

ABSTRACT

Background: HIV-1 infection is associated with increases in T cell proliferation and declines in telomere restriction fragment length (TRFL) and T cell receptor excision circles (TRECs), Treatment with HAART leads to increases in TRFL and TRECs. These results have been interpreted to suggest that the thymus plays a major role in immune reconstitution following HAART, although decreased proliferation can also account for these observations. We examined the relationship between changes in proliferation and the changes in TRFL and TRECs.

Method: Proliferation rates (ex vivo BrdU incorporation), TREC contents (PCR) and/or TRFL (Southern blotting) were measured longitudinally in 12 protease inhibitor-naïve patients following initiation of HAART and in 21 patients voluntarily terminating HAART.

Results: Changes in CD4+ T cell proliferation rates correlated with changes in viral load and were inversely correlated with changes in CD4+ T cell TREC levels in patients initiating HAART (n=9, r=-0.95, p<0.0001) as well as in patients terminating HAART (n=15, r=-0.60, p=0.02). TREC levels were found to increase in naïve (CD45RO-CD27+) CD4+ T cells following HAART suggesting the thymus is still functioning in these patients. TRFL were significantly increased in CD4+ (n=9, p=0.03) and CD8+ (n=9, p=0.03) T cells in patients receiving HAART for 52 weeks. Higher CD4 counts at the start of therapy were associated with greater increases in TRFL (p=0.01). Similar to the results for TREC levels, changes in CD4+ proliferation was inversely correlated with changes in TRFL following HAART (n=6, r=-0.83, p=0.04).

Conclusions: Our data suggests that TRECs, as well as TRFL, can be a measure of either the replicative history of a T cell or thymic function. In vivo BrdU labeling of T cells has shown that HIV-1 infection induces an accumulation of a pool of T cells with high turnover rates which most likely consists of cells with shorter TRFL and lower TREC content. Thus, increases in CD4+ T cell TREC levels and TRFL following HAART are likely the net result of increased thymic output, reduced T cell proliferation and increased clearance of a pool of rapidly dying cells that contain shorter TRFL and lower TREC levels.

INTRODUCTION

HIV-1 infection is characterized by high viral loads and depletion of CD4⁺ T cells in the circulating blood of patients. Controversy exists as to whether this T cell loss is related to the destruction of CD4⁺ cells at an accelerated rate, failure to effectively regenerate new cells or both (1,2). Highly active antiretroviral therapy (HAART) has extended patients lives and helped to improve quality of life by drastically reducing viral loads and partially restoring immune function. Researchers generally agree that immune reconstitution may occur through a combination of thymic dependent generation and peripheral expansion of naïve and memory T cells. Controversy, however, exists as to the role each of these processes plays in immune reconstitution. Recent studies evaluating lymphocyte turnover rates in HIV-infected patients during therapy and after cessation of HAART have shown that suppression of viral replication by HAART is accompanied by a direct increase in T cell proliferation. Patients voluntarily removed from therapy rapidly show the inverse, with viral loads rebounding (3). BrdU incorporation has been used to evaluate the effects of HIV-1 infection on T cell turnover and has been combined with the study of T cell excision circles (TRECs) in order define the role of the thymus in reconstituting the immune system. These studies reveal a profound inverse correlation between changes in naïve T cell proliferation and changes in TREC levels suggesting that the thymus may play a lesser role in immune reconstitution during HAART (4,5).

New insight into the pathogenesis of AIDS has also been gained by studying telomere dynamics. Telomeres consist of repetitive DNA sequences bound by protein complexes. They function in protecting chromosome ends from degradation and also promote chromosomal end replication. Telomere shortening is correlated with cellular senescence, aging, cancer and other diseases. The telomere and its role in HIV-1 disease have recently received attention from researchers. The bulk of this work, however, has focused on whether HIV disease alters telomere length in T cell subpopulations as a measure of the cell's replicative history (6,7).

Few studies have combined direct (BrdU, TREC) and indirect (TRFL and T cell counts) methods for measuring T cell turnover. With this in mind, we have examined changes in mean telomeric restriction fragment length (TRFL) as a measure of T cell replicative history and have correlated this data with changes in T cell subpopulations, T cell proliferation as measured by BrdU incorporation and TREC levels in HIV-1 infected patients undergoing HAART. We present evidence showing that HAART induces a pool of CD4⁺ T cells with increased TRFLs, increased TREC levels and reduced T cell proliferation. These results suggest that the thymus may play less of a role in immune constitution following HAART than previously thought.

Methods

TRF Length Analysis

Telomeric terminal restriction fragment (TRF) length was measured using a modification of the procedure by Feng et. al. (8). Briefly, equal amounts of DNA were digested with Alu I and HinF I and restriction fragments resolved by pulsed-field gel electrophoresis. DNA was transferred to a nylon membrane by southern blotting and hybridized with an alkaline phosphatase-conjugated telomeric probe (Life Technologies, Rockville, MD). Hybridized probe was detected following chemiluminescence (Whatman BioScience, Newton Center, MA) and exposure to Kodak Biomax MR 2 film (Sigma Chemical Company, St Louis, MO). The digitized lumigraph was analyzed using LabWorks Gel Analysis Software (UVP Laboratory Products, Upland, CA) and average TRF length was calculated using weighted mean calculations that normalize the signal intensity relative to the size of each digestion product.

