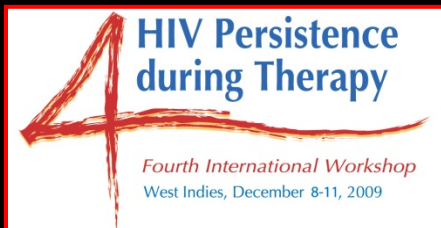




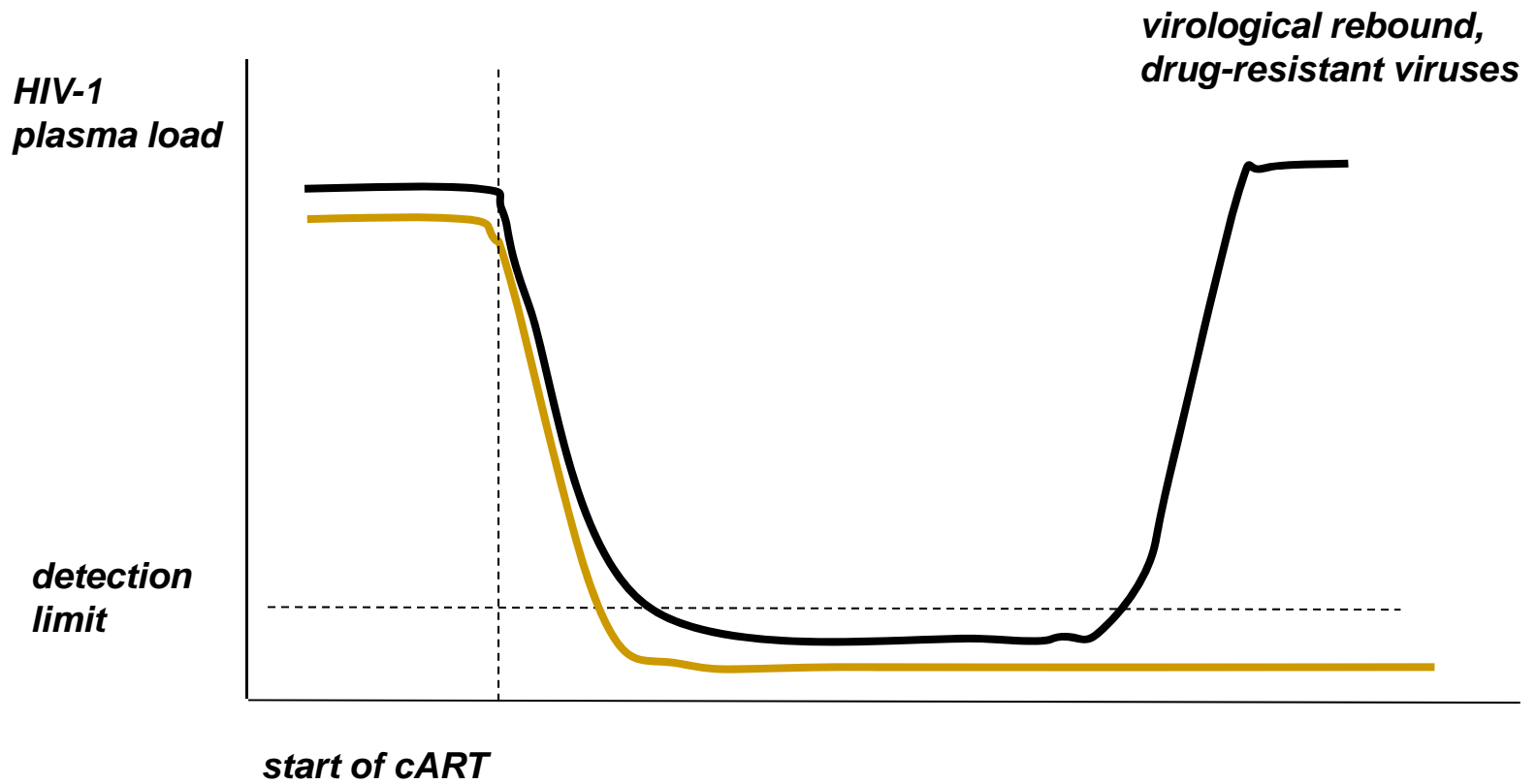
Cellular Levels of HIV-1 Unspliced RNA from Patients on cART with Undetectable Plasma Viremia Predict the Therapy Outcome

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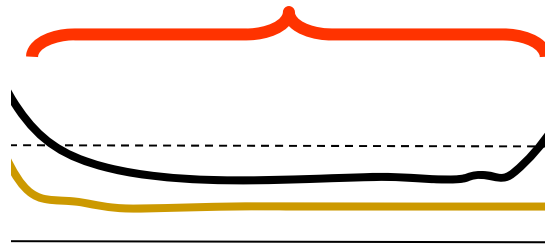


The problem is simple...

