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How Genotypic Resistance Test in PBMC (Proviral DNA) May Help to Identify Hidden Resistance in patients with Low Level or Undetectable HIV-RNA?

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Background

- The presence of resistant HIV strains is routinely verified in plasma samples, since circulating virus variants are considered representative of the viral population escaping the drug pressure.
- Nevertheless, archival HIV DNA present in peripheral blood mononuclear cells (PBMCs) might represent the reservoir of additional drug-resistant viral variants.
- PBMC sequences may be particularly important as a diagnostic tool in patients on antiretroviral therapy (ART) with low or undetectable virus load to plan drug switches for intolerance, toxicity or simplification.

Aim

To characterize the resistance mutations in PBMCs and compare their prevalence in plasma, in order to evaluate the added value in routine clinical practice of genotypic resistance tests (GRTs) executed in PBMCs.

Methods

Selection of Patients

- Patients attending four Italian reference clinical centers and selected by the caring physician for treatment change despite low or undetectable HIV-RNA were tested for PBMC GRT.
- For all patients included in the study, the results of GRT on PBMCs were collected.
- Moreover, all the results of previous or concomitant GRTs on plasma were collected, when available.

Evaluation of Resistance Mutations

- The number and type of major resistance mutations (MRM) (IAS-USA list 2014 and Stanford data base) in PBMCs were evaluated and were compared to those detected in the last available plasma GRT or in cumulative plasma GRTs (for patients with ≥ 2 plasma GRTs).

MRM reported in the IAS-USA list (2014) and/or the HIV Drug Resistance Stanford Database considered for the study:

- i. NRTI mutations: M41L, A62V, K65R, K65E, K65N, D67N, T69ins, K70E, K70R, L74V, V75I, F77L, Y115F, F116Y, Q151M, M184I, M184V, L210W, T215F, T215Y, K219E, K219Q.
 - ii. NNRTI mutations: L100I, K101E, K101P, K103N, K103S, V106A, V106M, V108I, E138A, E138G, E138K, E138Q, E138R, V179L, Y181C, Y181I, Y181V, Y188C, Y188H, Y188L, G190A, G190S, H221Y, P225H, F227C, M230I, M230L.
 - iii. PI mutations: D30N, V32I, M46I, M46L, I47A, I47V, G48V, I50L, I50V, I54L, I54M, Q58E, T74P, L76V, V82A, V82F, V82L, V82S, V82T, N83D, I84V, N88S, L90M.
 - iv. INSTI mutations: T66I, T66A, T66K, E92Q, F121Y, E138A, E138K, G140S, Y143R, Y143H, Y143C, S147G, Q148H, Q148K, Q148R, N155H.
- Multivariable logistic regression analysis was performed in order to assess factors associated with detection of MRM in PBMCs.

Results

Number of Patients Included in the Study and Genotypic Resistance Tests (GRTs)

Genotypic resistance test for protease/reverse transcriptase

- **468** patients were included in the study; for all of them a GRT from PBMCs was available
- **303/468** patients had ≥ 1 plasma GRT
- **149/303** patients had ≥ 2 plasma GRTs (cumulative GRT)

Genotypic resistance test for integrase

- **102/468** a GRT for the integrase from PBMCs was available
- **25/102** patients had ≥ 1 plasma GRT
- **9/25** patients had ≥ 2 plasma GRTs

