

TREC Response to Antiretroviral Therapy in HIV-infected Children in the PENTA 5 Trial

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Background & Objectives

Several studies have outlined differences between adults and children in the pattern of peripheral immune repopulation following antiretroviral therapy (ART). In children repopulation occurs mainly with naive T cells (CD45 RA+) and there is only a small rise in memory T cells (CD45 RO+). The substantial increase in naive T cells could be due to expansion and/or prolongation of survival of peripheral CD4 naive cells, or increases in thymic output.

During intrathymic T-cell differentiation, progenitor cells undergo rearrangement of the T cell receptor, resulting in the formation of episomal DNA by-products, termed T-cell receptor rearrangement excision circles (TREC). Since TREC do not replicate with mitosis and are thus diluted with cellular division, their detection in peripheral blood cells has been proposed as a marker to evaluate the thymopoietic capacity.

To investigate the contribution of the thymus to repopulation, we therefore analysed changes in TREC and CD45 immunophenotypes after initiation of ART in previously untreated children in the PENTA 5 trial¹.

PENTA 5 trial & substudy design

128 ART-naïve children were randomised to ZDV+3TC or ZDV+ABC or 3TC+ABC. 33 children (n=8, 13, 12 respectively) had sequential cellular samples available for TREC analysis in PBMC at baseline and then at 4, 12, 24, 48, and 96 weeks after initiation of ART. TREC were measured in purified CD4 cells in 12 children with adequate samples available.

Children with early disease (n=17) were also randomised to receive NFV or NFV placebo (Part A: 10 NFV, 7 NFVp); and children with more advanced disease (n=16) received open label NFV (Part B).

At baseline median age was 7.1 years, CD4% was 17% (IQR 9-24) and mean HIV-1 RNA was 5.0 log₁₀ copies/ml (SD 0.8); 4 children (12%) had AIDS. None of the 7 children on double NRTI only changed from dual therapy during the course of the trial, and all but one had HIV-1 RNA decline to below 400 copies/ml. Overall more than 99% of time was spent taking 2 or more antiretrovirals, and 73% taking triple therapy.

CD4 CD45RA/RO and CD8 CD45RA/RO immunophenotyping was performed in real time in a subset of 17 of these children, plus a further 9 children (total 26).

Laboratory methods

In brief, TREC levels were analysed by real-time quantitative PCR assay². 10 µl of cell lysate, equivalent to 80,000 cells, were used as template for PCR amplification. The reaction volume was 50 µl containing 1.0 x TaqMan Universal PCR Master Mix (PE Applied Biosystems, California, USA), 300nM of each primer (forward, 5'-CACATCCCTTCAACACATGCT-3'; reverse, 5'-GCCAGCTGCAAGGGTTAGG-3') and 100 nM of the fluorogenic probe (5'-ACACCTCTGGTTTGTAAAGGTGCCACT-3') conjugated with the fluorophores FAM (5-carboxyfluorescein) at the 5'-end, and TAMRA (6-carboxytetramethylrhodamine) at the 3'-end. The PCR primers and the fluorogenic probe were specifically designed for the detection of human TREC. The thermal cycling conditions were 2 min at 50°C, 10 min at 95°C, and 45 cycles each of 95°C for 15 sec and 60°C for 1 min. The reaction was performed in a spectrofluorometric thermal cycler (ABI PRISM 7700 Sequence Detector, PE Applied Biosystems).

For each run, a standard curve was generated from duplicate samples of five-fold serially diluted known copies of plasmid DNA obtained by inserting a human signal joint TREC fragment in the pCR-Blunt Vector. The assay was sensitive enough to detect 2.5 copies of TREC, and showed a dynamic range of at least 5 log₁₀ (from 2.5 copies to 2x10⁶ copies)². Each sample was run in duplicate. To normalise for cell equivalents, the β-actin gene was quantified under real-time PCR conditions similar to those used for TREC quantification; the primer and probe concentrations were as follows: 300 nM of the forward primer (5'-TCCACCCACATGTCGCCATCTACGA-3'), 600 nM of the reverse primer (5'-CAGCGAACCCTCATTCGCCATGG-3') and 200 nM of the fluorogenic probe (5'-ATCCCTCCCATGTCGCCATCTGCGT-3') conjugated with the fluorophore VIC at the 5'-end, and TAMRA at the 3'-end. The β-actin standard curve was obtained from five-fold serial dilutions of DNA extracted from the BE51 cell line.

