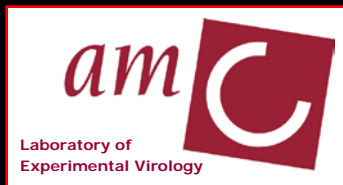


**Cell-associated HIV-1 unspliced to multiply spliced RNA ratio at 12 weeks ART  
correlates with markers of immune activation and apoptosis  
and predicts the CD4<sup>+</sup> T-cell count at 96 weeks ART**

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## Background

Combination antiretroviral therapy (ART) suppresses HIV replication and improves immune function

Some (10-20%) of HIV<sup>+</sup> individuals receiving ART fail to increase CD4<sup>+</sup> T-cell counts sufficiently: associated with increased morbidity and mortality

Clinically, a biomarker predicting immunological response would be useful for early identification of patients at risk for immunological failure

For better understanding of HIV-1 pathogenesis and improved design of curative strategies, it is important to determine whether viral persistence is associated with host biomarkers of immune dysfunction

Therefore, we were interested to study early predictors of immunological response to ART

## Study design and methods

We studied longitudinal samples from 28 Amsterdam Cohort Study patients starting ART with baseline CD4<sup>+</sup> counts <350 cells/mm<sup>3</sup>, baseline pVL >1000 copies/ml, and virological suppression in plasma by 48 weeks of ART

Samples were taken at 0, 12, 24, 48, and 96 weeks of ART

Virological markers (total and episomal [2-LTR circles] HIV-1 DNA, unspliced and multiply spliced [total and tat/rev] cell-associated HIV-1 RNA) – by seminested qPCR

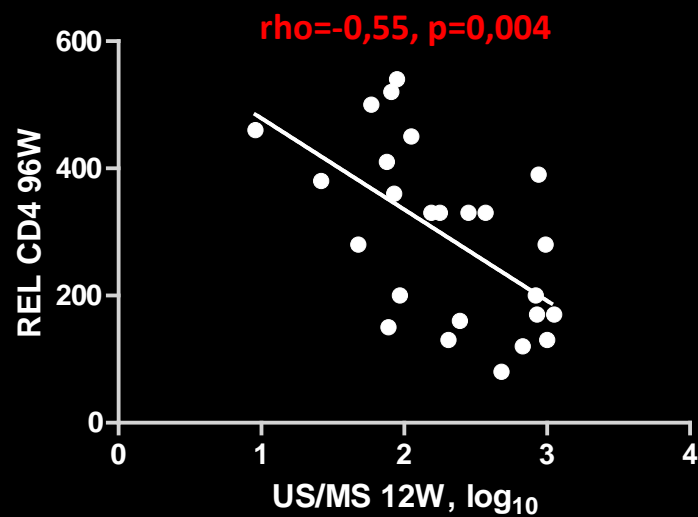
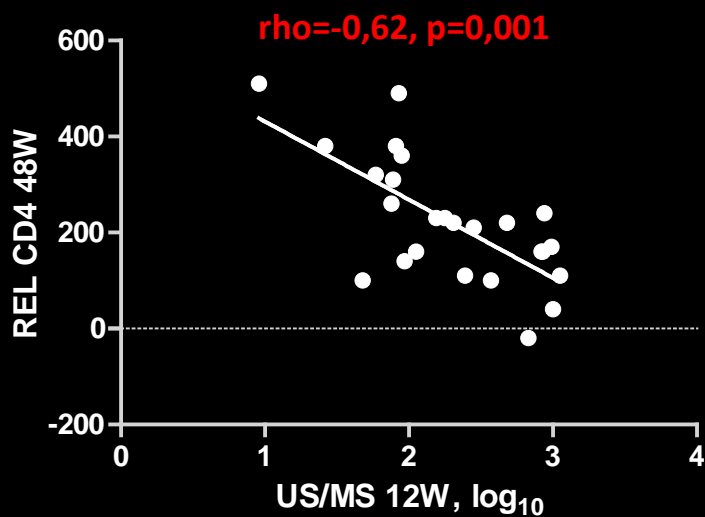
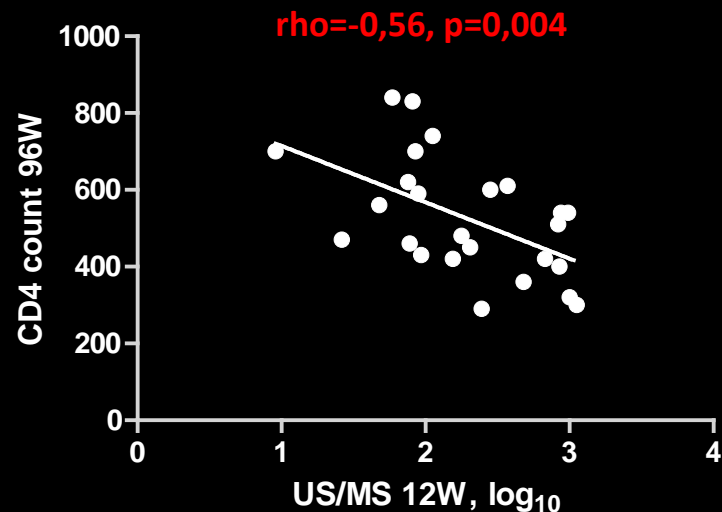
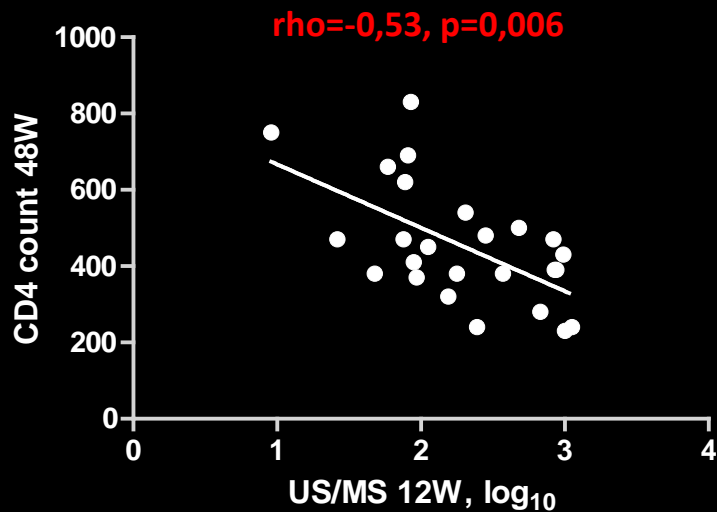
Detectability 100% for total DNA, 98% for US RNA, and 76% for MS RNA

