Evaluation of HIV-1 DNA resistance burden through NGS in highly treatment-experienced multi-resistant individuals under virological control enrolled in the PRESTIGIO Registry

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D. Armenia has nothing to disclose

PRESTIGIO

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OP 9

BACKGROUND (I)

- A fraction of heavily treatment-experienced (HTE) people living with HIV-1 (PLWH) harbor multidrug drug resistant (MDR) HIV [1-3] and require specialized care and management strategies

- Previous studies on *viremic* HTE PLWH with 4-class drug resistance showed that NGS performed on HIV-DNA from peripheral blood mononuclear cells (PBMC) can detect most DRMs listed in cumulative HIV-RNA genotype and additional DRMs [4]
  - At 5% threshold, detection of 71% DRMs detected in cumulative HIV-RNA genotype and an additional 15% of previously unknown DRMs [4]
  - At 1% threshold, detection of 76% DRMs detected in cumulative HIV-RNA genotype and an additional 19% of previously unknown DRMs [5]

1. Armenia at al 2020; 2. Yagai et JAC 2021; 3. Rossetti EACS 2022; 4. Armenia et al., Int JAA, 2022; 5. Hoffmann et al., CROI 2022

AIM

This study aimed to clarify whether PBMC DNA NGS might be useful for resistance assessment in *virologically suppressed* HTE individuals with MDR

MATERIALS AND METHODS

Participants' characteristics were retrieved from the database of the PRESTIGIO Registry.

Inclusion criteria:

- ✓Documented resistance to the 4 classes of antiretroviral drugs (NRTI, NNRTI, PI, INSTI);
- ✓HIV-RNA<50 copies/mL since >6 months
- ✓Plasma HIV drug-resistance, demographic, clinical, laboratory and therapeutic data available before PBMC sampling.

HIV-DNA sequencing & quantification:

HIV-1 DNA PR/RT/IN and V3 Next Generation Sequencing (NGS) was performed through MiSeq platform (Illumina Inc).

Total HIV-DNA quantification was performed on PBMC using QX200™ Droplet Digital™ PCR System (ddPCR, Biorad, Hercules, CA, USA).

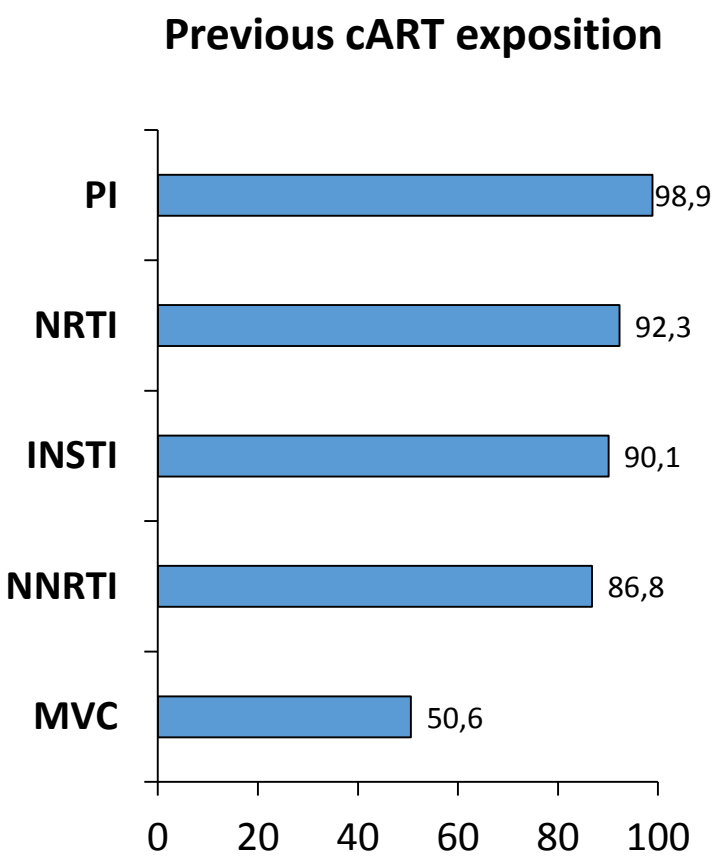
Resistance evaluation

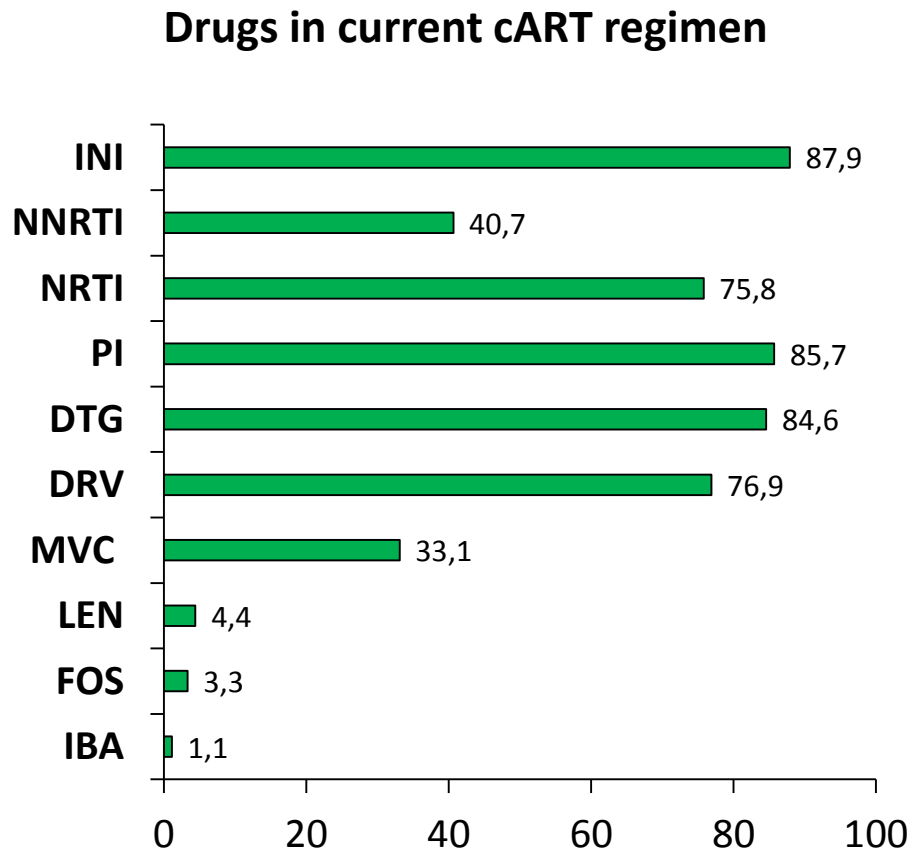
- Major resistance mutations (MRM) and APOBEC editing estimation (APOBEC mutations [APO-M]; stop codons) were evaluated through HIVdb algorithm.
- NGS cut-offs at ≥1%, ≥5% and ≥20% were tested.
- Minority MRM with frequency ranging 1-5% (mV1%) and 5-20% (mV5%) and majority MRM (frequency >20%, mV20%) were compared to plasma RNA historical-GRT (h-GRT).
- Variants distribution was compared between individuals who experienced virological rebound after NGS-GRT and those who maintained virological control.

