

# **Immune reconstitution in HIV-infected children following anti-retroviral therapy**

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# ABSTRACT

**Background:** Immune reconstitution following antiretroviral therapy (ART) in human immunodeficiency virus (HIV) infected patients may result from recovery of thymic function, peripheral redistribution or decreased T cell destruction. In the present study we have investigated thymic function by estimating the levels of T cell receptor excision circles (TRECs). In addition to this, activation and naïve status of T cells were studied to investigate their role in immune reconstitution.

**Methods:** This study consists of 23 HIV infected patients (age range 2.32 – 20.6 years, median 11.95 years) who are on antiretroviral treatment for >24 months. TRECs were estimated by TaqMan real time PCR assay. Surface markers for naïve and activated phenotype were estimated by multi-color flow cytometry. The correlation between different parameters was analyzed by Spearman Rank correlation analysis.

**Results:** In this study we found that there was a significant positive correlation between TREC levels and absolute CD4 numbers ( $r=0.7$ ,  $P,0.001$ ), and the CD4/CD8 ratio ( $r=0.42$ ,  $P=0.03$ ). These parameters showed a negative correlation with viral load [CD4 abs ( $r= -0.56$ ,  $P=0.02$ ), CD4/CD8 ratio ( $r= -0.7$ ,  $P<0.001$ )]. The level of T cell apoptosis marker CD95 and the level of memory CD4 population negatively correlated with TREC ( $r= -0.42$ ,  $P=0.04$  and  $r= -0.55$ ,  $P=0.004$  respectively) while CD4+CD45RA+62L+ levels positively correlated with TREC levels ( $r=0.55$ ,  $P=0.004$ ). Plasma virus load negatively correlated with %CD8+CD28+ ( $r= -0.77$ ,  $P<0.001$ ) and positively correlated with %CD8CD38+HLA-DR= ( $r=0.8$ ,  $P<0.001$ ). %CD8+CD28+ had a positive association with TREC and %CD8+CD38+HLA-DR+ had a negative association with TREC.

**Conclusion:** The results of this study suggest that peripheral T cell reconstitution is a cumulative effect of active thymic output and decreased activation status of peripheral T cells which thereby decreases peripheral T cell destruction following effective viral suppression.

# INTRODUCTION

- ◆ The thymus plays an important role in the development of the T cells providing an inductive microenvironment in which bone marrow derived progenitors undergo proliferation, T cell receptor gene rearrangement and thymocyte differentiation into mature T cells expressing CD4 or CD8 receptors
- ◆ Assessment of the thymic function, which previously was dependent upon either autopsy measurement of thymic size, or radiographic assessment of thymus size or immunophenotyping of T cells for naïve and memory markers, is now possible by quantitation of recent thymic emigrant (RTE) cells
- ◆ TRECs are formed by rearrangement of individual gene segments (V, D, and J) which encode for TCR during intrathymic development. This process involves cleavage of DNA at the recombination signal sequences that flank TCR gene segments in their germline configuration. When the intervening stretches of DNA are excised, then the coding ends are joined to form a functional TCR gene in the chromosomal DNA, and the signal ends join to form extrachromosomal DNA circles termed TRECs. The TCR- $\delta$  locus is embedded in the TCR- $\alpha$  locus, so TCR- $\delta$  sequences are specifically deleted in all  $\alpha\beta$  T cells (Fig 1)

- ◆ Several lines of evidence suggest that impaired thymic output plays a role in the CD4+ T-cell decline observed in HIV-1 infection as evidenced by the loss of phenotypic “naïve” T cells. With potent antiretroviral therapy patients show significant increases in CD4 T cell numbers.
- ◆ Immune reconstitution following antiretroviral therapy in HIV infected patients may result from recovery of thymic function, peripheral redistribution or decreased T cell destruction
- ◆ Importance of analysing the role thymus and the role of naïve and activation status of T cells in immunological recovery have been receiving increasing attention
- ◆ TREC levels estimated in periphery are not only representative of thymic out put but can be affected by changes occurring in periphery such as destruction and activation (proliferation) of T cells

# OBJECTIVES

- ◆ To determine TREC levels in a subset of HIV infected children and adolescents in relation to virologic response to antiretroviral therapy
- ◆ To correlate changes in TRECs with measures of quantitative immune reconstitution
- ◆ To evaluate changes in TRECs in relation to stabilization of peripheral T cell compartment

# MATERIALS AND METHODS

## Study Cohort

23 HIV infected patients, age range 2.3 to 20.6 years, median 11.9 years

All patients were on 3-4 anti-retroviral drugs for >24 months

**Response to therapy:** Patients were designated as

- ◆ **Responders n=11:** undetectable <400 copies/ml and sustained for  $\geq 6$  months
- ◆ **Non-responders n=12:** No change or decrease of viral load by  $<0.5 \log_{10}$  but not undetectable levels

**Table:** TREC levels, virus load and T cell subsets after 6 months of therapy

	<i><b>Non-responder (n=12)</b></i>	<i><b>Responder (n=11)</b></i>
Plasma virus load	5.09 (3.10-5.88)	<400
% CD4	21.3 (3.1-37)	37 (25-45)
Absolute CD4	637 (14-2038)	1120 (396-3656)
% CD8	45.3 (18.4-58)	25 (14.6-47)
Absolute CD8	1080 (258-1833)	659 (392-1828)
Ratio CD4/CD8	0.45 (0.05-1.89)	1.44 (0.8-2.21)
TREC levels	3,480 (21-56,513)	34,959 (3,606-194,000)

## **Immunophenotyping:**

Peripheral blood was drawn in heparinized tubes and Flow Cytometry was performed using the FACScan cytofluorometer for enumeration of CD4+, CD8+, CD45RA+62L+, CD45RA-62I-, CD45RA-62L+ cell subsets.

## **TREC levels**

TREC levels were estimated in PBMC by TaqMan real time PCR assay

## **Plasma HIV RNA copies**

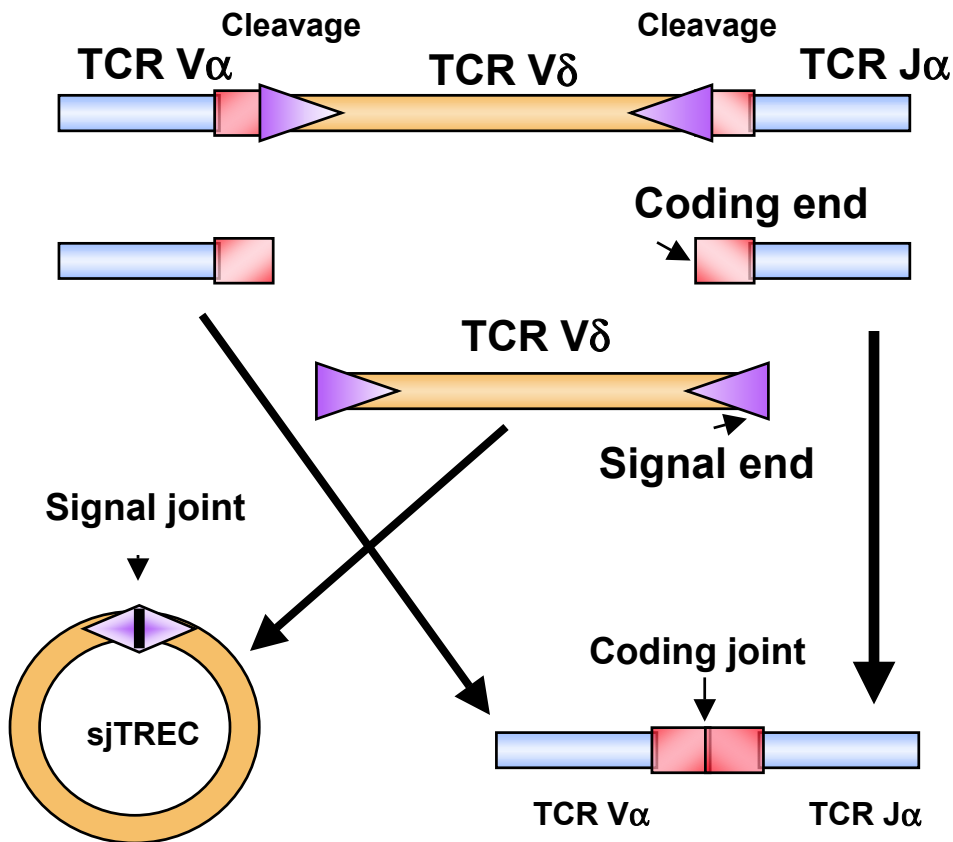
Plasma HIV RNA copies were determined in a commercial laboratory by Roche Amplicor assay