Immunological markers (CD4<sup>+</sup> and CD8<sup>+</sup> T-cell activation, proliferation, senescence, apoptosis, exhaustion, thymic migration, Treg/Th17, CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets [naïve, central memory, effector memory, transitional memory])

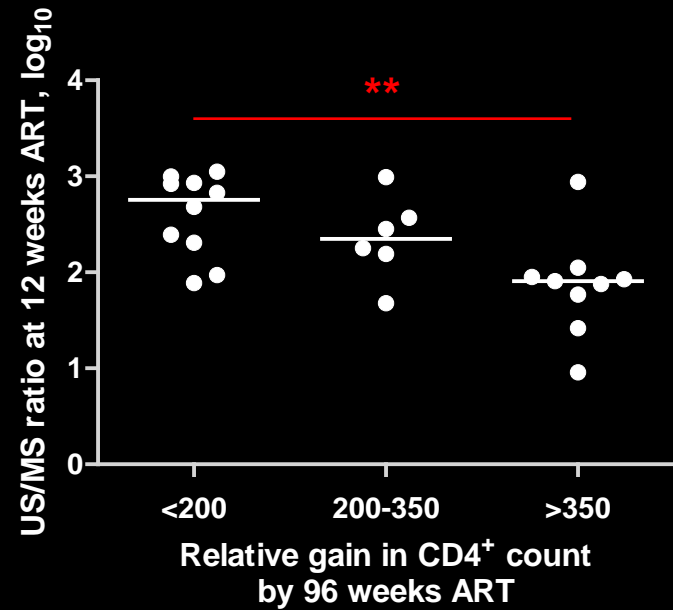
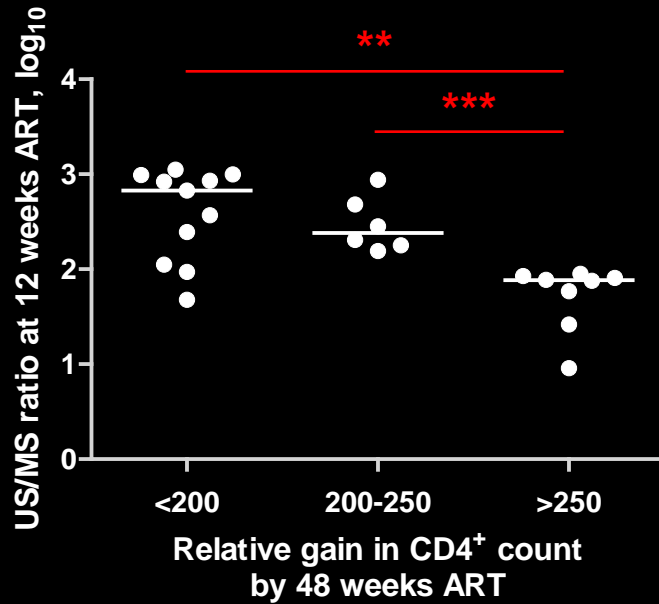
Microbial translocation markers