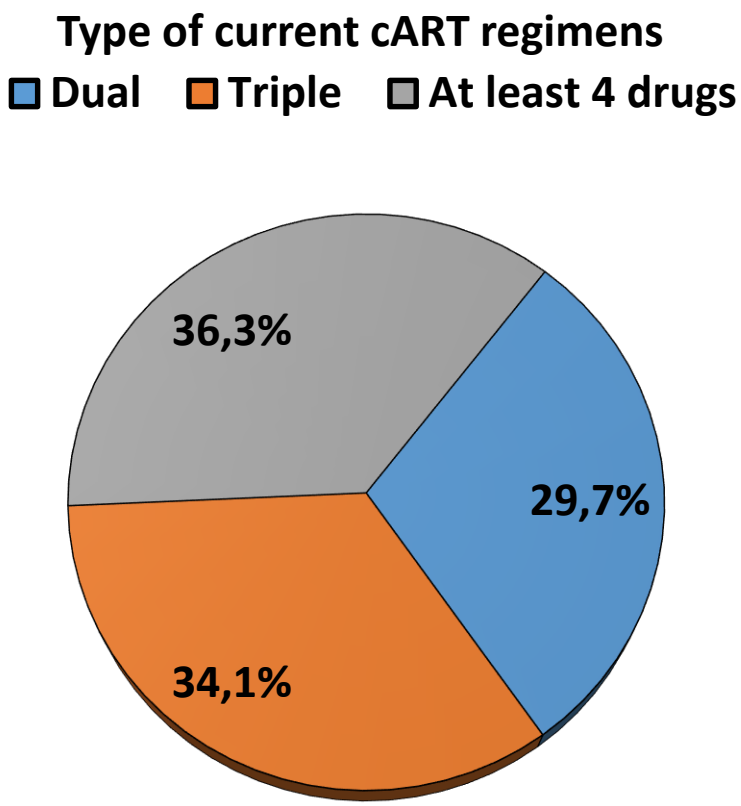
Participants' characteristics at sample collection

Characteristics	Overall (n=91)
Age, years, median (IQR)	54 (50-59)
Male, n (%)	70 (76.9)
Duration of HIV infection, years, median (IQR)	27 (23-31)
Time from HIV diagnosis to ART start, months, median (IQR)	24 (3-73)
Duration of ART, years, median (IQR)	23 (21-25)
Duration of last therapy, months, median (IQR)	33 (18-44)
Nadir CD4, cells/mm^3, median (IQR)	123 (36-213)
Target not detected (TND) at viral load determination, n(%)	42 (46.15)
CD4+ T-cells, cells/mm^3, median (IQR)	655 (479-890)
CD8+ T-cells, cells/mm^3, median (IQR)	961 (715-1237)
CD4+/CD8+ ratio, median (IQR)	0.71 (0.48-0.97)
Total HIV-1 DNA, copies/million CD4+ T-cells, median (IQR)	2377 (1274-4949)
Duration of virological suppression, years, median (IQR)	3 (2-5)
X4 tropism, n (%)	56 (61.5)

Participants experienced a median (IQR) number of previous regimens of 15 (10-19), showed complex treatment history with 87% who experienced at least 5 drug classes among NRTI, NNRTI, PI, INSTI, FI and EI.