## **Statistics**

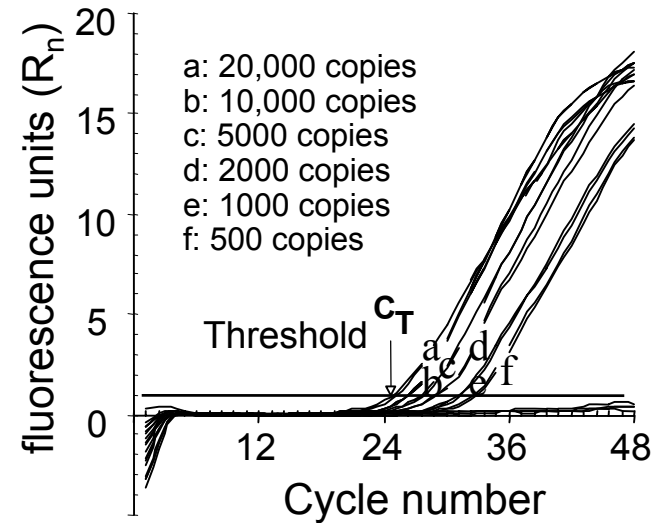
Correlation between different parameters were analyzed by Spearman Rank Correlation analysis. Change in different parameters over time was calculated by linear regression analysis. All statistical analyses were performed by SigmaStat software (SPSS Inc)

# Quantitation T cell receptor (TCR) gene rearrangement: formation of TCR excision circles (TRECs)

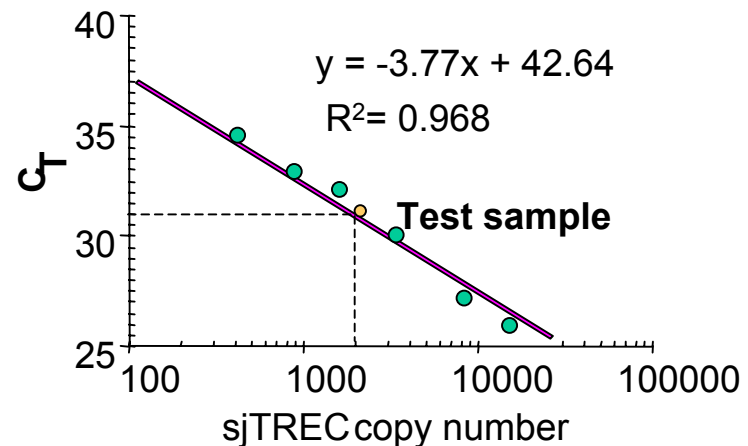
## Recombination signal sequence



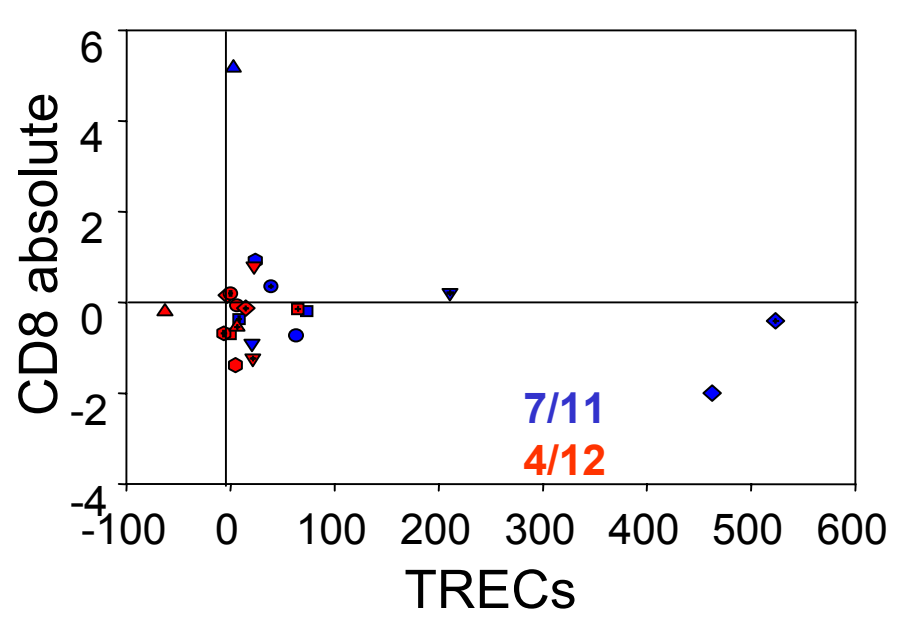
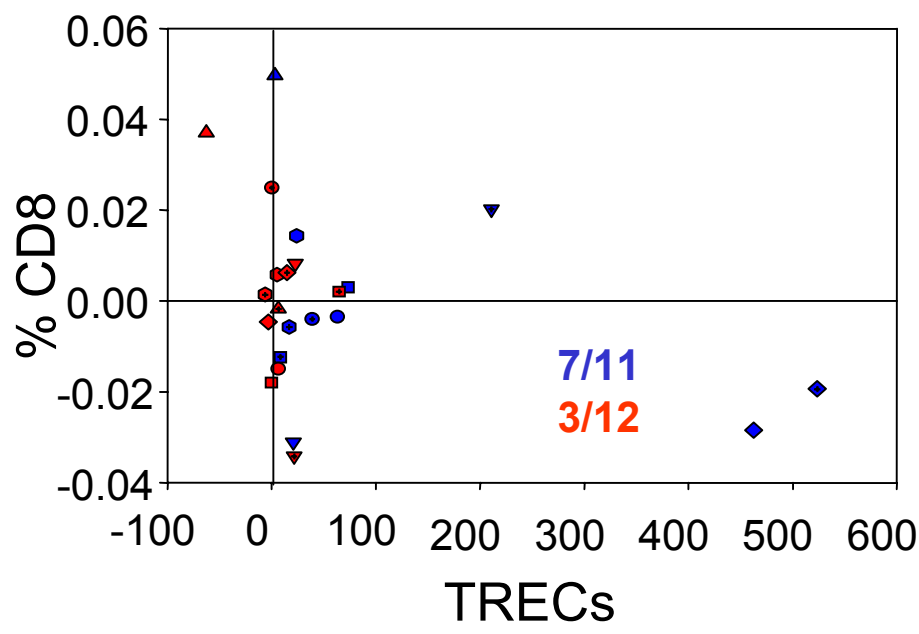
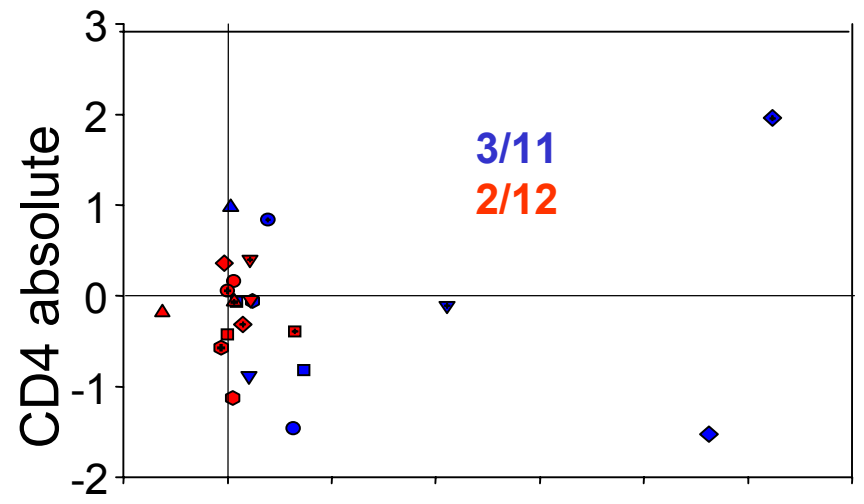
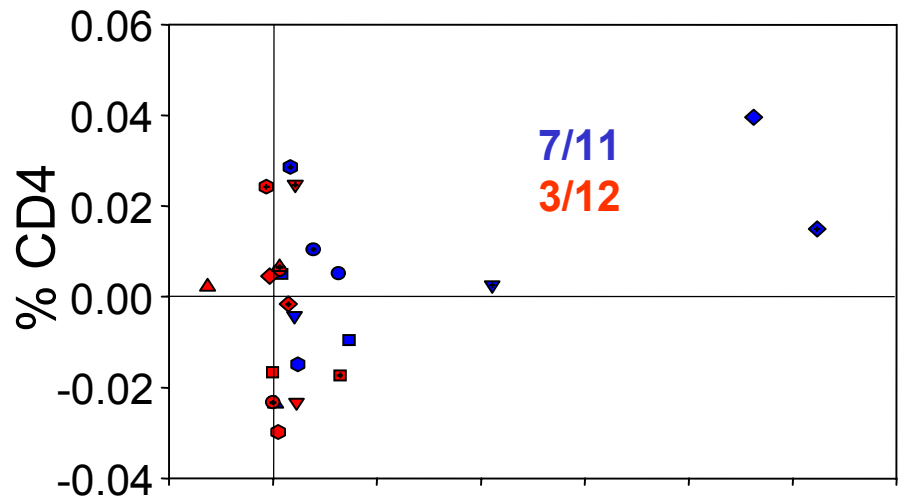
## Amplification of sjTREC containing plasmid