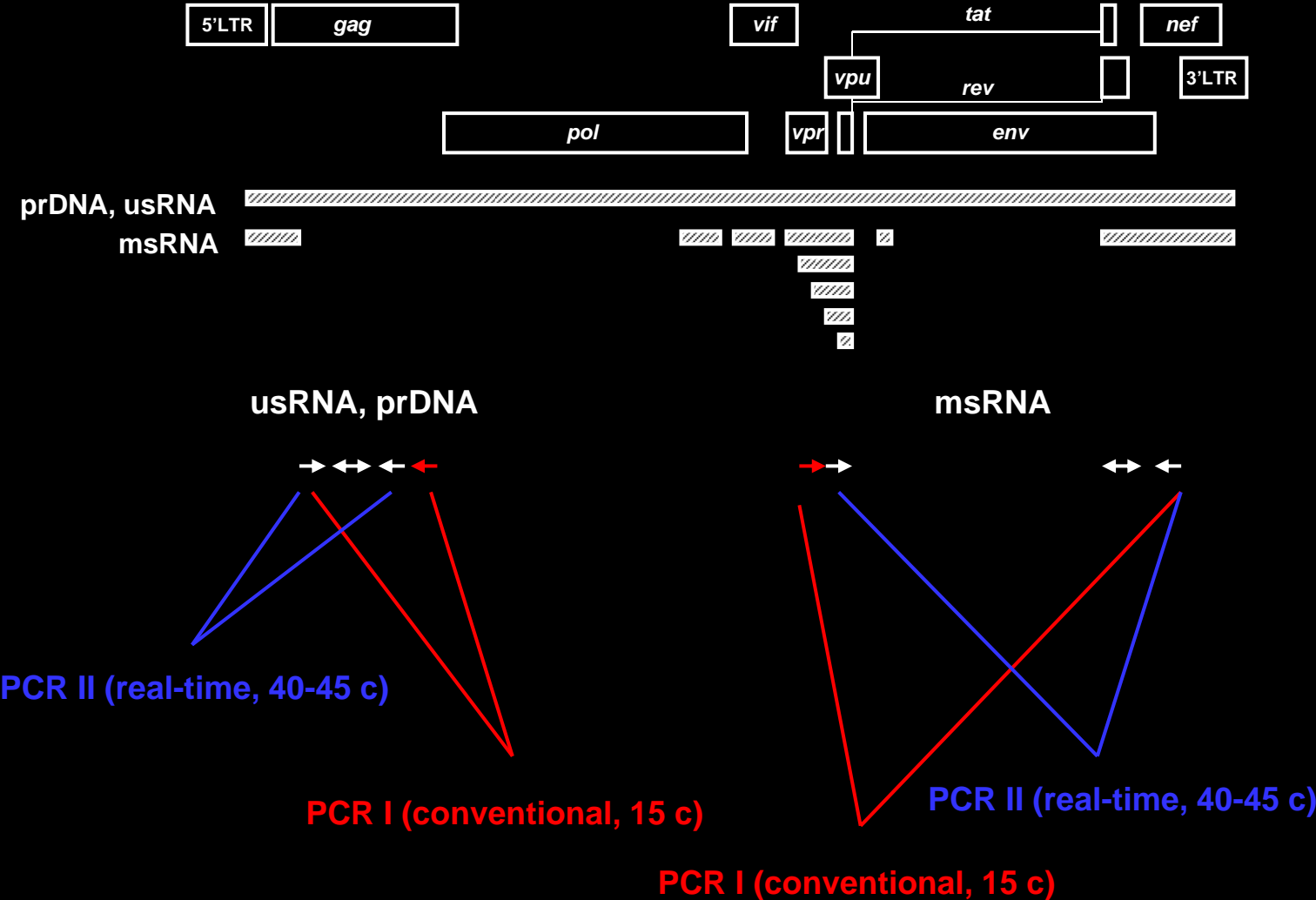


The problem is simple...

What happens here?

