GIORNATE INFETTIVOLOGICHE
"LUIGI SACCO"

Milano, 14 - 15 Giugno 2018
Ospedale Luigi Sacco Polo Universitario - ASST Fatebenefratelli Sacco
Aula Magna Polo Lita

Ricerca delle mutazioni conferenti resistenza nel DNA: correlati virologici e clinici

A. Lai
Dipartimento di Scienze Biomediche e Cliniche
Università degli Studi di Milano

DNA Resistant testing in Guidelines

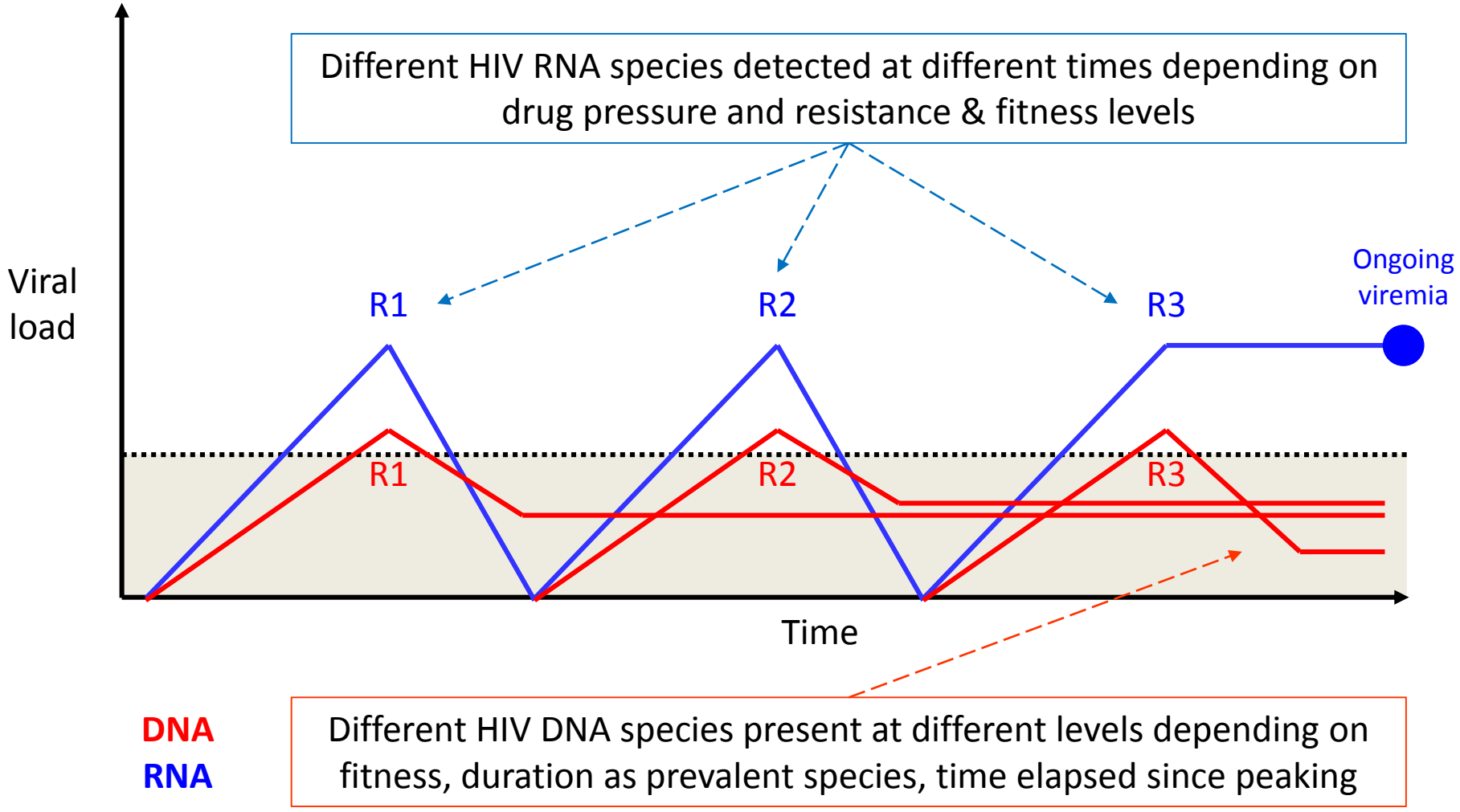
Tabella 5 - Valutazione e interpretazione dei test di resistenza ai farmaci antiretrovirali.

IMPIEGO	RACCOMANDAZIONE (FORZA/EVIDENZA)	RIFERIMENTI BIBLIOGRAFICI
Valutazione di ciascun test di farmaco-resistenza alla luce dei test precedenti (se disponibili), data la possibilità che alcune mutazioni siano non più rilevabili dal plasma, ma permangano archiviate nelle sequenze provirali dei reservoir cellulari [nota 1].	[AII]	[18]
I risultati dei test di resistenza dovrebbero essere interpretati tramite algoritmi gestionali con il supporto di esperti che possano avvalersi anche di dati immuno-virologici e terapeutici e di parametri aggiuntivi (es. aderenza).	[AI]	
Una valutazione della presenza di farmaco-resistenza a livello del DNA provirale appare utile in situazioni in cui la determinazione su RNA plasmatico non fornisca risultati dirimenti [nota 2].	[BIII]	[26-28]
Una valutazione della presenza di farmaco-resistenza a livello del liquor appare utile (al fine di un'impostazione terapeutica mirata) in pazienti in cui sia stata effettuata la puntura lombare a seguito di chiari segni e sintomi di impegno neurologico dell'infezione di HIV. Laddove siano presenti, nel virus liquorale, segni di resistenza ai farmaci antivirali, eventualmente associati anche ad una concentrazione virale più alta nel liquor rispetto al plasma, l'impostazione terapeutica dovrebbe tenere conto della situazione virologica liquorale.	[BIII]	[3]
L'uso e l'interpretazione dei test di resistenza per i sottotipi non-B di HIV-1 non si discostano (attualmente) da quelli in uso corrente per i sottotipi B.	[BI]	[20]
Nota 1: I riscontri di precedenti fallimenti virologici o di mutazioni di resistenza emerse in precedenti test rappresentano indicazioni utili, consentendo di evitare l'impiego di farmaci potenzialmente inefficaci, anche in caso di mancato rilievo di resistenze nell'ultimo test, per ragioni talvolta connesse con la sensibilità del metodo utilizzato. **Nota 2**: Il test genotipico da DNA provirale può fornire informazioni utili sulla resistenza presente nei reservoir, soprattutto per quei pazienti con viremia bassa o al di sotto delle 50 copie/mL (quindi in successo virologico) per i quali è ritenuta appropriata, da un punto di vista clinico, una semplificazione terapeutica.		