## Standard curve for sjTREC

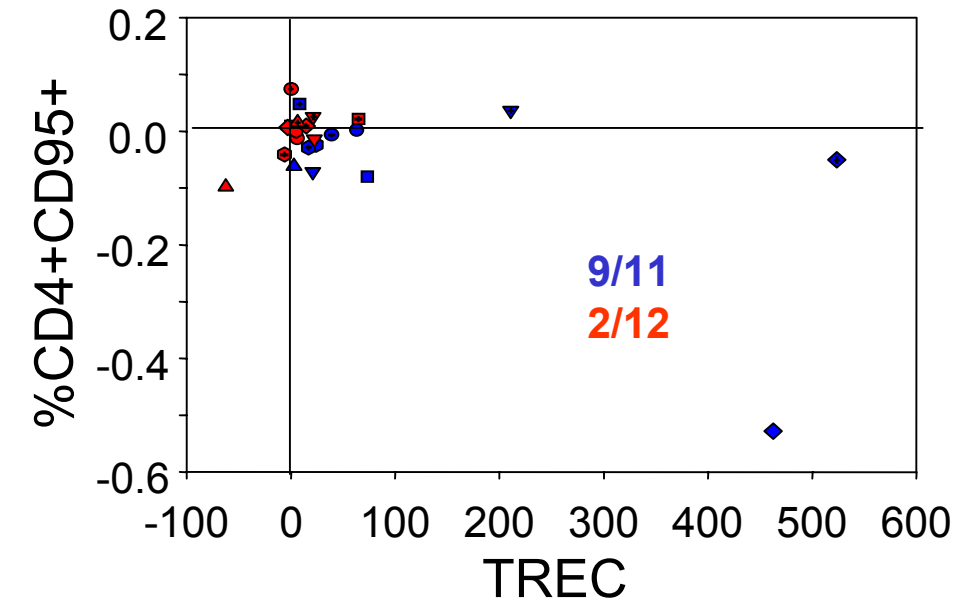
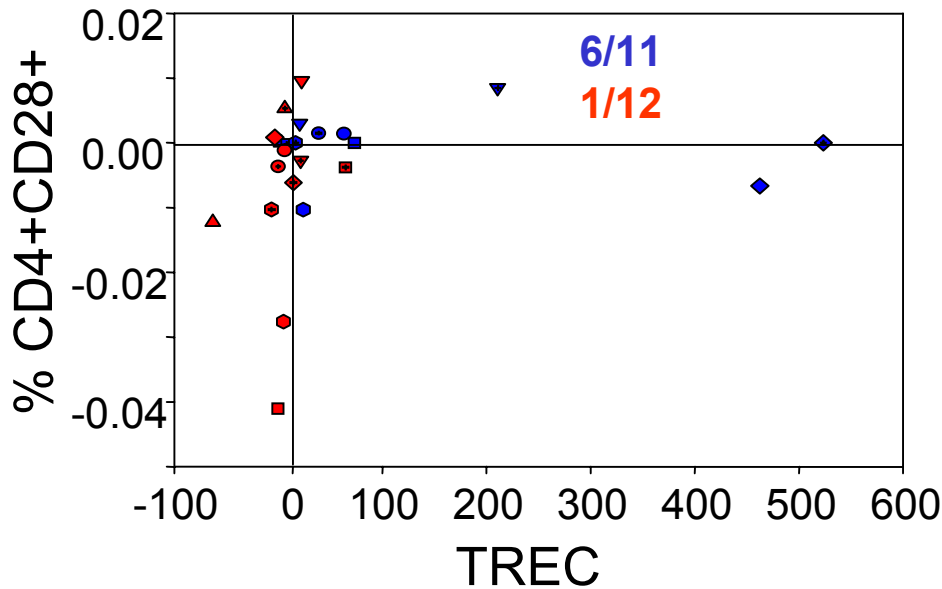
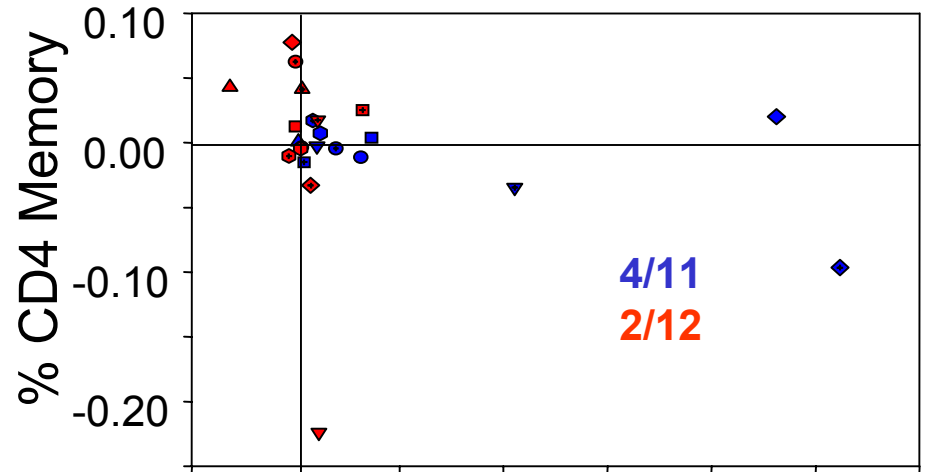
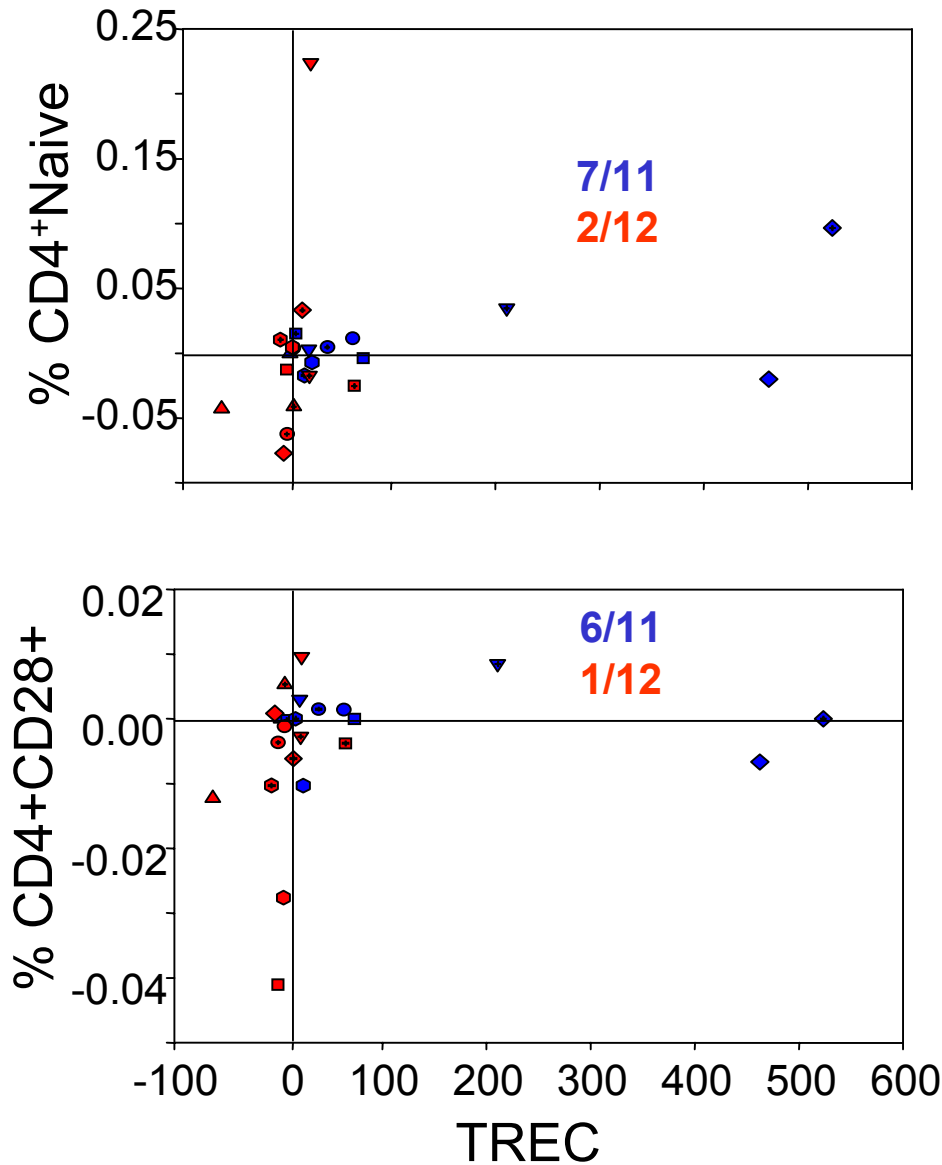


# Relationship between change in TREC and changes in % & absolute CD4 and CD8 T cells in Responders (blue) and Non-responders (red)