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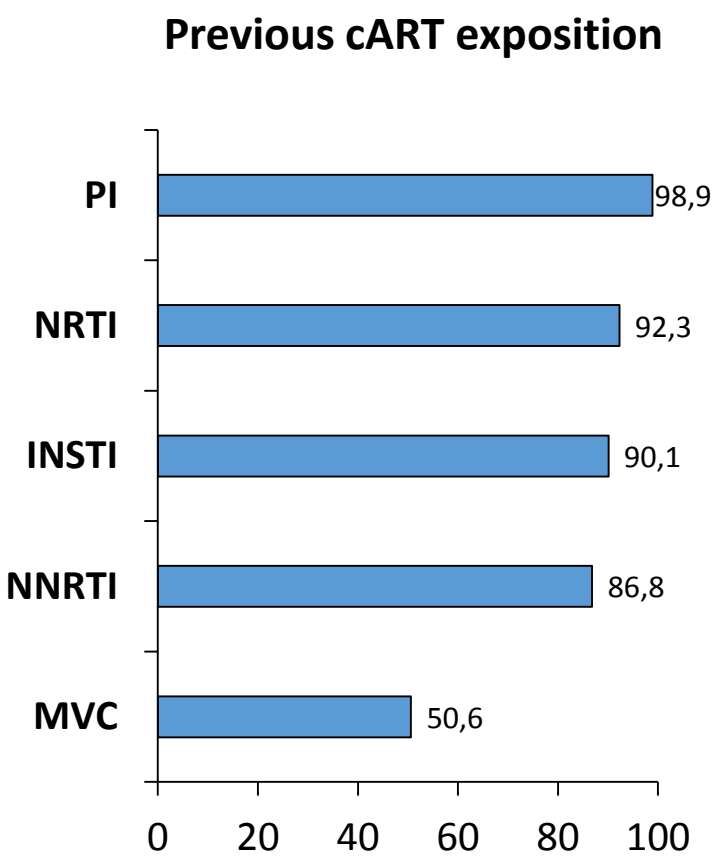
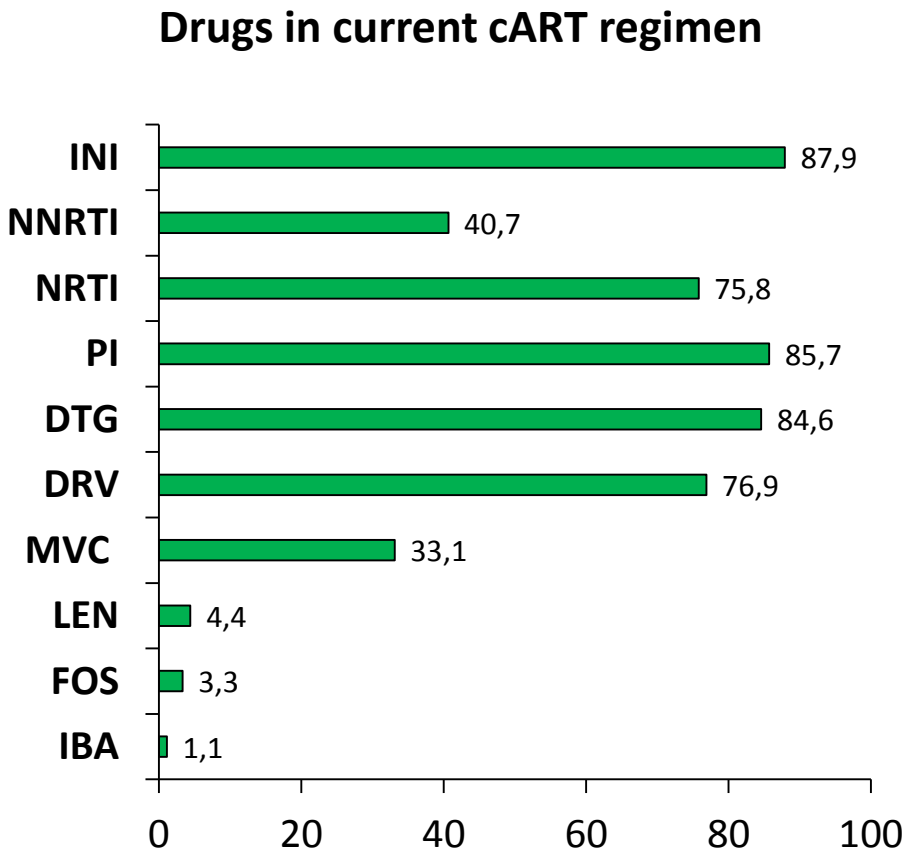
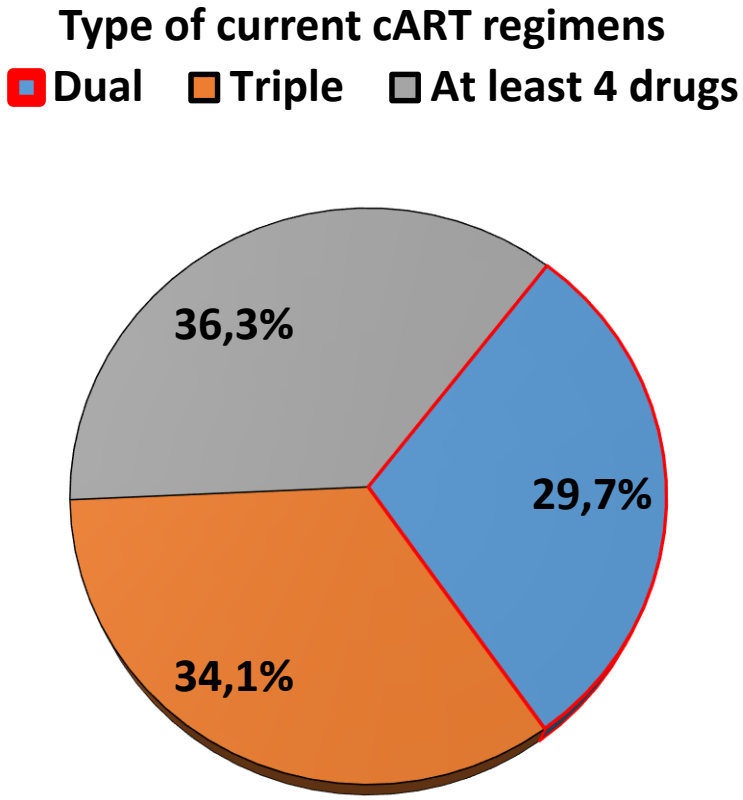


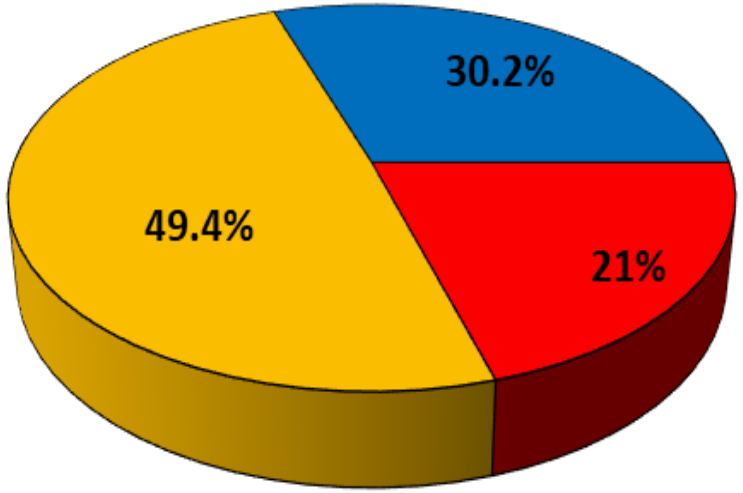




22 out 27 (81%) participants under a dual regimen received boosted DRV plus DTG

Overall, by merging data from h-GRT and NGS (1% cut-off), a total of 1,772 MRM were detected. Around 20% of MRM were detected only in h-GRTs. NGS detected a considerable proportion of MRM already present in the past, but also several new MRM never seen before.

