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.
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 as the age EBV was first detectable. Infants were considered to have a peak EBV load when EBV DNA reached the first detectable. Infants were considered to have detectable EBV DNA when EBV DNA was detected at least twice. Infants were considered to have no detection of EBV DNA when EBV DNA was not detected at least twice. Infants were considered to be at risk for EBV infection once they began ART. 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 suppression of EBV infection did not persist after adjusting for other factors. 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.

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, 3.3–4.6). The 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 1A). 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 log10 EBV DNA copies/mL (IQR, 2.0–2.9). Peak EBV DNA levels were similar between treatment groups (P = .7; Figure 1B).

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 1C). 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, 0.91–6.8]; 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 suppression of EBV infection did not persist after adjusting for other factors.
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-kB 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 copyrighted. 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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