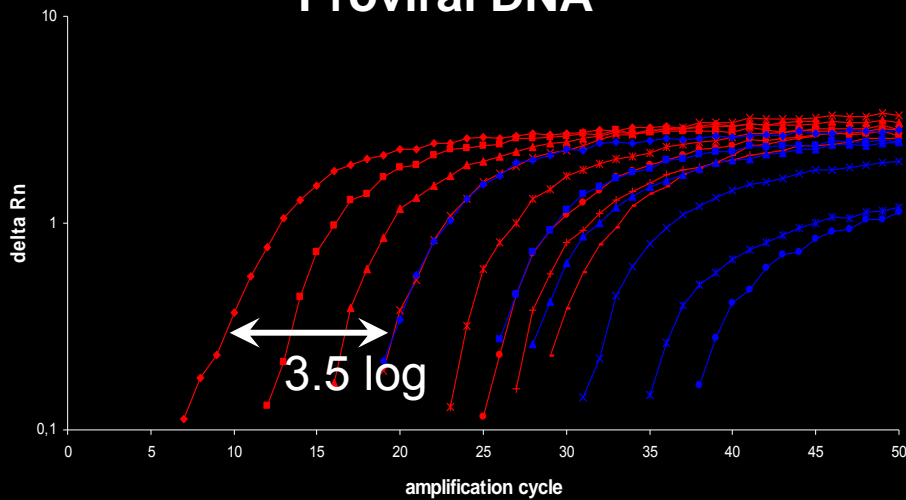


Development of sensitive seminested real-time PCR methods

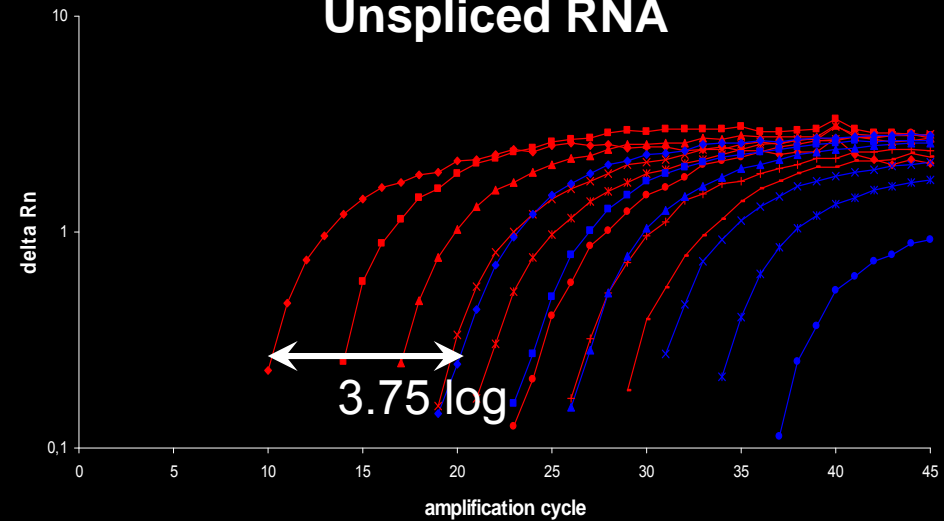


Pre-amplification results in 3-4 log stronger input signal

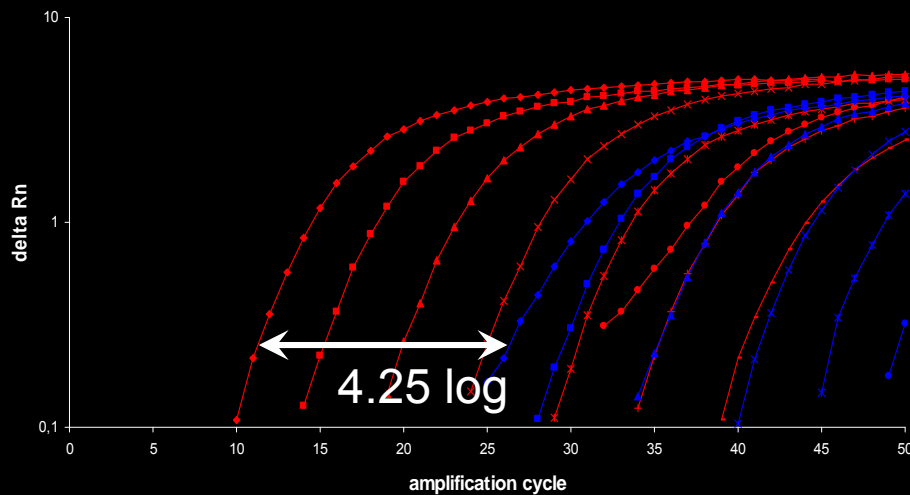
Proviral DNA



Unspliced RNA



Multiply spliced RNA

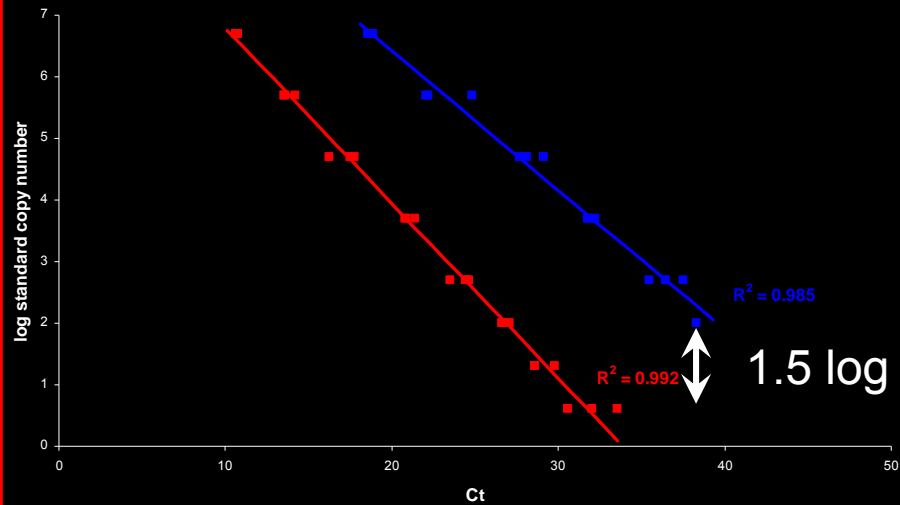


Seminested real-time PCR

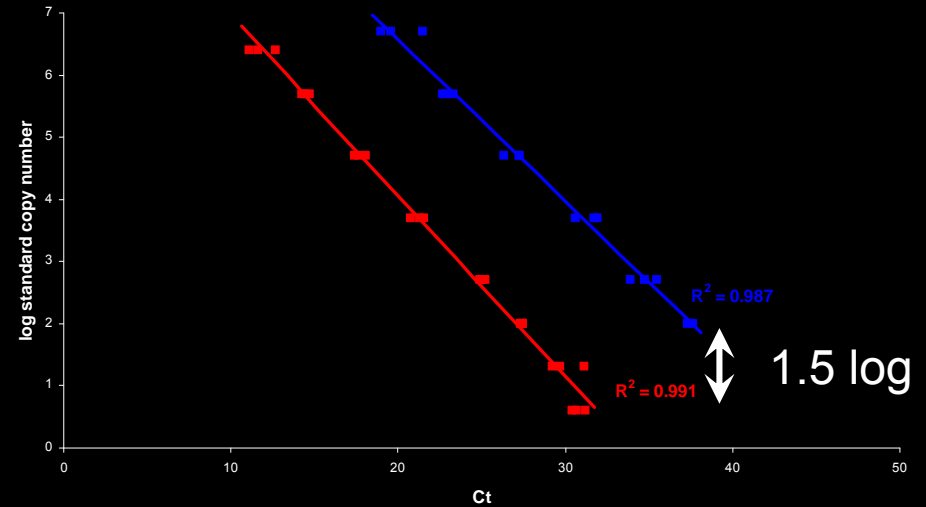
