjects for at least 48 weeks after the final participant was randomized (21). Trial 1 enrolled HIV-infected women who had received sdNVP for PMTCT ($n = 243$); trial 2 enrolled HIV-infected women with no history of NVP exposure ($n = 502$) (21). In both trials, the subjects were stratified by screening $CD4^+$ cell count (<50 cells/μl and ≥ 50 cells/μl) and randomized in a 1:1 ratio to receive either LPV/r-based ART or NVP-based ART. All subjects also received tenofovir disoproxil fumarate and emtricitabine (in addition to LPV/r or NVP), which is consistent with World Health Organization) guidelines (38). Subjects had regularly scheduled study visits (at weeks 2, 4, 8, 12, 16, and 24 and then every 12 weeks), during which the patient's health was assessed, and any clinical diagnoses were recorded. Subjects could also report to the study site for medical care as needed.

Study population. A5208 enrolled women older than 13 years of age (or older as dictated by the study site's Institutional Review Board [IRB]) with a $CD4^+$ cell count of <200 cells/mm^3 obtained within 90 days prior to study entry. The study was conducted at 10 ACTG-sponsored sites in sub-Saharan Africa: one each in Botswana, Malawi, Uganda, Zambia, and Zimbabwe, two in Kenya, and three in South Africa. Enrollment criteria are described in detail elsewhere (21). We used data from subjects in both trials from the five countries where malaria transmission is known to occur: Kenya (two sites), Malawi, Uganda, Zambia, and Zimbabwe. We excluded one participant (randomized to NVP in trial 2) who had probable clinical malaria at baseline, according to the definition below.

Exposure and outcome. The main exposure was the therapeutic regimen to which the participant was randomized (i.e., LPV/r-based ART or NVP-based ART). The primary outcome was the incidence of clinical malaria and included both confirmed and probable diagnoses. Confirmed malaria in study subjects was defined in ACTG appendix 60 as both a "compatible clinical syndrome" and the identification of *Plasmodium* sp. on a peripheral blood smear (2). Probable malaria was defined as a "compatible clinical syndrome" in subjects who received or who were recommended to receive antimalarial treatment (this group included subjects in which no peripheral blood smear was obtained and in those with a negative peripheral blood smear) (2). "Compatible clinical syndrome" was determined by on-site clinicians, typically either a physician or clinical officer, and was based on clinical judgment alone with no standardized documentation required.

Statistical analysis. All analyses were intent to treat. In our primary analysis, we only considered the first episode of malaria. We counted time-on-treatment from randomization until the date of malaria diagnosis (either confirmed or probable), death, study drop-out, or study completion. We used the hazard ratio (HR) as a measure of association and the 95% confidence interval (CI) as a measure of precision. To estimate the HR, we fit pooled logistic regression models, which approximate Cox proportional hazards models as long as the event proportion in all time periods is less than 10% (7). In our study, the largest event proportion was 6%. Pooled logistic regression is appropriate when the data are collected in intervals, similar to the scheduled study visits in A5208 (7). Time-on-treatment was modeled using a 5-knot restricted cubic spline (15) to allow a flexible nonlinear association between time and clinical malaria. All models included indicators for the specific combination of three design variables: trial, study site, and screening $CD4^+$ cell count stratum (<50 cells/μl or ≥ 50 cells/μl). Because few subjects had >165 weeks of follow-up, we administratively censored subjects still at risk at 165 weeks.

Because the risk of malaria can have marked seasonality and concomitant medications given to HIV-positive individuals may possess antimalarial activity, seasonality and concomitant medications were examined as possible modifiers of the HR by including them in the models in conjunction with their product terms with treatment. Using climate data from the National Oceanic and Atmospheric Administration (http://www7.ncdc.noaa.gov/CDO/cdo) and evidence from the literature (1, 8, 25, 34), we created a time-varying dichotomous variable denoting the rainy season (indicating a higher risk of malaria transmission). We also created a time-varying dichotomous variable that indicated current use of any of these concomitant medications known to possess antimalarial activity: azithromycin, clindamycin, diaminodiphenylsulfone (dapsone), doxycycline hydrochloride, doxycycline monohydrate, and trimethoprim-sulfamethoxazole. We examined the proportional hazards assumption by visually inspecting a plot of the log cumulative hazard by time and by testing the null hypothesis which included all individual product terms for each time variable and treatment. This did not improve model fit, as measured by the log likelihood (alpha was set at 0.1). We also explored the effects of LPV/r versus NVP in trial 1 and trial 2 separately. Finally, we generated crude plots of clinical malaria-free survival by time for each exposure group.

