Background: Patients on ART have increases in CD4+ lymphocytes but persisting cell-associated HIV-1 DNA, suggesting ongoing thymic output; persistence of HIV-1 DNA is mainly sustained by infection of newly produced cells. In children on ART, maintenance of HIV-1 RNA suppression <50 copies/ml was poor in PENTA 5 trial.

Methods: In the PENTA 5 trial, 128 ART-naive children were randomised to 2yADT-3TC or ZDV-3TC or 3TC-ABC, 33 children (9, 13, 12 respectively) had sequential cellular samples available for analysis in PBMC at baseline and then at 4, 12, 24, 48 and 96 weeks after initiation of ART. Children with early death (n=17) were also randomised to 2yADT-3TC, 7 children with more advanced disease (n=16) received open label TDF/3TC (Part II).

Baseline DNA levels were determined using Real-time PCR (mean ± standard deviation (SD) = 2.5 ± 0.9 log10 copies/ml). The percentage of CD4+ cells expressing TREC was assessed using flow cytometry.

Results: In children with ART naive for 4 weeks or less, the percentage of CD4+ cells expressing TREC increased from median of 0.07% to 1.3% at 4 weeks (p<0.0001), and at 24 weeks increased further to 2.1% (p<0.0001). The rate of increase in TREC was positively associated with CD4% and HIV-1 DNA, and inversely associated with RNA (p<0.001).

Figure 1: Relationship between TREC, DNA, CD4%, RNA and age at baseline (initiation of ART)

Figure 2: Response to ART

Baseline TREC had higher CD4% and TREC

1. Baseline, children with higher TREC had higher CD4% and TREC and lower HIV-1 RNA, and were younger (all p<0.05) (Figure 1).

2. In contrast, whilst children with higher HIV-1 DNA had higher TREC (p=0.002), age, CD4% and HIV-1 RNA were not independent predictors (p>0.05).

3. AIDS status at baseline did not add independent information to any relationship (p>0.15) – virtually identical results using absolute CD4 count rather than CD4 percentage.

4. To further study, in a univariate analysis children with higher CD4% had lower HIV-1 RNA and were younger. However, the multivariate analysis showed that this was the result of the differential relationship between HIV-1 RNA and age, CD4%, and that neither higher HIV-1 DNA nor age independently predicted CD4 since the TREC level was known.

Predicators of HIV-1 DNA response to ART

We then evaluated predictors of changes in HIV-1 DNA (Figure 3).

1. Overall, children with the greatest increases in HIV-1 DNA had lower values at baseline and subsequent decline to lowest point (initial decline) (p<0.0001, Figure 2(a) and (b)).

2. A greater increase in TREC per 106 CD4+ cells (estimated) was associated with a lower HIV-1 RNA decline (p<0.0001, Figure 2(a) and (b)).

3. For every 0.5 log10 greater increase in TREC per ml, DNA decline was 0.11 log10 copies/ml smaller (p=0.01).

4. Changes in TREC were the strongest predictor of changes in DNA.

5. Changes in CD4% and TREC were associated with changes in DNA.

6. Pre-virional RNA decay into initial RNA decline to lowest point

7. With maintenance of HIV-1 RNA suppression <50 copies/ml the TREC increase was greater in TREC responders compared to non-responders (p=0.002).

8. However, the relationship between changes in cell-associated HIV-1 DNA and changes in thymic output was virtually identical results using absolute CD4 count rather than CD4 percentage.

9. The relationship between changes in HIV-1 DNA and TREC varied according to the stage of HIV-1 suppression (p=0.003). During the initial RNA decline to lowest point (initial decline), TREC increased 0.5 log10 for every 1 log10 decrease in HIV-1 RNA per ml, and that higher HIV-1 DNA (p=0.02) and age independently predicted CD4 since the TREC level was known.

Relationship between HIV-1 DNA and TREC

1. There was no significant association between changes in HIV-1 RNA and DNA (p=0.62).

2. However, maintenance of HIV-1 RNA suppression <400 copies/ml was associated with greater TREC increase (p=0.001).

3. Children with continuously maintained full suppression, and several children experienced transient viraemia with or without subsequent persistent rebound >50 copies/ml.

4. Therefore, classified into 4 groups.

5. In children with stable suppression, there was a greater increase in TREC per 106 CD4+ cells (estimated) than during virological non-response and transient viraemia respectively (p<0.001).

6. The inverse relationship between decline in HIV-1 DNA and increase in TREC was stronger during non-response and transient viraemia than during virological non-response.

7. The decline in HIV-1 DNA burden during ART depends on both HIV-1 RNA levels in plasma and TREC changes in TREC, viral suppression in plasma and no increase in thymic output lead to the greatest decrease, while transient viraemia and increase in thymic output lead to the slowest decrease in HIV-1 DNA burden.

8. These findings together with the evidence that stable viral suppression was associated with smaller increases in TREC, suggests that:

9. During non-response or transient viraemia, CD4 depletion in the periphery leads to increased thymic output, and persistent TREC increase inversely sustained by newly infected cells during virological non-response; CD4 cells survive longer in the periphery, so thymic output is not increased much; persistence of HIV-1 DNA may be mainly sustained by infected cells having a longer survival.

9. The decline in HIV-1 DNA burden during ART depends on both HIV-1 RNA levels in plasma and TREC changes in TREC, viral suppression in plasma and no increase in thymic output lead to the greatest decrease, while transient viraemia and increase in thymic output lead to the slowest decrease in HIV-1 DNA burden.

10. These findings together with the evidence that stable viral suppression was associated with smaller increases in TREC, suggests that: