



# Potential Pre-treatment Interruption (TI) Immunological Predictors for CD4 Count Change Off Therapy in Chronically HIV-infected Subjects Having Various Pre-TI Viral Loads (VL)



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## Abstract

**Background:** To analyze whether pre-TI proliferative capacity and cell surface markers predicted CD4 count change during TI in chronically HIV-infected subjects.

**Methods:** HIV-infected subjects (n=27) with various VL outcomes on treatment with combination antiretroviral therapy (ART) who underwent a TI lasting more than 2 months were examined in this retrospective study. Peripheral blood mononuclear cells (PBMCs) from a time point (designated as the baseline time point) within 6 months before the TI were tested using the CFSE dilution assay to detect proliferation to a Gag p55 peptide pool, CMV lysate, staphylococcus enterotoxin B, and anti-CD3. Percent and median fluorescent intensity (MFI) of CD28 and CD57 expression on CD4+ and CD8+ T-cells were measured.

**Results:** The CD4 count change from baseline to nadir during TI was a median (interquartile range [IQR]) of -100cells/mm<sup>3</sup> (-25,-154). CD4+ and CD8+ T-cells proliferation to CMV lysate, and per cent CD4+CD57+ T-cells correlated negatively with CD4 decline during TI (r = -0.575, r = -0.397, and r = -0.493; p<0.05 for all analyses; Spearman correlation). Upon dividing the group dichotomously based on the median change in CD4 count into good and poor CD4 responders, good responders had a median (range) CD4 change of -26 cells/mm<sup>3</sup> (-17,-83) and poor responders a median CD4 change of -154 cells/mm<sup>3</sup> (-117,-313). The good responders had less CD4+ and CD8+ proliferative potential to CMV lysate and a lower percentage and MFI of CD4+CD57+ T-cells versus poor responders (median 1.19 vs. 8.79%, 0.54 vs. 6.86%, 2.05 vs. 4.43%, and 56.74 vs. 76.21 MFI, p<0.05 for all analyses; Mann-Whitney test).

**Conclusions:** In treated HIV-infected subjects in the chronic phase of infection having a spectrum of pre-TI VL before undergoing TI, the lack of proliferation to CMV lysate and lower expression of CD57 on CD4+ T-cells at baseline were associated with a smaller drop in CD4 count during TI. Pre-TI immune parameters may have predictive value for CD4 count decline during TI.

## Introduction

•TI is a strategy to reduce toxicity, costs, and time on ART

•Major clinical concern regarding TI:  
•Fall of CD4 count during TI could result in opportunistic infections  
•Hard to restore pre-TI CD4 count after re-initiation of HAART

•An estimated 19% of patients who initiate ART in chronic phase of HIV infection interrupts treatment

•It would be useful to be able to predict who will experience a clinically important fall in CD4 count during TI for patients with various baseline VL

## Methods: Flow Cytometry-based Assay

1. 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (CFSE) dilution assay



•CFSE spontaneously and irreversibly couples to both intracellular and cell surface proteins by reactions with lysine side chains and other available amine groups  
•Stimuli for CFSE: Gag p55 peptide pool, CMV viral lysate, SEB, and anti-CD3

2. Immunophenotyping

•PBMCs was analyzed for the following cell populations:  
-CD28+CD8+, CD28+CD4+, CD57+CD8+, CD57+CD4+

•**CD28:** co-stimulatory molecule, loss of expression associated with disease progression

•**CD57:** associated with clonal exhaustion

## Study Population

n=27  
Inclusion criteria:  
•Availability of frozen peripheral blood mononuclear cell (PBMC) samples from time points before TI  
•Must have received at least a 3-drug HAART regimen for a minimum of 6 months prior to TI  
•Duration of the TI must be greater than 2 months

**Patients with chronic viral suppression:**  
n=14  
•Must have viral suppression while on the combination as defined by a plasma HIV RNA < 500 copies/mL measured on 2 occasions at least 4 weeks apart  
•Median baseline CD4 count: 412 cells/μl IQR (340, 695)  
•Median baseline VL: <50 HIV RNA copies/ml IQR (49, 49)

**Patients with virologic failure:**  
n=13  
•Must have virologic failure while on the combination as defined by a plasma HIV RNA > 5000 copies/mL measured on 2 occasions at least 4 weeks apart  
•Median baseline CD4 count: 171 cells/μl IQR (56, 264)  
•Median baseline VL: 24 719 HIV RNA copies/ml IQR(12 581, 161 918)