Genetic markers

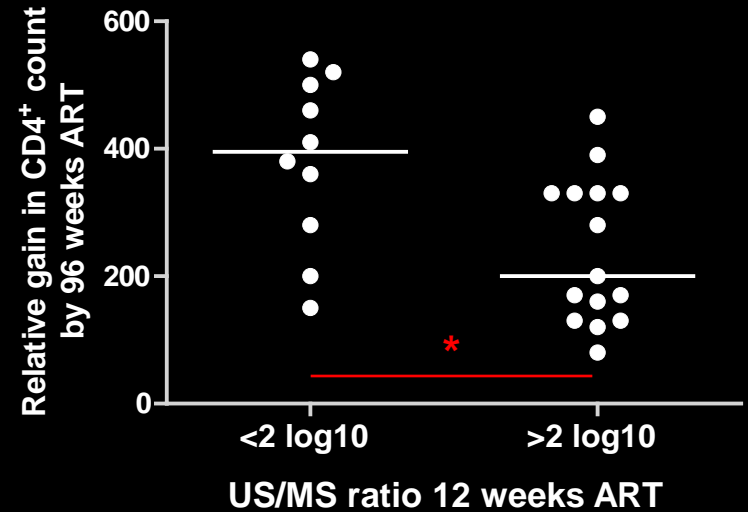
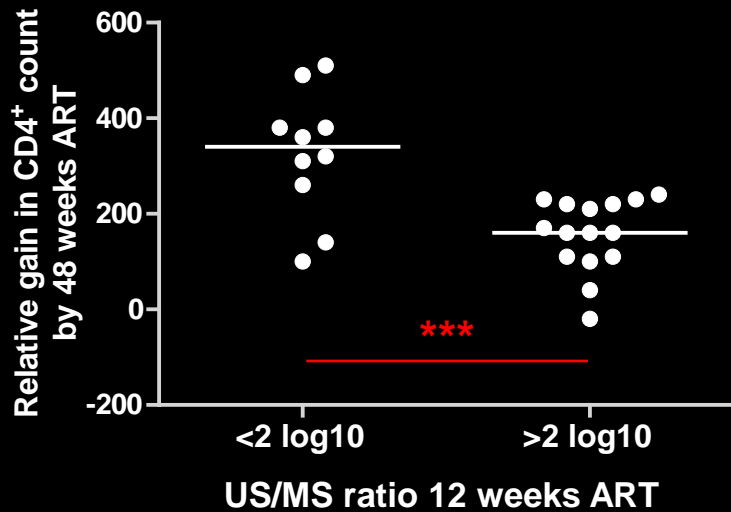
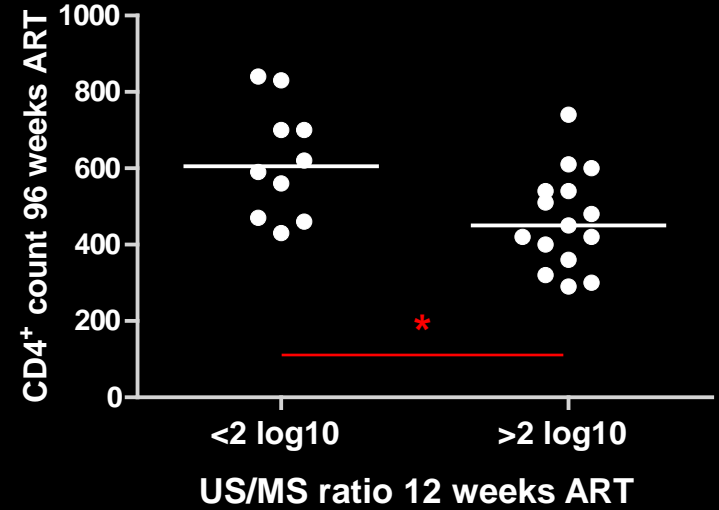
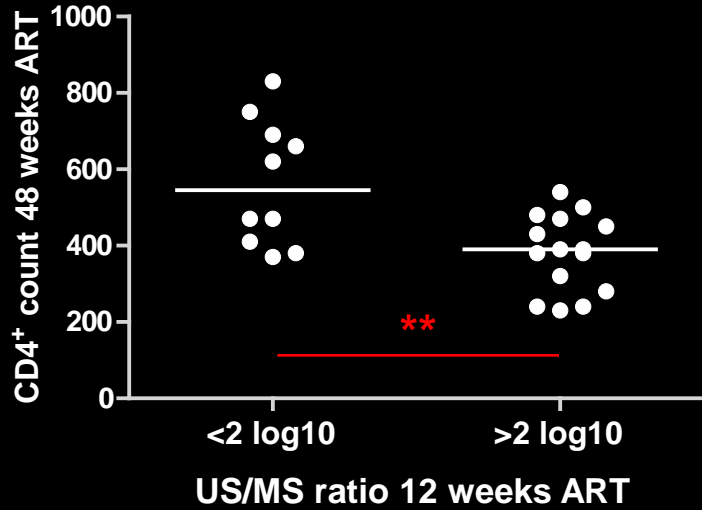
# Cell-associated HIV-1 unspliced to multiply spliced RNA ratio at 12 weeks ART inversely correlated with the absolute and relative CD4<sup>+</sup> T-cell count at 48 and 96 weeks