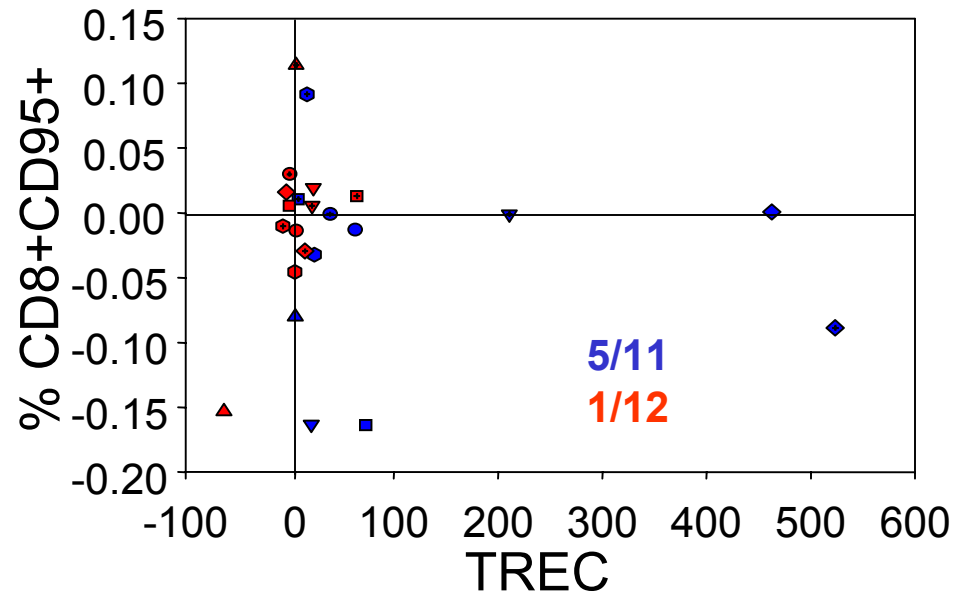
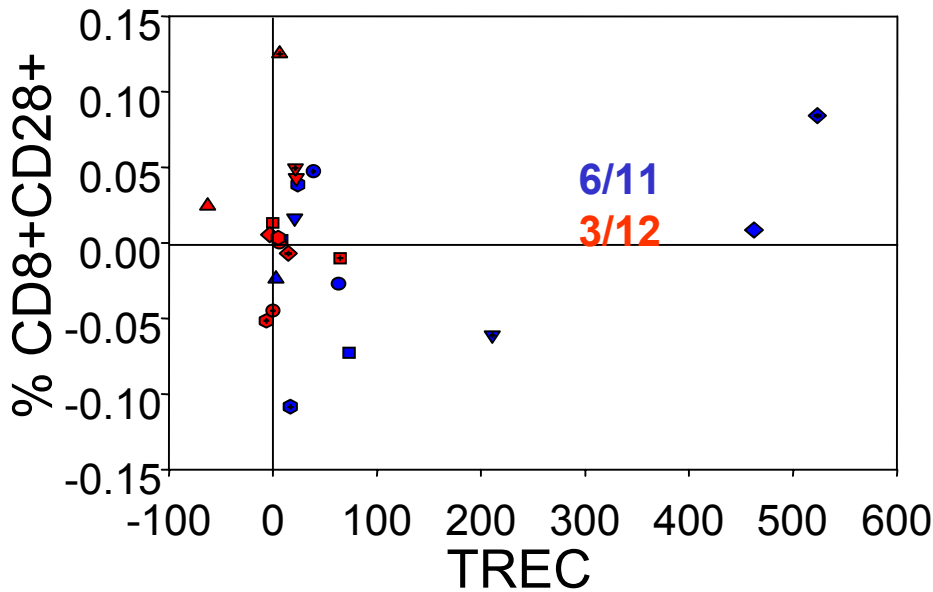
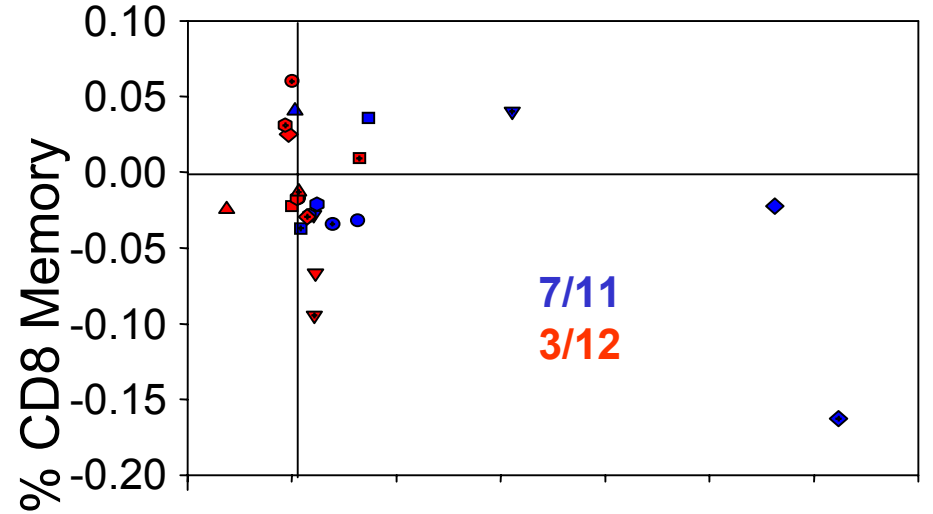
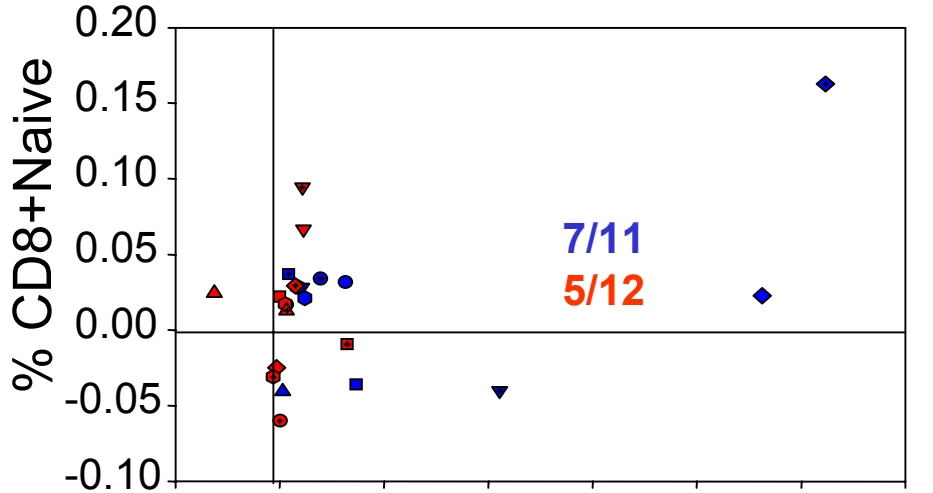
- Changes in TREC levels and changes in T cell subsets over the period of study were estimated by linear regression analysis. Each patient is represented individually as symbols.
- All the responders showed steady state level or positive change in TREC levels, whereas 7 out of 12 non-responders also showed steady state levels or positive change in TREC levels but these changes were significantly smaller in comparison to responders ( $p < 0.05$ )
- Changes in TREC levels correlated positively with changes in %CD4 T cells. Seven responders out of 11 have a positive slope for TREC levels and positive slope for % CD4 T cells. Same responders showed reciprocal association between change in TREC levels and changes in % and absolute CD8 T cells.

# Relationship between change in TREC levels and changes in CD4 T cell subsets in Responders (blue) and Non-responders (red)