Single-step real-time PCR

Sensitivity is increased 1.5 log without losing the linearity

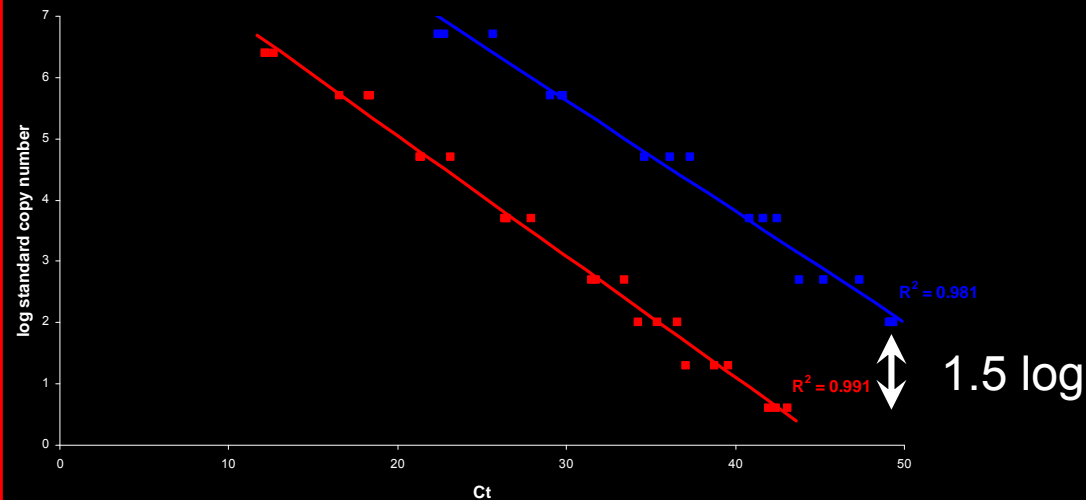
Proviral DNA



Unspliced RNA



Multiply spliced RNA

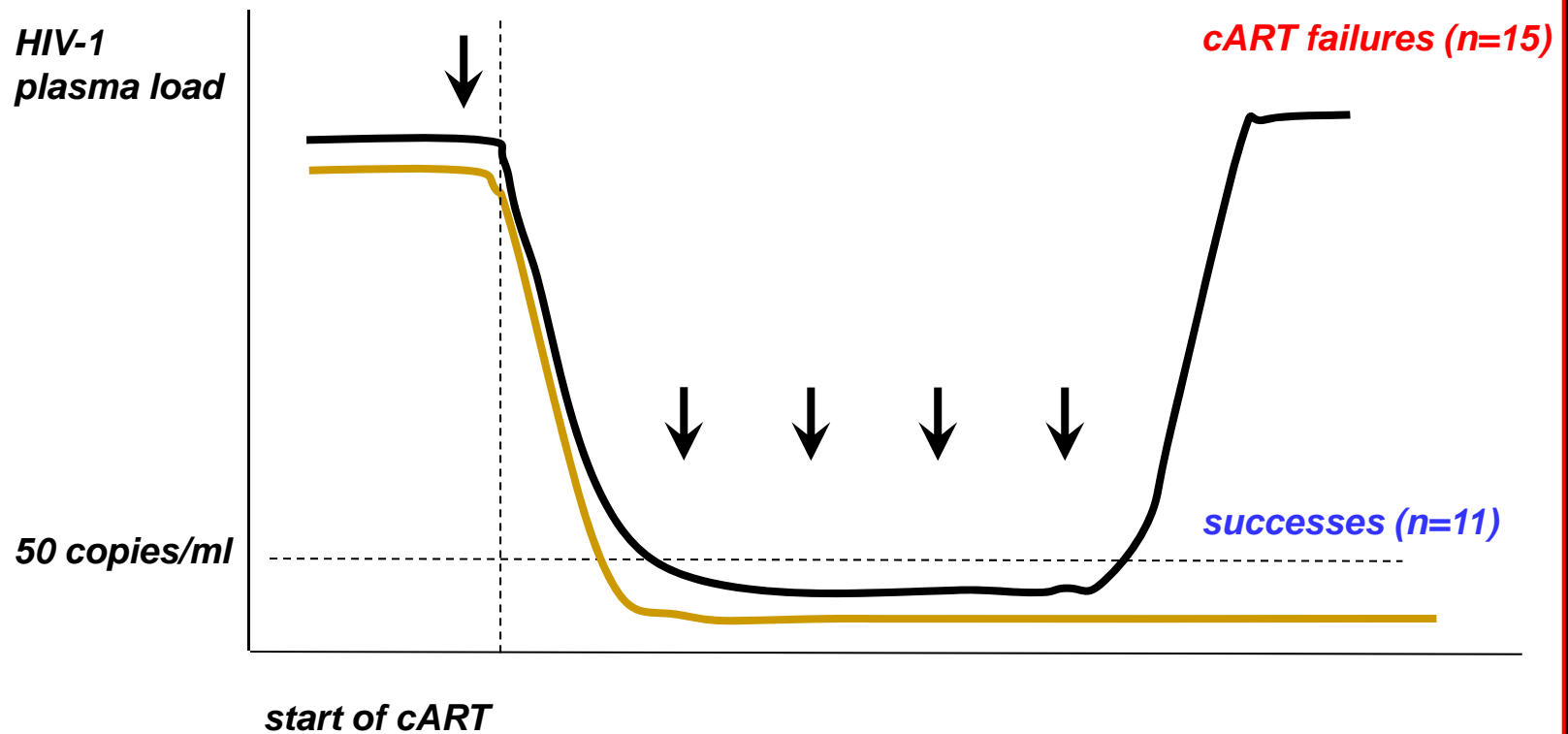


Seminested real-time PCR

Single-step real-time PCR

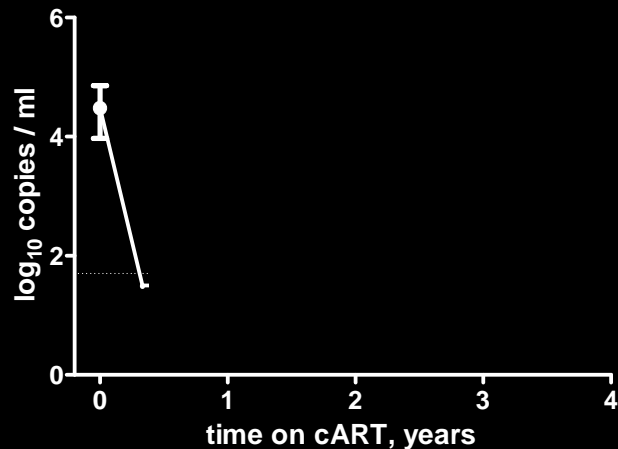
PBMC were isolated at baseline and at multiple time points during therapy