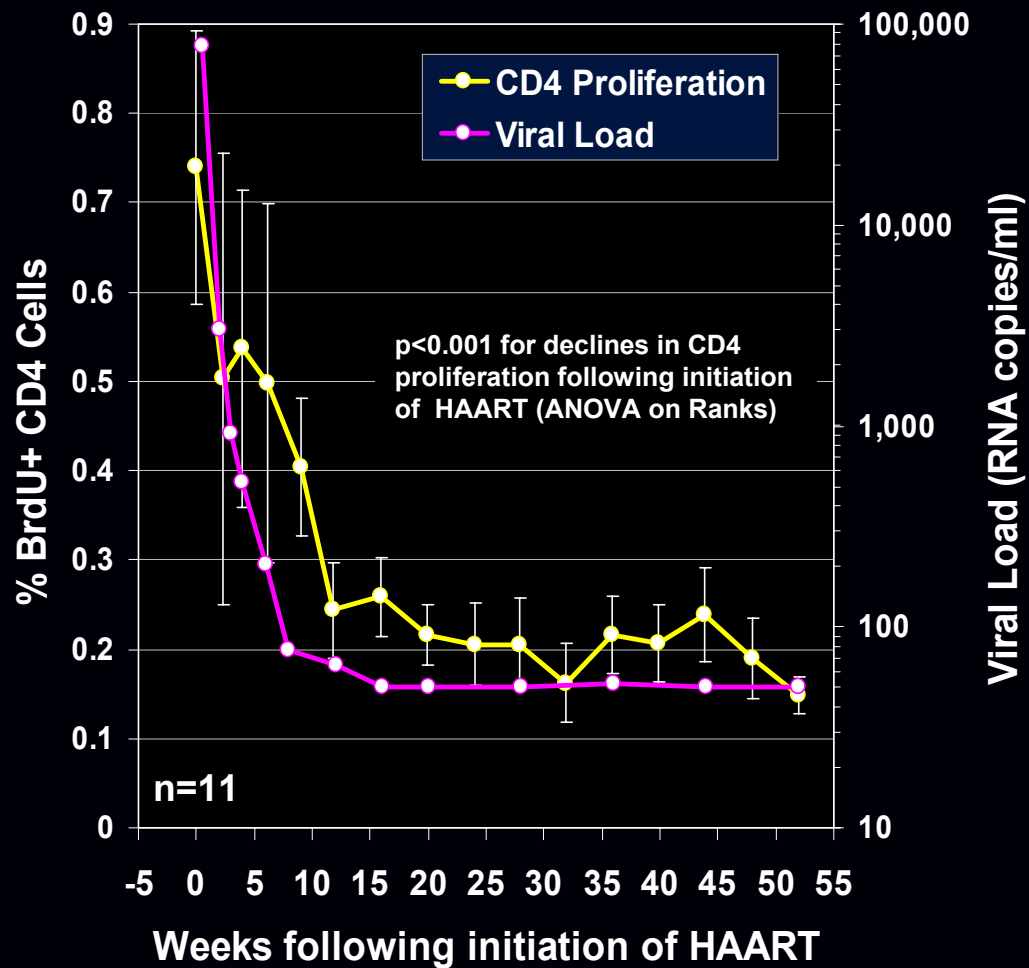
Ex Vivo BrdU Labeling

Ex vivo BrdU labeling was performed using modifications of the procedure reported by Tough and Sprent (9). CD4⁺ and CD8⁺ T cell suspensions were incubated with 10 μ M BrdU (Sigma). CD4 and CD8-specific cell surface staining (PharMingen) was performed, cells were lysed and fixed with 1% paraformaldehyde and 1% Tween-20 in 1x PBS. Cellular DNA in the permeabilized cells was partially digested, stained with an anti-BrdUrd FITC (Becton Dickinson) antibody and subjected to flow cytometric analysis. Samples were analyzed in parallel with unlabeled cells from the same individual, and this value was subtracted from the value obtained for BrdUrd-labeled cells. Data are presented as the percent of cells in the specific lymphocyte pool that are BrdUrd⁺ or as the number of BrdUrd⁺ T cells/ μ l of blood.