We conducted two sensitivity analyses to address possible bias from outcome misclassification. First, we fit a model in which only confirmed malaria cases were considered to have experienced a malaria episode (a person with a diagnosis of probable malaria was not censored at the time of diagnosis). Second, we explored the consequences of the imperfect specificity of clinically diagnosed malaria on the effects of treatment assignment by calculating risk ratios (RR), first from the observed data and again after correcting the observed numbers of cases, using a sensitivity analysis method described by Greenland (13).

We conducted a separate analysis to assess the treatment effect on all episodes of clinical malaria within the study period (as described above, combining confirmed and probable). In the analysis of recurrent malaria infections, subjects had to have at least one visit with no clinical malaria diagnosis (neither confirmed nor probable) between episodes. This was done in an effort to reduce potential misclassification of recurrent episodes as treatment failures as opposed to the acquisition of a new malarial infection.

We again used pooled the logistic regression to estimate HR and 95% CI values. We used a person-spell-week data structure (5, 36). Subjects remained in the first spell until they experienced clinical malaria, death, drop-out, or study completion. If a subject experienced clinical malaria, she remained in the first spell until death, drop-out, study completion, or a visit in which no clinical malaria was present. If the latter, the participant moved immediately into the second spell, indicating that the participant was again at risk for clinical malaria; this process was repeated until completion of follow-up. We used generalized estimating equations with an exchangeable covariance matrix to efficiently estimate robust standard errors that account for within-participant correlation. We modeled time-within-spell by using a 5-knot restricted cubic spline (15). We included trial, study site, and screening CD4 cell count in this model; we also included indicator variables to adjust for spell. As in the primary analysis, we examined seasonality and concomitant medication as possible effect measure modifiers; we also assessed interaction between treatment and spell.

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The nonrandom movement from one spell to the next in this analysis (i.e., the data cannot be considered randomized after the first spell) may have introduced bias, the direction and magnitude of which is difficult to predict, and therefore the results need to be interpreted cautiously.

A5208 was approved by all appropriate local and U.S. IRBs and ethics committees, and all participants provided written informed consent. This secondary analysis included only de-identified data and was determined to be exempt from IRB review. All analyses were conducted using SAS statistical software (version 9.2; SAS Institute, Cary, NC).

RESULTS

There were 145 women from trial 1 included in this analysis. A total of 72 were assigned LPV/r and 73 were assigned NVP. The baseline demographics, including mean age, CD4 lymphocyte count, and plasma HIV-1 RNA levels, were comparable between the arms (Table 1). The subjects in trial 1 were monitored for up to 184 weeks; eight were administratively censored at 165 weeks. The mean duration of follow-up was 89 weeks. In trial 1, 53 subjects (37%) received a clinical diagnosis of confirmed or probable malaria at least once during follow-up (Table 2). Of these 53, 12 subjects (23%) had confirmed malaria as their first episode (Table 2), and 18 subjects (34%) had one or more additional episodes of confirmed or probable malaria.

There were 300 women from trial 2 included in this analysis. A total of 154 were assigned LPV/r, and 146 were assigned NVP (Table 1). The mean ages and median viral loads were similar across treatment arms within trial 2 (Table 1). The subjects in trial 2 were followed for up to 192 weeks; 27 were administratively censored at 165 weeks. The mean duration of follow-up was 93 weeks. In trial 2, 84 subjects (28%) received a confirmed or probable diagnosis of malaria at least once during follow-up (Table 2). Eighteen of these 84 (21%) had confirmed malaria. Of the 84 subjects with a confirmed or probable diagnosis of malaria, 37 (44%) had more than one episode.