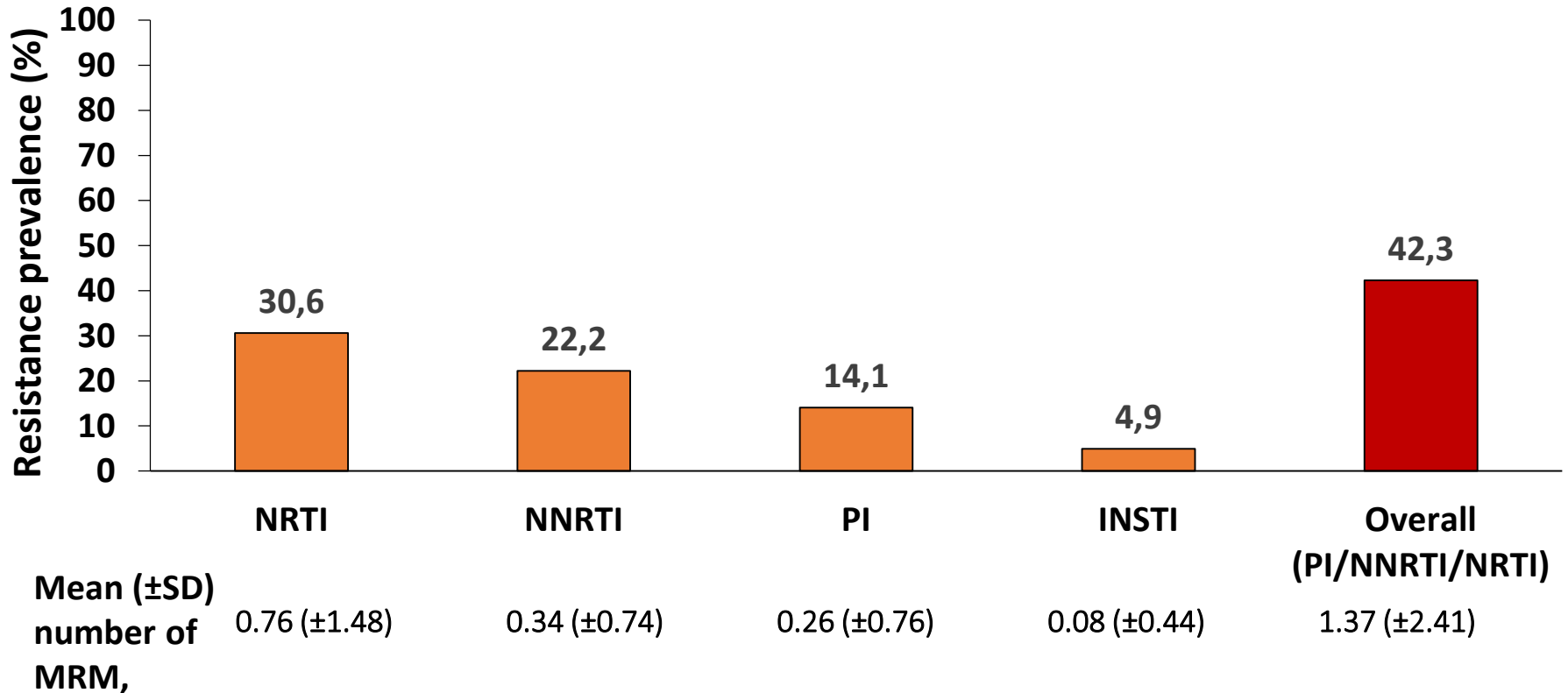
Characteristics of 468 Patients Included in the Study

Variables

Male, n (%)	329 (70.3)
Age, Median (IQR) years	50 (44-54)
Non-B subtype, n (%)	87 (18.6)
Risk factor, n (%)	
<i>Heterosexual</i>	162 (34.6)
<i>Homosexual</i>	104 (22.2)
<i>Intravenous drug user</i>	136 (29.1)
<i>Other/unknown</i>	66 (14.1)
CD4 Nadir, Median (IQR) cells/mm ³	160 (61-290)
CD4 count at DNA test, Median (IQR) cells/mm ³	499 (497-599)
HIV viral load at DNA test, n (%) copies/mL	
<50	289 (61.8)
51-200	114 (24.4)
201-500	24 (5.1)
501-1000	41 (8.7)
Years if antiretroviral treatment, Median (IQR)	8 (2-14)
No. of regimens, Median (IQR)	4 (2-7)
No. of antiretrovirals administered, Median (IQR)	7 (5-10)
No. of previous plasma GRT, median (IQR) ^a	2 (2-3)

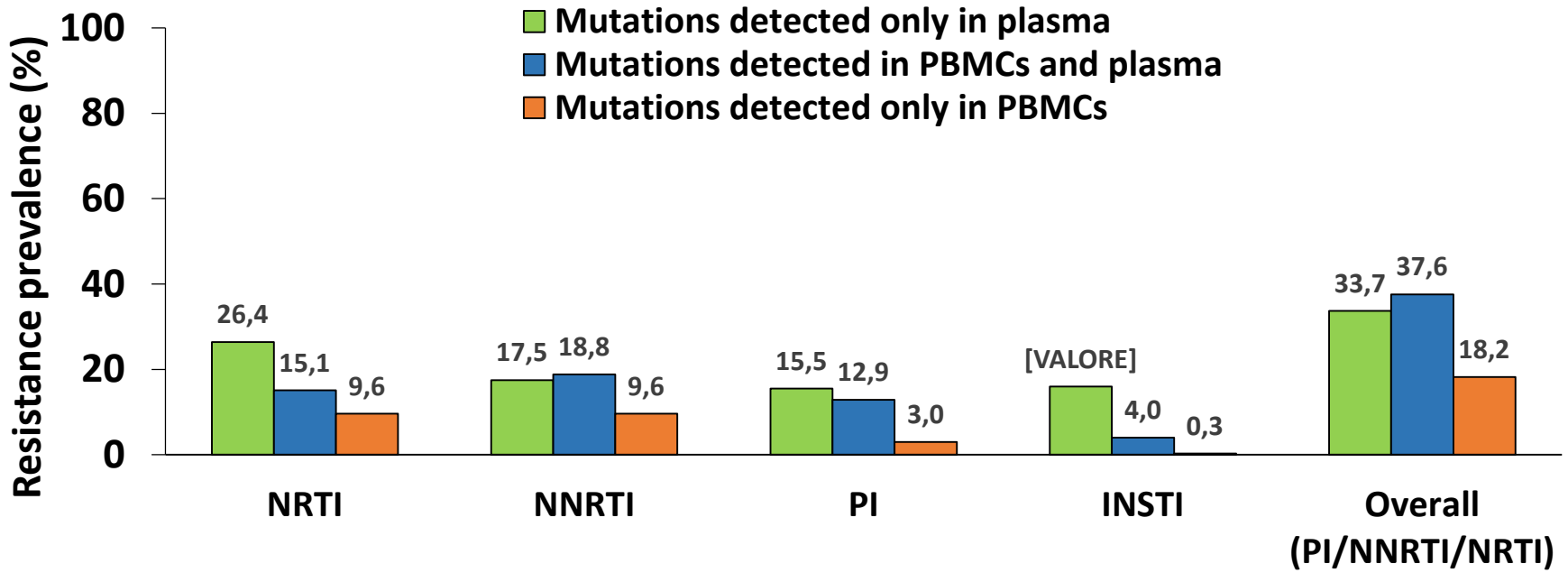
a: calculated on 149 patients with at least 2 plasma GRTs available. GRT: Genotypic resistance test; cART: Combined antiretroviral therapy.

Overall Proportion of Patients with MRM Detected in PBMCs (Proviral DNA – 468 Patients, 102 for INSTI)