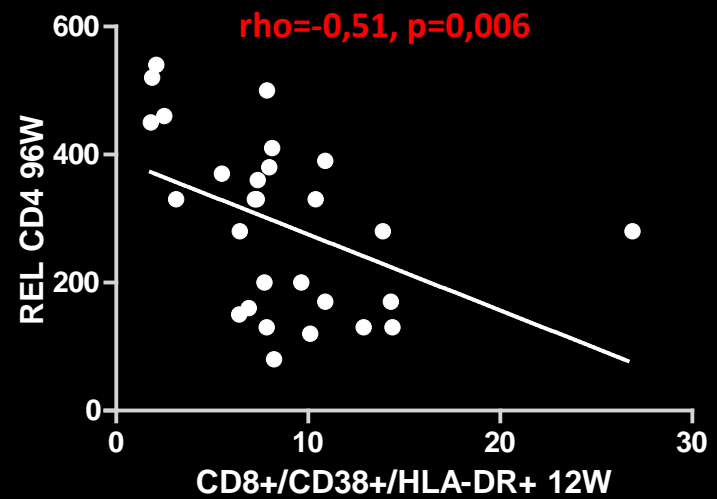
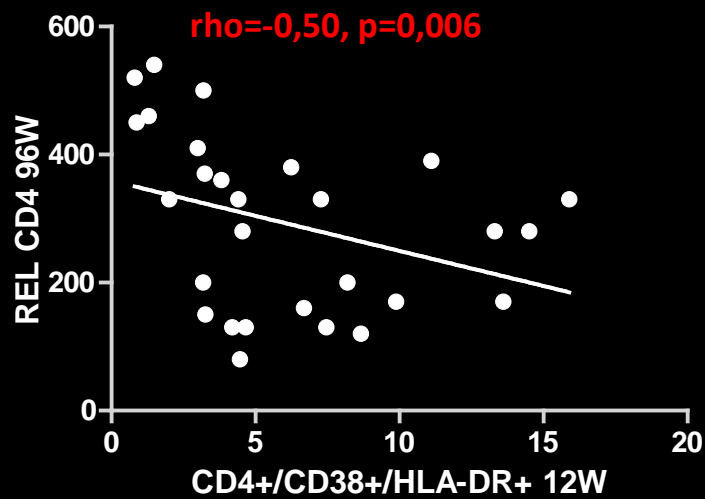
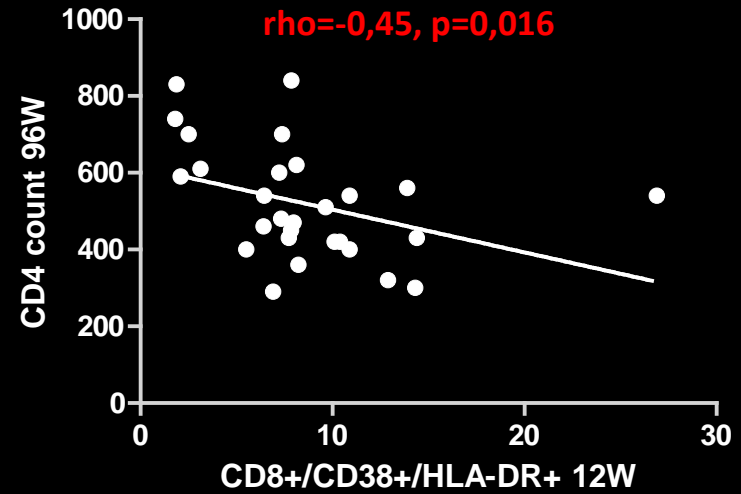
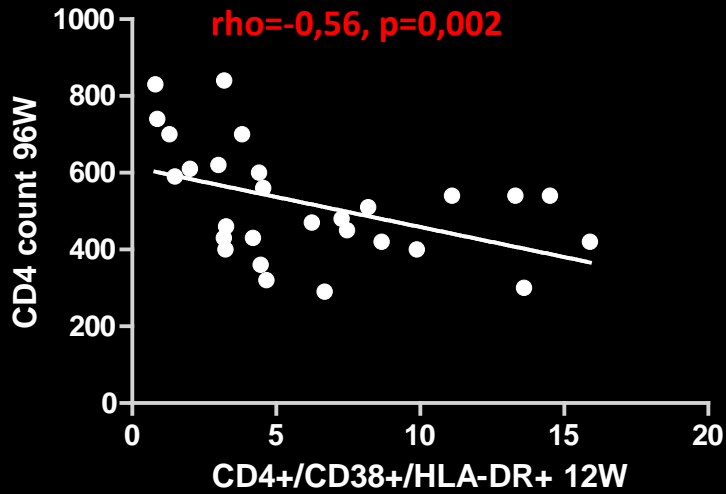
Patients with better immunological response at 48 and 96 weeks ART had lower cell-associated HIV-1 unspliced to multiply spliced RNA ratios at 12 weeks