prDNA	100%	94%
usRNA	100%	94%
msRNA	83%	16%

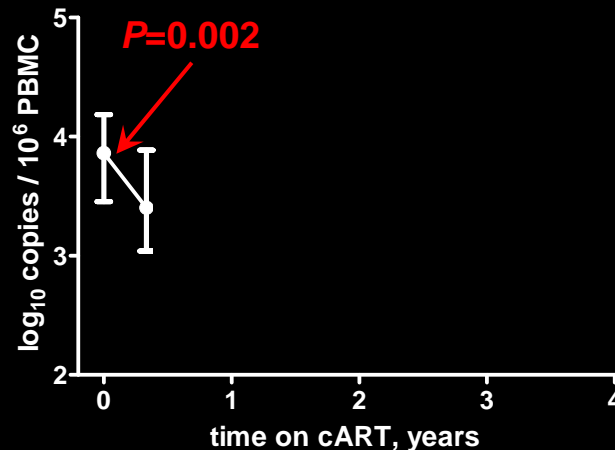


Longitudinal trends of virological parameters and CD4⁺ count in patients on cART

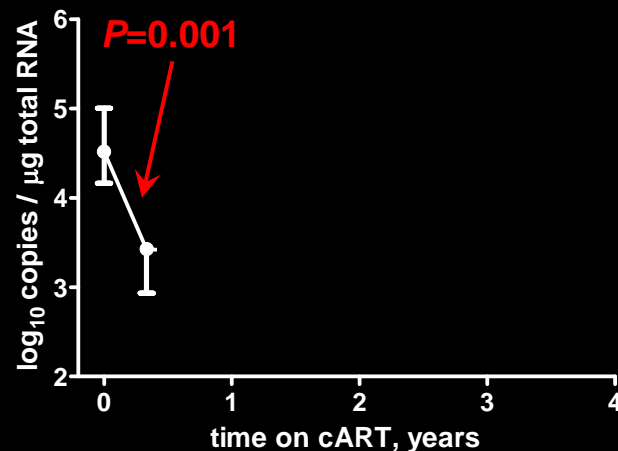
Plasma RNA



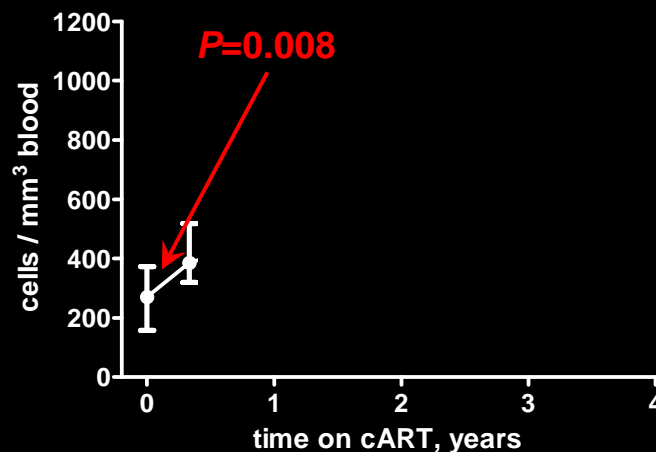
Proviral DNA



Unspliced RNA

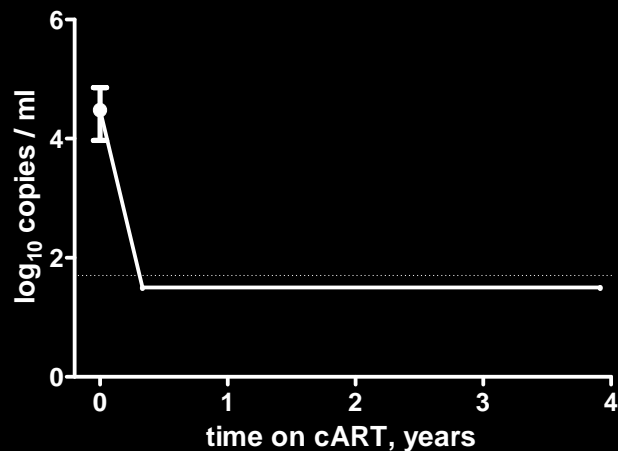


CD4⁺ T cell count

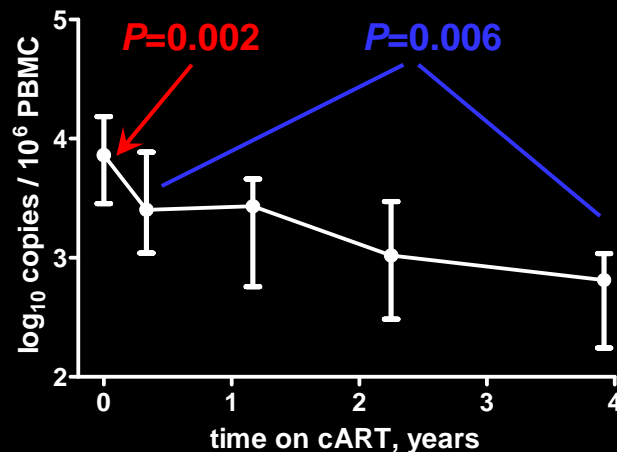


Longitudinal trends of virological parameters and CD4⁺ count in patients on cART

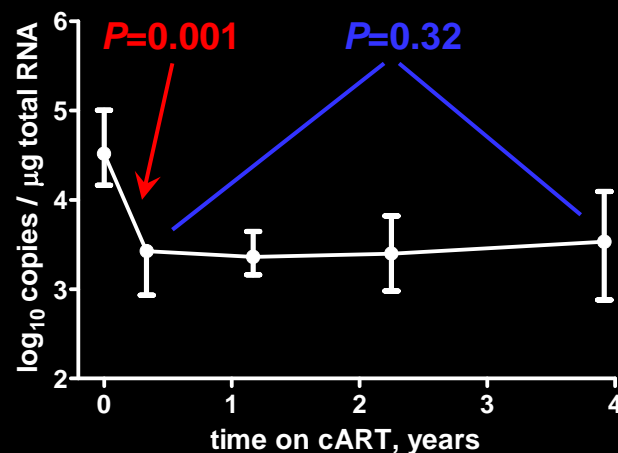
Plasma RNA



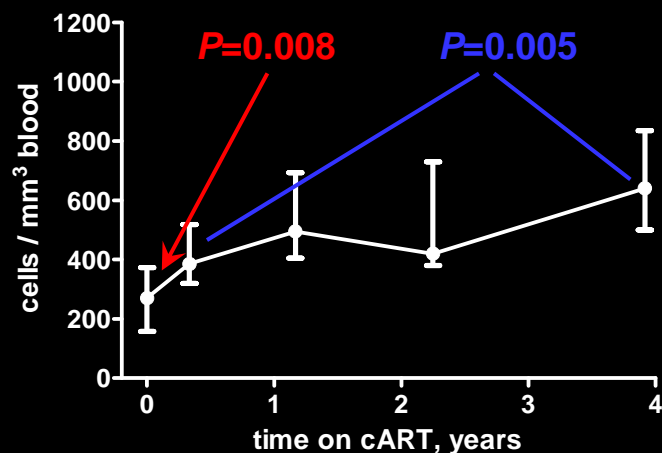
Proviral DNA



Unspliced RNA



CD4⁺ T cell count