References

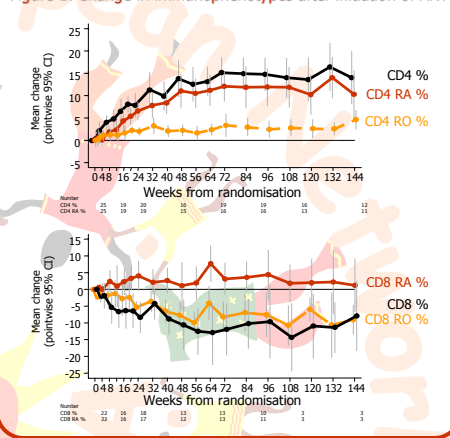
1. PENTA 5. A randomised trial evaluating three NRTI regimens with and without nevirapin in previously untreated HIV-infected children: 48 week follow-up from the PENTA 5 trial. Lancet 2010 (in press)
2. Ometto L, De Forni D, Patiri F, et al. Immune reconstitution in HIV-1-infected children on antiretroviral therapy: role of thymic output and viral fitness. AIDS (in press).

Immunophenotypes

The early increase in CD4 CD45RA+ cells was sustained beyond 2 years (Figure 1).

- the increase in CD4% plateaued at around 15% after 48 weeks (12% increase in CD45RA% & 3% increase in CD45RO%; both p=0.0001 over all follow-up)
- absolute values had reached CD4% around 30% (22% CD45RA% & 8% CD45RO%)
- the change in CD8% plateaued at around 8% decrease after week 48, with around 10% decrease in CD8 CD45RO% and 2% increase in CD8 CD45RA% (both p<0.0001)

Figure 1: Change in immunophenotypes after initiation of ART

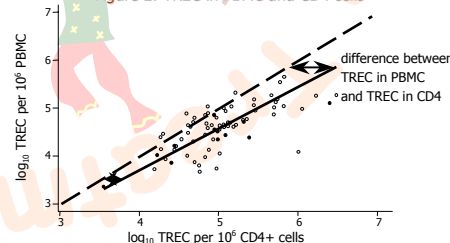


TREC in PBMC & CD4 cells

74 samples had TREC results in both PBMC and CD4 cells.

- as expected, TREC levels in PBMC were lower than TREC levels in CD4 cells (Figure 2), with an average 0.42 excess log₁₀ TREC in CD4 cells
- the overall agreement between log₁₀ TREC in PBMC and CD4 was high (Spearman's ρ=0.70, p<0.0001)

Figure 2: TREC in PBMC and CD4 cells

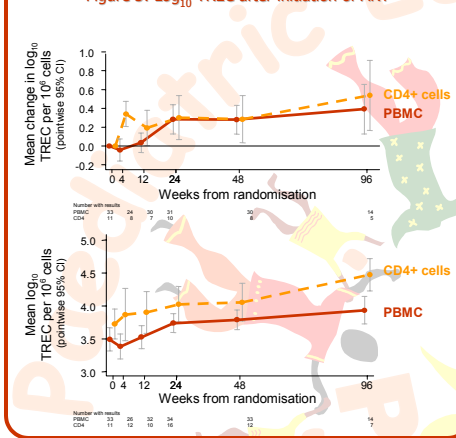


TREC response to ART

There was a significant increase in log₁₀ TREC in both PBMC and CD4 cells after baseline (p<0.0001 for both, Figure 3).

- changes in TREC were similar in PBMC and CD4 cells, with the exception of changes at week 4
- there was no evidence that the relative difference between TREC in CD4 and PBMC was larger at this stage (Figure 2)

Figure 3: Log₁₀ TREC after initiation of ART



Baseline predictors of TREC changes

- Univariately, TREC in PBMC at baseline were strongly related to baseline CD4% and inversely related to age at baseline. Including these factors and baseline HIV-1 RNA in a joint multilevel model, baseline log₁₀ TREC was found to be independently and inversely related to baseline log₁₀ HIV-1 RNA (p=0.008), as well as positively related to baseline CD4% (p=0.002), and inversely related to age at baseline (p<0.0001). There was no independent effect of AIDS status at baseline on baseline TREC (p=0.83).

- However, neither age (p=0.65) nor baseline log₁₀ HIV-1 RNA (p=0.32) were associated with subsequent increases in log₁₀ TREC; nor was there any effect of AIDS status at baseline (p=0.27).

- The only baseline factor found to be a significant independent predictor of subsequent changes in log₁₀ TREC was baseline CD4%

- higher baseline CD4% was associated with smaller changes in log₁₀ TREC subsequently (on average 0.04 smaller change in log₁₀ TREC for each 5% higher baseline CD4%, 95% CI 0.00 to 0.07, p=0.05). This effect increased over time, with a 5% higher CD4% at baseline predicting a 0.00, 0.04, 0.08, 0.13 and 0.15 smaller change in log₁₀ TREC at 4, 12, 24, 48 and 96 weeks respectively (heterogeneity p=0.001, Figure 4).

- In the multilevel model, children with higher baseline TREC had smaller changes in subsequent TREC values. Thus, a 1 log₁₀ higher TREC at baseline was estimated to be independently associated with, on average, a 0.10, 0.64, 0.34 and 0.47 smaller change in log₁₀ TREC at weeks 4/12, 24, 48 and 96, respectively.

Subsequent predictors of TREC changes

- Larger changes in CD4% after baseline were associated with larger changes in log₁₀ TREC

- on average 0.08 larger change in log₁₀ TREC for each 5% larger change in CD4% (95% CI 0.04 to 0.13 p=0.0006)

- HIV-1 RNA suppression below 50 c/ml was independently inversely related to change in log₁₀ TREC

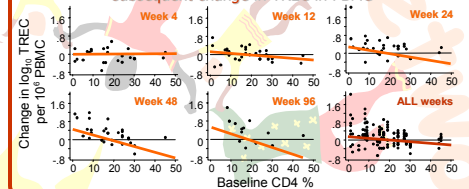
- there was a 0.12 smaller change in log₁₀ TREC (95% CI 0.01 to 0.24, p=0.04) when HIV-1 RNA was below 50 c/ml, after adjusting for change in CD4%
- there was no evidence for an additional effect of absolute log₁₀ HIV-1 RNA or change in log₁₀ HIV-1 RNA at each assessment week (p=0.28 and p=0.16 respectively)

- 17 children with TREC data also had immunophenotypes

- change in CD45RA% (of total lymphocytes) predicted change in TREC similarly to total CD4% (on average 0.13 larger change in log₁₀ TREC for each 5% larger change in CD45 RA%, 95% CI 0.06 to 0.20 p=0.0004)

- there was no significant effect of change in CD45RO% (on average 0.04 smaller change in log₁₀ TREC for each 5% larger change in CD45RO%, 95% CI 0.16 smaller to 0.08 larger change, p=0.52).

Figure 4: Variation in the effect of baseline CD4% on subsequent change in TREC in PBMC



Summary

- Initiation of ART is associated with significant increases in TREC, correlating with baseline & change in CD4% but not age
- Increase in naive CD4CD45RA cells, but not memory CD4 CD45RO cells, was closely related to TREC changes
 - thymic output appears to be the main source of CD4 cell repopulation in children on ART
 - recovery of thymic function is independent of age, and influenced by the status of peripheral CD4 cell depletion and HIV-1 suppression
- The association between HIV-1 RNA suppression and lower thymic output supports the concept that unless full viral suppression occurs, higher thymic output is required to compensate for greater peripheral CD4 depletion in children with detectable circulating virus

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