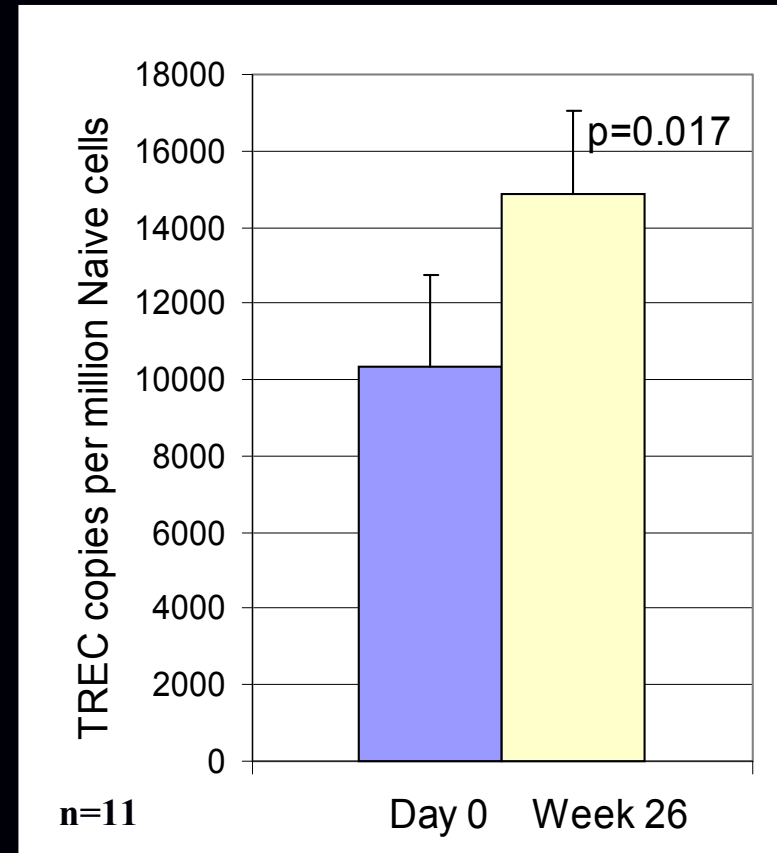
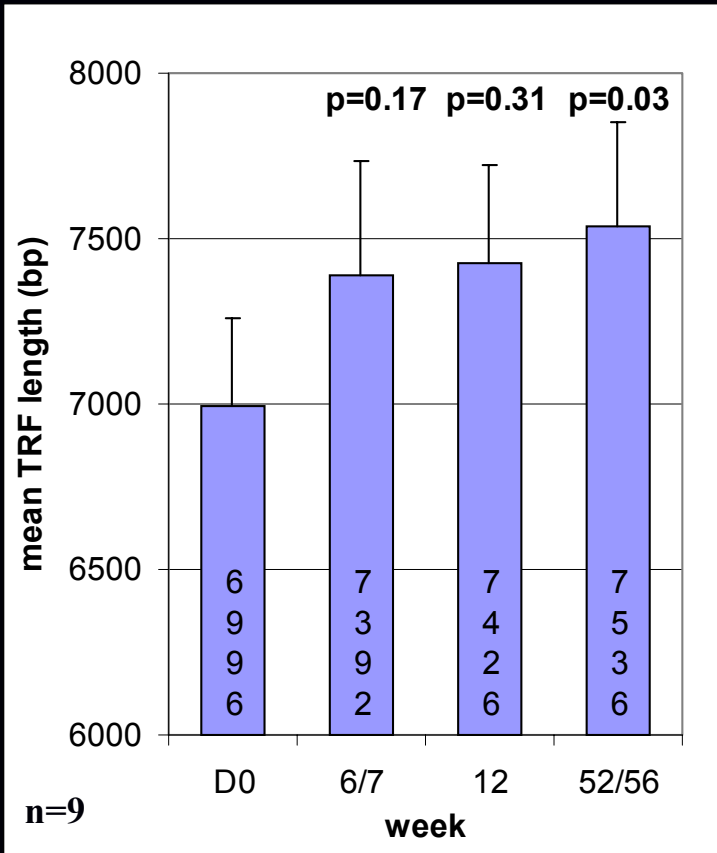
T Cell Receptor Rearrangement Excision Circle (TREC) Measurements

TREC levels in CD4⁺ and CD8⁺ cells were measured by quantitative competitive PCR based on the procedure described by Douek et al. Competitive internal standard DNA was added to equal aliquots of DNA from patient samples and amplified for 35 cycles. PCR products were labeled with ³²P-dCTP during amplification and separated using polyacrylamide gel electrophoresis. Radioactivity was quantitated by phosphorimaging. TREC copy numbers were determined by using a linear regression equation of the ratio of PSL for the patient sample to internal standard versus the copy number of the competitive internal standard.