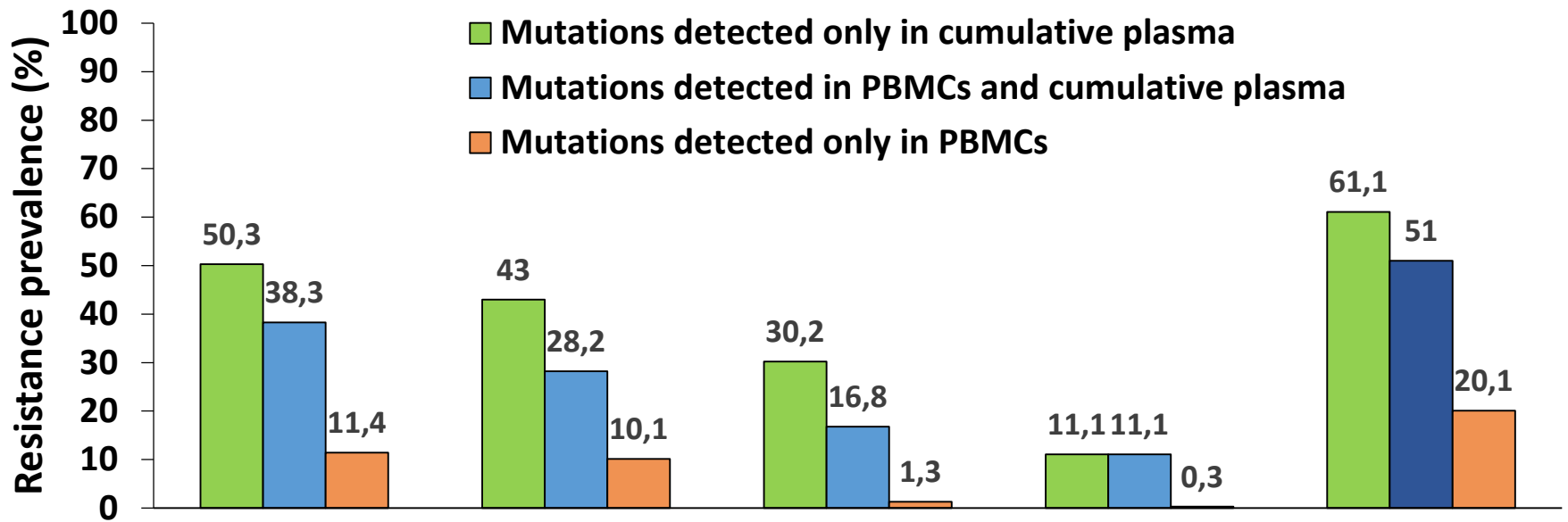
MRM: Major Resistance Mutations

Proportion of Patients with MRM in PBMCs and Plasma GRT (303 Patients with DNA GRT and Last Plasma GRT, 25 Patients for INSTI)



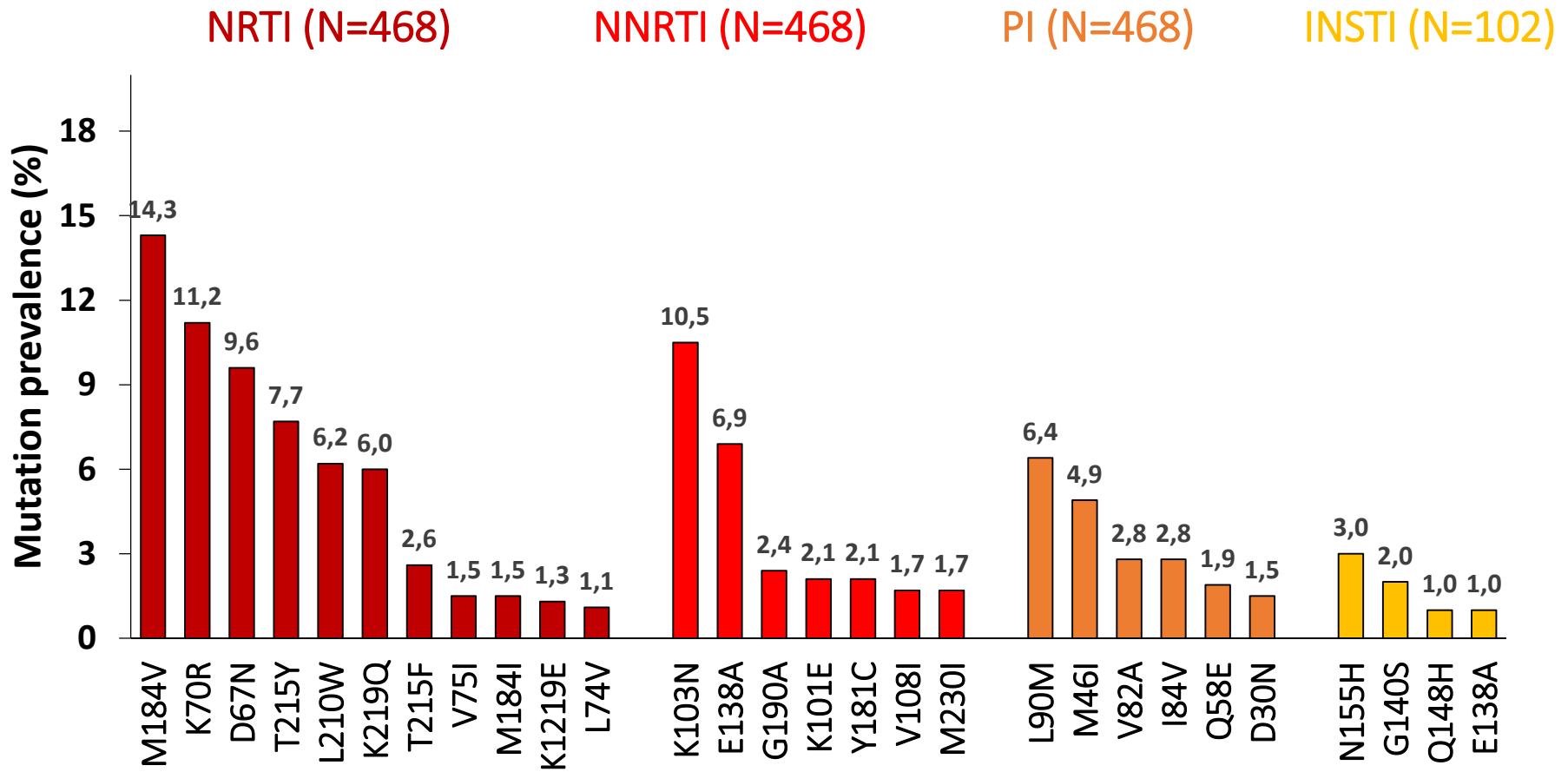
Mean (\pm SD)	■ 0.29 (\pm 0.71)	0.33 (\pm 0.89)	0.16 (\pm 0.37)	0.57 (\pm 1.13)	1.19 (\pm 2.21)
number of	■ 0.23 (\pm 0.54)	0.25 (\pm 0.76)	0.20 (\pm 0.90)	0.66 (\pm 1.39)	1.15 (\pm 2.14)
MRM	■ 0.14 (\pm 0.47)	0.05 (\pm 0.29)	0.00 (\pm 0.00)	0.13 (\pm 0.44)	0.31 (\pm 0.91)