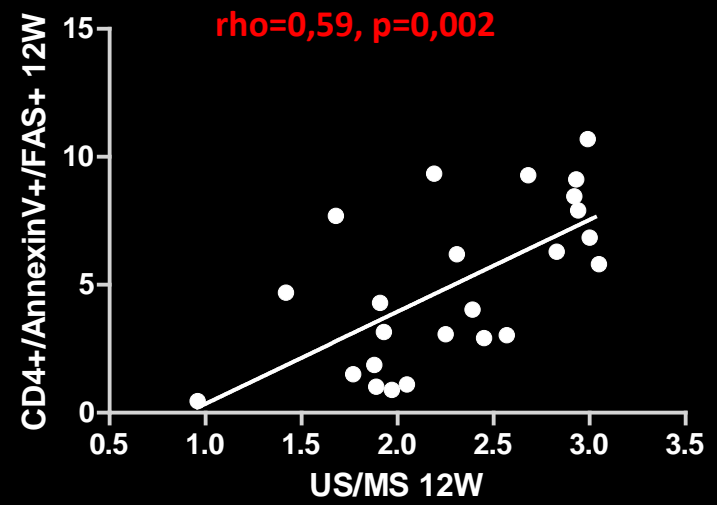
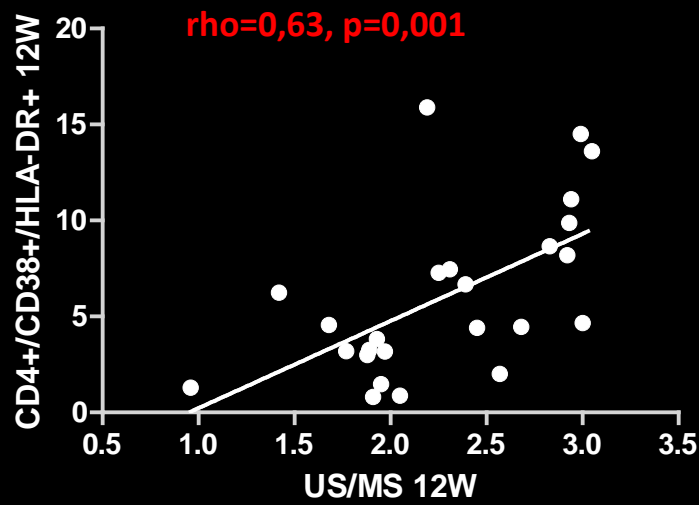
# Cell-associated HIV-1 unspliced to multiply spliced RNA ratio at 12 weeks ART was predictive of the relative CD4<sup>+</sup> T-cell count gain by 48 and 96 weeks



