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Institute of Virology
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P291

Longitudinal analysis of proviral HIV-DNA

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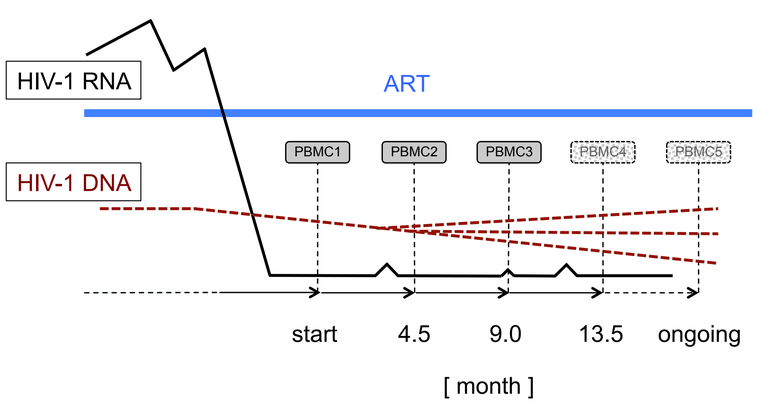
Background: HIV replication can be measured as viral load in plasma and is a suitable and approved marker for monitoring treatment success. Sustained plasma viral load below the limit of detection is the goal in treatment strategy of HIV patients. In time of successful long-term therapies and upcoming cure strategies there is need for a further marker to analyse the influence of a certain drug combination on the proviral reservoir and also for the long-term monitoring of the proviral reservoir. In order to analyse the dynamics of proviral load (pVL) and evolution of proviruses in patients with undetectable plasma viral load we are collecting and examining longitudinal collected samples from 72 patients. Here we present the preliminary results of this ongoing analysis.

Methods: Nucleid acid extraction from 50 µL buffy coat was performed using the 'DNA and viral NA large volume kit' (Roche) for the automated MagNA Pure 96 system. HIV-1 DNA were quantified using the Versant HIV-1 RNA 1.5 Assay (kPCR) (Siemens). For exclusive detection of proviral HIV copies the enzyme mix was incubated 5 minutes at 90° C. In parallel b-globin copies were determined with an inhouse system to calculate the proviral load (pVL). PRRT region was amplified and analysed by NGS with respect to sequence distance (Mega7) between the 3 samples and the detection of resistance mutations (RAMs) and APOBEC associated RAMs, respectively.

Study design



Three consequent samples from each patient (n=72) were collected every 4.5 months. DNA from PBMCs was extracted and the HIV-1 DNA (proviral load; pVL) and PBMC count were measured in a real-time PCR to calculate pVL (log_{10} total HIV-1 DNA / Mio wbc).

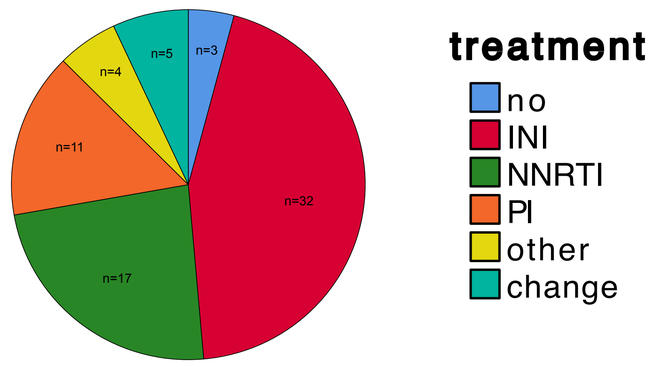
Patients' characteristics

n	72
CD4 (median [IQR])	790 [589 ; 1051]
age (median [IQR])	49 [39 ; 55]
male (%)	64 (89)
female (%)	8 (11)
log_{10} total HIV-1 DNA / Mio wbc (median [IQR])	2,42 [1,99 ; 2,82]
treatment naïve	3 (4%)
time with supressed viral load in month (median [IQR])	49 [20 ; 96]
< LOD/LOQ* [<20 cop/mL (%)]	45 (63)
LLV# [20 cop/mL – 500 cop/mL (%)]	13 (18)
viraemic [>500 cop/mL (%)]	14 (19)