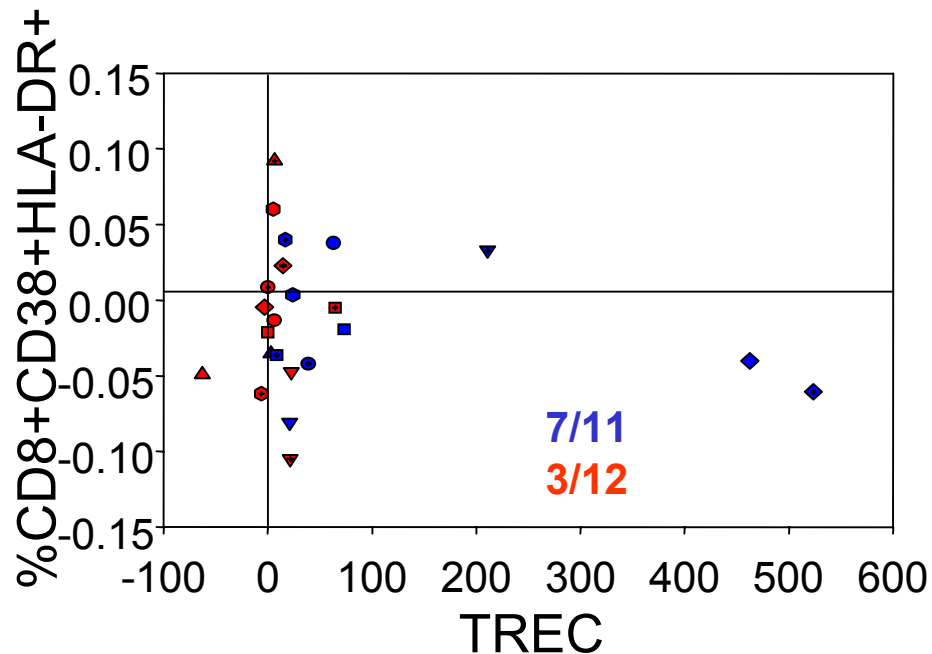
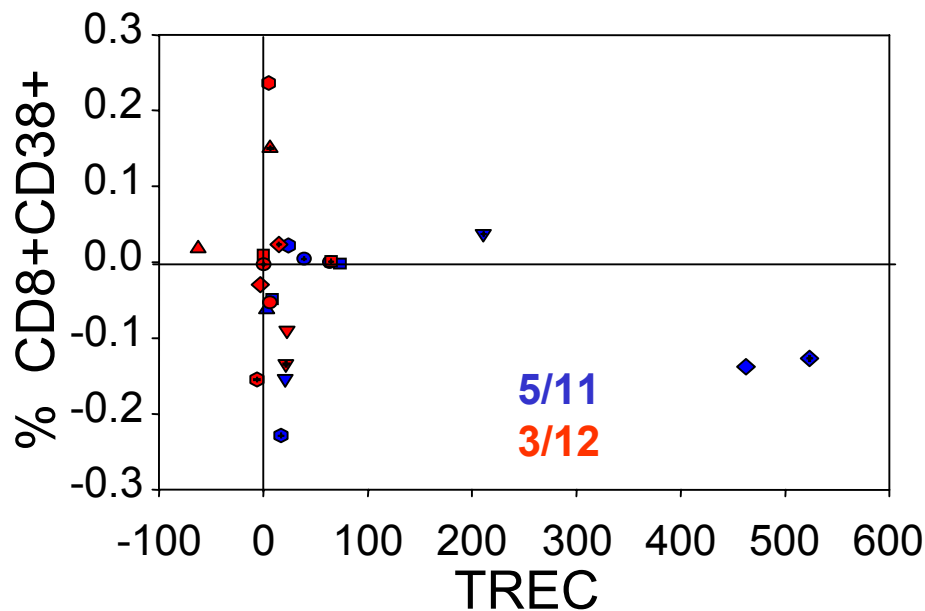
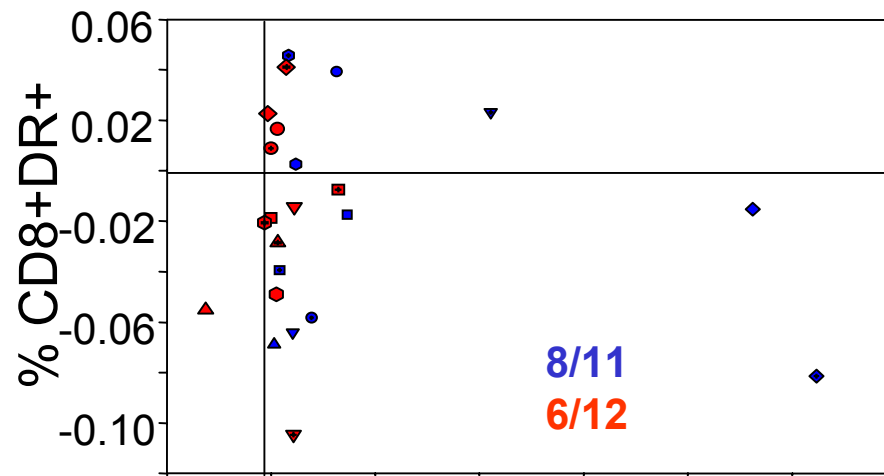
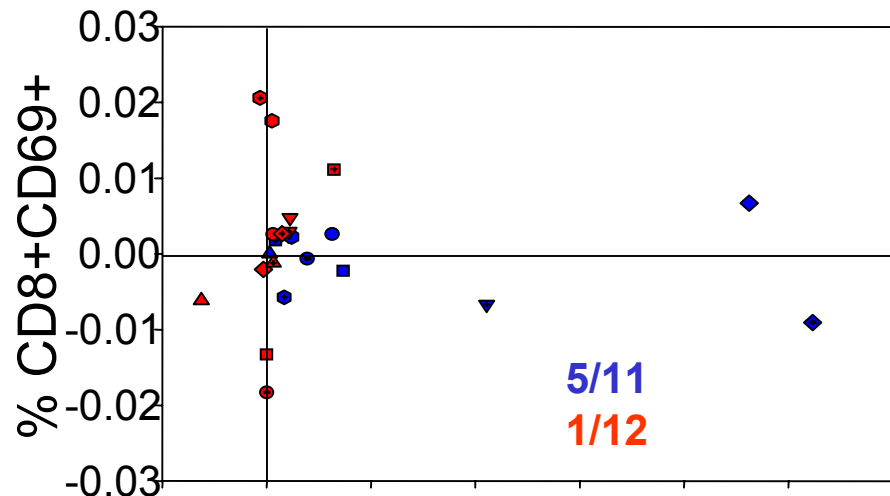
- Changes in TREC levels and changes in T cell subsets over the period of study were estimated by linear regression analysis. Each patient is represented individually as symbols.
- Slope of naïve CD4 T cells was positive for 7/11 responders and in 3/12 non-responders and reciprocal effect was seen with CD4 memory T cells.
- Slope of CD4+CD28+ T cells was positive for 9/11 responders and it was negative for 9/12 non-responders
- Slope of CD4+CD95+ T cells was negative for 9/11 responders and only for 2/12 non-responders
- Change in TREC levels of 7/11 responders and 6/11 responders showed a positive association with % of CD4+ naïve subset while in 9/11 responders a negative association was observed between change in TREC levels and the change in % of CD4+CD95+ cells
- In majority of the non-responders a negative slope in % CD4+ naïve T cells and % CD4+CD28+ was associated with a negative slope of TREC. The relationship between change in TREC and change in % of CD4+ memory cells was not appreciable

# Relationship between change in TREC and changes in CD8 T cell subsets in Responders (blue) and Non-responders (red)



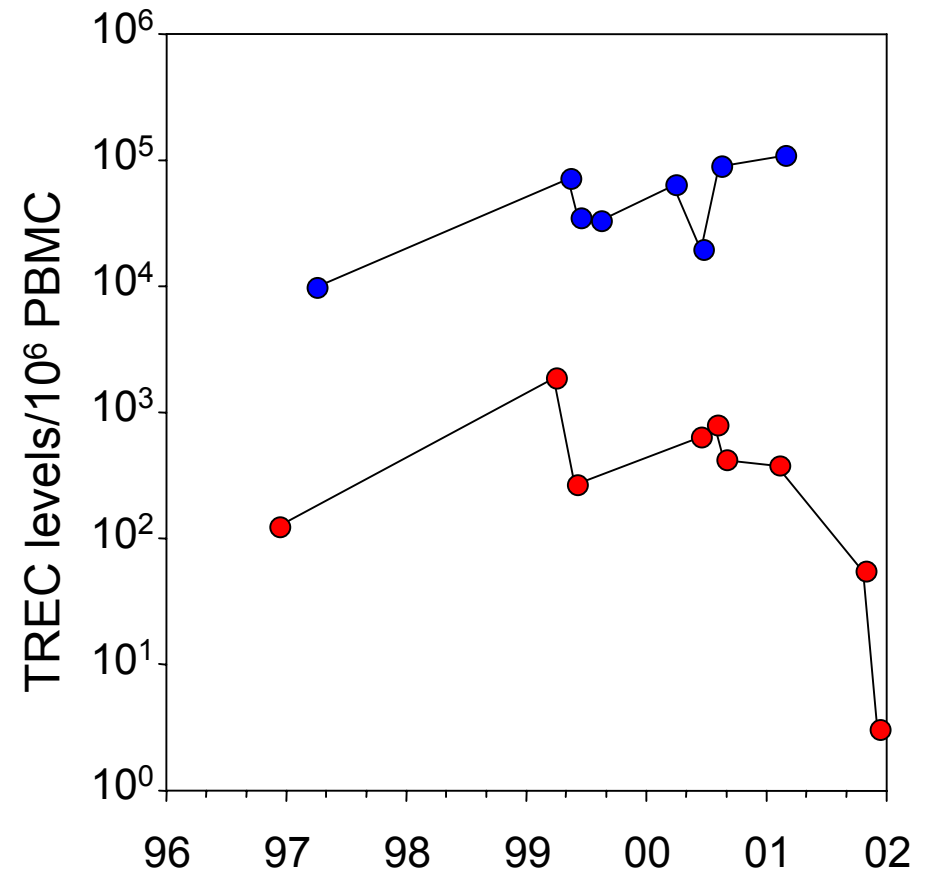
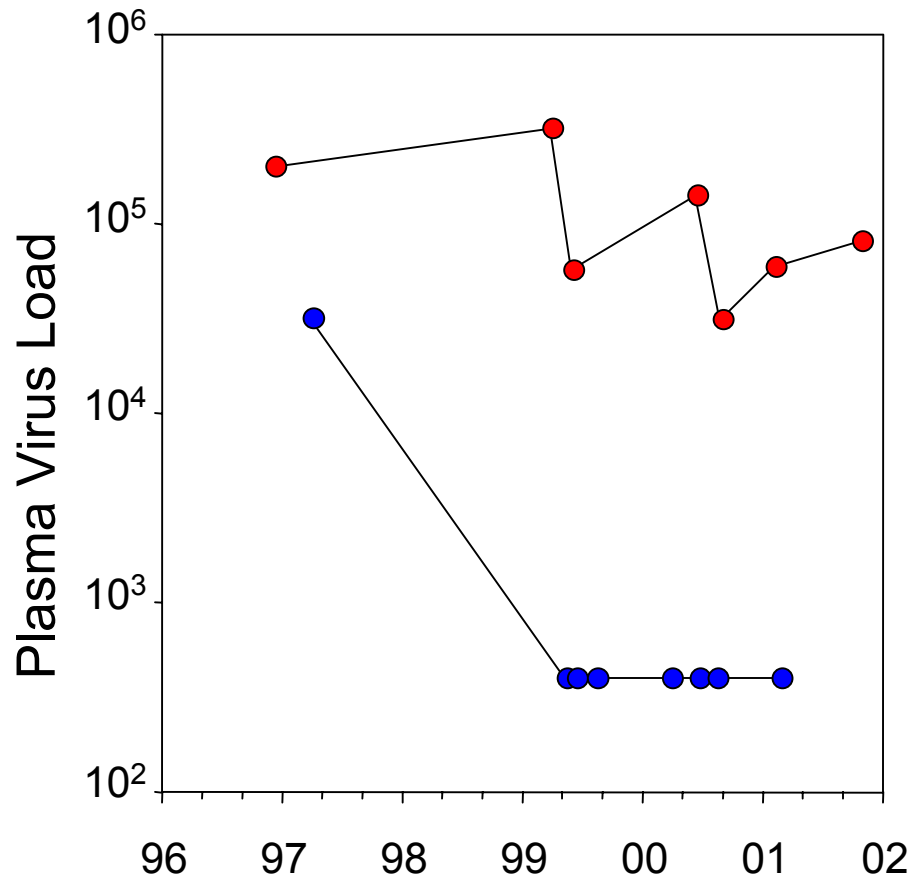
- Changes in TREC levels and changes in T cell subsets over the period of study were estimated by linear regression analysis. Each patient is represented individually as symbols.
- A positive slope for CD8+ naïve T cells was observed in 7/11 responders and 6/12 non-responders and this was reciprocal in the CD8+ memory T cells
- 8/12 non-responders had positive slope for % CD8+CD28+ and 7/12 non-responders showed a positive slope for % CD8+95+
- In the responder group 5/11 had a positive slope for % CD8+CD28+ which was positively associated with a positive slope in TREC levels.
- 5/11 responders had a decrease in the slope for % CD8+CD95+ with an increase in the slope for TREC levels
- The positive association observed between the slope for CD8+ naïve T cells and slope of TREC in 7/11 responders had a reciprocal relationship between slope in CD8+ memory T cells and slope of TREC levels