Based on the plot of the log cumulative hazard as well as the *P* value for the testing of the benefit of the addition of product terms of treatment assignment and time ($P = 0.63$), we concluded the proportional hazards assumption was acceptably met. Assignment to LPV/r-based therapy ($n = 226$) was not consistent with a decrease in the hazard of clinical malaria (HR for LPV/r versus NVP = 1.11 [95% CI = 0.79 to 1.56]) (Table 3). Kaplan-Meier curves are presented in Fig. 1. The HRs estimated for the analyses stratified by trial were similar (Table 3). Trial-specific curves are presented in Fig. 2. Strong modification of the HR by seasonality or use of concomitant medications was not apparent (the *P* value for the product term of treatment and seasonality was 0.83; the *P* value for the product term of treatment and concomitant medications was 0.65).

When we considered only confirmed cases of malaria, the results were not substantially different. Although there was an increase in the hazard of malaria among the LPV/r treatment group, this estimate was imprecise and compatible with no association (HR for LPV/r versus NVP = 1.34 [95% CI = 0.64 to 2.79]) (Table 3).

When we corrected the observed number of cases for specificity of the outcome to <100%, we found that, at reasonably high specificities, the lack of an observed protective effect of LPV/r was robust (at 90% specificity, the RR = 1.03; at 80% specificity, the RR = 1.05). However, at specificities of 70% or less, the number of "true" cases was quite small (<1), making the estimate of the effect unstable.

TABLE 1 Subjects in ACTG A5208 by trial and treatment group from sites where malaria is endemic[a]

Parameter	Trial 1 ($n = 145$)		Trial 2 ($n = 300$)		Total ($n = 445$)	
	LPV/r ($n = 72$)	NVP ($n = 73$)	LPV/r ($n = 154$)	NVP ($n = 146$)	LPV/r ($n = 226$)	NVP ($n = 219$)
Mean age (yr) ± SD	30.5 ± 5	30.7 ± 5	35.8 ± 8	34.7 ± 7	34.1 ± 7	33.4 ± 7
Site (no. of subjects)						
Eldoret, Kenya	9	8	25	22	34	30
Kericho, Kenya	14	13	23	23	37	36
Lilongwe, Malawi	10	14	22	22	32	36
Kampala, Uganda	9	8	21	21	30	29
Lusaka, Zambia	12	12	21	19	33	31
Harare, Zimbabwe	18	18	42	39	60	57
Baseline CD4, mean cells/mm^3 ± SD	126 ± 57	134 ± 62	123 ± 78	127 ± 82	124 ± 71	130 ± 76
Baseline CD4 <50, no. (%)	5 (6.9)	8 (11.0)	22 (17.5)	19 (13.0)	27 (12.0)	27 (12.3)
Baseline plasma HIV-1 RNA, median copies/ml (IQR)	157,453 (299,030)	161,630 (318,221)	131,175 (315,188)	112,387 (288,626)	143,628 (309,244)	129,514 (292,436)
Concomitant medication[b] use at study entry, no. (%)	54 (75.0)	55 (75.3)	120 (77.9)	112 (76.7)	174 (77.0)	167 (76.3)
Trimethoprim-sulfamethoxazole use at study entry, no. (%)	52 (72.2)	55 (75.3)	115 (74.7)	104 (71.2)	167 (73.9)	159(72.6)

[a] LPV/r, lopinavir boosted with ritonavir-based ART; NVP, nevirapine-based ART; IQR, interquartile range.

[b] This reflects the concomitant use of therapies with antimalarial effects: azithromycin, clindamycin, diaminodiphenylsulfone (dapsone), doxycycline hydrochloride, doxycycline monohydrate, or trimethoprim-sulfamethoxazole.

TABLE 2 First episodes of clinically diagnosed malaria among subjects of ACTG A5208

Subject category	No. of subjects			
	Trial 1 ($n = 145$)		Trial 2 ($n = 300$)	
	LPV/r ($n = 72$)	NVP ($n = 73$)	LPV/r ($n = 154$)	NVP ($n = 146$)
All malaria[a]				
Eldoret, Kenya	5	5	5	8
Kericho, Kenya	11	6	12	8
Lilongwe, Malawi	6	6	7	8
Kampala, Uganda	3	1	6	8
Lusaka, Zambia	4	6	12	8
Harare, Zimbabwe	0	0	1	1
Total	29	24	43	41
Confirmed malaria[b]	7	5	10	8

[a] All malaria, defined as a compatible clinical syndrome and prescription of antimalarials.
[b] Confirmed malaria, defined as a compatible clinicalsyndrome and a positive blood smear.

