No Differences Between Lopinavir/Ritonavir and Nonnucleoside Reverse Transcriptase Inhibitor–Based Antiretroviral Therapy on Clearance of *Plasmodium falciparum* Subclinical Parasitemia in Adults Living With HIV Starting Treatment (A5297)

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Background: HIV protease inhibitors anti-*Plasmodium falciparum* activity in adults remains uncertain.

Methods: Adults with HIV CD4+ counts >200 cells/mm3 starting antiretroviral therapy (ART) with P. falciparum subclinical parasitemia (*Pf* SCP) were randomized 1:1 to (step 1) protease inhibitor lopinavir/ritonavir (LPV/r)-based (arm A) or nonnucleoside reverse transcriptase inhibitor (nNRTI)-based ART (arm B) for 15 days. In step 2, participants received nNRTI-based ART and trimethoprim/sulfamethoxazole prophylaxis for 15 days. *P. falciparum* SCP clearance was measured by polymerase chain reaction. The Fisher exact test [95% exact confidence interval (CI)] was used to compare proportions of *P. falciparum* SCP clearance (<10 parasites/μL on 3 occasions within 24 hours) between LPV/r and nNRTI arms at day 15. The Kaplan–Meier method and log-rank test were used to compare time-to-clearance.

Results: Fifty-two adults from Kenya, Malawi, and Uganda with a median age = 31 (Q1, Q3: 24–39) years, 33% women, with baseline median $CD4^+$ counts of 324 (259–404) cells/mm^3, median HIV-1 RNA viremia of 5.18 $\log_{10}$ copies/mL (4.60–5.71), and median estimated *P. falciparum* density of 454 parasites/μL (83–2219) enrolled in the study. Forty-nine (94%) participants completed the study. At day 15, there was no statistically significant difference in the proportions of *P. falciparum* SCP clearance between the LPV/r (23.1% clearance; 6 of the 26) and nNRTI (26.9% clearance; 7 of the 26) arms [between-arm difference 3.9% (95% CI, −21.1% to 28.4%; $P = 1.00$)]. No significant difference in time-to-clearance was observed between the arms ($P = 0.80$).

Conclusions: In a small randomized study of adults starting ART with *P. falciparum* SCP, no statistically significant differences were seen between LPV/r- and nNRTI-based ART in *P. falciparum* SCP clearance after 15 days of treatment.

Key Words: malaria, subclinical parasitemia, HIV, ART, protease inhibitors

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INTRODUCTION

In sub-Saharan Africa, adults living with HIV also live in malaria-endemic regions.[1] Sub-Saharan Africa remains heavily affected by HIV where 61% of worldwide daily

infections occurred in 2019.[2] In 2018, there were an estimated 213 million cases of malaria in Africa, 93% of cases worldwide.[3] The interaction between HIV and malaria is bidirectional and synergistic with each disease increasing the pathogenesis of the other.[4] HIV protease inhibitors (PIs), part of second-line antiretroviral therapy (ART) throughout sub-Saharan Africa,[5] have demonstrated clinical activity against malaria in children.[6,7]

There are limited clinical data regarding the antimalarial activity of PIs in adults or children with HIV.[6–10] A5297 was a prospective, open-label, randomized, multicenter trial testing the hypothesis that PIs have antimalarial activity in adults with HIV infection in malaria-endemic regions in Kenya, Malawi, and Uganda.[1–3] If PIs have marked benefit in clearance of subclinical malaria, such information would be valuable to clinical researchers and local Ministries of Health in designing future larger-scale clinical trials of optimal first-line therapies in regions with high malarial prevalence.

METHODS

Study Design and Participants

A5297 was a phase I/II, open-label, proof of concept, 2-step, 2-arm, randomized clinical trial testing the superiority of lopinavir/ritonavir (LPV/r)-based ART compared with non-nucleoside reverse transcriptase inhibitor (nNRTI)-based ART for *Plasmodium falciparum* subclinical parasitemia (SCP) clearance. Participants enrolled from 5 sites across 3 countries: Kericho, Kisumu, and Eldoret (Kenya); Kampala (Uganda); and Blantyre (Malawi). In step 1, participants were randomized 1:1 to LPV/r-based ART (arm A) or nNRTI-based ART (arm B) with efavirenz for 15 days. Participants unable to take efavirenz were offered nevirapine. In step 2, all participants received nNRTI-based ART plus trimethoprim/sulfamethoxazole (TMP/SMX) for 15 days. Participants included men and women aged 18 years and older with HIV and with *Pf* SCP initiating ART with $CD4^+$ count >200 and ≤ 350 or ≤ 500 cells/mm^3 based on World Health Organization ART guidelines at the time of enrollment.[11] Participants were recruited through the abovenamed sites of the AIDS Clinical Trials Group (ACTG) network using outreach in both community and hospital settings.