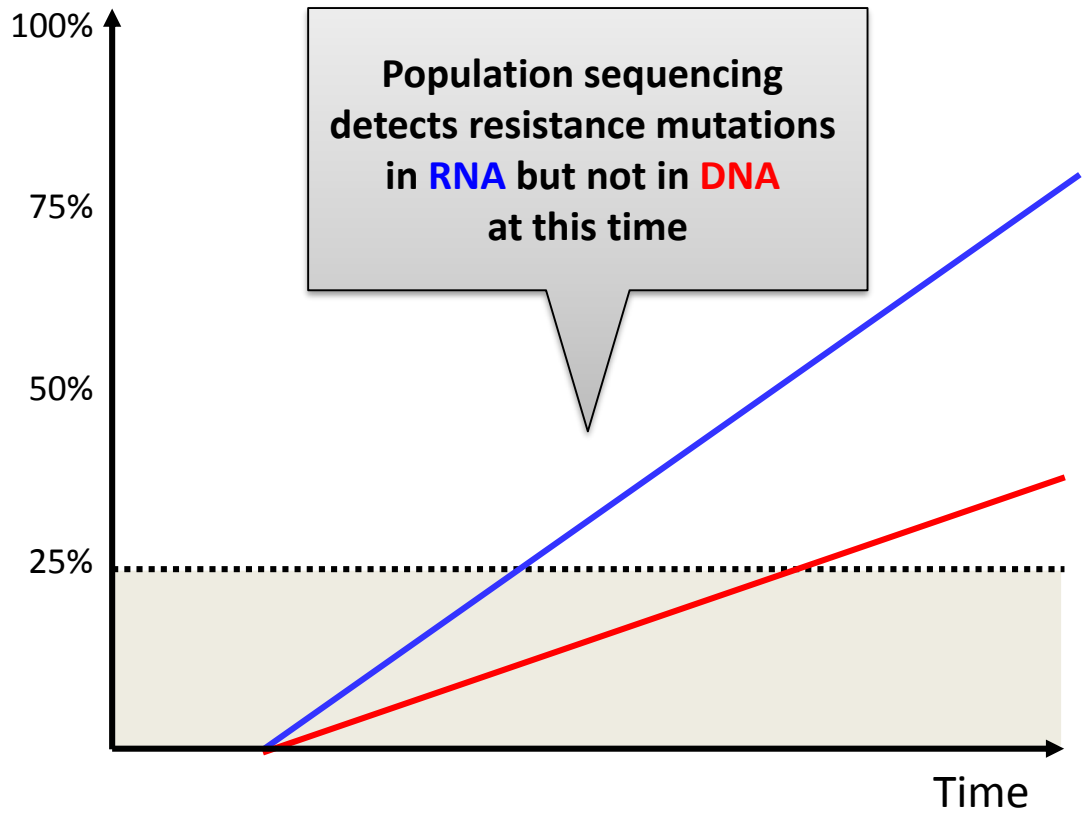
Tabella 11 – Valutazione della presenza della farmaco-resistenza nel DNA provirale in pazienti in soppressione virologica.

IMPIEGO	RACCOMANDAZIONE (FORZA/EVIDENZA)	RAZIONALE/NOTE	RIFERIMENTI BIBLIOGRAFICI
1. Pazienti in successo virologico	[BIII]	Nei pazienti con viremia al di sotto delle 50 copie/mL (quindi in successo virologico) per i quali è ritenuta appropriata, da un punto di vista clinico, una semplificazione terapeutica il test genotipico da DNA provirale può fornire informazioni utili sulla resistenza presente nei reservoir.	[26-28]

Kinetics of drug resistant mutants in plasma HIV RNA and cellular HIV DNA

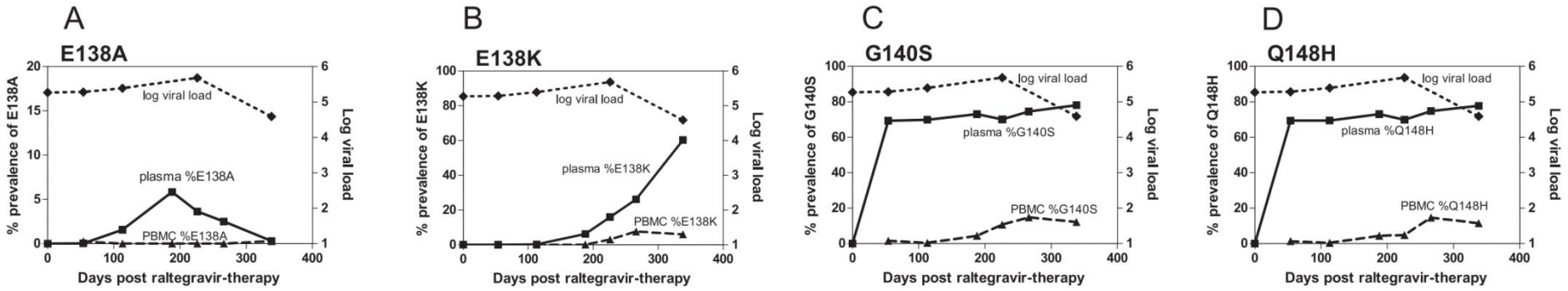


Kinetics of drug resistance mutations in plasma RNA vs. PBMC DNA
At treatment failure