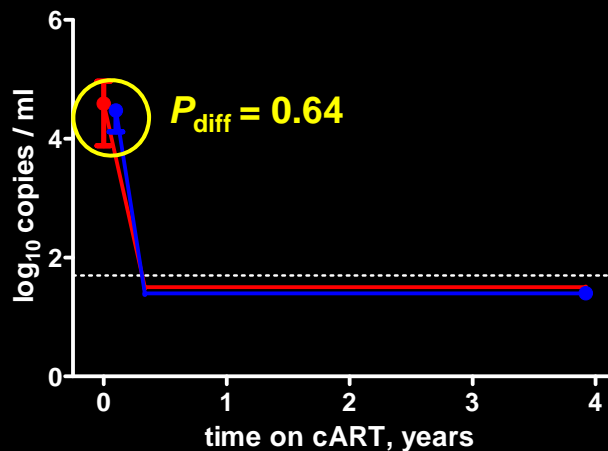


Levels of virological parameters and CD4⁺ count in patients on cART

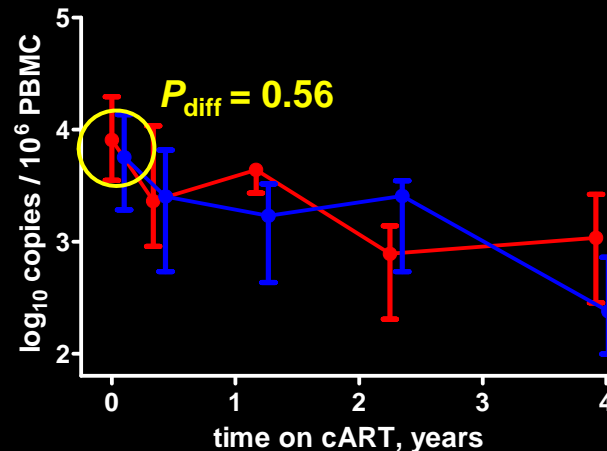
future failures (n=15)

successes (n=11)

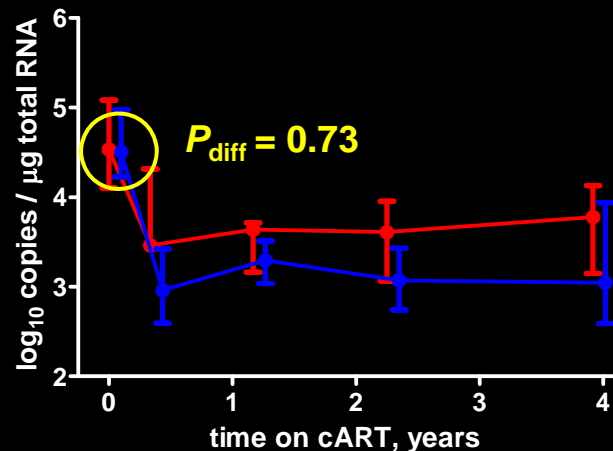
Plasma RNA



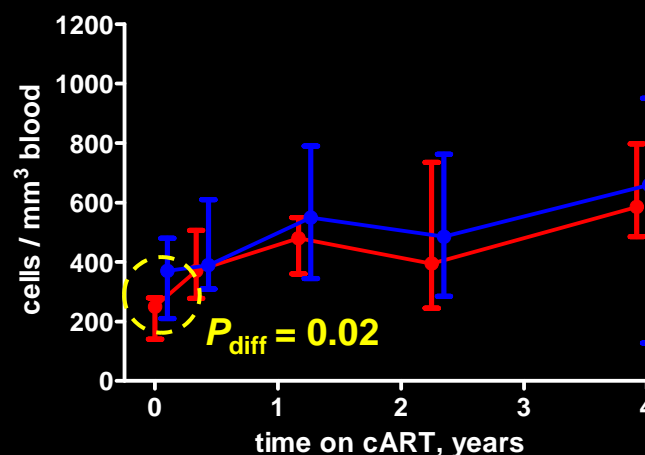
Proviral DNA



Unspliced RNA



CD4⁺ T cell count

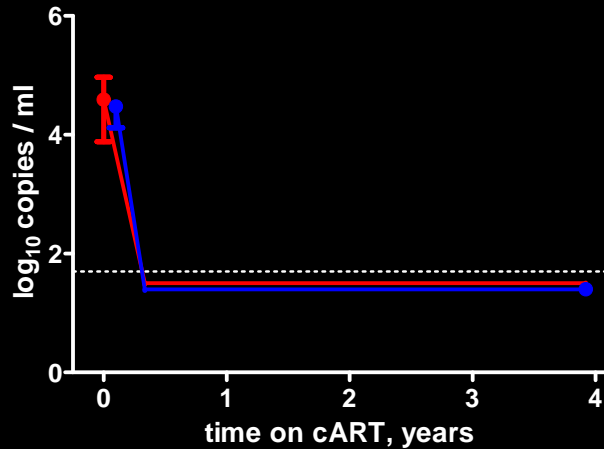


Levels of virological parameters and CD4⁺ count in patients on cART

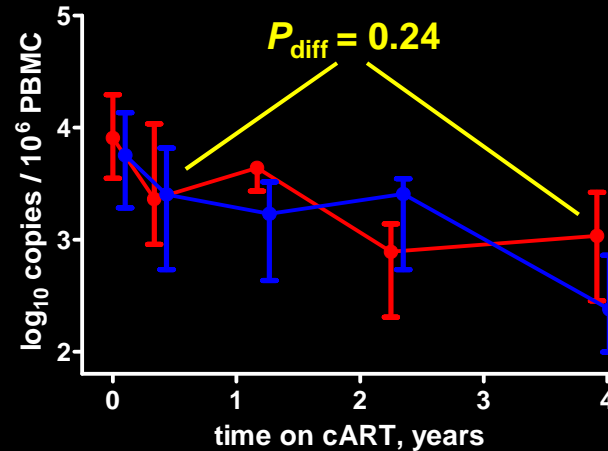
future failures (n=15)

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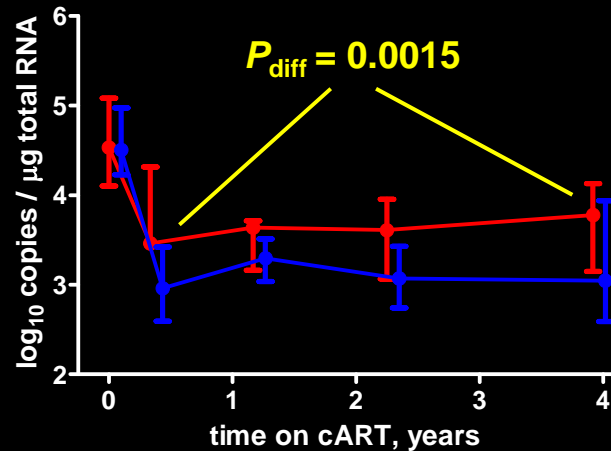
Plasma RNA



