

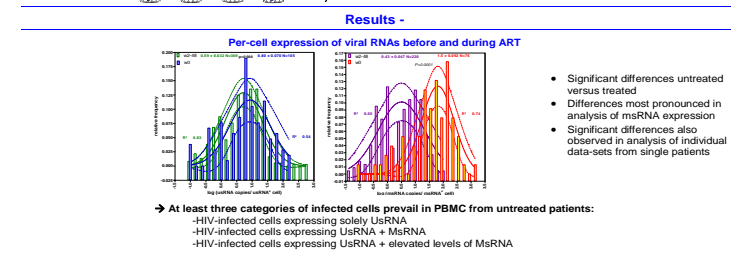
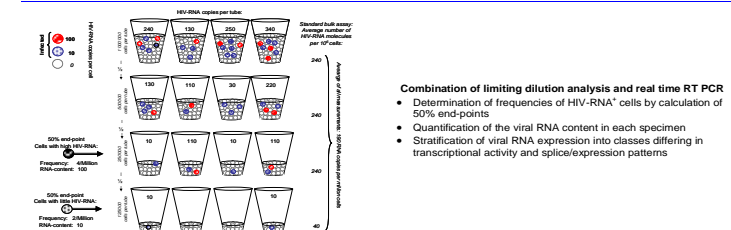
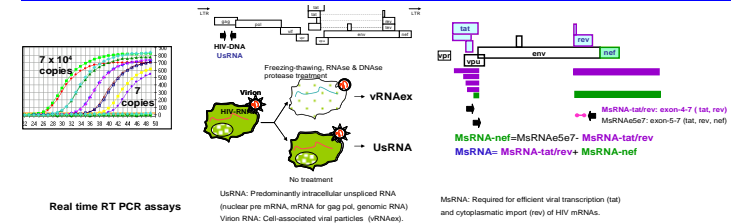
# Rapid Turnover of Non-productively HIV-1 Infected PBMC Following Initiation of Antiretroviral Therapy

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**Background**  
 Antiretroviral therapy (ART) results in 2-3 phased decay of HIV-1 plasma viremia. Mathematical models based decay kinetics of plasma viremia concluded that the first phase may reflect decay of productively infected T-cells, whereas later phases likely mirror decline of cells with lower productivity or of reactivated latently infected cells. To verify and refine this model, HIV<sup>+</sup>PBMC of different viral load activity were monitored during ART

**Methods**

- 5 Patients (#103, 104, 110, 111, 112); initially therapy naive, then ART for 1 year using LPV/AZT/3TC, study visits at baseline, and weeks 2, 4, 8, 12, 24, 48.
- Sequencing of primer sites in gag and env for each patient at baseline to adjust PCR primers and probes to the predominant viral quasi-species.
- Limiting dilution of multiple specimens of PBMC at each visit. Measurement of viral RNAs in each specimen to determine frequencies of HIV-RNA positive cells and their viral RNA burden.



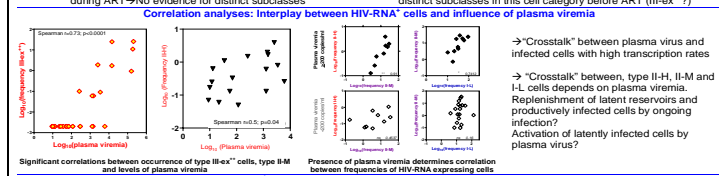
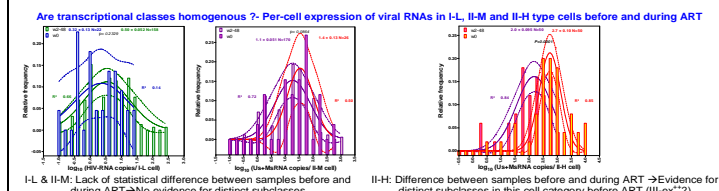
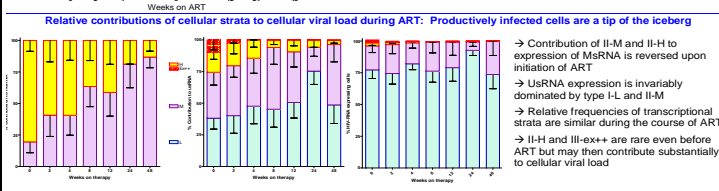
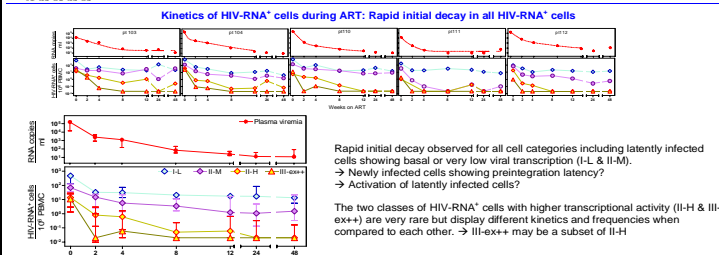
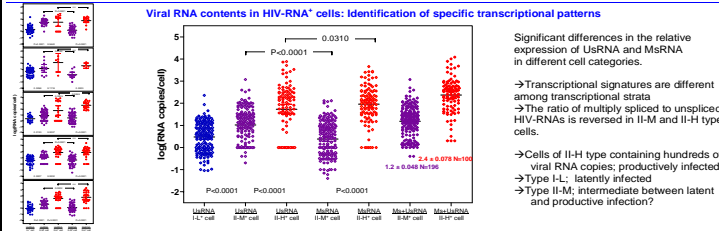
**Definition of transcriptional classes of HIV-RNA<sup>+</sup> cells**

