Do minority resistant variants in HIV-DNA predict virological rebound in highly treatment experienced PLWH under virological control? Data from the PRESTIGIO Registry

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Background

A fraction of heavily treatment-experienced (HTE) people living with HIV-1 (PLWH) harbours a multidrug drug resistant (MDR) virus and requires specialized care and management strategies. Previous studies showed that next generation sequencing (NGS) performed on HIV-DNA from peripheral blood mononuclear cells (PBMC) can detect most drug resistance mutations (DRM) listed in cumulative HIV-RNA genotype and additional DRM [1-2]. Despite this, the sequencing accuracy of HIV-DNA NGS is still debated due to the potential biases related to unusual mutations and/or APOBEC hypermutations. Finally, technical and clinical NGS cut-off are still not standardized and no data on the impact of minority species detected in HIV reservoir on the maintenance of virological control in HTE-MDR PLWH are available.

Aim

This study aimed to clarify whether HIV-DNA NGS ~~might~~ could be useful for resistance assessment and to evaluate the role of HIV-DNA minority species in the maintenance of virological suppression in HTE individuals with MDR.

Methods

HTE PLWH with 4 drug class resistance enrolled from the PRESTIGIO Registry under virological suppression (HIV-RNA <50 copies/mL) for >6 months, with an available PBMC sample and complete drug-resistance and ~~therapeutic~~ treatment history were included.

Major resistance mutations (MRM) and APOBEC editing estimation (APOBEC mutations [APO-M]; stop codons) were evaluated through HIVdb algorithm (HIVdb Ver 9.4.0). Samples with at least one stop codon or APO-M were considered affected by APOBEC signal. NGS cut-offs at ≥1%, ≥5% and ≥20% were tested.

Mutational load was calculated by multiplying the variant frequency detected through NGS per the HIV-DNA levels (copies/million CD4+ cells) measured by digital droplet PCR approach (Biorad, Hercules, CA, USA).

Minority MRM with frequency ranging 1-5% (mV1%) and 5-20% (mV5%) and majority MRM (frequency >20%, mV20%) were compared to plasma RNA historical-GRT (h-GRT) and according to virological response.

Kaplan-Meyer curves were used to evaluate the probability of virological rebound (VR, 2 consecutive viremia >50 copies/mL or 1 viremia >1000 copies/mL) after NGS resistance assessment according to the detection of minority resistant variants and APOBEC editing signal.

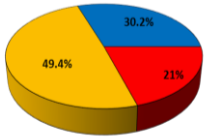
Participants' characteristics

Characteristics	Overall (n=91)
Age, years, median (IQR)	54 (50-59)
Male, n (%)	70 (76.9)
Duration of ART, years, median (IQR)	23 (21-25)
Duration of last therapy, months, median (IQR)	33 (18-44)
Nadir CD4, cells/mm³, median (IQR)	123 (36-213)
Current CD4+ T-cells, cells/mm³, median (IQR)	655 (479-890)
Total HIV-1 DNA, copies/million CD4+ T-cells, median (IQR)[a]	2377 (1274-4949)
Duration of virological suppression, years, median (IQR)	3 (2-5)
X4 tropism, n (%)	56 (61.5)
Previous exposure to 6 ARV classes	45 (49.5)
Current dual therapy	26 (28.6)
Current triple therapy	32 (35.2)
Current complex regimen (>3 drugs)	33 (36.3)
DTG in current regimen	77 (84.6)
DRV in current regimen	70 (76.9)
MVC in current regimen	21 (23.1)
FOS/IBA/LEN in current regimen	7 (7.8)

[a]performed trough digital PCR approach. DRV: darunavir; DTG: dolutegravir; MVC, maraviroc; FOS: fostemsavir; IBA: Ibalizumab; LEN: lenacapavir

Around half of MRM detected with a considerable frequency in HIV-DNA were already found in h-GRT. However, 30% of those detected at lower frequency/mutational load were never observed before.

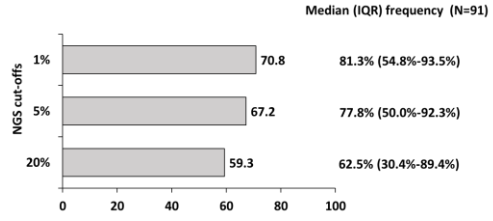
Distribution of total MRM detected by NGS (cut-off 1%) and historical GRTs (h-GRT) according with median frequency and mutational load.