Dynamics of INSTI resistance mutations

Subject #3501



# Patient	INSTI DRM	Days on RAL	% in RNA	% in DNA
3180	Q148H/G140S	177	100	1
3242	N155H	224	100	36
3501	Q148H/G140S	338	78	11
3508	Y143R	197	100	0

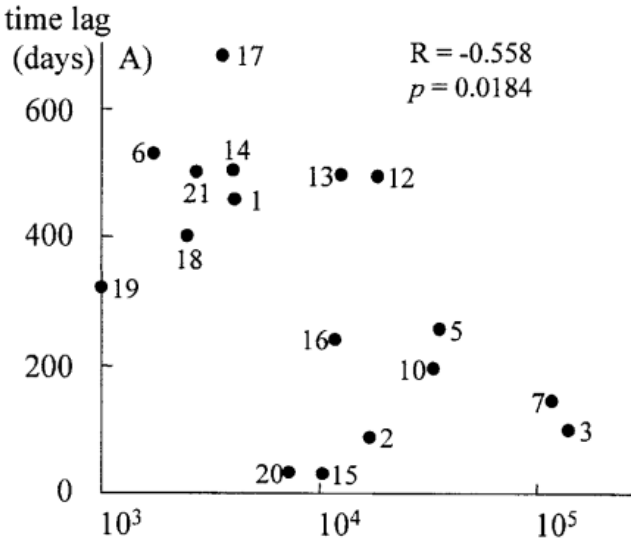
Lee et al., PLoS ONE 2012

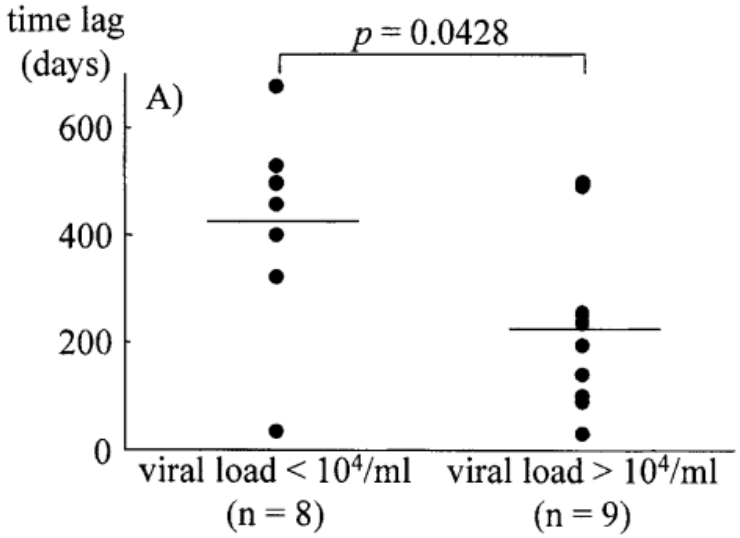
Dynamics of PI resistance mutations

- 275 plasma RNA and 211 PBMC DNA HIV-1 PR sequences from 22 patients on PI therapy

Viral Load in Plasma (Copies/mL)	Number of Patients	Mean Number of Mutations in		*P* Value[a]
		Plasma	Peripheral Blood Mononuclear Cells	
$<10^4$	10	4.19	1.78	0.0076
$>10^4$	12	5.24	4.02	0.0216
Total	22	4.76	3.00	0.0004

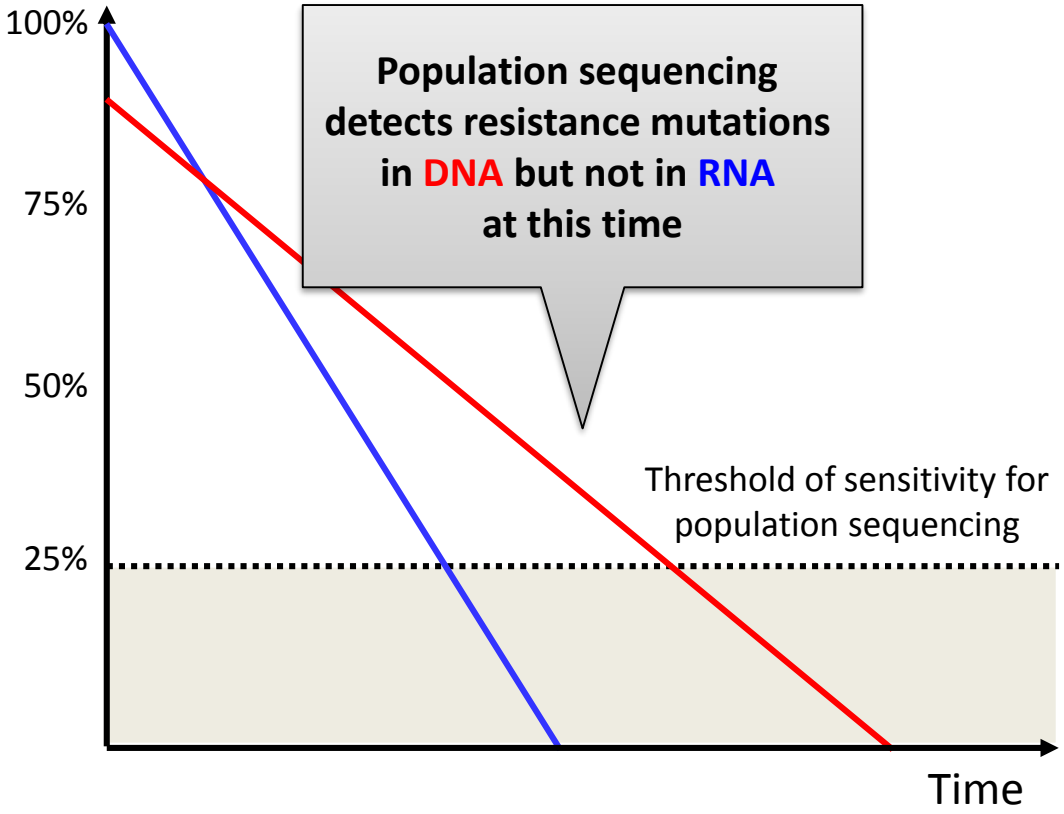
[a]Wilcoxon signed rank test.