P. falciparum SCP was defined by the following conditions within 72 hours of study entry: (1) microscopy-confirmed parasitemia, (2) oral temperature $\leq 37.5°C$, and (3) absence of $\geq$ grade 2 signs or symptoms believed to be related to clinical malaria (ie, headache, malaise or fatigue, abdominal discomfort, muscle or joint pain, fever, chills, perspiration, anorexia, and vomiting). Before enrollment, participants had confirmed hemoglobin level of ≥ 7.0 g/dL, estimated creatinine clearance of ≥ 60 mL/min, aspartate aminotransferase and alanine aminotransferase $\leq 2.5 \times$ ULN, and negative results for hepatitis B surface antigen. Participants of reproductive potential had negative serum or urine pregnancy results from testing performed within 72 hours before entry.

The following conditions rendered volunteers ineligible: history or current use of ART; medication use with antimalarial activity within 14 days of entry; confirmed or clinically suspected opportunistic infections (eg, tuberculosis, clinical malaria, *Pneumocystis jiroveci* pneumonia); other untreated pulmonary or gastrointestinal infections more than 30 days before enrollment or with signs and symptoms of during screening; breastfeeding; known allergy/sensitivity to components of study drugs or their formulation; active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements; serious illness requiring systemic treatment and/or hospitalization within 30 days before entry; and results suggestive of active pulmonary disease from a chest x-ray performed within 30 days before study entry.

The primary outcome was *P. falciparum* SCP clearance defined by quantitative reverse transcription polymerase chain reaction (qRT-PCR) to an estimated density of <10 parasites/μL on 3 consecutive occasions within a 24-hour period within 15 days after treatment initiation. Secondary outcomes included time to confirmed *P. falciparum* SCP clearance (ie, 3 consecutive negative samples), *P. falciparum* parasite density, and uncomplicated clinical malaria (ie, fever/symptoms and parasitemia).

The study was approved by all relevant local institutional review boards and ethics committees. Participants provided written informed consent. All authors affirm the correctness of the data and results presented. Coauthors who were also site investigators collected data. The pharmaceutical sponsors provided study treatment (Abbott: LPV/r, Aluvia, Gilead: Emtricitabine/Tenofovir Disoproxil Fumarate, Truvada, and Merck: Efavirenz, Stocrin), participated in the study team but did not participate in the analysis of the data. All authors made the decision to submit the manuscript for publication.

Study Monitoring and Safety

The study was monitored by a Clinical Management Committee consisting of study leadership and by an independent Study Monitoring Committee under the ACTG. Predefined safety signals identified severe malaria, *P. jiroveci* pneumonia, and infectious diarrhea resulting in hospitalization or prolonged hospitalization. Such events could have resulted in study discontinuation. Low accrual rates and targets could have resulted in early termination. All participants with clinical malaria received local standard of care treatment (artemether/lumefantrine). At the end of the study, participants with *P. falciparum* SCP received artemether/lumefantrine treatment.

Data Collection, Follow-Up, and Laboratory Analyses

Step 1 study visits occurred every 3 ± 1 days (1, 3, 6, 9, 12, and 15). Step 2 study visits occurred every 5 ± 1 days (20, 25, and 30). Participants had a blood draw and/or finger stick sample collected twice daily separated by 8 ± 3 hours except for days 15 and 30 when samples were collected 3 times separated by at least 5 hours. Real-time microscopy and laboratory analyses were conducted at Division of

AIDS–approved site laboratories. All laboratories participated in a Division of AIDS–supported proficiency testing program for malaria microscopy. The primary outcome was conducted at the University of Washington ACTG Virology Specialty Laboratory using a validated qRT-PCR assay targeting the *P. falciparum* 18S rRNA.[12]

