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Biphasic decay of cell-associated HIV-1 DNA in HIV-1-infected children on antiretroviral therapy

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Cell-associated HIV-1 DNA was quantified in 33 HIV-1-infected children followed for 96 weeks after the initiation of antiretroviral therapy. HIV-1 DNA decay was biphasic, being 10-fold more rapid during the first 4 weeks, and was inversely associated with the baseline plasma HIV-1-RNA level, but unrelated to HIV-1-RNA suppression. HIV-1 DNA decay per absolute CD4 cell number was lower than per 106 CD4 cells, suggesting an ongoing infection of newly produced CD4 cells, despite HIV-1 suppression in plasma.

It is well established that despite the strong inhibition of viral replication in patients on antiretroviral therapy (ART), HIV-1 may persist in peripheral blood cells and lymphoid tissues. We assessed the relationship between changes in cell-associated HIV-1 DNA and plasma HIV-1 RNA in a subset of 33 ART-naive children initiating therapy in the PENTA 5 trial [1], with a median age of 7.1 years (range 0.3-15.5), CD4 cell percentage 17% (interquartile range 9-24%), and mean HIV-1-RNA level 5.01 $\log_{10}$ copies/ml (SD 0.76) at baseline. Children were randomly assigned to receive zidovudine/lamivudine (n = 8), zidovudine/abacavir (n = 13) and lamivudine/abacavir (n = 12). Sixteen children with more advanced disease also received nelfinavir; and 17 with asymptomatic disease received nelfinavir (n = 10) or placebo (n = 7) in a second randomization.

Cell-associated HIV-1 DNA was measured in peripheral blood mononuclear cells (PBMC) at baseline, and at 4, 12, 24, 48 and 96 weeks. HIV-1-DNA quantification was performed using real-time polymerase chain reaction [2]. HIV-1 DNA copy numbers were normalized to the number of β-actin genes, and expressed relative to 10^6 PBMC (2×10^6 β-actin copies), and to 10^6 CD4 cells, by attributing the HIV-1-DNA load to the CD4 cell fraction, given that these cells are the main target of HIV-1 infection. Children in PENTA 5 experienced substantial increases in CD4 cell counts during the trial [1], so HIV-1-DNA copy numbers per cell were also transformed to copy numbers per millilitre of blood by taking into account the total number of CD4 cells per millilitre. HIV-1-RNA levels were determined using the Roche Amplicor assay (version 1.5; Roche Diagnostic Systems, Inc.,

Branchburg, NJ, USA). Normal interval regression [3] was used to estimate the mean absolute levels of and change in $\log_{10}$ HIV-1 DNA and HIV-1 RNA, replacing undetectable values with the interval in which the true value could lie (the interval up to the cut-off), and adjusting for multiple measurements on each child [4].

Overall, there was a significant decrease in $\log_{10}$ HIV-1-DNA copies in PBMC and CD4 cells after baseline (both $P < 0.0001$, Fig. 1(a) and (b)). Declines in HIV-1 DNA per 10^6 PBMC and 10^6 CD4 cells were 0.06 [95% confidence interval (CI) 0.02-0.10; $P = 0.003$] and 0.09 (0.05-0.13; $P < 0.001$) $\log_{10}$ copies per week, respectively, to week 4; followed by slower declines of 0.005 (0.002-0.007; $P = 0.001$) and 0.008 (0.004-0.012; $P < 0.001$) $\log_{10}$ copies per week in PBMC and CD4 cells, respectively, from 4 to 96 weeks. Expressing HIV-1-DNA copies per millilitre of blood to incorporate changes in CD4 cells, decreases of 0.06 $\log_{10}$ copies per week to week 4 (0.02-0.10; $P = 0.005$) and 0.005 $\log_{10}$ copies per week from 4 to 96 weeks (0.002-0.008; $P < 0.001$) were observed (Fig. 1(c) and (d)), which were lower than that estimated per 10^6 CD4 cells.

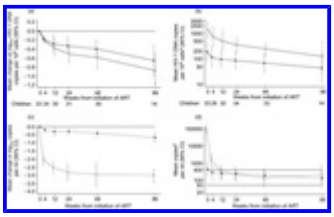

Fig. 1

Higher baseline plasma HIV-1-RNA levels were associated with a faster decline in HIV-1 DNA in the first 4 weeks (0.06 steeper slope for 1 $\log_{10}$ higher baseline HIV-1-RNA level; $P = 0.04$), but not after 4 weeks ($P = 0.99$). At week 4, no child had a plasma HIV-1-RNA level of less than 50 copies/ml, and only eight children (33%) had HIV-1-RNA levels of less than 400 copies/ml. Thirty children (91%) achieved HIV-1-RNA levels of less than 400 copies/ml and 19 (58%) less than 50 copies/ml at some time during follow-up. There was no evidence of a greater decline in HIV-1-DNA levels at timepoints when children had HIV-1-RNA levels of less than 50 copies/ml; if anything, changes in the HIV-1-DNA level were 0.07 $\log_{10}$ copies per millilitre smaller at these assessments ($P = 0.63$; 95% CI 0.37 smaller to 0.22 greater change from baseline at timepoints with HIV-1-RNA levels < 50 copies/ml). A higher baseline CD4 cell percentage was not associated with a decline in HIV-1-DNA level per 10^6 CD4 cells ($P = 0.58$), but was marginally associated with a faster decline in the HIV-1-DNA level per millilitre in the first 4 weeks (0.01 steeper slope for 5% higher baseline CD4%; $P = 0.11$). This is consistent with smaller increases in CD4 cell counts during ART in children with higher baseline CD4 cell percentages [2].

The overall change in plasma HIV-1 RNA per millilitre was 0.60 $\log_{10}$ copies per week to 4 weeks (95% CI 0.51-0.68; $P < 0.0001$), and 0.007 $\log_{10}$ copies per week from 4 to 96 weeks (0.001-0.010; $P = 0.03$). Although higher baseline HIV-1-DNA levels were associated with less steep initial and subsequent slopes in plasma HIV-1-RNA levels, neither interaction was statistically significant (both $P = 0.16$). However, children with higher baseline HIV-1-DNA levels had a lower chance of subsequently achieving plasma HIV-1-RNA levels of less than 50 copies/ml (odds ratio 0.54 for 1 $\log_{10}$ higher baseline HIV-1-DNA level; 95% CI 0.29-1.00; $P = 0.05$), whereas there was no significant effect of the baseline plasma HIV-1-RNA level ($P = 0.25$).

In conclusion, our data show that after the initiation of ART the decay of cell-associated HIV-1 DNA is biphasic, with a rapid decrease during the first 4 weeks followed by a slower decline, similar to that observed for HIV-1 RNA, and in contrast with previous

observations in adults [5]. We found that the higher the baseline HIV-1-RNA level the faster the initial decline in the HIV-1-DNA level, but that there was no association between the suppression of plasma HIV-1-RNA and HIV-1-DNA levels during ART. The decrease in the HIV-1-DNA level per millilitre of blood was lower than that estimated per 10^6 CD4 cells, mostly because of ongoing increases in CD4 cells, which are the main target of HIV-1 infection, and did not differ significantly in children with or without HIV-1-RNA suppression. The persistence of HIV-1 DNA may result both from latently infected cells with a long half-life and newly infected cells [6,7]. Together with the evidence that the peripheral increase in CD4 cells mainly consists of naive cells [1,8], these data strongly suggest that viral replication and the infection of newly produced CD4 cells occur in children on ART, regardless of their apparent HIV-1 suppression in plasma.

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