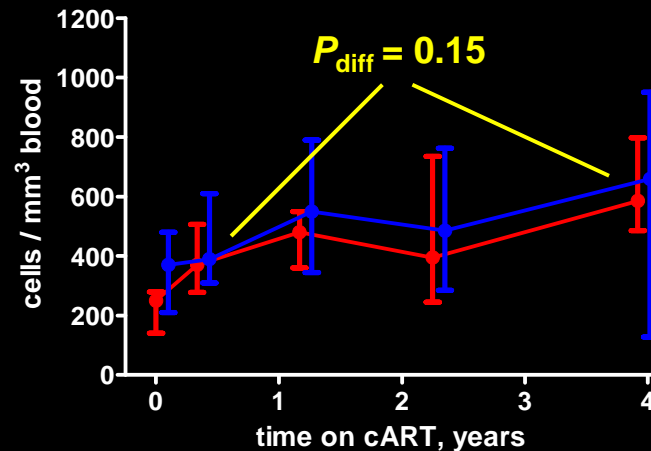
Proviral DNA



Unspliced RNA



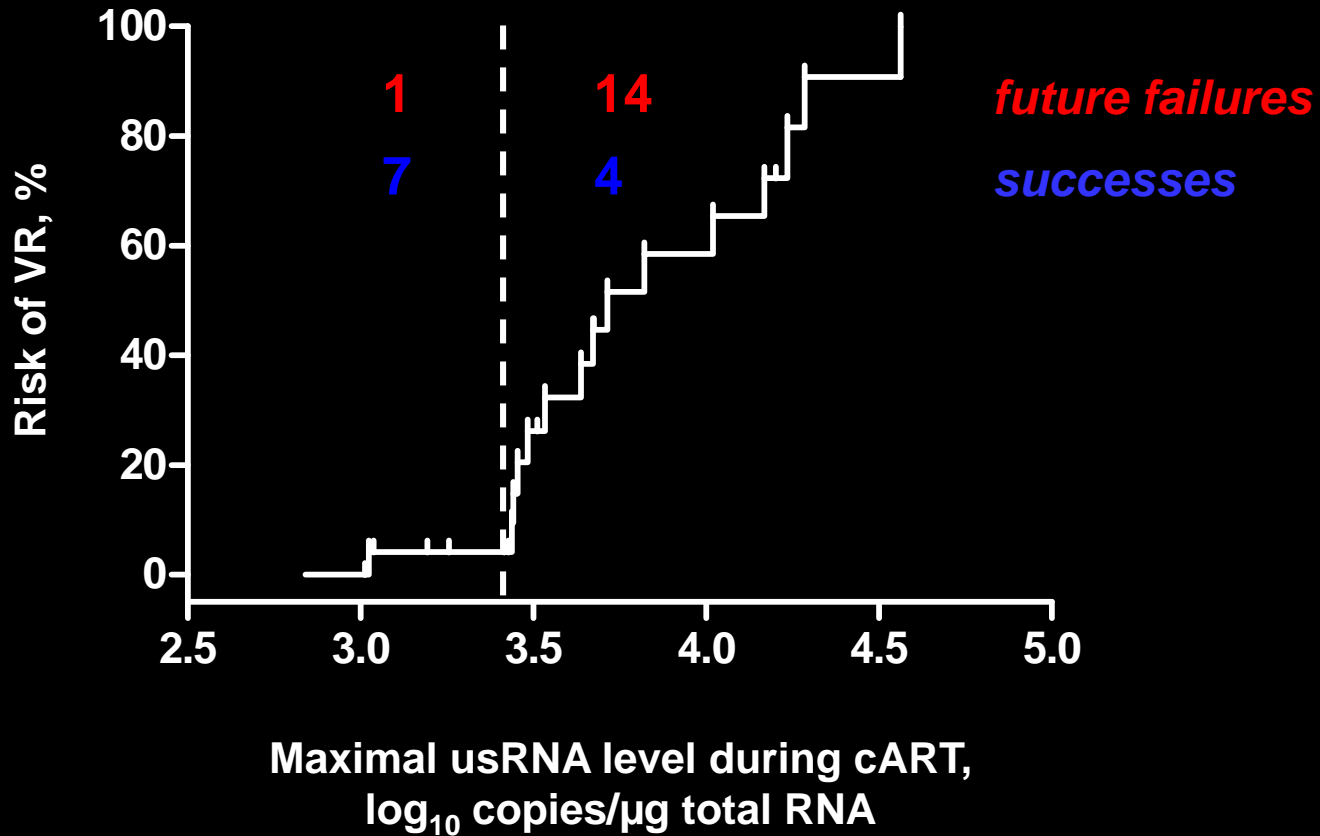
CD4⁺ T cell count



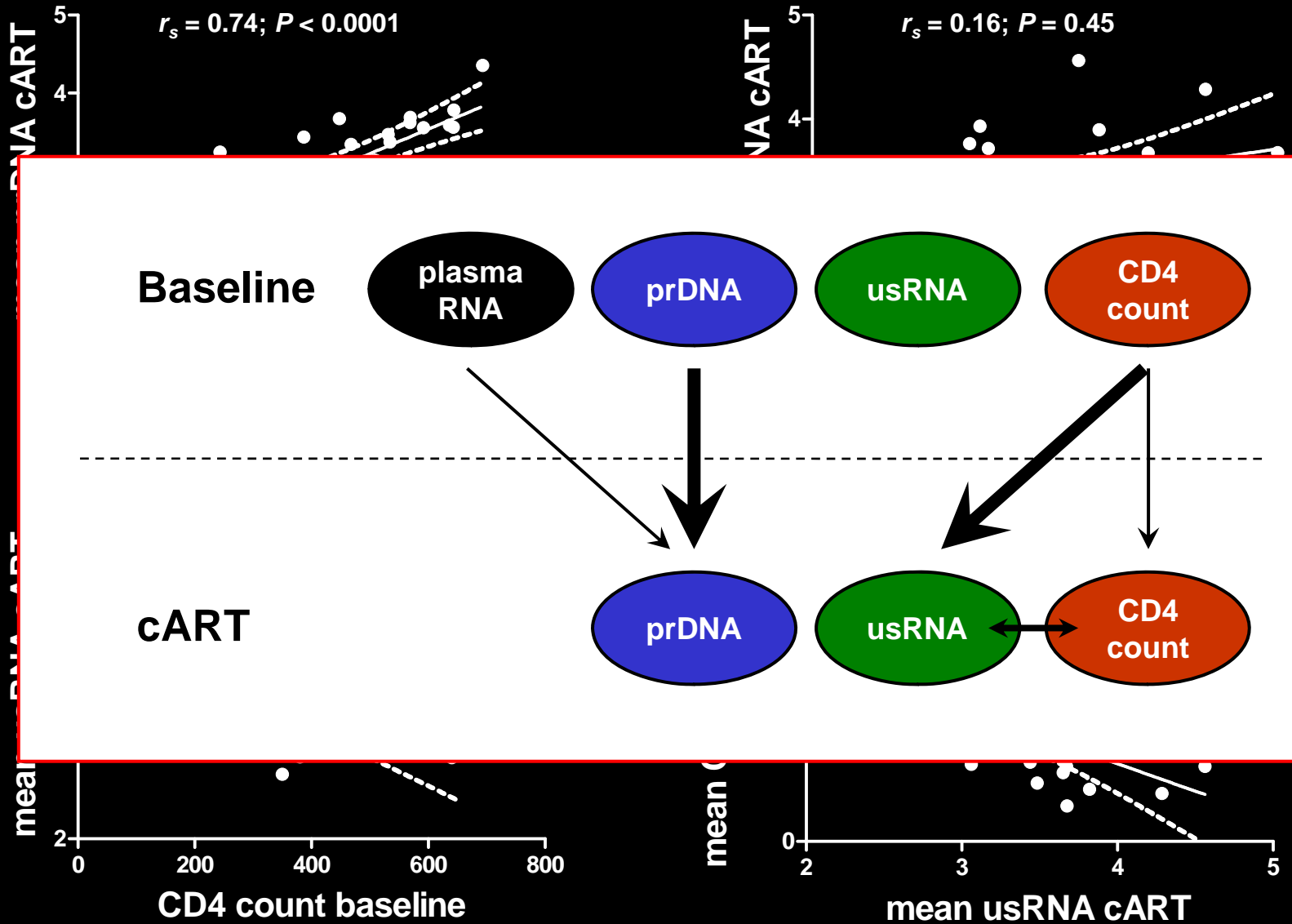
The risk of virological rebound according to the maximal usRNA value during cART

$P=0.0033$ (univariate)

$P=0.024$ (multivariate)



Correlations of virological parameters and CD4⁺ count



Conclusions

Levels of usRNA in PBMC in patients on cART with undetectable plasma viremia are strongly predictive of the subsequent outcome of therapy.

Levels of prDNA gradually diminished during the therapy period in both patient groups

Levels of usRNA dropped sharply after the start of therapy, but subsequently reached the plateau, with the difference between the usRNA levels of future cART failures and successes established early after the start of cART and persisting throughout the therapy period.

Levels of usRNA under cART strongly inversely correlated with baseline CD4⁺ count, but did not correlate with either baseline usRNA levels or prDNA levels under therapy.

Discussion