Xiuqiong et al., JAIDS 2003

Kinetics of drug resistance mutations in plasma RNA vs. PBMC DNA
Following drug removal



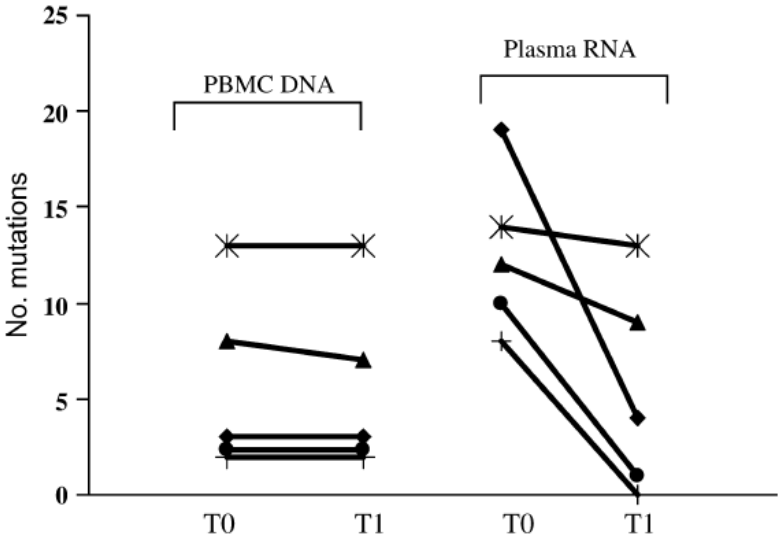


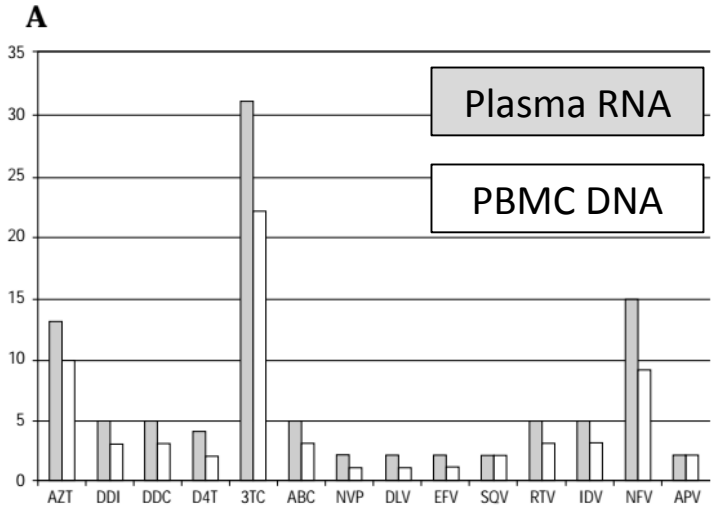
FIGURE 4. Number of drug-resistant mutations in plasma RNA and in PBMC DNA before (T0) and after treatment interruption (T1).

Turriziani et al., Clin Science 2007

Kinetics of drug resistance mutations in plasma RNA vs. PBMC DNA

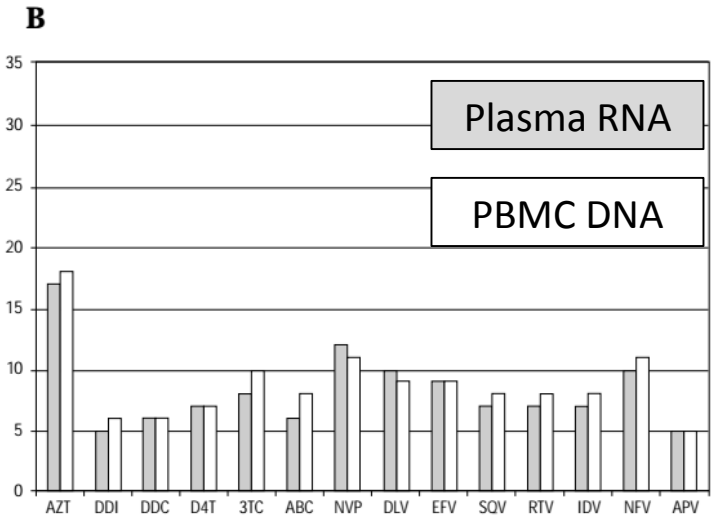
OFF vs. ON therapy

A. 58 patients failing their first ART after reaching undetectable viraemia for at least 6 months



- More drug resistance in **plasma RNA** than in PBMC DNA in the **on-therapy** group (*p=0.004*)

B. 50 patients after a median of 18.6 weeks after treatment interruption following at least 2 (median 4, range 2-11)



- More drug resistance in **PBMC DNA** than in plasma RNA in the **off-therapy** group (*p=0.04*)