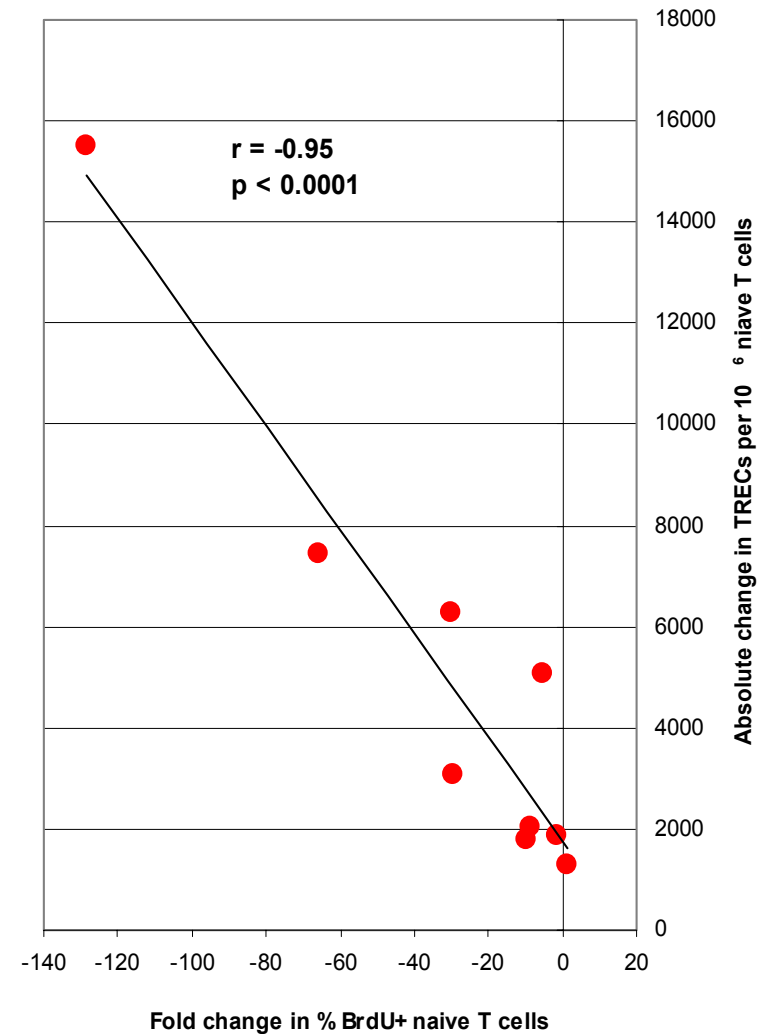
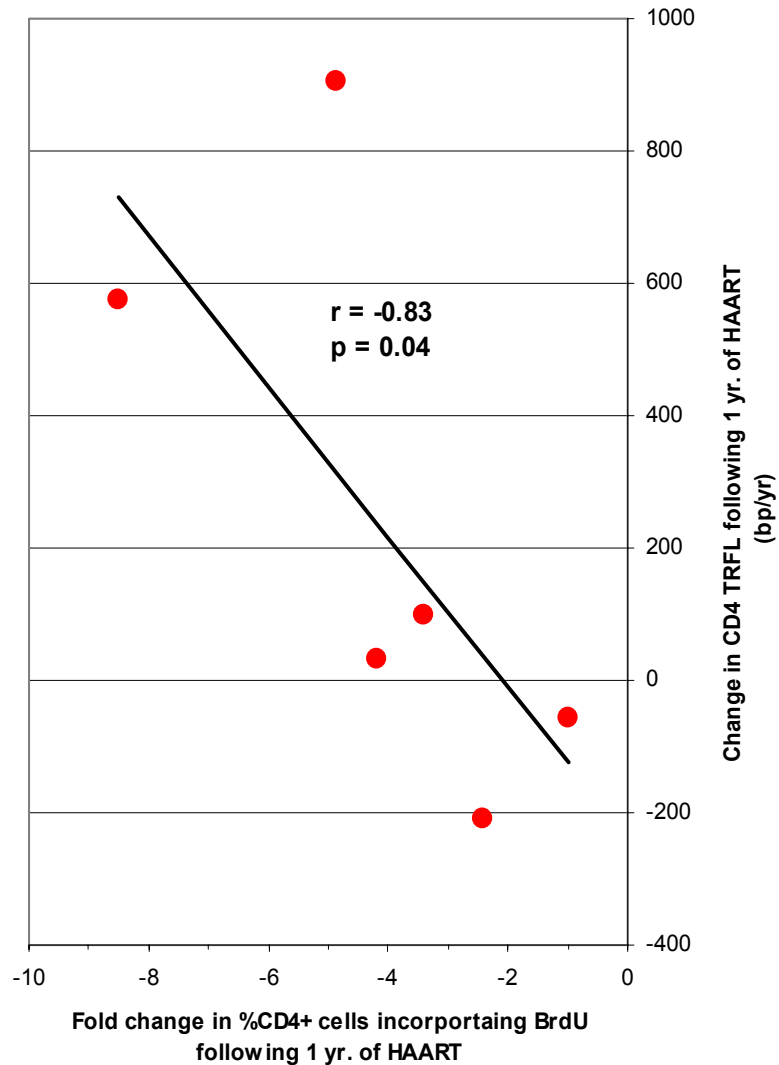
Significant declines in CD4 proliferation are seen following the initiation of HAART