Proportion of Patients with MRM in PBMCs and Cumulative Plasma (149 Patients with DNA GRT and ≥2 Plasma GRTs, 9 Patients for INSTI)



	NRTI	NNRTI	PI	INSTI	Overall (PI/NNRTI/NRTI)
Mean (\pm SD)	1.29 (\pm 1.67)	0.74 (\pm 1.00)	0.77 (\pm 1.39)	0.11 (\pm 0.33)	2.79 (\pm 3.26)
number of	1.03 (\pm 1.66)	0.38 (\pm 0.72)	0.34 (\pm 8.84)	0.44 (\pm 1.33)	1.75 (\pm 2.57)
MRM,	0.13 (\pm 0.37)	0.14 (\pm 0.47)	0.02 (\pm 0.18)	0.00 (\pm 0.00)	0.29 (\pm 0.71)

MRM Most Frequently Detected in PBMCs (Frequency $\geq 1\%$)

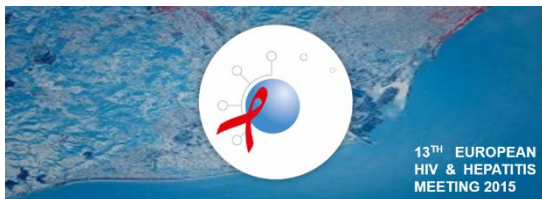


Logistic Regression for Association with MRM in PBMCs

Variables	OR (95% C.I.)	P value	AOR (95% C.I.)	P value
Female gender	0.72 (0.48-1.08)	NS		
Age (by year)	1.03 (1.01-1.05)	0.005	1.01 (0.99-1.04)	NS
IDU as HIV risk Factor	1.32 (1.05-1.67)	0.017	1.37 (1.07-1.75)	0.013
N. of regimens (each)	1.18 (1.12-1.24)	<0.001	1.16 (1.09-1.24)	<0.001
CD4 nadir <200/mmc	1.72 (1.18-2.52)	0.005	1.22 (0.80-1.85)	NS
Years of ARV treatment (per one year increase)	1.05 (1.02-1.09)	<0.001	1.00 (0.96-1.03)	NS
HIV-RNA <50 copies/ml	0.91 (0.62-1.33)	NS		
Non-B HIV subtype	0.34 (0.04-3.04)	NS		
CD4 <200/mmc at PBMC GRT	1.46 (0.91-2.34)	NS		

Conclusions

- GRT in PBMCs (proviral DNA) is able to detect potential hidden resistance.
- Indeed, about 20% of patients harbored mutations not detected in cumulative plasma GRT.
- GRT in PBMCs can improve the results of the resistance test in plasma.
- Further investigations on the impact of major resistance mutations detected only in PBMCs to virological response are needed.



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