Differences in longitudinal trends between prDNA and usRNA can be explained by the fact that prDNA levels reflect the size of the proviral archive, whereas levels of usRNA reflect the amounts of productively infected cells at every moment in time.

The observed difference in the usRNA levels between failures and successes may be shaped by the difference in the relative amounts of latently infected resting CD4⁺ T cells that become reactivated to the productively infected phenotype and escape immunological clearance.

The relative amount of productively infected cells at any moment during cART (reflected in the usRNA level) might be defined, to a large extent, by the immunological state of the patient before therapy (reflected in the CD4⁺ count), and not simply by the size of viral reservoir (reflected in the levels of prDNA).

The observation that patients with higher levels of usRNA in PBMC were more prone to failing cART might link higher cellular HIV-1 RNA load in these patients with virus replication under therapy, which resulted in selection of drug-resistance mutations.

Acknowledgements

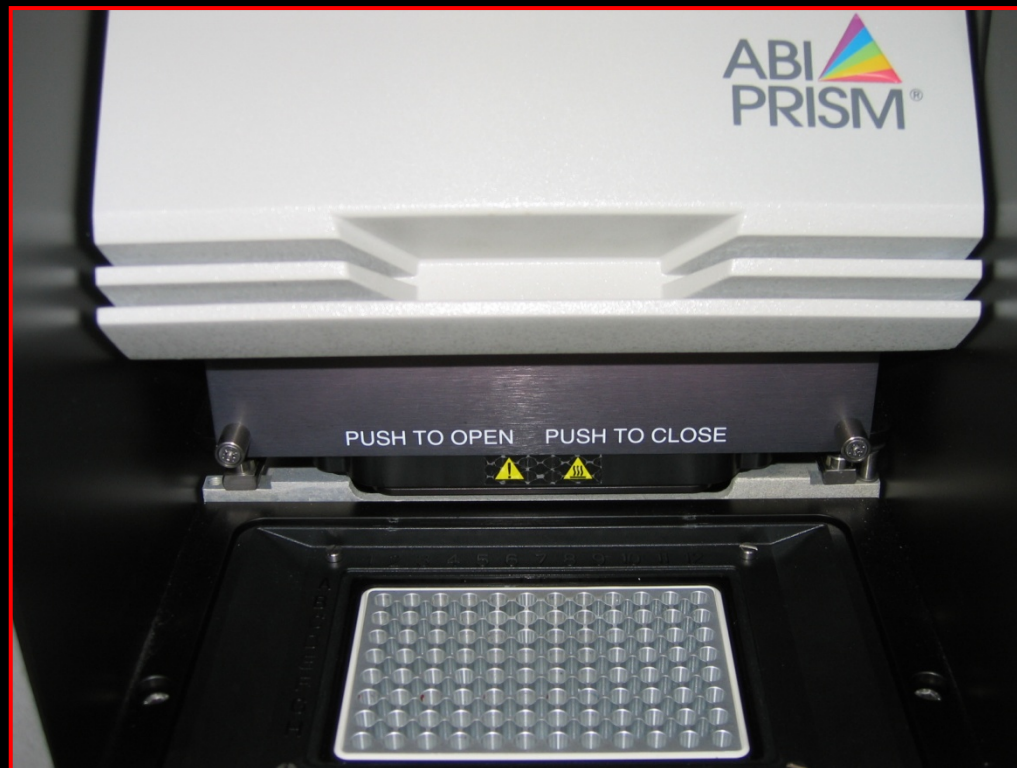
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