Accelerated Suppression of Primary Epstein-Barr Virus Infection in HIV-Infected Infants Initiating Lopinavir/Ritonavir-Based Versus Nevirapine-Based Combination Antiretroviral Therapy

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We compared primary Epstein-Barr virus (EBV) infection and suppression between Kenyan human immunodeficiency virus–infected infants starting nevirapine-based vs lopinavir/ritonavir-based antiretroviral regimens. Although the rate of EBV infection was similar between groups, infants receiving lopinavir/ritonavir suppressed EBV more rapidly. Our findings suggest that specific antiretrovirals may potentially impact the risk of future EBV-associated malignancies.

Keywords. Epstein-Barr virus; primary infection; HIV; infant; antiretroviral therapy.

Epstein-Barr virus (EBV) is associated with several human immunodeficiency virus (HIV)–associated malignancies [1]. Risk factors for EBV-associated lymphomas include B-cell activation, unsuppressed HIV type 1 (HIV-1), low CD4 count, and starting antiretroviral therapy (ART) late [2–7]. ART initiation prior to EBV acquisition may have the potential to influence later development of EBV-associated malignancy via delayed infection, better EBV suppression, or limiting the latent EBV reservoir.

An increasing body of evidence suggests that HIV-1 protease inhibitors have broad off-target effects, including inhibition of γ-herpesvirus replication and antitumor activity [8,9]. We compared rates of primary EBV infection and suppression between infants initiating ART regimens containing nevirapine (NVP) vs ritonavir-boosted lopinavir (LPV/r).

MATERIALS AND METHODS

Study Design and Cohort

The study was approved by the Institutional Review Board of the University of Washington and the Ethics and Research Committee of the University of Nairobi. This is a retrospective analysis of specimens collected during a randomized clinical trial with a 2-year prerandomization phase (NCT00428116 [10]); this report includes only the prerandomization period. Enrollment criteria were age <5 months, residence in Nairobi, no prior ART (other than for prevention of mother-to-child HIV transmission), retention in the study beyond ART initiation, and baseline specimen availability. ART was initiated shortly after enrollment; children with prior NVP exposure were initiated on an LPV/r-based regimen, and all others started NVP. Infants were assessed monthly, with quarterly blood collection [10]. We excluded 14 infants who initiated NVP-based regimens despite prior NVP exposure, whose specimens were reserved for ART resistance studies.

EBV Diagnostics

EBV DNA levels were measured from cryopreserved plasma until 24 months after ART initiation [11]. The lower limit of detection was 50 copies/mL. Infants with no EBV DNA detection received serologic testing of their final specimen collected at >6 months of age to determine infection status using enzyme-linked immunosorbent assay as previously described [11].

Statistical Analysis

Stata SE version 11.2 for Macintosh (StataCorp, College Station, Texas) was used for analysis. All tests were 2-tailed with $\alpha = .05$. Characteristics were compared between groups using either the χ^2 test for categorical variables, or the Mann-Whitney *U* test for continuous variables.

Time to EBV infection and suppression were estimated using Kaplan-Meier survival analysis, and the log-rank test was used

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to compare survival probabilities. Infection time was estimated as the age EBV was first detectable. Infants were considered to be at risk for EBV infection once they began ART; we thereby excluded infants who exited or died before starting ART or had EBV infection at enrollment. For EBV-infected infants, suppression was defined as the first time point at which an infant became EBV DNA undetectable for 2 consecutive visits. For all time-to-event analyses, infants not experiencing the event of interest were censored at death or last study visit.

Generalized estimating equations with a binomial link function and robust standard errors were used to estimate odds ratios (ORs) for EBV suppression. All models include time since EBV infection. For both unadjusted and adjusted models, ART regimen is time-updated.

RESULTS

Cohort Characteristics

Among the 100 infants enrolled in the trial, 64 met criteria for inclusion in the EBV study (Supplementary Figure 1). Characteristics of the infants and their caregivers are provided in Supplementary Table 1. Median age at enrollment was 3.6 months (interquartile range [IQR], 3.0–4.0), and infants were followed for a median of 24 months (IQR, 3.8–24). Mean infant CD4 percentage was low at enrollment (20% [SD, 8.3%]). ART was started at a median age of 4.1 months (IQR, 3.3–4.6), with 34 (53%) initiating LPV/r and 30 (47%) initiating NVP. All baseline infant and caregiver characteristics were similar between infants receiving LPV/r- and NVP-based regimens.