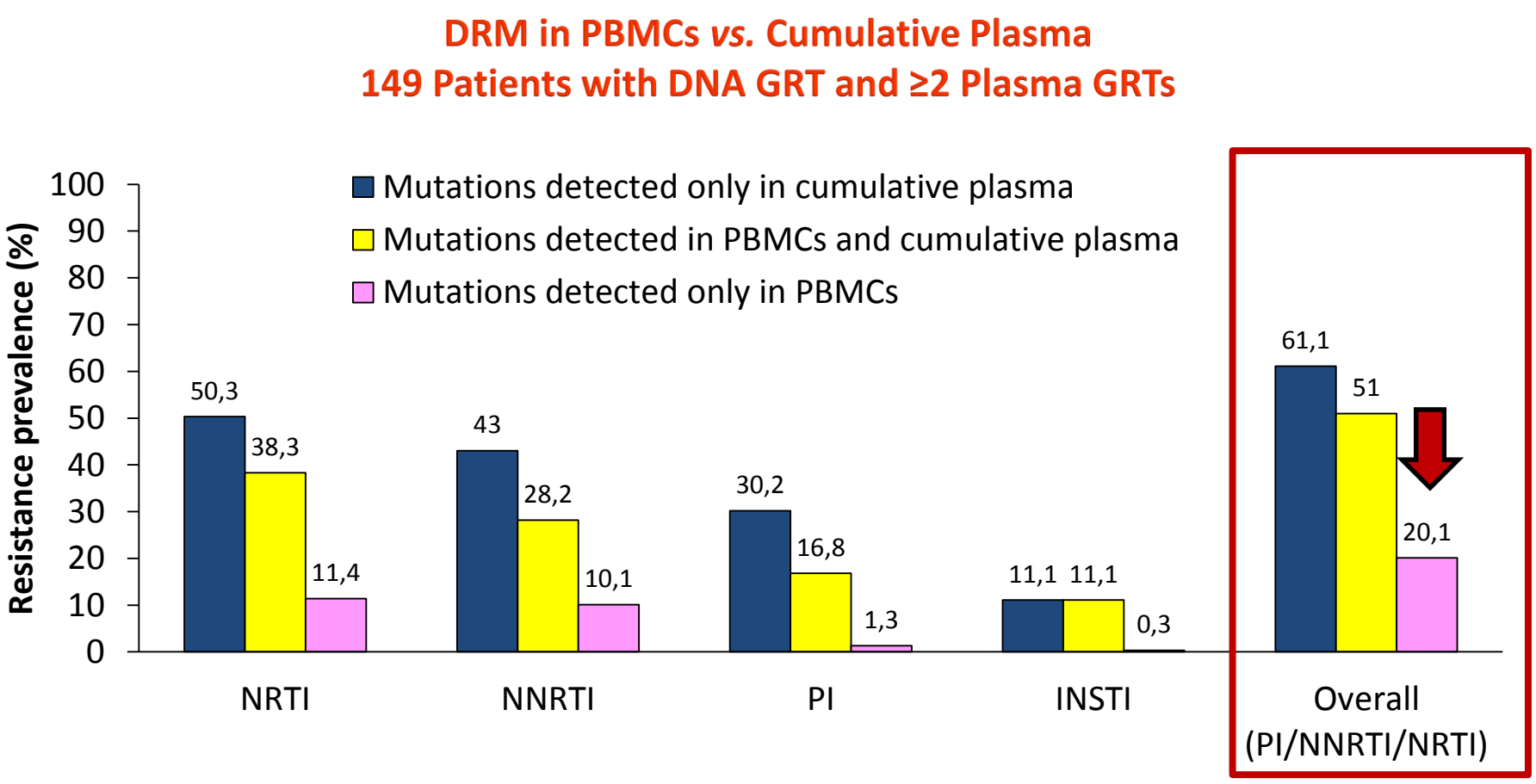
Venturi et al., Antivir Ther 2002

Use of HIV DNA rather HIV RNA for drug resistance genotyping in drug-naïve patients?

Study	No Patients	Major No of detected DRM
Lubke 2015	48	DNA
Steegen 2007	10	RNA
Vicenti 2007	169	RNA
Parisi 2007	288	RNA
Bon 2007	29	DNA
Chew 2005	11	RNA
Total	**555**	

- ✓ **DRMs in RNA than in DNA**
- ✓ However, **cases with resistance detected only in plasma RNA or only in PBMC DNA** were documented
- ✓ Potential predictors of DNA vs. RNA discrepancy include time from diagnosis, viral load, type of mutation
- ✓ **PBMC DNA could better integrate, rather than replace, plasma RNA testing in drug-naïve patients**

Genotypic resistance test in proviral DNA can identify resistance mutations never detected in historical genotypic test in patients with low level or undetectable HIV-RNA



Zaccarelli, J Clin Virol 2016

Genotypic resistance test in proviral DNA can identify resistance mutations never detected in historical genotypic test in patients with low level or undetectable HIV-RNA

Table 2
Factors associated with detection of resistance in PBMCs.