A total of 137 subjects experienced at least one episode of clinical malaria, and 55 experienced a second episode of clinical malaria while on the study. Since only 14 subjects experienced three or more episodes of clinical malaria, we limited the recurrent events analysis to the first two episodes. These results were also compatible with no reduction in the hazard of clinical malaria and assignment to LPV/r between treatment assignment and the hazard of clinical malaria (Table 3). There did not appear to be a meaningful interaction between treatment and spell ($P = 0.43$). Strong modification of the HR by seasonality or use of concomitant medications was also not apparent in this model (the P value for the product term of treatment and seasonality was 0.64, and the P value for the product term of treatment and concomitant medications was 0.76).

DISCUSSION

The results reported here are consistent with no or little protective effect from LPV/r versus NVP-based ART regimen on the incidence of clinical malaria. Subjects randomized to LPV/r-based ART were as, or more, likely to develop clinically diagnosed malaria as subjects randomized to receive a NVP-based ART regimen. The results were similar across both trials of ACTG A5208

TABLE 3 Summary of treatment assignment and hazard of clinical malaria in ACTG A5208

Primary analysis[a]	Hazard ratio[b] (95% CI)
Overall	1.11 (0.79–1.56)
Stratified	
Trial 1	1.28 (0.74–2.22)
Trial 2	1.02 (0.66–1.57)
Confirmed malaria only	1.34 (0.64–2.79)
Recurrent events analysis	1.20 (0.90–1.59)

[a] Survival analysis in which subjects were censored upon first episode of malaria.
[b] For lopinavir boosted with ritonavir-based ART compared to nevirapine-based ART.

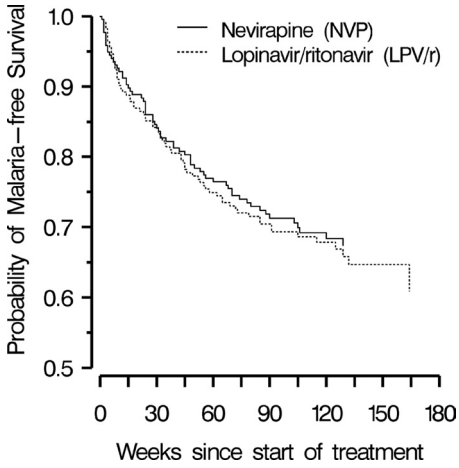

FIG 1 Malaria-free survival in ACTG A5208 subjects, by treatment assignment. NVP, $n = 219$; LPV/r, $n = 226$.

and when the analysis was restricted to only confirmed cases of malaria.

These results are inconsistent with laboratory evidence supporting an antimalarial effect of HIV PIs. Skinner-Adams et al. demonstrated the inhibitory effects of ritonavir, saquinavir, and indinavir on the *in vitro* growth of *P. falciparum*, whereas the NNRTI NVP had no such inhibitory effect (33). Additional studies using murine models of malaria have also demonstrated the antimalarial effect of certain HIV PIs. After infection with *P. chabaudi*, for example, mice exposed to LPV/r had a delayed onset of parasitemia by 2 days and a decrease from 20 to 4% in median parasitemia (3). Evidence from a different murine model infected with *P. yoelii* has suggested that HIV PIs, including lopinavir (LPV), can also inhibit the growth of pre-erythrocytic-stage parasites at concentrations effective to inhibit HIV-1 replication (17).

The absence of a protective effect of LPV/r on the incidence of clinical malaria seen in the present study needs to be considered in the context of the study design of the trials. Women who experienced virologic failure in either trial, as well as those with adverse reactions to their assigned medications, were eligible to switch their antiretroviral regimen and receive the treatment available in the other study arm. Among the 445 women included in this secondary analysis, 50 (11%) did so; almost all of these subjects switched from a NVP-based to a LPV/r-based ART regimen. Although this could have potentially influenced the results of the study, the small percentage of subjects who actually did switch treatment may have limited the effect that switching could have had on our results.