Sample Size and Statistical Analyses

For sample size calculation, nNRTI-based ART clearance rate was assumed 20% given lack of data suggesting nNRTI antimalarial activity but with recognition for potential ART activity. A clinically relevant LPV/r-based ART clearance rate of 50% was selected for treatment effect, resulting in LPV/r-based ART clearance of 70%. Assuming a 10% loss-to-follow-up, enrolling 52 participants (26 per arm) would provide 90% power to detect a clinically meaningful difference in *P. falciparum* SCP clearance between arms using the Fisher exact test with 2-tailed alpha = 0.05. The primary analyses were based on intent-to-treat principles and included all randomized participants. Secondary analyses were per protocol. All statistical tests were 2-sided, using a 5% significance level, without adjustment for multiple comparisons. In the primary analysis (comparison between arms of the proportions of *P. falciparum* SCP clearance after 15 days of therapy), the difference in proportion was evaluated using the Fisher exact test. Analysis for the secondary outcome of time-to-clearance used the Kaplan–Meier method and the log-rank test. Sensitivity analyses were conducted around missing data: one where missing data were assumed to have cleared and one where missing data were excluded. Sensitivity analyses were also conducted using less stringent definitions of clearance: one where participants were considered cleared if any 2 of the 3 samples had <10 estimated parasites/μL whole blood at day 15 by qRT-PCR and one where participants were considered cleared if any 1 of the 3 samples had <10 parasites/μL at day 15 by qRT-PCR.

RESULTS

In total, 166 volunteers were screened. Common reasons for ineligibility included *P. falciparum* SCP absence and out-of-range $CD4^+$ count. Fifty-two participants (26 per arm) enrolled between January 10, 2014, and May 20, 2016, with 49 (94%) from Kenya. Fifty-one participants completed the primary outcome (step 1) visit, and 49 completed the protocol as specified. The treatment completion status for the 2 steps aligned with the study completion status (ie, 51 and 49, respectively). The study population was mostly men (67%) with a median age of 31 (Q1, Q3 = 24–39) years. The median baseline CD4+ count was 324 cells/mm^3 (259–404), and the median baseline log_{10} (HIV-1 RNA) was 5.18 copies/mL (4.60–5.71). The median baseline parasite density was 454 parasites/μL of whole blood (83–2219) (Table 1).

For the primary outcome at day 15, there was no statistically significant difference in the proportions of *P. falciparum* SCP clearance between the LPV/r (23.1% clearance; 6/26) and nNRTI (26.9% clearance; 7/26) arms {between-arm difference 3.9% [95% confidence interval (CI), −21.1% to 28.4%; $P = 1.00$]}. In all sensitivity analyses (ie, missing data and clearance definitions), the difference in clearance between the treatment arms remained nonsignificant.

Secondary objective analyses included time-to-*P. falciparum* parasite clearance, between-arm parasite densities over time, and safety outcomes. No statistically significant differences in time-to-*P. falciparum* parasite clearance were observed between arms ($P = 0.80$, Fig. 1). The comparison was in participants who received 15 days of treatment, excluding one on LPV/r arm who only attended the entry visit and one on nNRTI arm who had no parasite data available. The mean *P. falciparum* parasite densities through the 30-day study window were examined. Although overall, a trend was observed in decreasing *P. falciparum* parasite densities in both arms throughout the study, there was no difference between the 2 treatment arms during days 1–15 [LPV/r arm median = −172 parasites/μL (Q1, Q3 = −2195, 0) and nNRTI arm −135 parasites/μL (−1221, 5)] or days 15–30 [LPV/r arm −35 parasites/μL (−334, −2) and nNRTI arm −64 parasites/μL (−587, −1)]. Overall, *P. falciparum* parasite density decreased between entry and day 30 [−462 (−2219, −40)]; at the same time, the median CD4+ counts increased (101 cells/mm^3) and median log_{10} HIV RNA decreased (−2.48 copies/mL) with no significant between-arm differences.

ART and TMP/SMX adherence was >90% during the study. During the study period, there were few adverse events and no hospitalizations. Three cases of uncomplicated, grade

TABLE 1. Demographic and Baseline Characteristics by Treatment (ACTG A5297)

	Treatment Arm		
	LPV/R-Based ART (N = 26)	**nNRTI-Based ART (N = 26)**	**Total (N = 52)**
Baseline age, yrs			
Median (Q1, Q3)	31 (24–38)	31 (24–39)	31 (24–39)
Sex			
Female	9 (35%)	8 (31%)	17 (33%)
Male	17 (65%)	18 (69%)	35 (67%)
Race			
Black African	26 (100%)	26 (100%)	52 (100%)
Country			
Kenya	24 (92%)	24 (96%)	49 (94%)
Malawi	1 (4%)	1 (4%)	2 (4%)
Uganda	1 (4%)	0 (0%)	1 (2%)
IV drug history			
Never	26 (100%)	26 (100%)	52 (100%)
Baseline CD4+ count			
Median (Q1, Q3)	360 (265–407)	302 (256–389)	324 (259–404)
Baseline HIV RNA viral load (Log10 copies/mL)			
Median (Q1, Q3)	5.11 (4.65–5.51)	5.37 (4.56–5.76)	5.18 (4.60–5.71)
Baseline parasite density (parasites/μL)			
Median (Q1, Q3)	488 (119–2501)	324 (53–1842)	454 (83–2219)