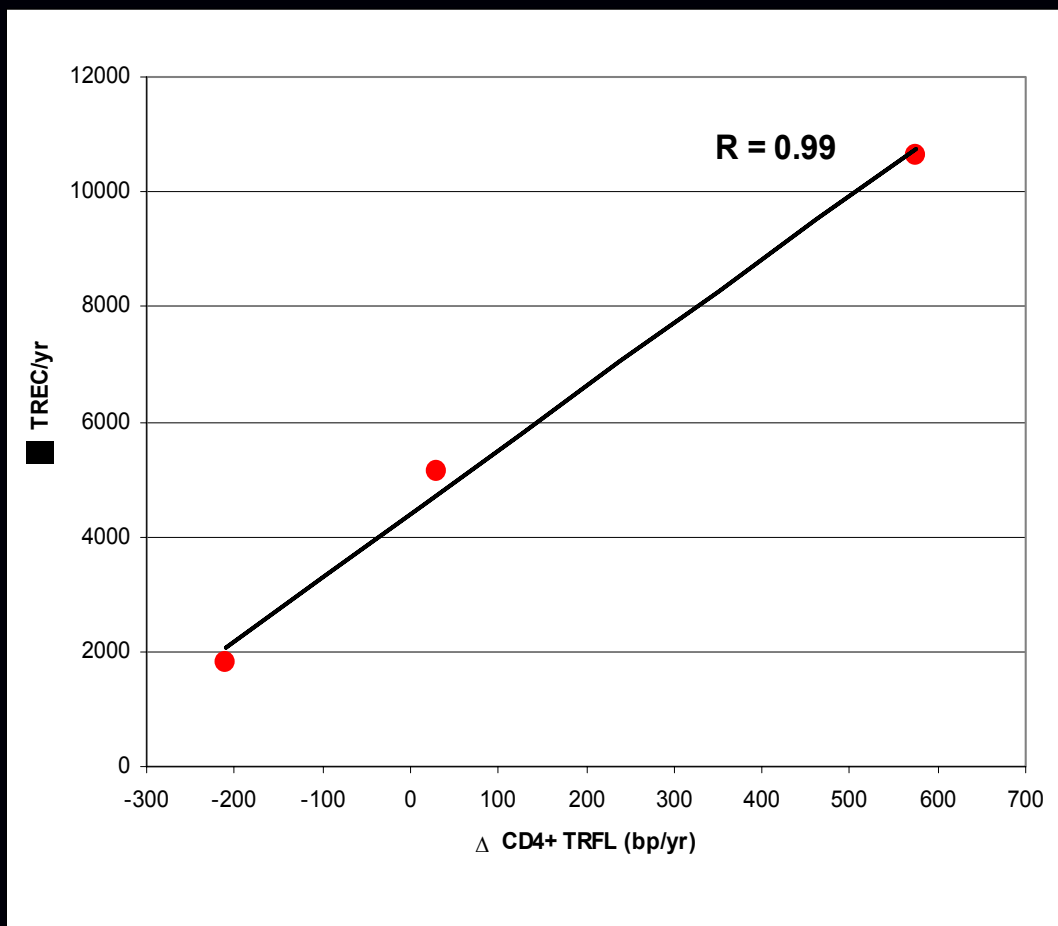
Significant increases in CD4 TRFLs and TREC levels are seen following the initiation of HAART



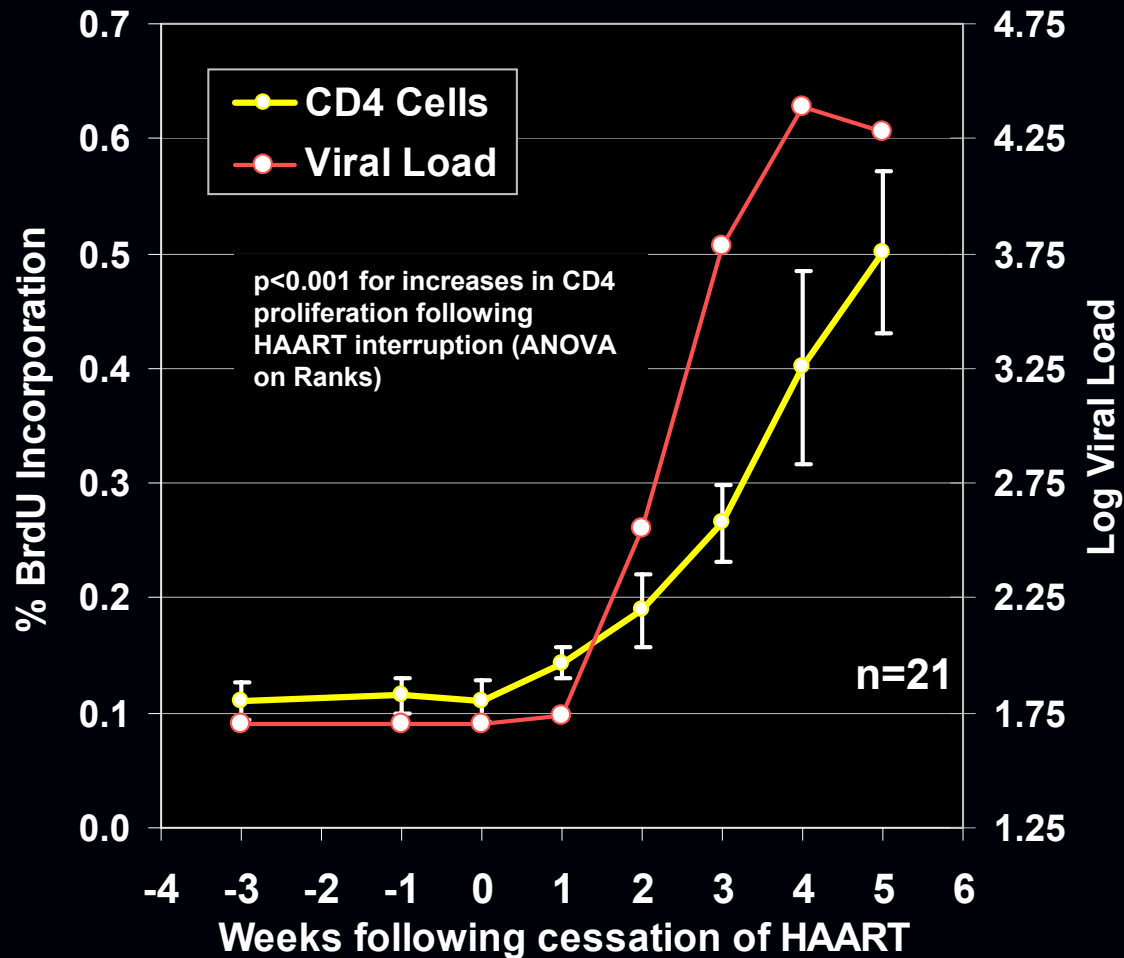
Changes in TREC levels and TRFLs inversely correlate with changes in CD4 BrdU incorporation 1 year following the initiation of HAART