# CD4<sup>+</sup> and CD8<sup>+</sup> T-cell activation markers at 12 weeks ART inversely correlated with the absolute and relative CD4<sup>+</sup> T-cell count at 96 weeks



# Cell-associated HIV-1 US/MS RNA ratio at 12 weeks ART positively correlated with markers of CD4<sup>+</sup> T-cell activation and apoptosis





**Cell-associated HIV-1 US/MS RNA ratio at 12 weeks ART  
was the only predictor of the relative CD4<sup>+</sup> T-cell count gain by 48 and 96 weeks**

**48 weeks**

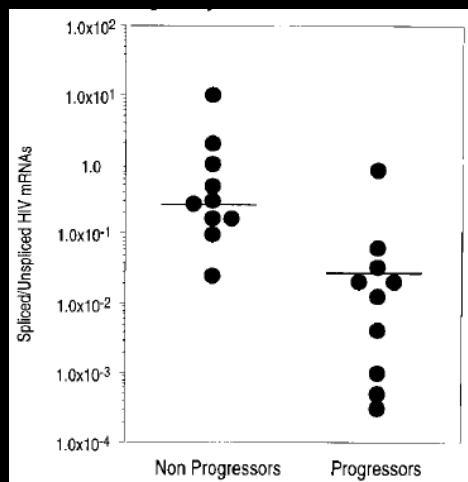
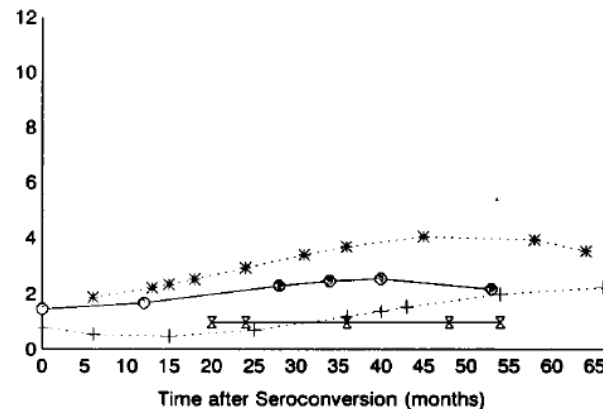
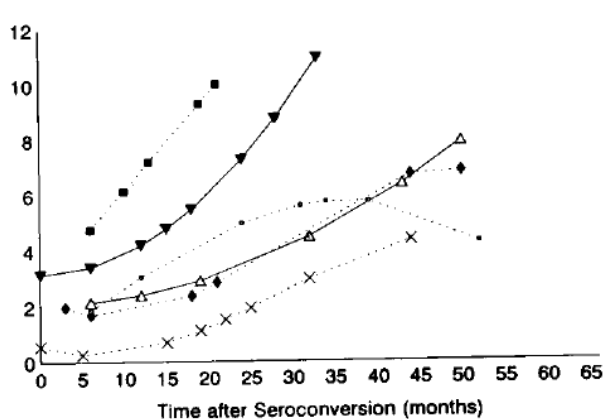
<b>Variable</b>	<b>Univariate P</b>	<b>Multivariate P</b>
<b>US/MS 12 W</b>	<b>0.001</b>	<b>0.002</b>
<b>CD4<sup>+</sup>/CD38<sup>+</sup>/HLA-DR<sup>+</sup> 12W</b>	<b>0.042</b>	<b>0.53</b>
<b>CD8<sup>+</sup>/CD38<sup>+</sup>/HLA-DR<sup>+</sup> 12W</b>	<b>0.041</b>	<b>0.44</b>