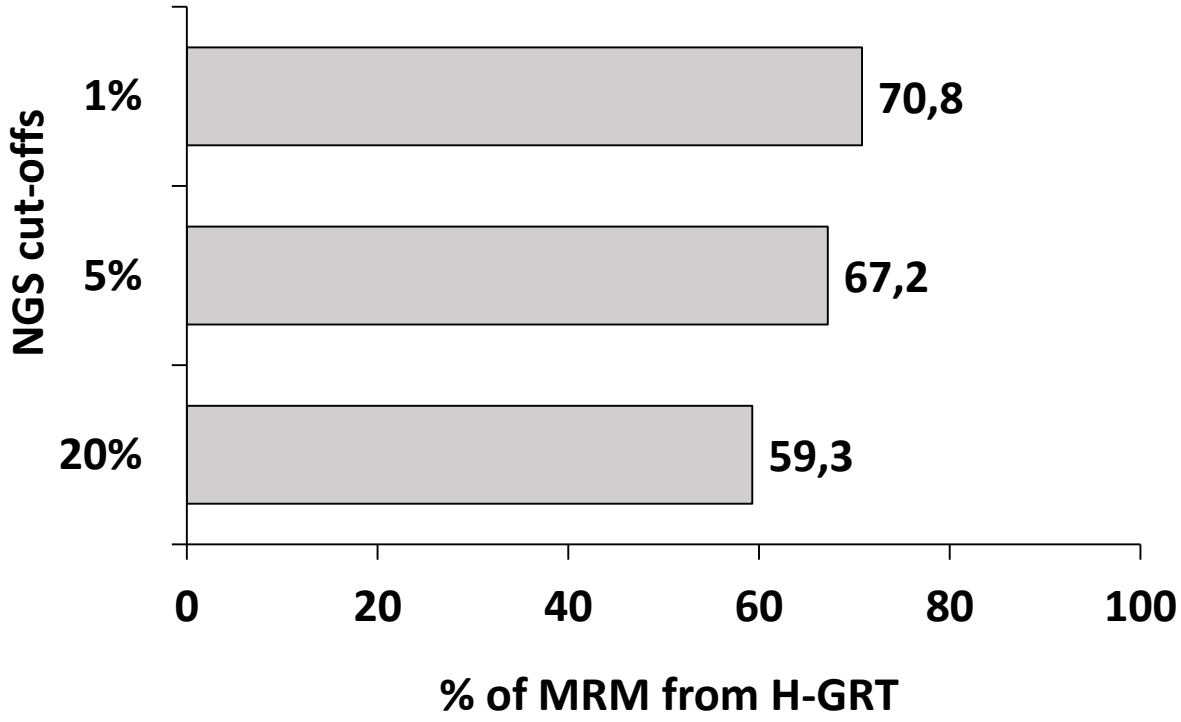
Overview of MRM detected in historical-GRT or NGS at 1% (N=1,772)



	Intra-patient 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)

As expected, by considering the amount of historical resistance detected by DNA-NGS the highest detection rate was obtained by using a cut-off of 1%

Rate of historical resistance detected by NGS set at different thresholds



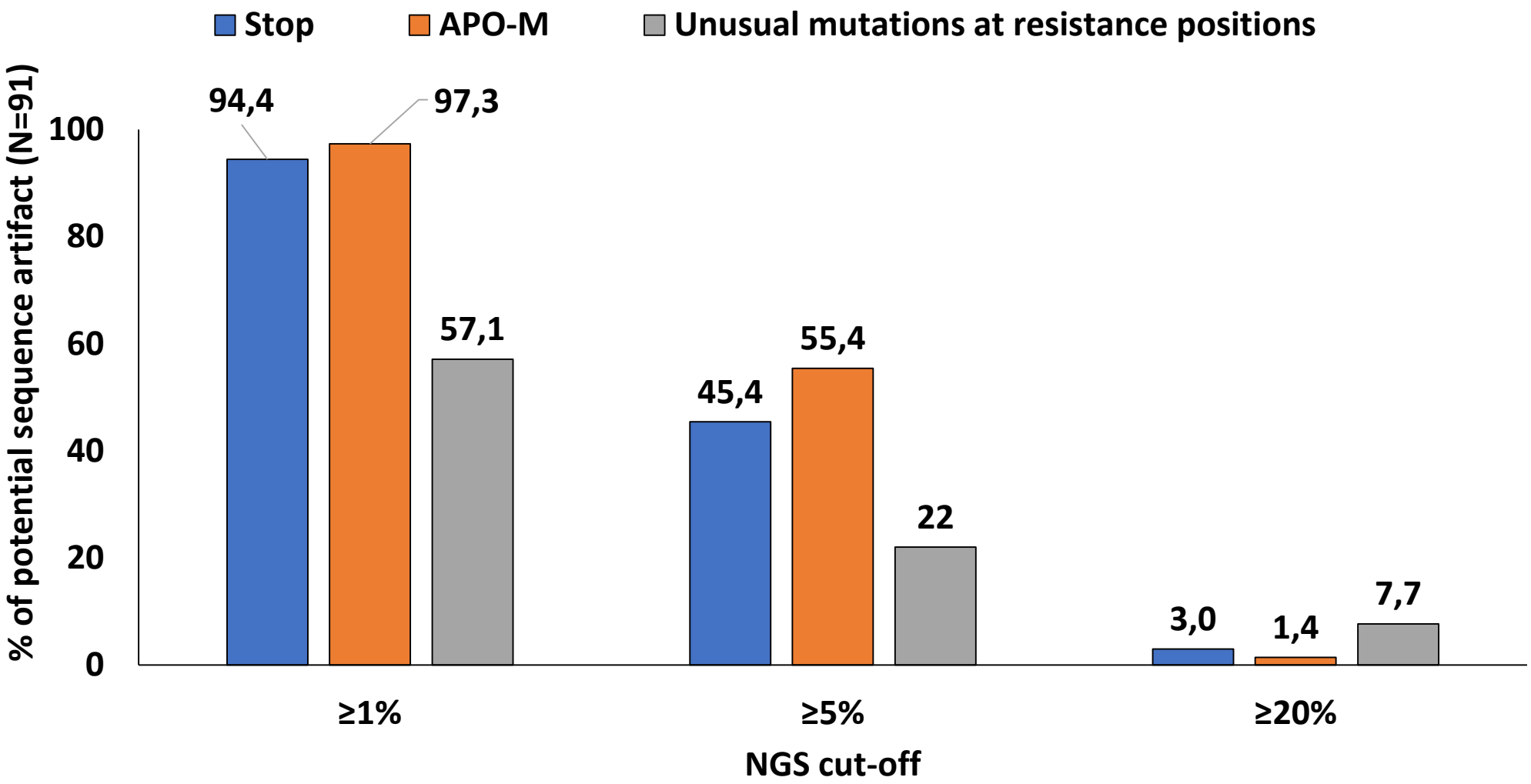
Median (IQR) frequency (N=91)