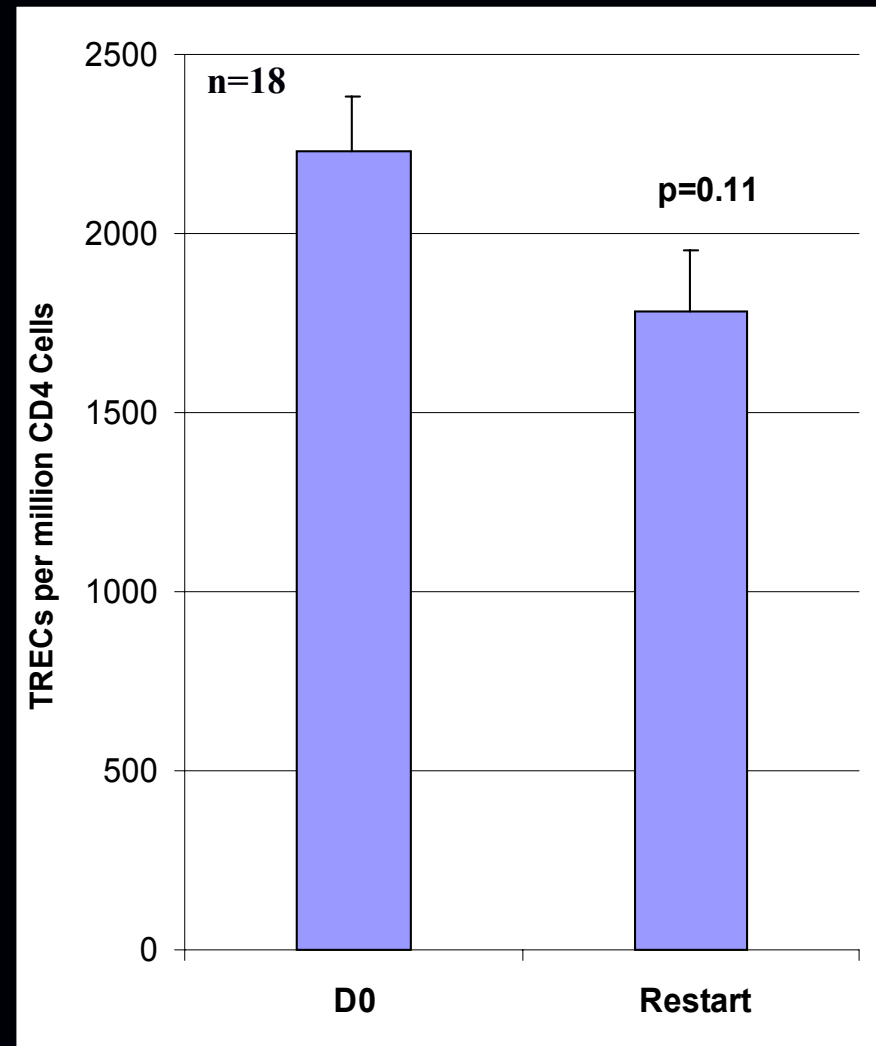
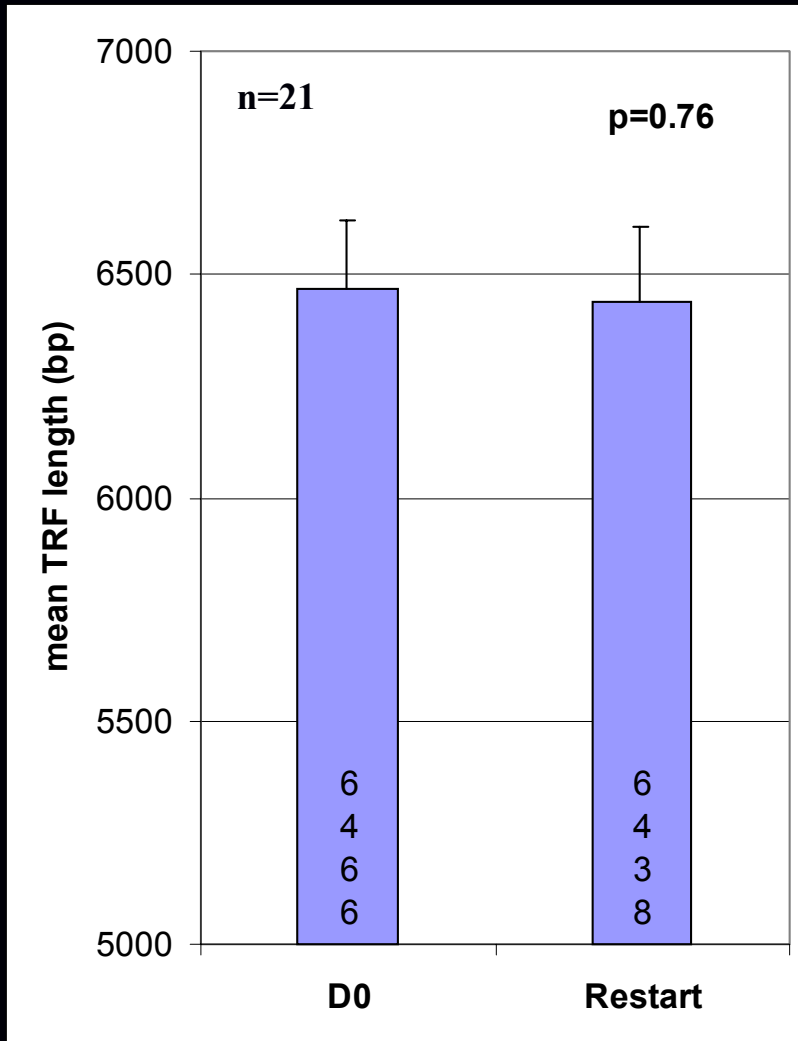
Changes in TREC levels correlate with changes in TRFLs 1 year following the initiation of HAART