# Relationship between change in TREC and changes in CD8 T cell activation markers in Responders (blue) and Non-responders (red)

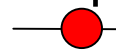


- Changes in TREC levels and changes in T cell subsets over the period of study were estimated by linear regression analysis. Each patient is represented individually as symbols.
- 5/11 responders had a negative slope for % CD8+CD69+ and % CD8+CD38+ markers whereas, 8/11 and 7/11 responders had a negative slope for the activation markers % CD8+DR+ and % CD8+CD38+ HLA-DR+ respectively
- 7/12 and 6/12 non-responders had positive slope for % CD8+CD69+ and % CD8+CD38+ respectively. 8/12 non-responders showed a negative slope for % CD8+DR+ and % CD8+ HLA-DR+
- 5/12 non-responders and 6/11 responders expressed a positive association between slope of % CD8+CD69+ and TREC levels. Similar association was observed between slope of % CD8+CD38+ and slope of TRECs
- Slope of % CD8+DR+ for 8/11 responders and 6/12 non-responders negatively correlated with the slope of TREC
- In 7/11 responders a decrease in the slope of CD8+CD38+HLA-DR+ was associated with an increase in the slope of TREC

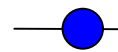
# Plasma virus load and TREC levels in Responder (blue) and Non-responder (Red) siblings over 2 year period



Sample Date



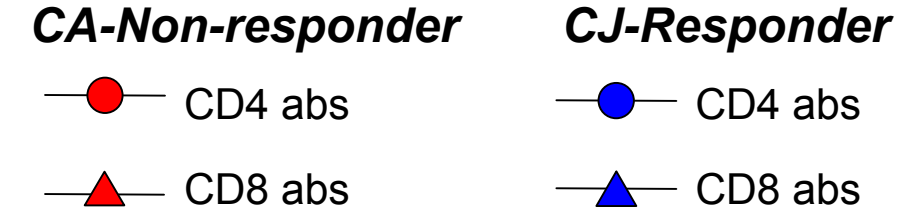
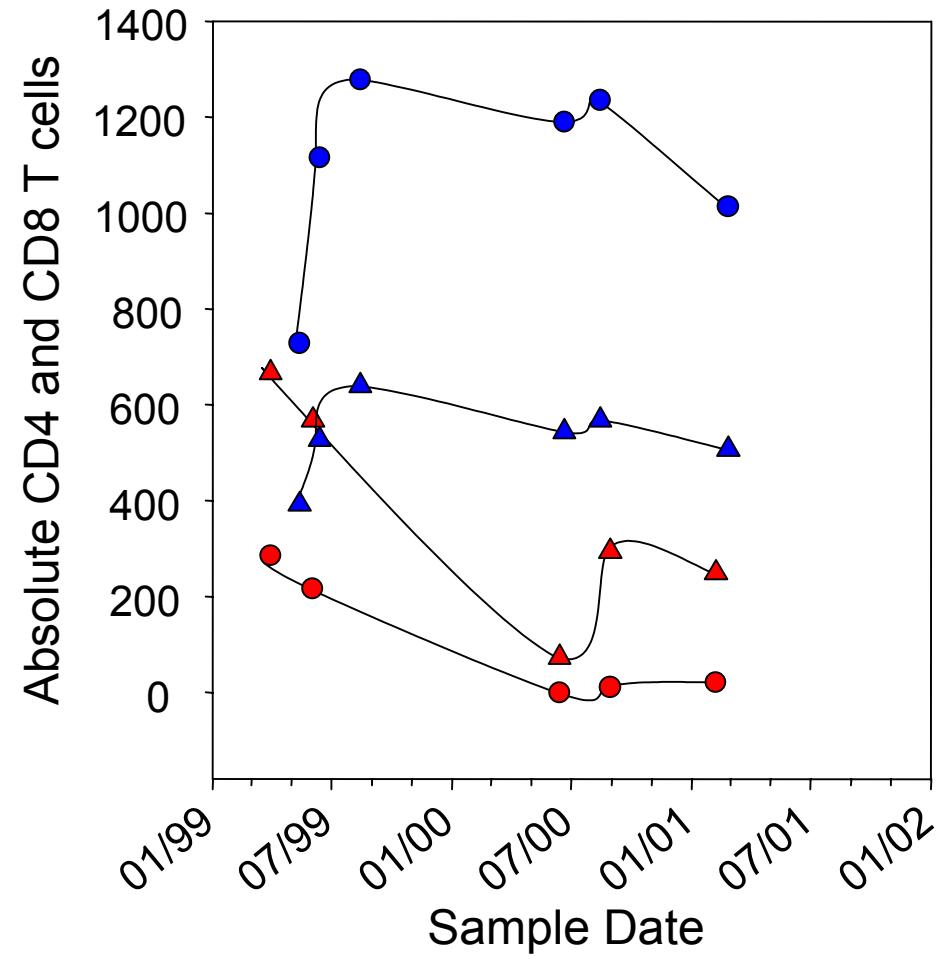
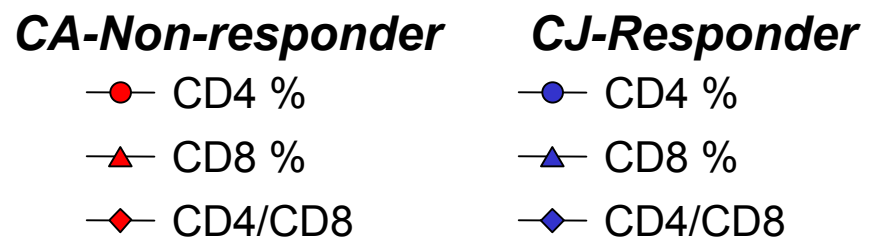
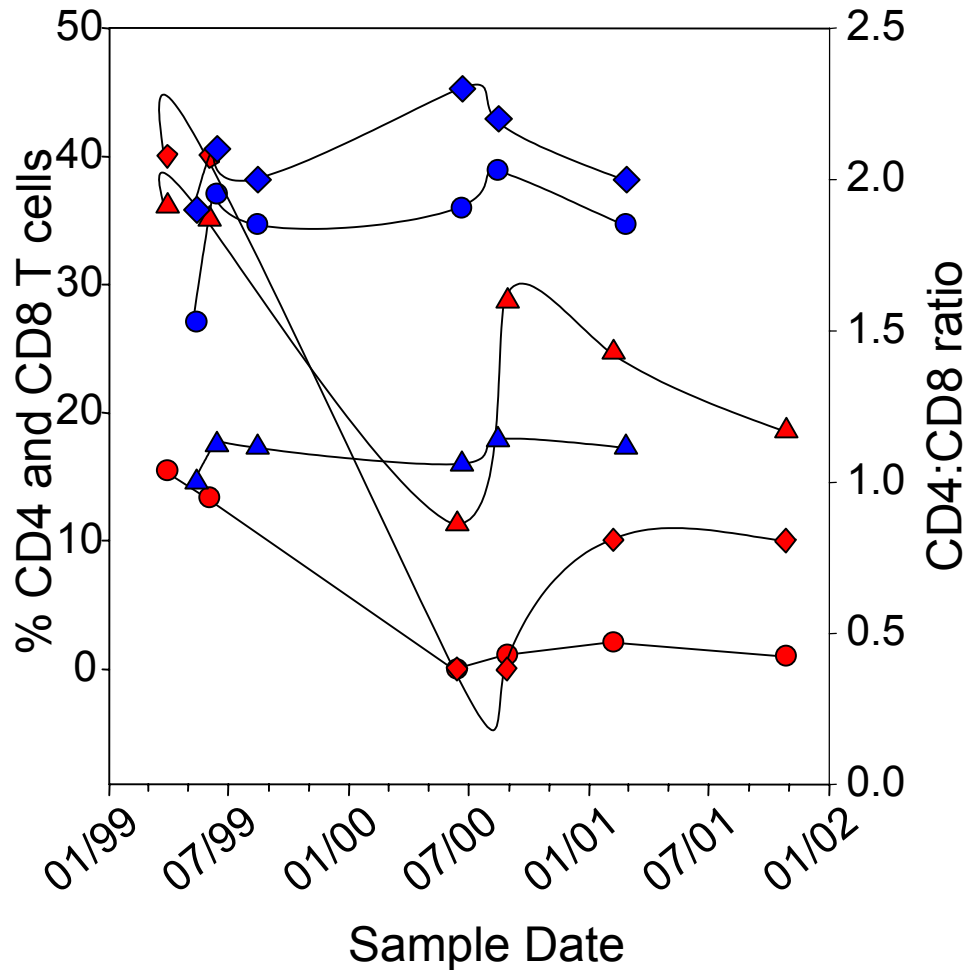
**CA-Non-responder**