81.3% (54.8%-93.5%)

77.8% (50.0%-92.3%)

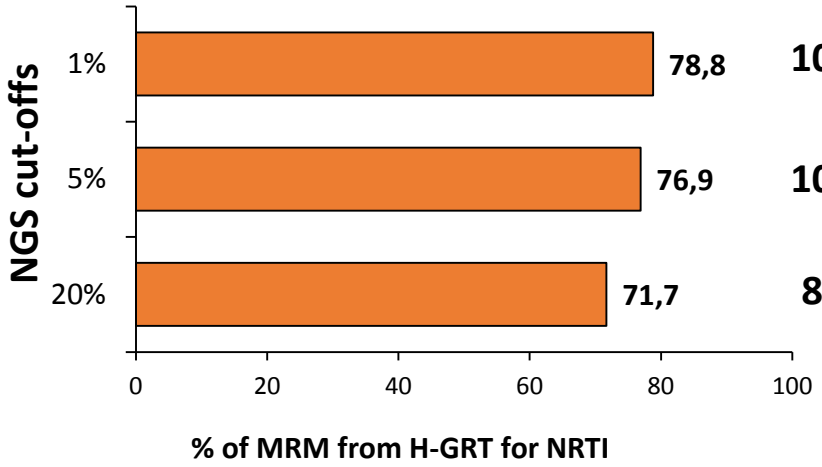
62.5% (30.4%-89.4%)

However, NGS set at 1% showed poor reliability. At this cut-off, almost all the samples were affected by APOBEC related hypermutations and a high number of unusual substitutions at resistance position were observed

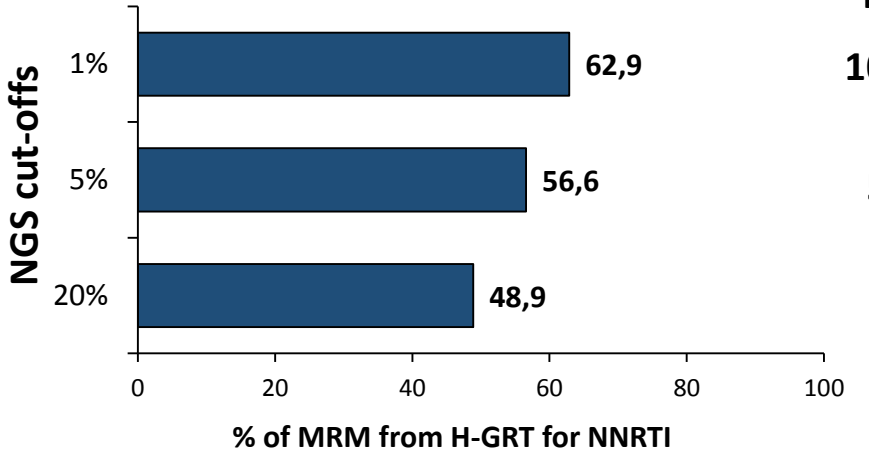


By considering specific drug classes, the detection rate of historical NRTI resistance was >70% regardless the NGS cut-off used

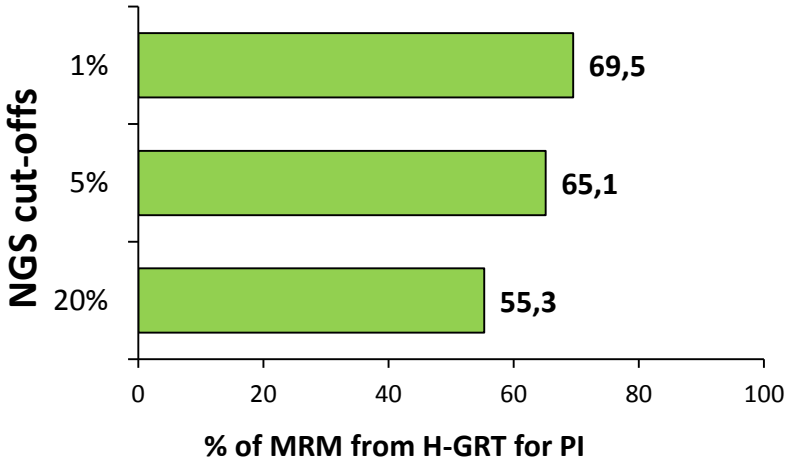
Rate of historical resistance detected by NGS set at different thresholds for each ARV class



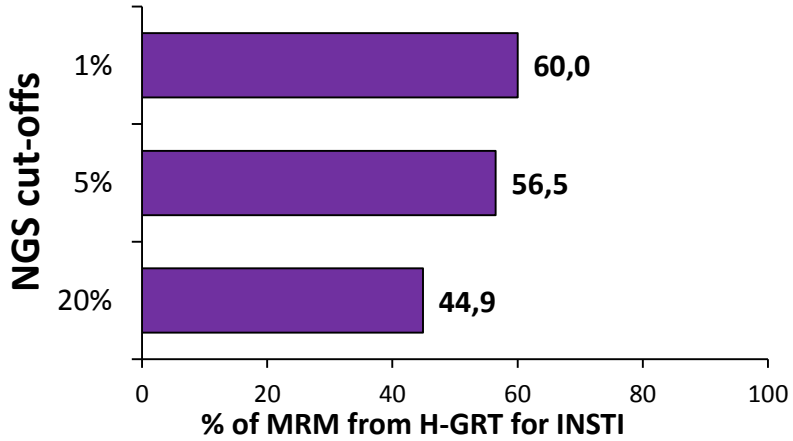
Median (IQR) frequency (N=91)
100% (63%-100%)
100% (60%-100%)
88% (50%-100%)



Median (IQR) frequency (N=87)
100% (50%-100%)
50% (0%-100%)
0% (0%-100%)