EBV Acquisition

A total of 18 infants were persistently EBV DNA negative throughout follow-up; 5 of these had specimens collected after 6 months of age, and 3 of these 5 were positive for EBV antibodies. Nine prevalent EBV infections were detected at enrollment (9/64 [14%]), prior to ART initiation; these infants had been enrolled at approximately 3 (n = 3) and 4 (n = 6) months of age. Overall, 77% of the infants we tested had evidence of EBV infection (49/64) and 72% had detectable EBV DNA (46/64).

The mean age at EBV infection was 8.8 months (95% confidence interval [CI], 6.6–11) overall; 7.8 months (95% CI, 5.0–11) in the LPV/r group, and 8.9 months (95% CI, 5.7–12) in the NVP group ($P = .6$; Figure 1*A*). The overall probability of EBV infection in ART-treated infants at 12 and 24 months was 0.65 (95% CI, .51–.79) and 0.93 (95% CI, .83–.98), respectively, and was similar between the LPV/r and NVP groups (data not shown).

EBV Viral Levels and Suppression

Overall, the median peak EBV load among infants with detectable EBV viremia was 2.5 $\log_{10}$ EBV DNA copies/mL (IQR, 2.0–2.9). Peak EBV DNA levels were similar between treatment groups ($P = .7$; Figure 1*B*).

Among the 46 EBV-infected infants with detectable EBV DNA, 27 (59%) later became undetectable for EBV DNA, at a mean time of 11 months (95% CI, 7.7–14; Figure 1*C*). Mean time to suppression was shorter in the LPV/r group (6.4 months [95% CI, 4.7–8.0]) compared with the NVP group (15 months [95% CI, 10–20]; log-rank $P = .02$).

Infants were grouped into 3 different patterns of EBV suppression; "good controllers" were EBV seropositive with no detection of EBV DNA, or had a single episode of EBV DNA detection followed by complete suppression, "poor controllers" had transient or persistent viremia lasting >3 months, and "unclassifiable" had EBV DNA detected only at their final study visit. The proportion of good controllers (54% in LPV/r vs 26% in NVP), poor controllers (35% in LPV/r vs 61% in NVP), and unclassifiable infants (12% in LPV/r vs 13% in NVP) differed significantly between the LPV/r and NVP groups ($P = .03$).

LPV/r, HIV-1 Viral Suppression, and CD4 Percentage

HIV-1 suppression <1000 copies/mL (OR, 4.9 [95% CI, 2.6–9.3]; $P < .001$), CD4 >25% (OR, 4.0 [95% CI, 1.8–8.8]; $P < .001$), and LPV/r use (OR, 3.1 [95% CI, 1.4–7.0]; $P = .006$) were associated with the odds of concurrent EBV suppression. LPV/r regimen remained significantly associated with EBV suppression when adjusting for HIV-1 suppression (OR = 3.1 [95% CI, 1.4–6.9]; $P = .006$) and retained a trend when adjusting for CD4 >25% (OR = 2.5 [95% CI, .91–6.8]; $P = .07$).

DISCUSSION

Our findings suggest that LPV/r-based ART may substantially accelerate EBV suppression compared with NVP-based regimens. As poor suppression of EBV infection is associated with EBV-related malignancies, our data suggest that initiation of LPV/r ART prior to infant EBV acquisition could potentially have implications for later risk of EBV-associated malignancy.

The probability of infant EBV infection, time to EBV acquisition, and peak EBV levels reported here were similar to those observed in a cohort of treatment-naive children from the same clinic [11]. Together, these data suggest that early infant ART does not afford significant protection from EBV acquisition or limit peak systemic viral load. However, ART-treated children had a shorter time to EBV suppression (11 months) compared with untreated infants (17 months) in the earlier study [11]. Importantly, infants initiating LPV/r regimens suppressed virus approximately 8 months earlier than infants receiving NVP regimens and were more likely to be good EBV controllers. The association between LPV/r regimen and accelerated