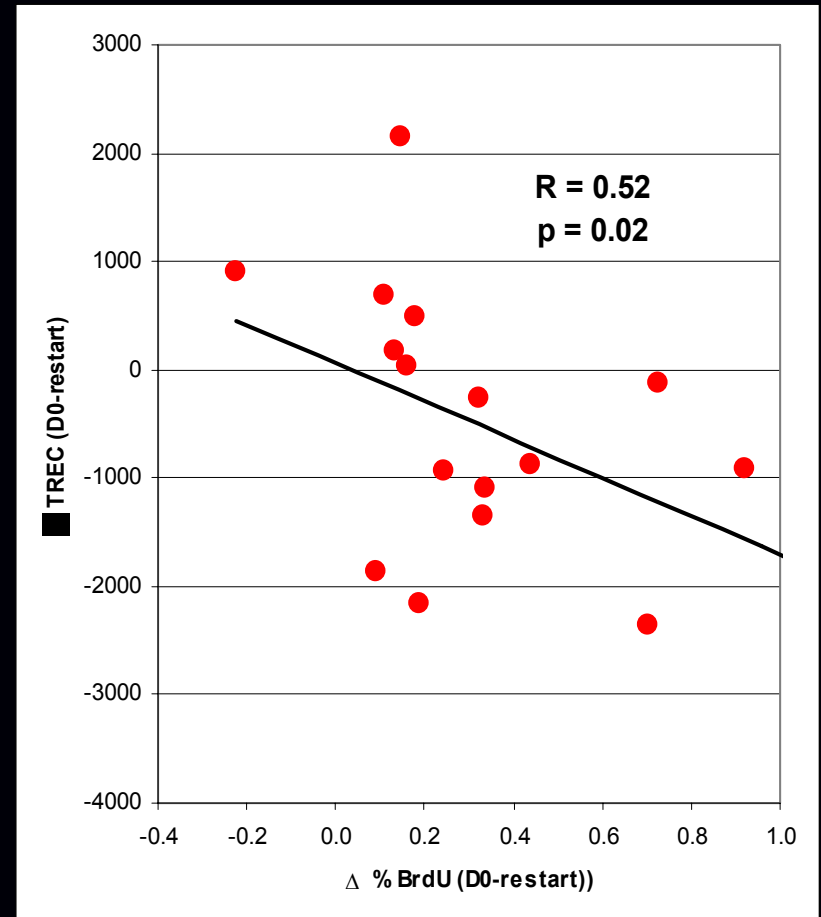
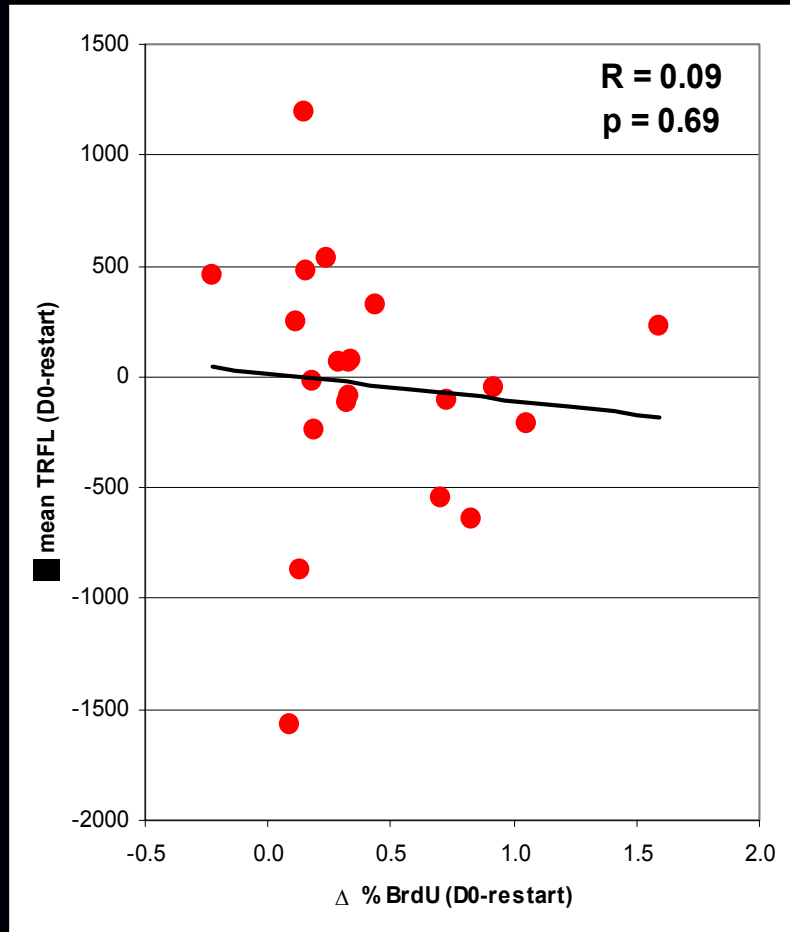
Significant increases in CD4 proliferation are seen following HAART Cessation