**96 weeks**

<b>Variable</b>	<b>Univariate P</b>	<b>Multivariate P</b>
<b>US/MS 12 W</b>	<b>0.004</b>	<b>0.027</b>
<b>CD4<sup>+</sup>/CD38<sup>+</sup>/HLA-DR<sup>+</sup> 12W</b>	<b>0.006</b>	<b>0.64</b>
<b>CD8<sup>+</sup>/CD38<sup>+</sup>/HLA-DR<sup>+</sup> 12W</b>	<b>0.006</b>	<b>0.22</b>

# Cell-associated HIV-1 US/MS RNA ratio was previously shown by several groups to correlate with rapid progression in untreated patients

Ratio of Unspliced/Spliced RNA Progressor      Ratio of Unspliced/Spliced RNA Non-Progressor

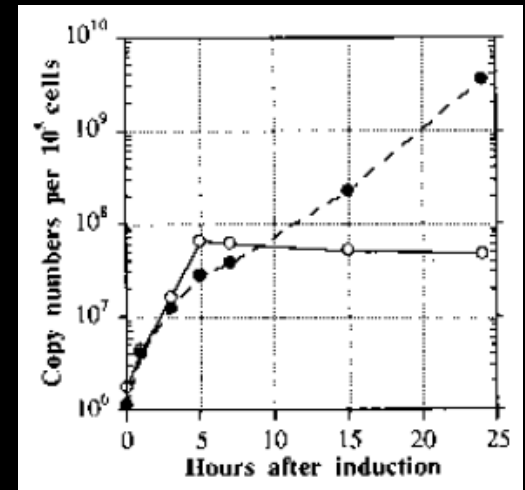
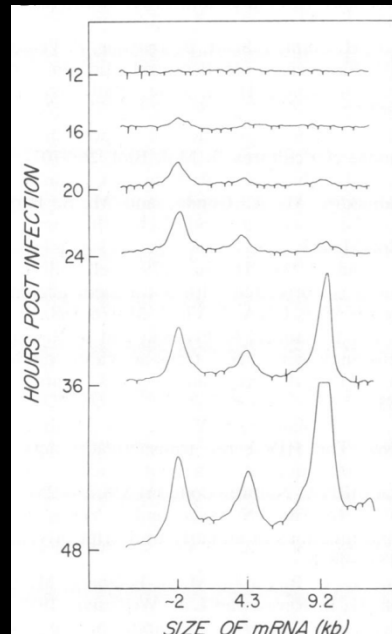
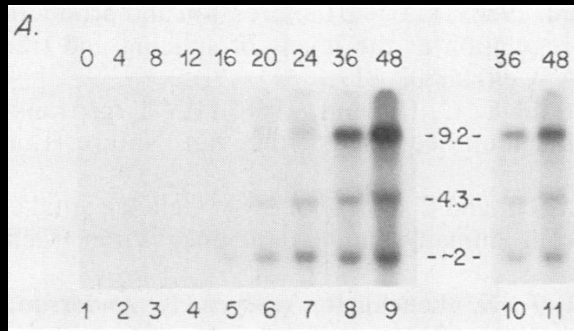


## How can we interpret the US/MS ratio on the cellular level?

Because HIV life cycle involves a temporal shift from the production of multiply spliced to the production of unspliced RNA species (observed both after infection of H9 cells with HIV and after stimulation of ACH-2 cells with PMA),

A higher US/MS RNA ratio in a patient might reflect the higher frequency of HIV-infected cells in the later stages of viral life cycle, which is characterized by expression of viral proteins and presentation of antigens (active reservoir).