* under limit of detection/quantification, # low level viraemia

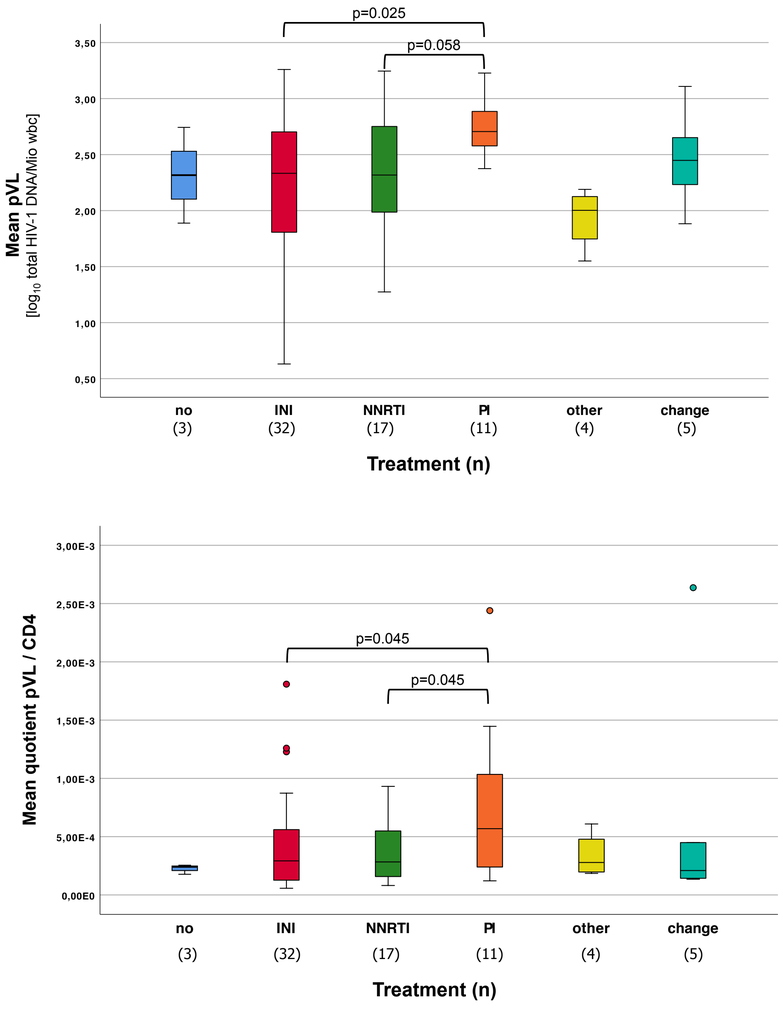
The data were analysed with regard to CD4 cell counts, age, sex, pVL, HIV treatment characteristics (naïve/combinations) and the period of undetectable plasma viral load. With respect to plasma viral load, patients were divided into 3 subgroups: <LOD, LLV and viraemic.

Treatment combinations



The patients were grouped according to their treatment regimen: without treatment (**no**, 4%), NRTI-**INI** (44%), NRTI-**NNRTI** (24%), NRTI-**PI** (15%), **other** combinations [NNRTI-PI, NNRTI-INI-PI, INI-PI, etc] (6%) and patients with treatment **change** (7%).

pVL in different treatment groups



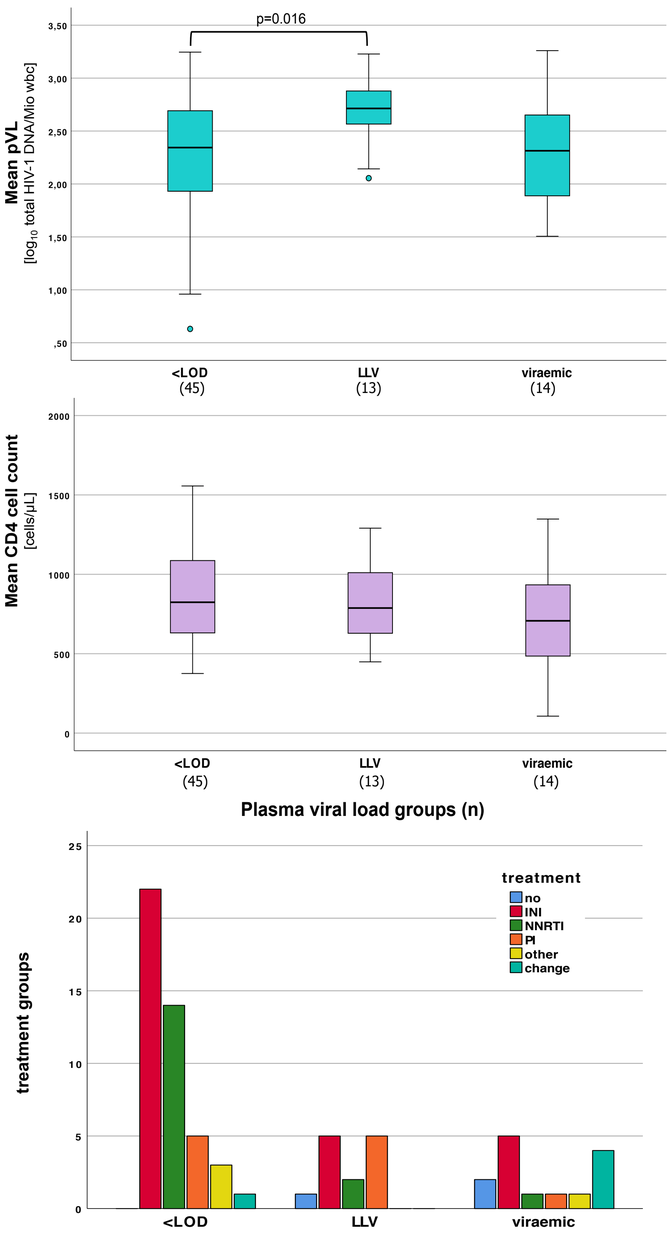
Results:

In our study the mean pVL was 2.36 log_{10} total HIV-1 DNA / Mio wbc (0.63-3.26).