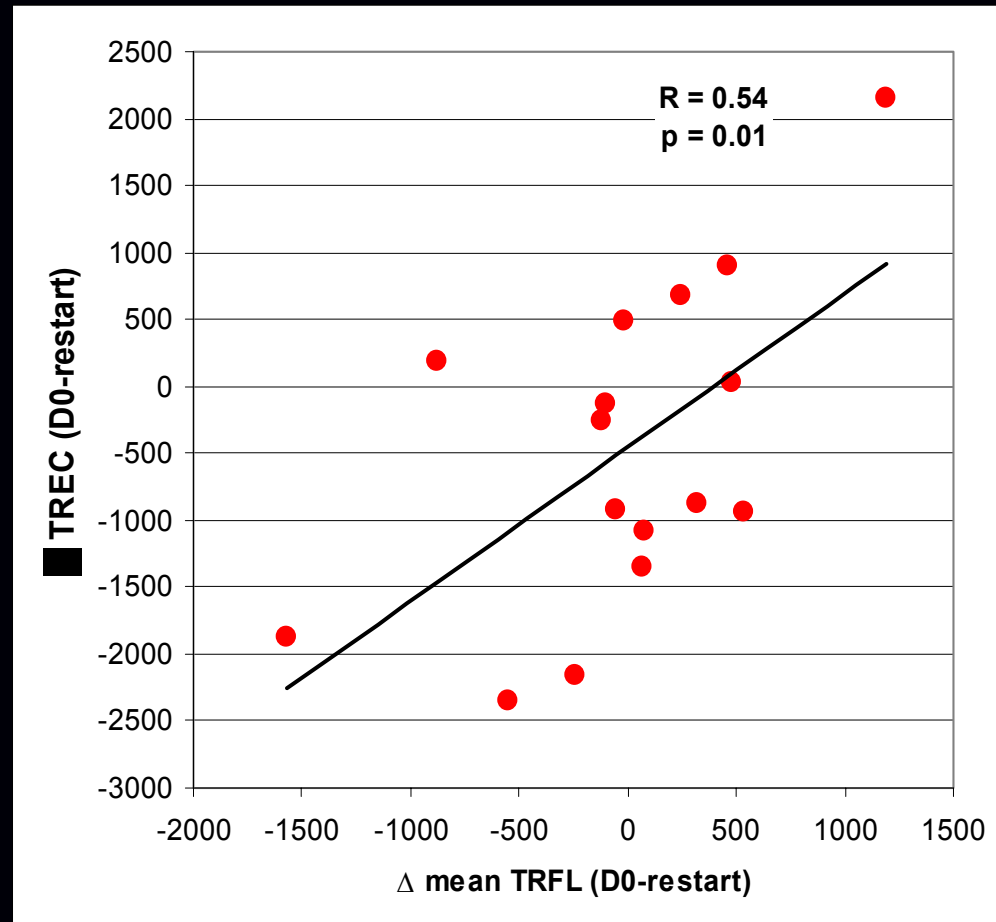
No significant changes in CD4 TRFLs or TRECs are seen following viral rebound after therapy interruption



Changes in TREC levels (but not TRFLs) inversely correlate with changes in CD4 BrdU incorporation following therapy interruption



Changes in TREC levels correlate with changes in TRFLs following HAART interruption



Patient Cohorts

- **Protease-Inhibitor (PI)-naïve Patients**

- 17 patients

- Ex vivo BrdU data collected pre- and weekly to monthly for 1 year following initiation of HAART
- T cell Receptor Excision Circle (TRECs) data collected pre- and 6 months post-initiation of HAART
- Telomere Restriction Fragment Length (TRFL) data was collected pre- and 6, 12 and 52 weeks post-initiation of HAART

- **Therapy Interrupted Patients**

- 21 patients: Long-term HAART recipients with viral loads <500 RNA copies/ml for >1.5 years who voluntarily terminated HAART. All patients had rebounds in viral load, most within 2-8 weeks of terminating therapy. All patients restarted therapy with a median time to restart of 7 weeks.

- Ex vivo BrdU, TREC and TRFL data was collected pre-termination of HAART and the day before restarting therapy during the phase of HAART termination and viral rebound.

Protease-Inhibitor (PI)-naïve Patients

Therapy Interrupted Patients

Conclusions

- **PI-naïve patients receiving HAART for 1 year showed significant increases in CD4 TREC and TRFL that inversely correlate with significant declines in CD4 proliferation**
- **Therapy interrupted patients showed high rebounds in viral load and CD4 proliferation. Increases in CD4 proliferation correlated with declines in TREC levels following HAART interruption**
- **These data strongly support the hypotheses that TREC levels and TRFL are measures of the replicative history of the cell and may be poor measures of thymic function**