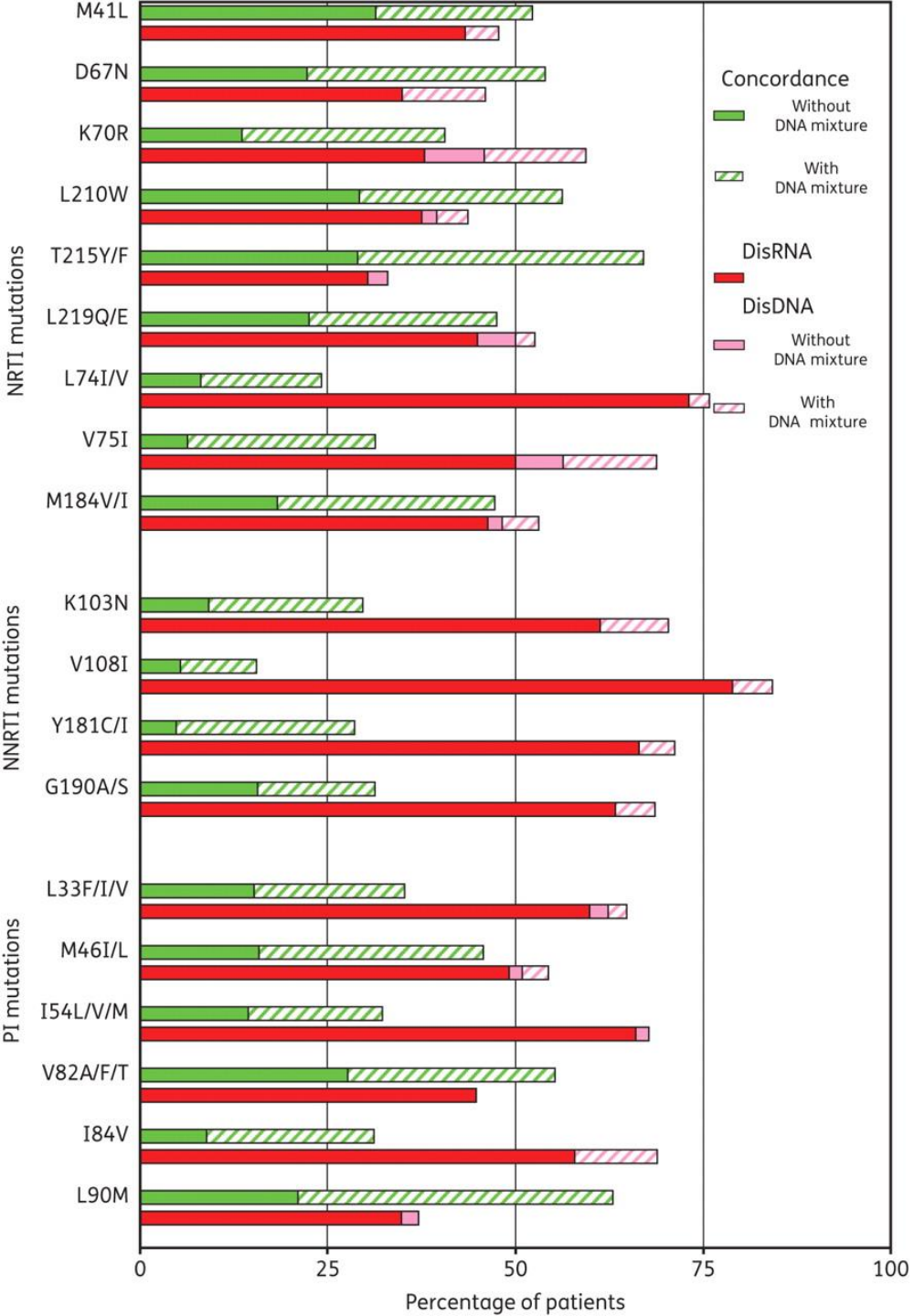
Variables	OR[a] (95% C.I.)	P value	AOR[b] (95% C.I.)	P value
Age (by year)	1.03 (1.01–1.05)	0.005	1.01 (0.99–1.04)	0.337
Gender (female vs. male)	0.72 (0.48–1.08)	0.111		
IDU as HIV Risk Factor	**1.32 (1.05–1.67)**	**0.016**	**1.36 (1.06–1.75)**	**0.016**
HIV-RNA < 50 copies/mL at PBMC genotyping	0.91 (0.62–1.33)	0.613		
CD4 count at PBMC genotyping (per 50 cell/mm^{3}increase)	**0.96 (0.93–0.99)**	**0.012**	**0.96 (0.92–0.99)**	**0.033**
CD4 count nadir (per 50 cell/mm^3 increase)	0.96 (0.91–1.00)	0.070	1.04 (0.98–1.11)	0.203
No. of previous regimens (each)	**1.18 (1.12–1.24)**	**<0.001**	**1.18 (1.04–1.33)**	**0.008**
Years of cART exposure (each)	1.05 (1.02–1.09)	<0.001	0.99 (0.97–1.03)	0.958
No. of drugs used (each)	1.18 (1.12–1.24)	<0.001	1.00 (0.87–1.14)	0.950

AOR: adjusted odd ratio; C.I.: Confidence interval; cART: combined antiretroviral therapy; IDU: injection drug user; OR: odd ratio.

[a] Univariable logistic regression.

[b] Multivariable logistic regression. Variables with a p value < 0.1 at univariable analysis were retained in multivariable model. In bold factors significantly associated to resistance detection by uni-multi variable logistic regression.