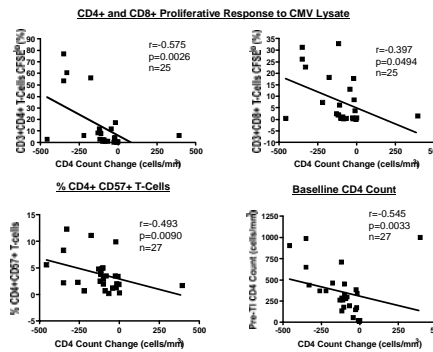
## Results:

**Table 1: Correlation between baseline immunological parameters and CD4 count change from baseline to nadir during TI**

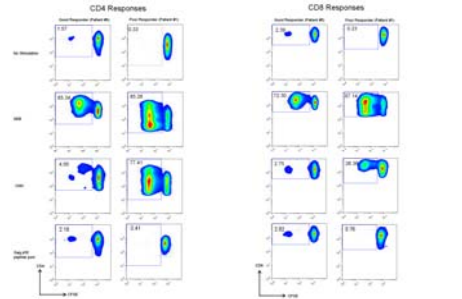
| T-cell compartment  | Proliferative Stimulus/ Phenotypic Markers Expression* | Correlation with CD4 count change from baseline to nadir during TI** |
|---------------------|--|--|
| CD4                 | anti-CD3   | 0.198 (p=0.378)  |
|                     | SEB  | 0.290 (p=0.222)  |
|                     | CMV  | -0.575 (p=0.026)***  |
|                     | Gag p55 peptide pool                                   | -0.144 (p=0.473)   |
|                     | %CD57+   | -0.493 (p=0.009)***  |
|                     | CD57+ MFI  | -0.338 (p=0.064)   |
| CD8                 | %CD28  | -0.232 (p=0.244)   |
|                     | CD28+ MFI  | -0.146 (p=0.467)   |
|                     | anti-CD3   | -0.322 (p=0.144)   |
|                     | SEB  | 0.0609 (p=0.777)   |
|                     | CMV  | -0.397 (p=0.0494)***   |
|                     | Gag p55 peptide pool                                   | -0.205 (p=0.306)   |
| Baseline CD4+ count | %CD57+   | 0.0312 (p=0.877)   |
|                     | CD57+ MFI  | 0.197 (p=0.326)  |
|                     | %CD28  | 0.0434 (p=0.839)   |
|                     | CD28+ MFI  | -0.205 (p=0.306)   |
| Baseline CD4+ count |  | -0.545 (p=0.0033)***   |
| Baseline viral load |  | 0.377 (p=0.0525)   |

\*CMV= cytomegalovirus lysate; SEB=streptococcus enterotoxin B; MFI=median fluorescence intensity  
\*\*nonparametric Spearman correlation coefficient (p-value)  
\*\*\*p<0.05

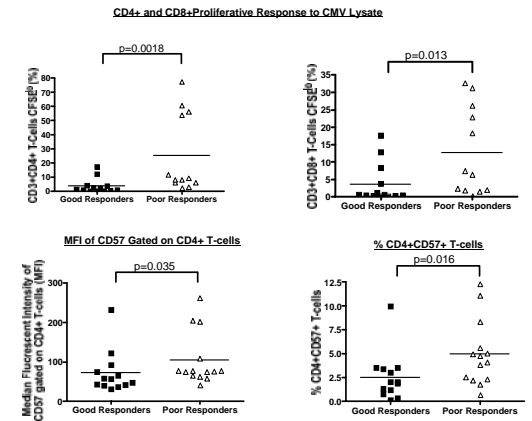
## Negative Correlation between baseline immunological variables and CD4 count change from baseline to nadir during TI



## Representative FACS contour plot showing CD4+ and CD8+ proliferative responses for a Good and Poor CD4 Responder



## Decreased CD4+ and CD8+ Proliferative Responses to CMV Lysate and CD4+CD57+ Expression for Good vs. Poor Responders



## Discussions

•95% of HIV-infected patients are also infected with CMV

•Presence of endogenous CMV could activate and expand CD4+ T-cells, rendering them permissive for HIV replication

•CD57 is a senescence marker and higher expression on CD4+ T-cells in poor responders defines a population of CD4+ T-cells with replicative exhaustion from chronic HIV antigen stimulation

## Conclusions

•In treated HIV-infected subjects in the chronic phase of infection having a spectrum of pre-TI VL before undergoing TI, the lack of proliferation to CMV lysate and lower expression of CD57 on CD4+ T-cells at baseline were associated with a smaller drop in CD4 count during TI.

•Pre-TI immune parameters may have predictive value for CD4 count decline during TI

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