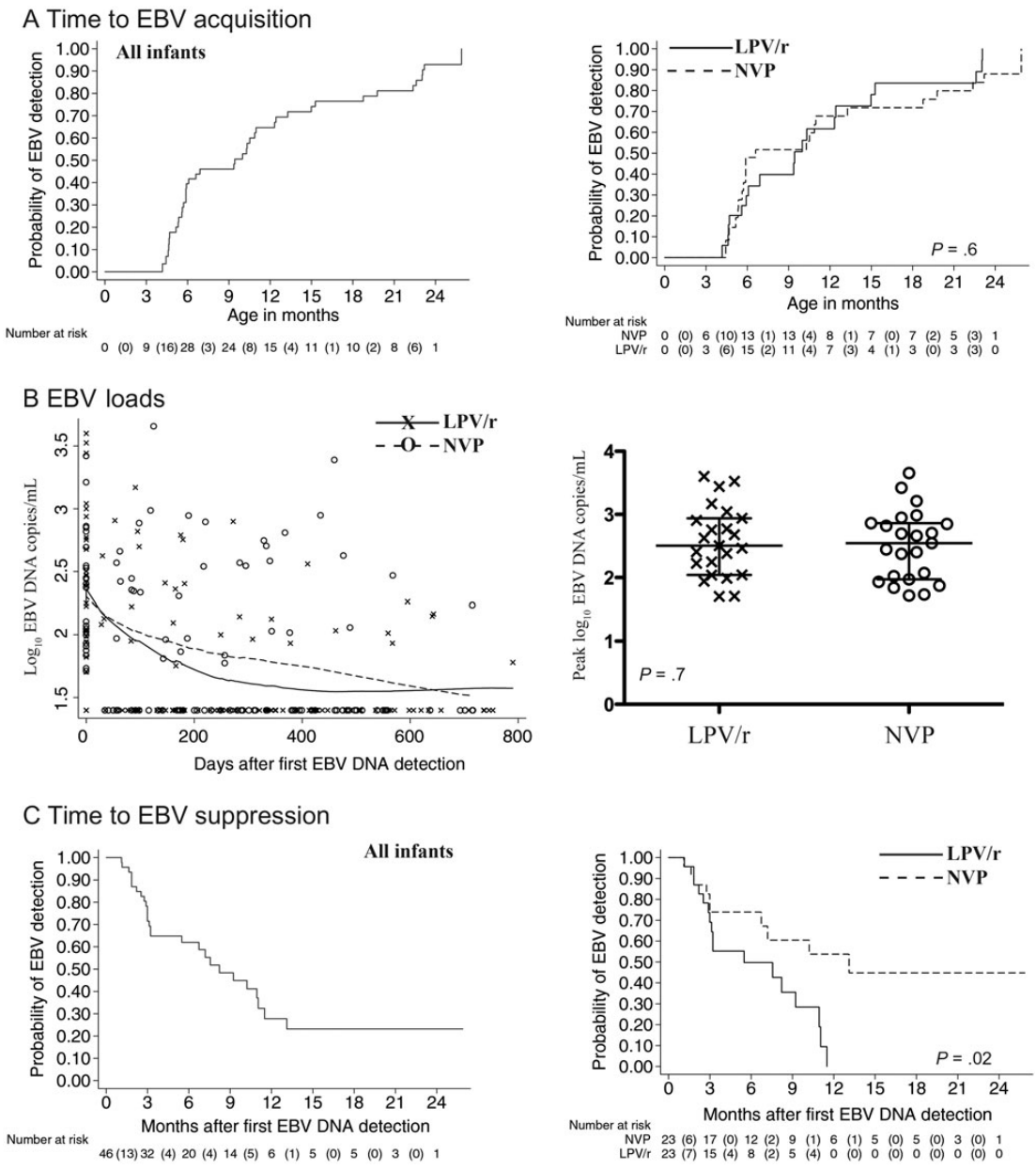

Figure 1. Epstein-Barr virus (EBV) acquisition, viral loads, and suppression. *A,* Kaplan-Meier–estimated functions showing time to EBV acquisition (infants enter analysis at antiretroviral therapy initiation). Risk tables show number remaining at risk, followed by number of events (in parentheses) at each time point. *B,* Loess curves fitted to EBV load measurements for infants receiving ritonavir-boosted lopinavir (LPV/r) and nevirapine (NVP) regimens. Median (middle lines) and interquartile ranges (end bars) of peak EBV load measurements for infants with EBV DNA detected, grouped by regimen. *C,* Kaplan-Meier–estimated functions showing time to EBV suppression (infants enter analysis at first EBV DNA detection).

EBV suppression does not appear to be mediated through better HIV-1 treatment responses in the LPV/r group; LPV/r was not associated with improved rates of HIV-1 suppression or CD4 reconstitution in the cohort (Benki-Nugent et al., manuscript in preparation), and the association between LPV/r and EBV suppression was independent of HIV-1 suppression. To our knowledge, the effect of LPV/r on EBV replication has not been studied, but several mechanisms could explain our observations, including better restoration of global lymphocyte function, a direct effect on EBV replication, or altered host B-cell cycling. Because there was no difference in EBV load between groups, a direct effect of LPV/r on EBV replication seems unlikely. Dewan and colleagues previously demonstrated that ritonavir inhibited EBV-immortalized lymphoblastoid cell line

(LCL) growth in vitro, and reduced LCL infiltration and growth in a mouse model by targeting NF-κB to induce cell cycle arrest and apoptosis [12], suggesting a potential effect of ritonavir on B cells undergoing lytic EBV replication.