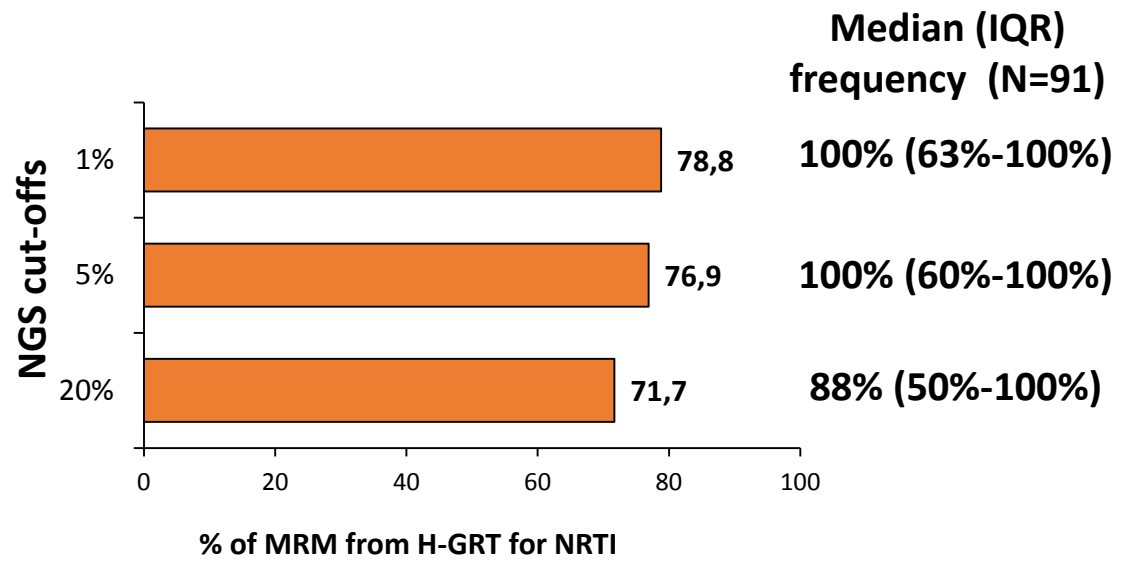
Median (IQR) frequency (N=91)
86% (40%-100%)
75% (23%-100%)
40% (0%-100%)



Median (IQR) frequency (N=77)
100% (0%-100%)
33% (0%-100%)
0% (0%-100%)

Differently, for other drug classes and especially for INSTI, the detection rate was affected by NGS cut-off.

Rate of historical resistance detected by NGS set at different thresholds for each ARV class



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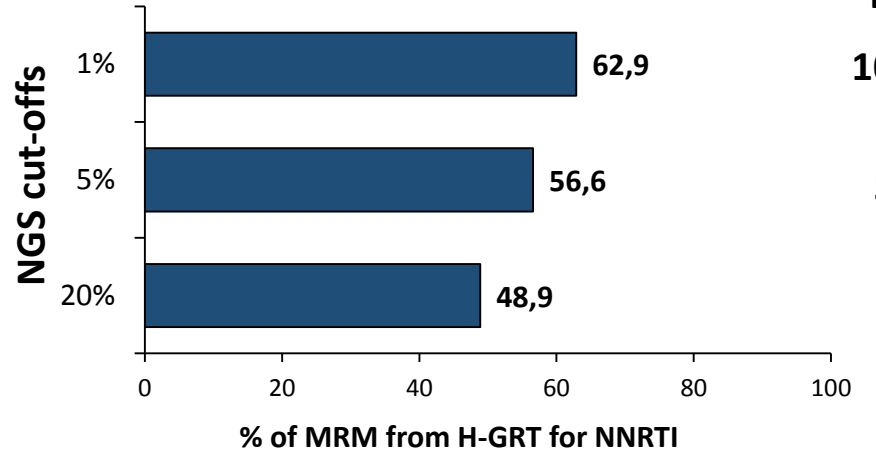

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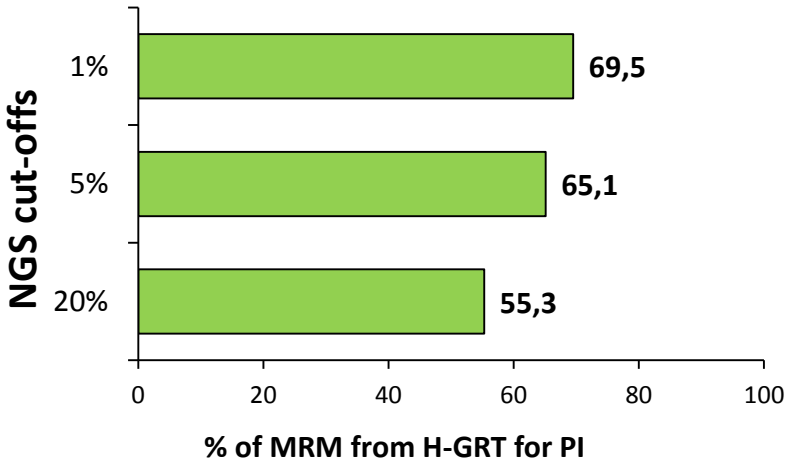

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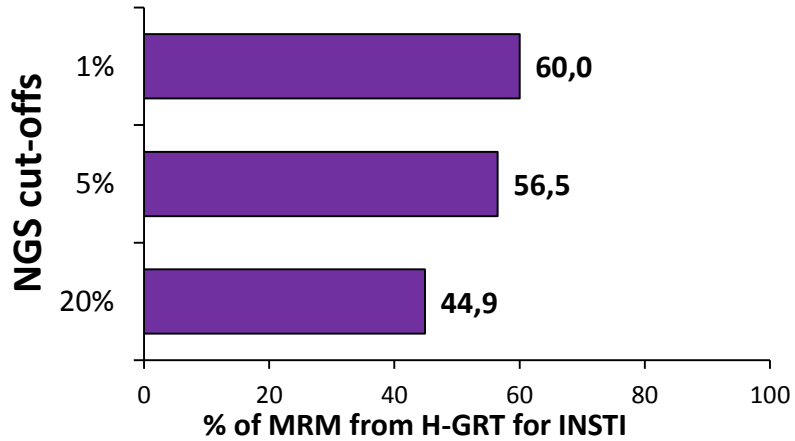