**Class: RNA-expression**  
**II-L** UsRNA<sup>+</sup> MsRNA<sup>-</sup> Basal expression of UsRNA (revlat & nef) < 2 copies/sample  
**II-M** UsRNA<sup>+</sup> MsRNA<sup>+</sup> Basal expression of MsRNA and UsRNA (revlat & nef) < 2 copies/sample  
**II-H** UsRNA<sup>+</sup> MsRNA<sup>+</sup> Significant expression of revlat and nef mRNA (revlat & nef) > 2 copies/sample  
**III-ex++** vRNAex<sup>+</sup> Significant expression of vRNAex (v1-3 copies/RNA<sup>+</sup> cell)

**Rationale for classification**  
 Repeated observation of specimens positive for UsRNA and negative for MsRNA  
 → Nuclear retention of multiply spliced HIV-1 RNA in resting CD4<sup>+</sup> T cells; Lassen et al. PLOS Pathog. 2:7, e60, 2006  
 Ongoing transcription & cytoplasmic RNA export resulting in increased levels of cytosolic viral RNAs  
 Abundant and high level expression of vRNAex at baseline, whereas on ART vRNAex expression occurs sporadically

→ Frequencies of these newly defined classes can be recalculated at each time-point for each patient  
**Calculation of specific viral RNA contents in HIV-RNA<sup>+</sup> cells by "bottom to top" subtraction**

**Class: UsRNA MsRNA**  
**II-L** UsRNA<sup>+</sup> MsRNA<sup>-</sup> UsRNA<sup>+</sup> MsRNA<sup>-</sup> Not present  
**II-M** UsRNA<sup>+</sup> MsRNA<sup>+</sup> UsRNA<sup>+</sup> MsRNA<sup>+</sup> Not present  
**II-H** UsRNA<sup>+</sup> MsRNA<sup>+</sup> UsRNA<sup>+</sup> MsRNA<sup>+</sup> Not present  
**III-ex++** UsRNA<sup>+</sup> MsRNA<sup>+</sup> vRNAex<sup>+</sup> UsRNA<sup>+</sup> MsRNA<sup>+</sup> vRNAex<sup>+</sup> Not present



**Summary: Decay kinetics of HIV<sup>+</sup> PBMC, plasma viremia and cellular viral RNA expression levels**

Stratum	Log2(UsRNA)	Log2(MsRNA)	Log2(vRNAex)	Log2(Plasma viremia)
II-L	1.0	0.0	0.0	0.0
II-M	1.0	1.0	0.0	0.0
II-H	1.0	1.0	1.0	1.0
III-ex++	1.0	1.0	1.0	1.0

**Presence of plasma viremia determines correlation between frequencies of HIV-RNA expressing cells**

**Conclusions**

- In accord with current mathematical models based on decay of plasma viremia, PBMC with vigorous viral transcription were rare and decayed swiftly to mostly undetectable levels. These productively HIV-infected cells were identified by their specific expression patterns showing abundant transcription and virus production.
- Two newly identified categories of HIV<sup>+</sup> cells displaying low HIV-transcription showed rapid initial decays followed by persistence. These cell classes might comprise subpopulations with distinct half-lives, e.g. cells in state of pre- or postintegration latency.
- Conversely, these latent HIV<sup>+</sup> PBMC showed no transcriptional inhomogeneity and correlation analyses suggested crosstalk with productively infected cells during ongoing viremia.
- Thus, rapid turnover of latently infected cells may be shaped by changes in their cellular and humoral environment during ART (decreases in anti-HIV immunity → lengthening of life spans of cells with basal antigen expression; reductions in viremia-associated inflammation → diminishing activation rates of latently infected cells towards virus production)
- Further characterization of the molecular mechanisms underlying the observed rapid declines of latent cells with basal HIV-transcription may help to identify strategies to attack latent HIV-reservoirs.
- Identification of transcriptional patterns distinguishing between latent and productive HIV-infection may offer a new tool to monitor the impact of ART at the cellular level.

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