Zaccarelli, J Clin Virol 2016

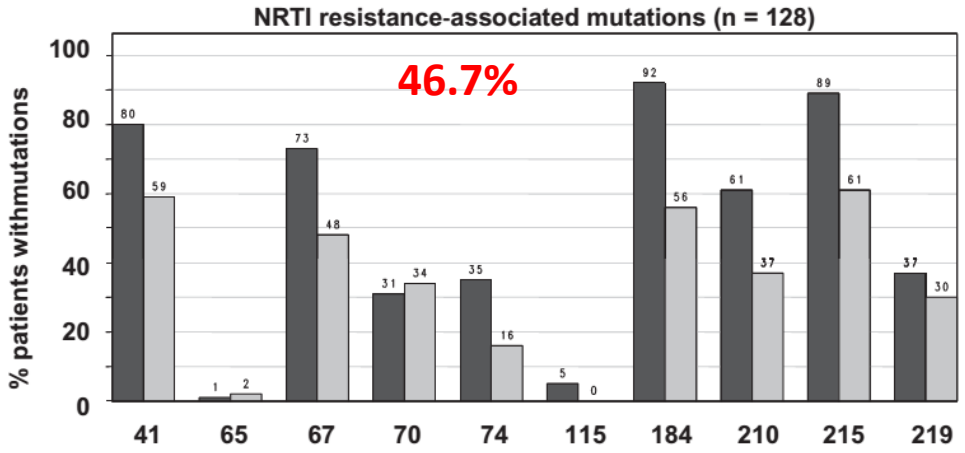


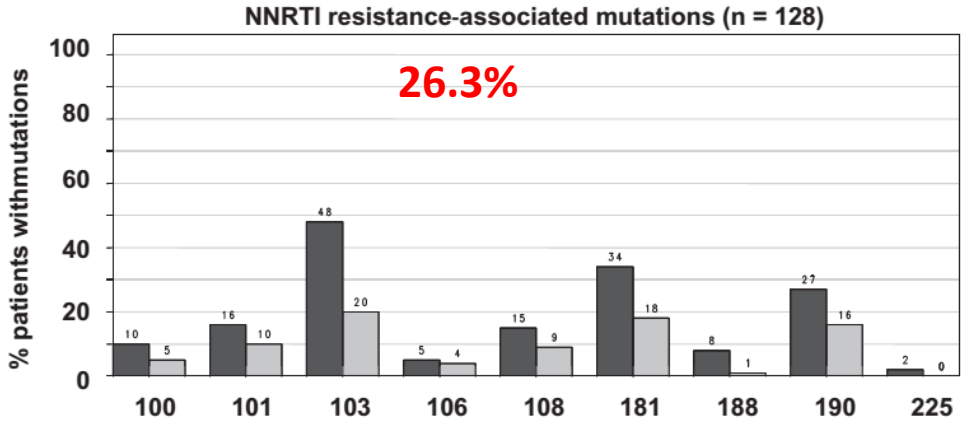
Historical HIV-RNA resistance test results are more informative than proviral DNA genotyping in cases of suppressed or residual viraemia

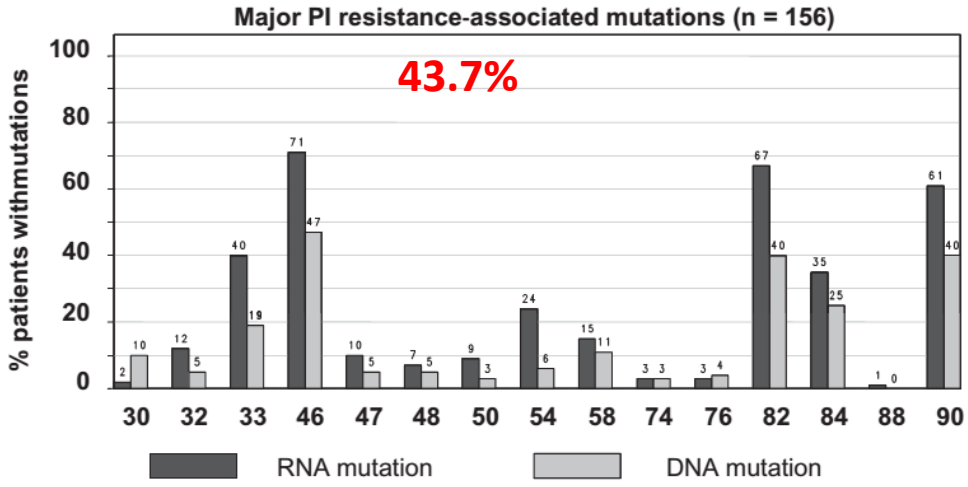
n=151

- ✓ RT: 134 current DNA compared with 443 past RNA
- ✓ PR: 141 DNA compared with 462 past RNA
- ✓ **Mutations detected more in past RNA genotypes than in current DNA genotype**
- ✓ DNA has more mixtures with respect to RNA

Wirden, JAC 2011





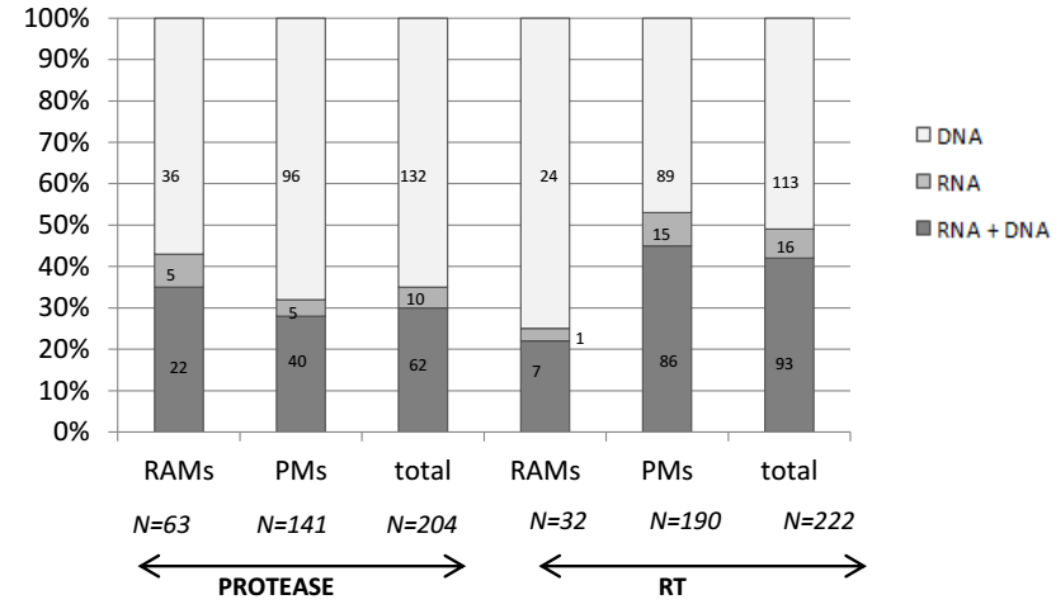


Historical HIV-RNA resistance test results are more informative than proviral DNA genotyping in cases of suppressed or residual viraemia