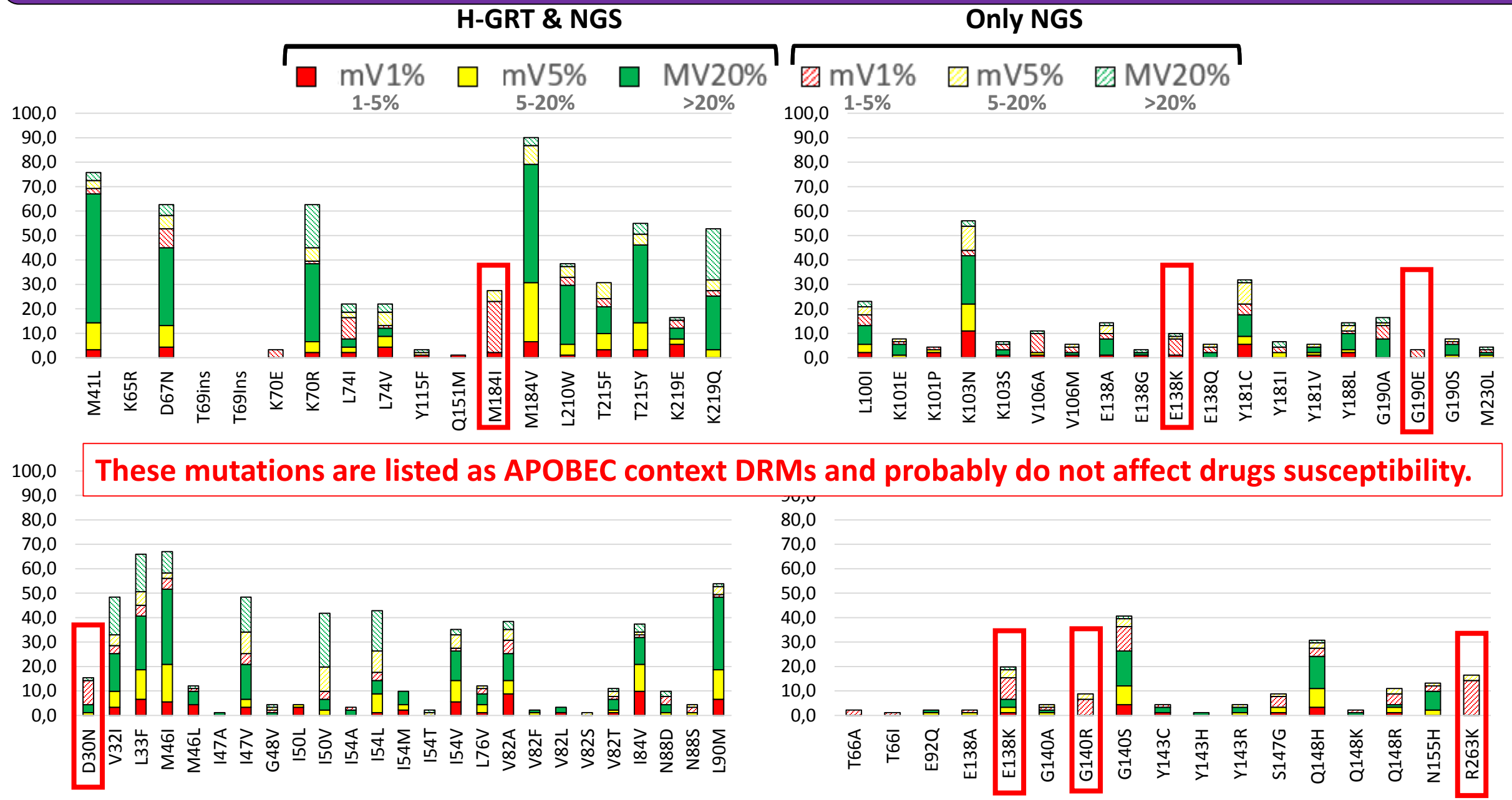
Median (IQR) frequency (N=77)

100% (0%-100%)

33% (0%-100%)

0% (0%-100%)

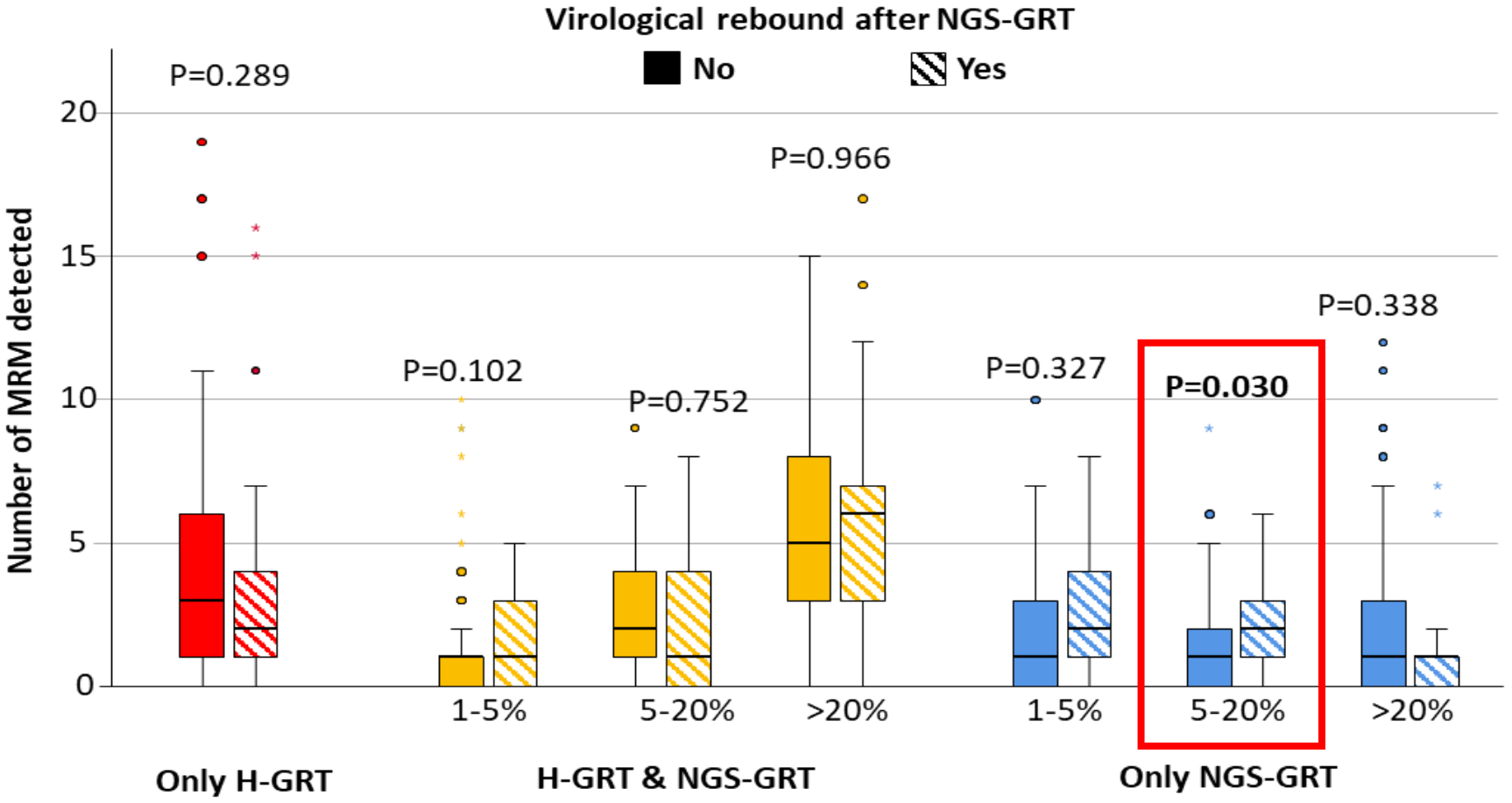
Among specific MRM detected by NGS, M184I, E138K and G190E in RT, D30N in PR and E138K, G140R and R263K in integrase were detected almost (>70%) exclusively by NGS as minority variants at 1-5% of frequency.