The mean pVL of the measurements from three consequent time points was significantly higher in the PI group compared to the INI group (p=0.025) and also by trend higher compared to the NNRTI group (p=0.058).

In order to include the CD4 cell count into the analysis of the pVL the quotient of pVL and CD4 cell count was determined. Here, also, the mean of the quotient was significantly higher in the PI group compared to the INI and NNRTI groups (p=0.045 each).

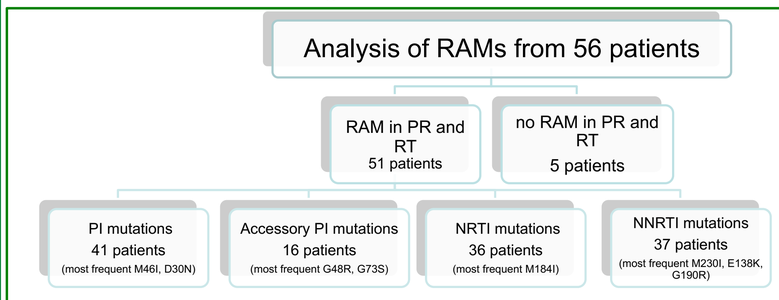
pVL depending on treatment success



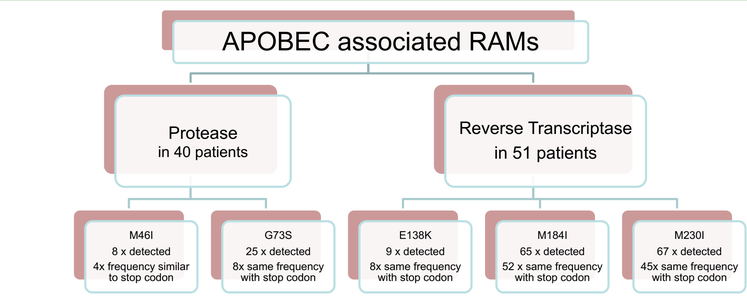
Results: To analyse the dependency of pVL according to treatment success, we compared the mean pVL in the 3 plasma VL subgroups (<LOD, LLV and viraemic). The mean pVL was significantly higher in the LLV compared to the <LOD group (p=0.016). No difference of the mean CD4 cell count was observed between the subgroups. In the <LOD subgroup the percentage of patients with INI treatment was by trend higher compared to other regimens (48.9% vs. 2.2%-31.1%).

HIV evolution under treatment

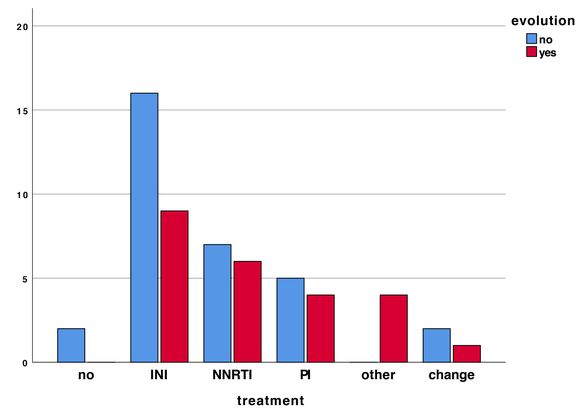
56 out of 72 patients were successfully amplified and sequenced in all 3 samples. Samples were screened for RAM in PR and RT using Stanford DB.



RAMs in proviral DNA: In 51 patients RAMs in PR and RT were detected. High frequency of APOBEC associated RAMs was observed amongst these.



Frequency of APOBEC associated RAMs: The most frequent APOBEC associated RAMs were M46I and G73S in PR and E138K, M184I, M230I in RT. The frequency of these RAMs often correlated with the frequency of adjacent stop codons hinting at the occurrence of non-replication competent strains.



Evolution: Sequences of PR/RT were analysed with respect to sequence distance using Mega7. Higher distances reflect larger differences between sequences. Evolution/alteration was assumed if sequence distance were >0. In the INI group there was the lowest percentage of evolution (36%) compared to NNRTI (46.2%) and PI (44.4%) regimens.

Conclusions: Based on the observation that pVL in patients receiving the standard treatment regimen NRTI-PI is higher compared to other therapy combinations, the role of PI-regimens in sustainment of proviral reservoir has to be determined. Patients treated with INI show low pVL, more frequently successful suppressed plasma viral load and lower evolution. Furthermore, there is also need to examine in detail which factors contribute to the evolution in proviral DNA inspite of suppressed plasma VL. Proviral load could be an easily to perform and useful readout in routine diagnostics.