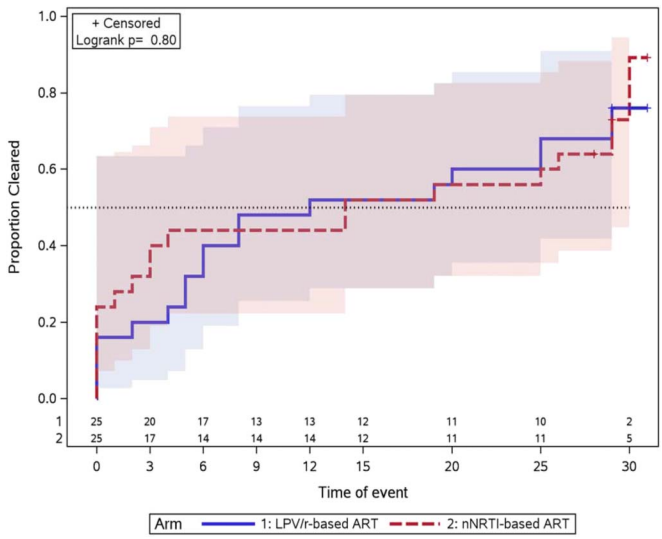

FIGURE 1. Kaplan–Meier plot of time to *P. falciparum* SCP clearance between LPV/r and nNRTI-based ART treatment arms (ACTG A5297)*. *The dotted line represents the median time to clearance. full color online

2 clinical malaria (LPV/r Arm n = 2, nNRTI Arm n = 1) occurred during step 1; all received standard of care artemether/lumefantrine. One participant in both treatment arms experienced grade 2 anemia (n = 2).

DISCUSSION

In vitro studies from the 2000s demonstrated antimalarial activity of HIV-1 PIs. PIs have shown antimalarial activity through several mechanisms including cell-mediated cytoadherence of *P. falciparum*-infected erythrocytes, inhibition of *P. falciparum* parasite growth both in vitro and in vivo, and limiting liver-stage parasite development.[13–18] Data from Thailand suggested PI-based ART demonstrated prophylactic activity against both *Plasmodium vivax* and *P. falciparum* malaria in regions where multidrug resistance is endemic.[19] Studies in adults and children have produced conflicting results. PI LPV/r–based ART compared with nNRTI-based ART reduced malaria recurrence in children in Uganda.[6] LPV/r combined with malaria treatment in HIV-infected children in Malawi was associated with a lower risk of recurrent parasitemia by blood smear microscopy but not clinical malaria.[7] The findings among children in Uganda and Malawi were likely a result of the pharmacokinetic interaction between LPV/r and lumefantrine rather than between efavirenz and artemether.[20] In adults, no difference in malaria incidence was observed in women receiving a PI-based vs. nNRTI-based ART.[8]

We did not find a statistically significant difference in parasite clearance rates nor in time-to-parasite clearance between PI and nNRTI treatment arms. Our sample size was relatively small based on a 50% PI treatment effect, which we believed would be necessary to ultimately affect policy changes and warrant additional, large-scale studies. Our results were notably robust in predefined sensitivity analyses. We observed a trend in decreasing *P. falciparum* parasite densities in both arms. This may have been due to TMP/SMX, ART, natural history, or a combination. The duration of asymptomatic malaria infections was previously described.[21] More recently, subclinical malaria epidemiology and treatment considerations were reviewed.[22] To our knowledge, our randomized clinical trial of adults with HIV beginning first-line ART with *P. falciparum* SCP is the first comparison between a PI (LPV/r) and nNRTI.

Our study provides a unique cohort testing the clinical impact of PIs in a setting without and with TMP/SMX. Although participants had CD4+ counts >200 cells/mm^3 given requirements for TMP/SMX in people with lower CD4+ counts, we observed no complicated malaria, Pneumocystis pneumonia, or diarrheal disease. We observed 3 cases of uncomplicated malaria during the first 15 days in the absence of TMP/SMX. This likely reflects fluctuations in SCP in the absence of TMP/SMX and occasional fever threshold densities being passed. Our findings contribute to discussions among clinical researchers and Ministries of Health regarding PI activity in adults with HIV and *P. falciparum* SCP, TMP/SMX safety data, and future research regarding *P. falciparum* parasite life cycles.

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