**CJ-Responder**

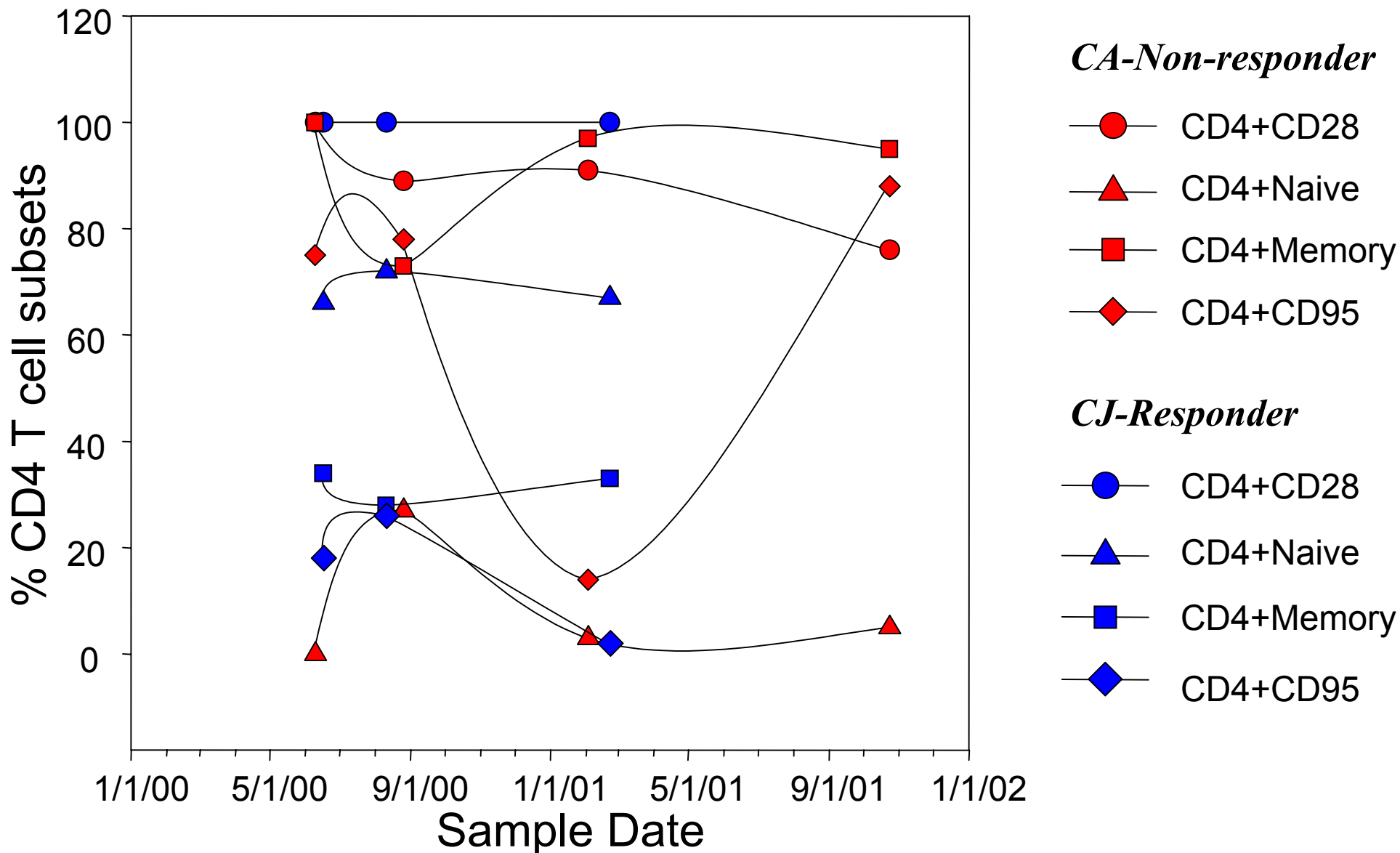
- TREC levels decreased over time in non-responder
- TREC levels maintained at steady state level in responder over time