There are several potential explanations for why LPV/r-based therapy was not associated with a decrease in the incidence of clinical malaria in the present study. First, Nathoo et al. (24) have proposed that the decrease in CD36 expression associated with exposure to HIV PIs, while perhaps being beneficial to the patient in controlling *P. falciparum* infection, may also make it more difficult for a patient's innate immune system to produce an effective immune response against *P. falciparum* infection through its inhibitory effect on the nonopsonic phagocytosis of parasitized red cells by macrophages. As a result, this reduction in the innate immune response to malaria may have offset any potential antiparasitic activity of LPV/r, resulting in little or no net protective benefit.

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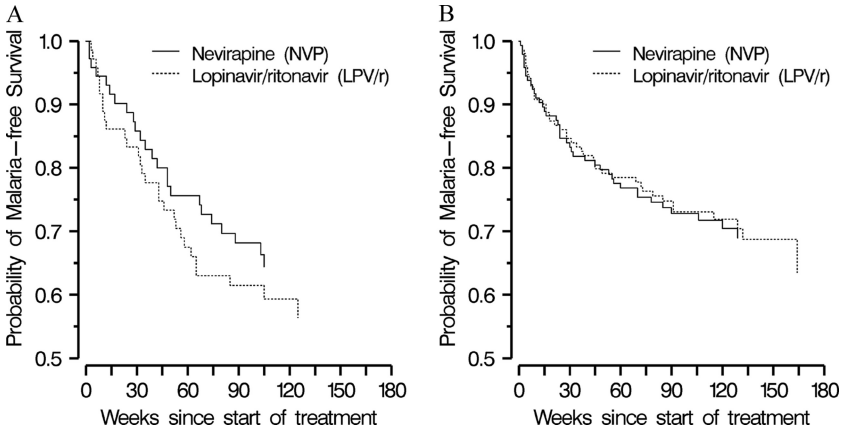

FIG 2 Malaria-free survival in ACTG A5208 subjects, by trial and treatment assignment. (A) Trial 1. NVP, $n = 73$; LPV/r, $n = 72$ (B) Trial 2. NVP, $n = 146$; LPV/r, $n = 154$.

In addition, the concentration of LPV in the patients' sera might not have achieved a level sufficient enough to exert an antimalarial activity. *In vitro* activities of the HIV PIs against *P. falciparum* are modest compared to standard antimalarial medications, including chloroquine (3); they are also much less active against malaria *in vitro* than HIV (26, 30). In addition, LPV is highly protein bound, and free drug concentrations in plasma are likely to be 100-fold less than measured total concentrations (9). Although the antimalarial activity of sera taken from patients treated with HIV PIs has been evaluated *ex vivo* to explore the effect of clinically relevant peak serum concentrations (29), it is unknown whether the parasites' drug exposures in these laboratory studies are truly equivalent to the drug concentrations one would anticipate in a patient on ART and, because there was no systematic sampling of LPV levels in subjects who initiated LPV/r therapy, we could not evaluate that in the present study.

Limitations to the present study include the imperfect sensitivity and specificity of relying on a clinical syndrome (6) and microscopy (37) for the diagnoses of malaria. This reduced our ability to determine the true incidence of malaria; however, the results from the sensitivity analyses indicate that the results were robust to moderate levels of outcome misclassification. Also, since the study sites are generally in areas of high malaria transmission and high preexisting immunity, they may not be generalizable to areas with low malaria transmission.

These results should be considered cautiously given the reliance on clinically diagnosed malaria as the primary outcome in the present study. Studies on the effects of PI's on laboratory-confirmed malaria in this study population are ongoing and will provide additional data. In addition to studies in adults that are designed to directly address whether PIs have a beneficial effect on malaria in regions where malaria is endemic and the prevalence of HIV-1 is high, further research is warranted to determine the effects of HIV PIs on malaria in children and pregnant women, two groups that are at greater risk for clinical malaria. In addition, since laboratory evidence suggests that the coadministration of HIV PIs with additional antimalarial agents, including chloroquine or mefloquine, may enhance the antimalarial activity of these drugs (16, 23, 31), the utility of such combinations also warrants further research. In conclusion, the results from our analyses, which were not consistent with large a decreased hazard of clinical malaria in subjects receiving LPV/r-based ART, provide evidence, albeit preliminary and imperfect, that supports the possibility of no or little effect of LPV/r-based therapy on clinical malaria.

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