Limitations of our study include its retrospective and observational design, lack of maternal EBV data, and short follow-up period. Our time-dependent analyses are limited by the high early mortality in the cohort, as many infants died before acquiring EBV or achieving suppression. Based on a small number of previous publications with longitudinal serology, we used 6 months of age to discriminate infant from maternal antibodies [13, 14]; although we are unable to completely rule out maternal antibodies, we would expect misclassification of outcome to be nondifferential with regards to ART exposure, and would therefore have the overall effect of underestimating differences between study groups. Selection of first-line ART regimen was based upon prior infant NVP exposure; whether prior NVP exposure, or some other unidentified confounder, would affect EBV suppression is unknown.

In conclusion, ART did not protect HIV-infected infants from EBV acquisition or limit peak viremia. However, LPV/r-ART was associated with accelerated suppression of primary EBV infection. It will be important to determine the mechanism underlying the association between LPV/r and improved EBV control. As many African countries are currently adapting their guidelines to enable earlier infant diagnosis and ART, strategic implementation of particular ART regimens could have population-level implications for EBV-associated malignancies.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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References

1. Carbone A, Cesarman E, Spina M, Gloghini A, Schulz TF. HIV-associated lymphomas and gamma-herpesviruses. Blood **2009**; 113:1213–24.
2. Pollock BH, Jenson HB, Leach CT, et al. Risk factors for pediatric human immunodeficiency virus-related malignancy. JAMA **2003**; 289:2393–9.
3. Righetti E, Ballon G, Ometto L, et al. Dynamics of Epstein-Barr virus in HIV-1-infected subjects on highly active antiretroviral therapy. AIDS **2002**; 16:63–73.
4. Kest H, Brogly S, McSherry G, Dashefsky B, Oleske J, Seage GR III. Malignancy in perinatally human immunodeficiency virus-infected children in the United States. Pediatr Infect Dis J **2005**; 24:237–42.
5. Bruyand M, Thiebaut R, Lawson-Ayayi S, et al. Role of uncontrolled HIV RNA level and immunodeficiency in the occurrence of malignancy in HIV-infected patients during the combination antiretroviral therapy era: Agence Nationale de Recherche sur le Sida (ANRS) CO3 Aquitaine Cohort. Clin Infect Dis **2009**; 49:1109–16.
6. Breen EC, Hussain SK, Magpantay L, et al. B-cell stimulatory cytokines and markers of immune activation are elevated several years prior to the diagnosis of systemic AIDS-associated non-Hodgkin B-cell lymphoma. Cancer Epidemiol Biomarkers Prev **2011**; 20:1303–14.
7. Grulich AE, Wan X, Law MG, et al. B-cell stimulation and prolonged immune deficiency are risk factors for non-Hodgkin's lymphoma in people with AIDS. AIDS **2000**; 14:133–40.
8. Chow WA, Jiang C, Guan M. Anti-HIV drugs for cancer therapeutics: back to the future? Lancet Oncol **2009**; 10:61–71.
9. Gantt S, Casper C. Human herpesvirus 8-associated neoplasms: the roles of viral replication and antiviral treatment. Curr Opin Infect Dis **2011**; 24:295–301.

10. Wamalwa D, Benki-Nugent S, Langat A, et al. Survival benefit of early infant antiretroviral therapy is compromised when diagnosis is delayed. Pediatr Infect Dis J **2012**; 31:729–31.
11. Slyker JA, Casper C, Tapia K, et al. Clinical and virologic manifestations of primary Epstein-Barr virus (EBV) infection in Kenyan infants born to HIV-infected women. J Infect Dis **2013**; 207:1798–806.
12. Dewan MZ, Tomita M, Katano H, et al. An HIV protease inhibitor, ritonavir targets the nuclear factor-kappaB and inhibits the tumor growth and infiltration of EBV-positive lymphoblastoid B cells. Int J Cancer **2009**; 124:622–9.
13. Biggar RJ, Henle W, Fleisher G, Bocker J, Lennette ET, Henle G. Primary Epstein-Barr virus infections in African infants. I. Decline of maternal antibodies and time of infection. Int J Cancer **1978**; 22:239–43.
14. Chan KH, Tam JS, Peiris JS, Seto WH, Ng MH. Epstein-Barr virus (EBV) infection in infancy. J Clin Virol **2001**; 21:57–62.