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 (98-7,840) copies/mL.

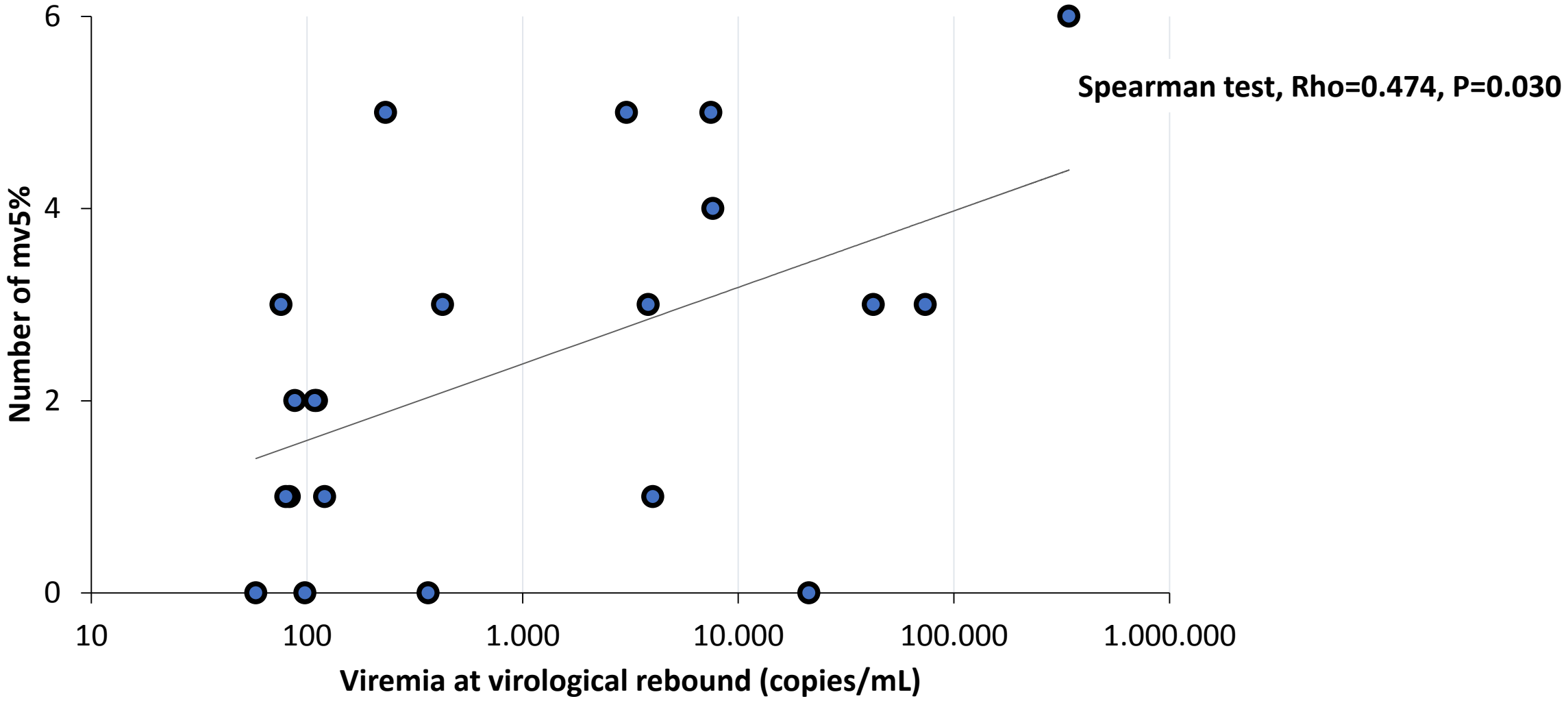
Among them, the median (IQR) number of mV5% detected exclusively by NGS-GRT was higher compared to those who maintained virological control. No significant differences in the number of mV1% and MV20% were observed.

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.



Differences in number of major resistance mutations (MRM) according with virological rebound were evaluated with Mann-Whitney test. P value<0.05 were indicated in boldface.

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



Conclusions

- In HTE MDR virologically suppressed individuals enrolled in the PRESTIGIO registry, NGS-GRT on HIV-1 DNA allows detection of around 60-70% historical MRM and detects considerable new resistance.
- In this population, 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 minority species correlates with loss of virological control and with viremia levels at virological rebound.

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The PRESTIGIO Registry

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