# Percent and Absolute CD4 and CD8 T cell levels in Responder (blue) and Non-responder (Red) siblings over 2 year period



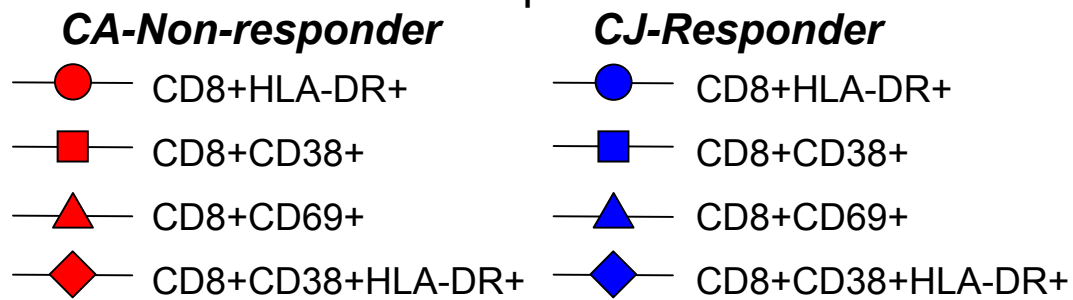
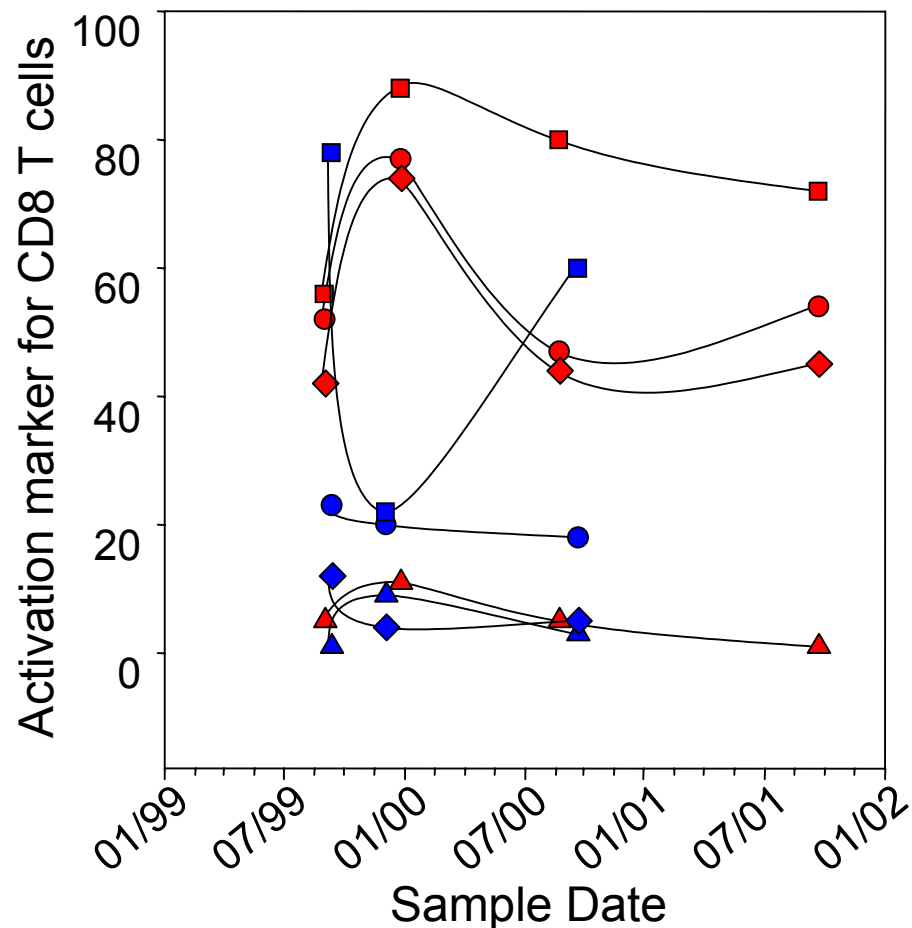
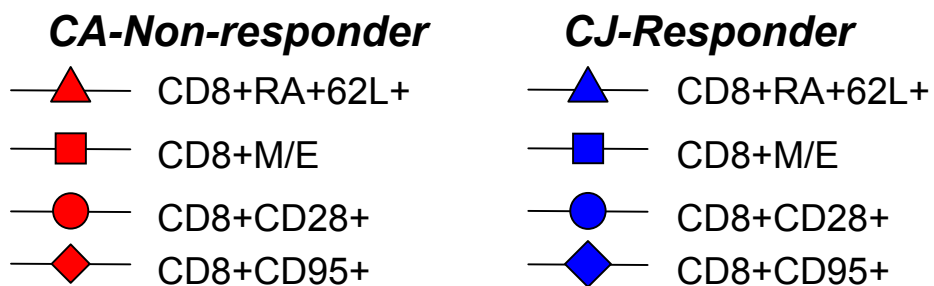
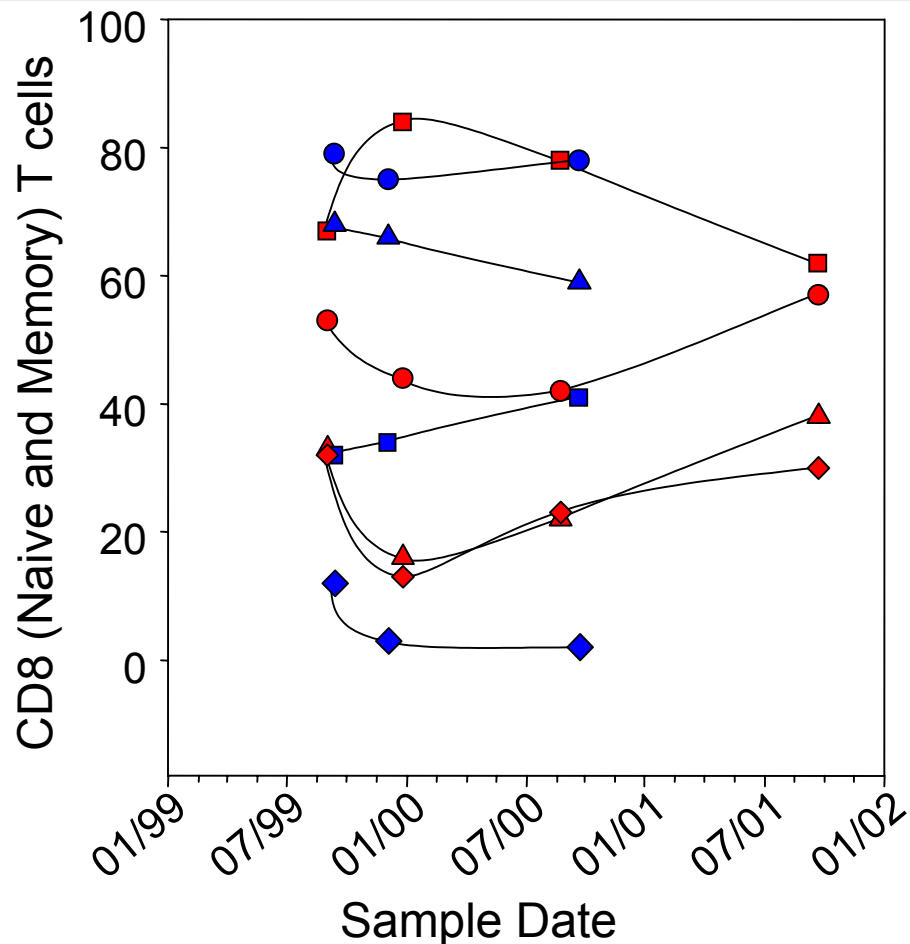
- Percent and absolute CD4 T cells were decreased over time in the non-responder, whereas they increased initially (with control of virus load) and was maintained at steady state level over time in the responder
- Percent and absolute CD8 T cells were decreased over time in the non-responder, whereas they increased initially (with control of virus load) and was maintained at steady state over time in the responder
- Ratio of CD4:CD8 T cells was decreased over time in the non-responder , whereas it increased initially (with control of virus load) and was maintained at steady state level over time in the responder

# Percent of CD4 T cell subsets in Responder (blue) and Non-responder (Red) siblings over 2 year period



- In the CD4+ subsets CD4+CD28+ and CD4+RA+62L+ levels were higher in the responder while the non-responder has higher levels of memory population and the activation marker CD95+
- CD4 T cells expressing naïve, memory and CD28 phenotypic markers were decreased over time in non-responder, whereas the CD4 T cells expressing these phenotypic markers were maintained at steady state in responder over study period.
- CD4 T cells expressing CD95 a marker of activation were increased over time in non-responder, whereas the CD4 T cells expression this phenotypic markers were maintained at steady state in responder over study period.

# Percent of CD8 T cell subsets in Responder (blue) and Non-responder (Red) siblings over 2 year period



- The responder has comparatively higher levels of CD8+CD28+ and CD8+RA+62L+ than the non-responder who had increase in the levels of CD8+ memory T cells and activation marker CD95+
- The levels of other activation markers, CD8+CD38+ HLA-DR+, CD8+HLA-DR+ and CD8+CD38+ were higher in the non-responder than the responder
- CD8 T cells expressing naïve, memory and CD28 phenotypic markers were decreased over time in non-responder, whereas the CD8 T cells expressing these phenotypic markers were maintained at steady state in responder over study period.
- CD8 T cells expressing a marker of activation CD95, CD69, CD38, HLA-DR, CD38 & HLA-DR together were increased over time in non-responder, whereas the CD8 T cells expressing these phenotypic markers were maintained at steady state in responder over study period.

# Summary

- In the entire cohort changes in TREC levels were correlated positively with changes in % CD4 T cells and naïve CD4 cells negatively with changes in % activated CD8 T cells
- TREC levels were high and increased gradually or maintained steady state over study period in all responders
- TREC levels were low but were maintained at steady state in majority of non-responders
- In responders positive changes in TREC levels was associated
  - positively with changes in % CD4 T cells, naïve CD4 T cells and naïve CD8 T cells
  - negatively with activated CD8 T cells
- In non-responders no such correlation was observed with changes in TREC levels

## Summary-contd...

- Majority of the responders showed a positive association between change in TREC levels and change in % CD4 T cells. The same responders showed reciprocal association between change in TREC levels and changes in % and absolute CD8 T cells
- Change in TREC levels of responders showed a positive association with change in % of CD4+ naïve subset and change in % of CD4+CD28+ cells
- A negative association was observed between change in TREC levels and the change in % of CD4+CD95+ in 9/11 responders
- In majority of the non-responders a negative slope in % CD4+ naïve T cells and % CD4+CD28+ was associated with a negative slope of TREC.
- Although most non-responders showed a negative slope for activation markers of CD8+ subset, they did not have a positive slope for TREC. But in responders a negative slope of activation markers was associated with a positive slope of TREC
- The relationship between change in TREC levels and change in naïve subsets of both CD4 and CD8 T cells were reciprocal in the respective memory subsets

# Conclusions

- The steady state levels of TRECs in responders and non-responder suggest active thymic function in both responders and non-responders
- The positive association of TREC levels with phenotypic markers of naïve T cells and its negative association with activation markers of T cells suggest that TREC levels can indicate the stabilization of peripheral compartment
- The increase in TREC levels or maintenance of steady state with control of plasma HIV RNA copies in responders and the association of TRECs with different cell markers of peripheral T cell compartment suggest the influence of peripheral changes on quantitation of TREC levels
- Additional studies are needed to validate the utility of TRECs as definitive markers of recent thymic emigrant cells