Such cells could exert pressure on the host immune system, causing persistent immune activation and apoptosis and contributing to poor immunological response to ART.



## However, this is not so simple...

Kaiser et al. J Virol 2007:  
(in ART-treated patients)

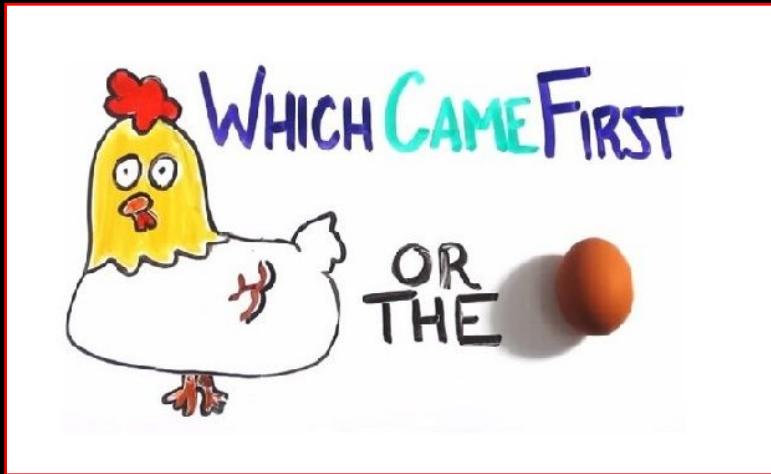
US/MS (resting CD4<sup>+</sup> T cells) = 1

US/MS (activated CD4<sup>+</sup> T cells) = 27

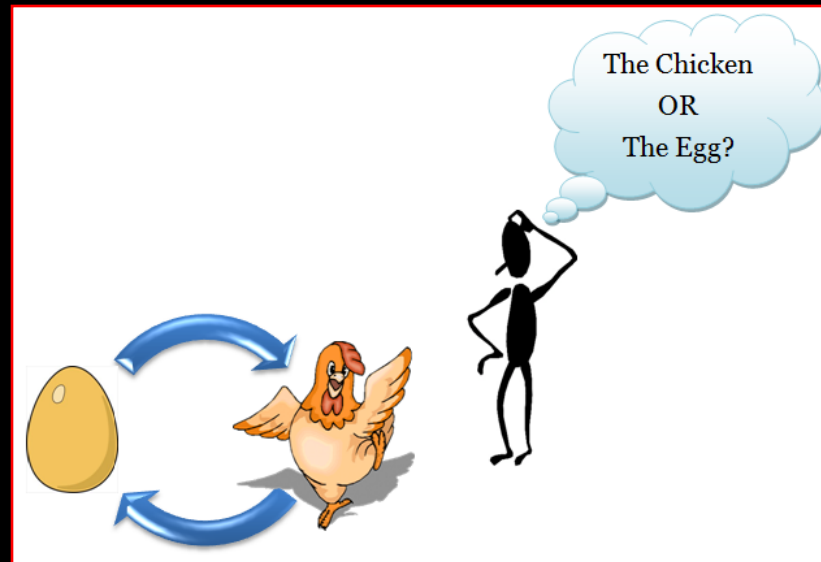
Therefore, the US/MS ratio in ART-treated patients might be an indicator of the relative abundance of (re-) activated (as compared to resting) HIV-infected CD4<sup>+</sup> T cells.

Persistence of such cells might be caused by increased immune activation.

## Chicken / egg conundrum?



So is persistence of active HIV reservoir a cause or a consequence of persistent immune activation and apoptosis?





# Acknowledgements



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