	Frequency, %, median (IQR)	Mutational load, copies/million CD4+ T-cells, median (IQR)
Only h-GRT (N=361)	-	-
h-GRT & NGS-GRT (N=875)	27.1% (10.3%-55.3%)	589 (172-1818)
Only NGS (N=536)	9.6% (2.8%-31.8%)	222 (52-726)

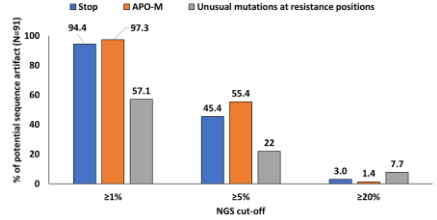
The highest detection rate of historical MRM was reached with NGS set at 1% also in terms of proportion of individual detection of historical resistance by HIV-DNA NGS.

Rate of historical resistance detected by NGS set at different thresholds.



However, 1% cut-off was affected by a considerable number of unusual substitutions and APOBEC hypermutations, suggesting technical concerns and poor clinical relevance.

Rate of historical resistance detected by NGS set at different thresholds.



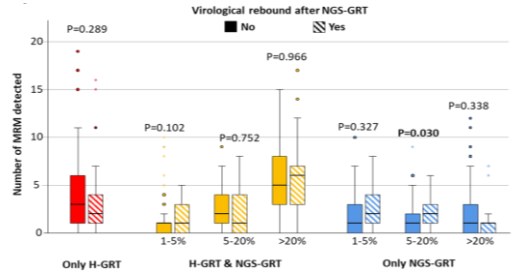
After NGS-GRT, 21 individuals underwent virological rebound in a median time of 23 (10-33) months with a median (IQR) viremia at rebound of 365 (98-7840) copies/mL.

The number of minority resistant variants (frequency 5-20%, mv5%) detected exclusively by NGS-GRT was higher in people who experienced VR compared to those maintaining virological control.

Number of MRM detected by NGS- and/or historical-GRT among 70 HTE individuals who maintained virological suppression compared to 21 HTE individuals who experienced virological rebound after NGS-GRT.

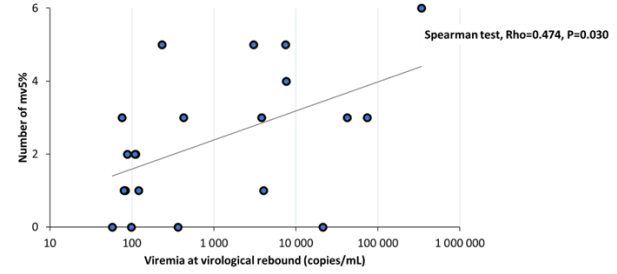
mv1%: minority variants with frequency ranging 1-5%
mv5%: minority variants with frequency ranging 5-20%
MV20%: minority variants with frequency >20%

Differences in number of MRM according to virological rebound were evaluated with Mann-Whitney test. P-value <0.05 is indicated in boldface.



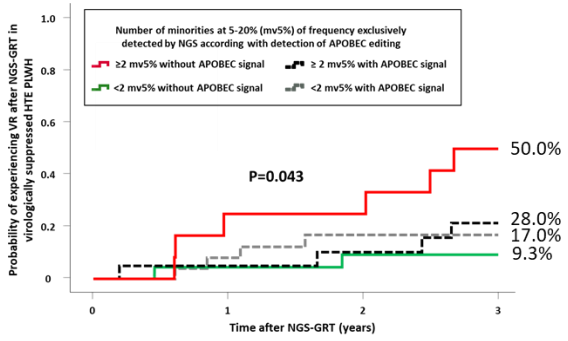
The number of mV5% newly detected by NGS in failing individuals positively correlated with plasma HIV-RNA levels at VR.

Correlation of number of mv5% exclusively detected by NGS and viremia levels observed at virological rebound in 21 individuals who lose virological control after NGS-GRT.



By 3 years after NGS-GRT, the probability of VR was 21.6%. Having ≥2 mv5% exclusively detected by NGS without APOBEC signal was associated with VR.

Impact of minority variants and ABOBEC signal detected through HIV-DNA NGS on the probability to experienced virological rebound after GRT.



Summary and Conclusions

- **In HTE MDR PLWH, setting NGS cut-off at 5% might be a good choice to obtain reliable sequence data that allows to detect a considerable proportion of historical resistance, but also new resistance with acceptable reliability.**
- **At 5% cut-off, an increased number of resistant minority species correlates with loss of virological control and with viremia levels at virological rebound.**
- **In absence of APOBEC signal, minority resistant variants exclusively detected by NGS might predict loss of virological control.**

References:
1. Armenia D et al. Int J Antimicrob Agents. 2022 doi: 10.1016/j.ijantimicag.2021.106492.
2. Hoffmann et al. CROI 2022

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