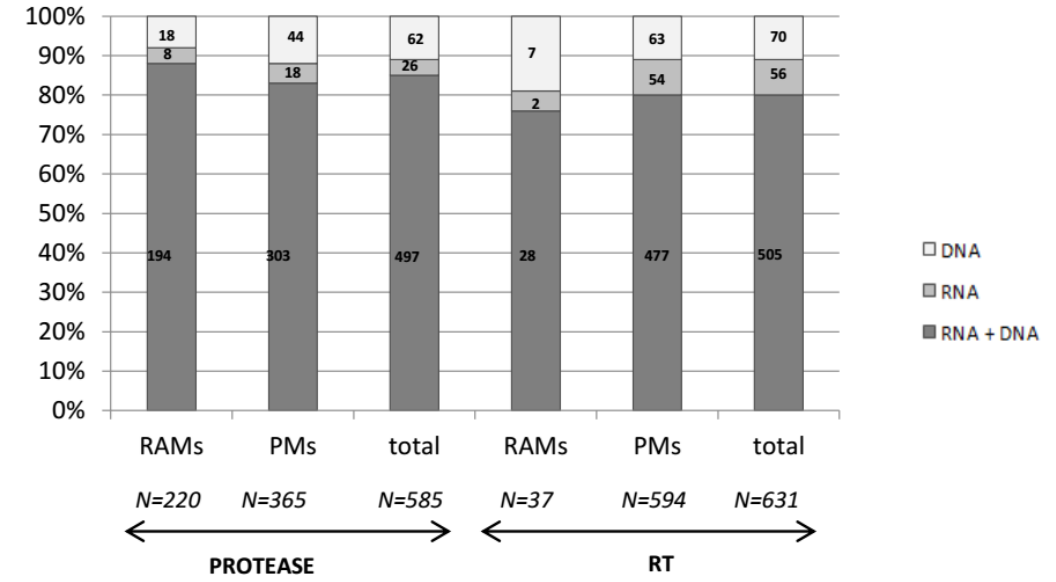
- ✓ 169 patients from the EASIER trial (RAL replacing T20)
- ✓ 716 RNA (Median 4) earlier genotypes vs. 1 DNA genotype
- ✓ Median RAMs in RNA and DNA:
  - ✓ 5 and 4 for NRTIs ($P < 0.001$)
  - ✓ 2 and 1 for NNRTIs ($P < 0.001$)
  - ✓ 10 and 8 for PIs ($P < 0.001$)
- ✓ Resistance exclusively in RNA or in DNA:
  - ✓ **63% RNA vs. 13% DNA - NRTI**
  - ✓ **47% RNA vs. 1% DNA - NNRTI**
  - ✓ **50% RNA vs. 7% DNA - PI**

Delaugerre, HM 2012

DNA data from defective viruses



DNA data from non defective viruses

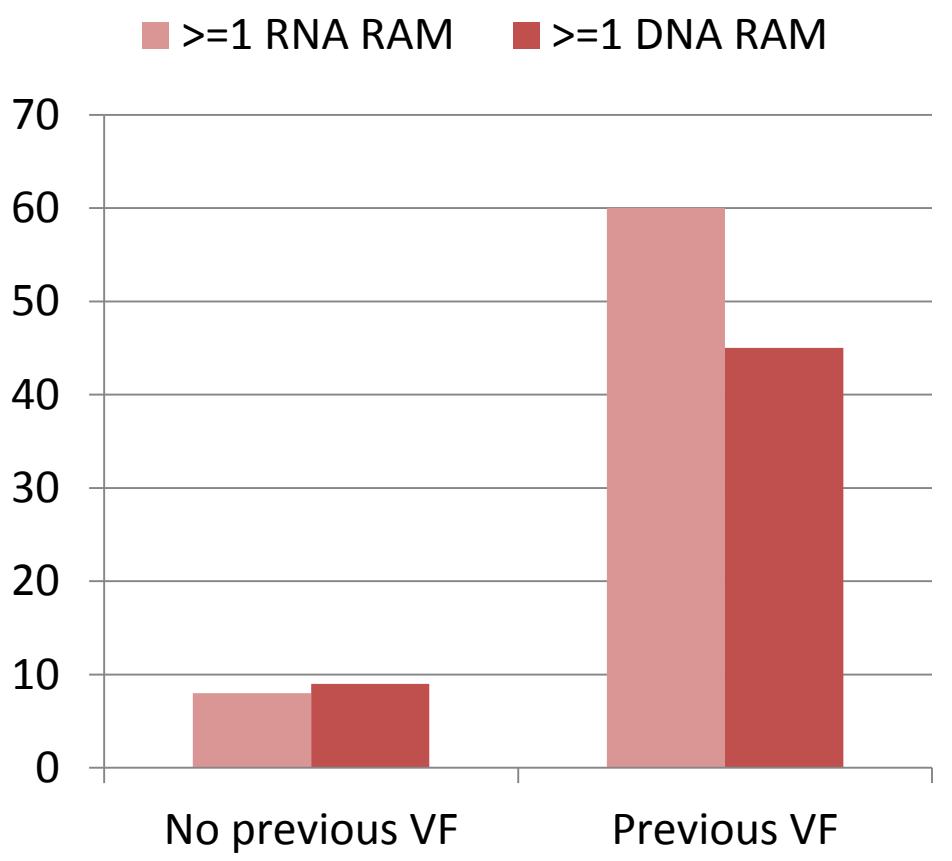


Historical HIV-RNA resistance test results are more informative than proviral DNA genotyping in cases of suppressed or residual viraemia

- ✓ 69 virologically suppressed patients with no history of virological failure
- ✓ DNA resistance compared with pre-ART plasma RNA resistance
- ✓ Median time between RNA and DNA testing 47 months (IQR 29-63)
- ✓ Stop codon in 23% (16/69) of DNA sequences
- ✓ Within the non-defective strains, high DRM concordance rate between RNA and DNA (protease (194/220 = 88%; reverse transcriptase 28/37 = 76%)

Allavena, J Virol Meth 2017

Usefulness of an HIV DNA resistance genotypic test in patients who are candidates for a switch to the TDF/FTC/RPV combination

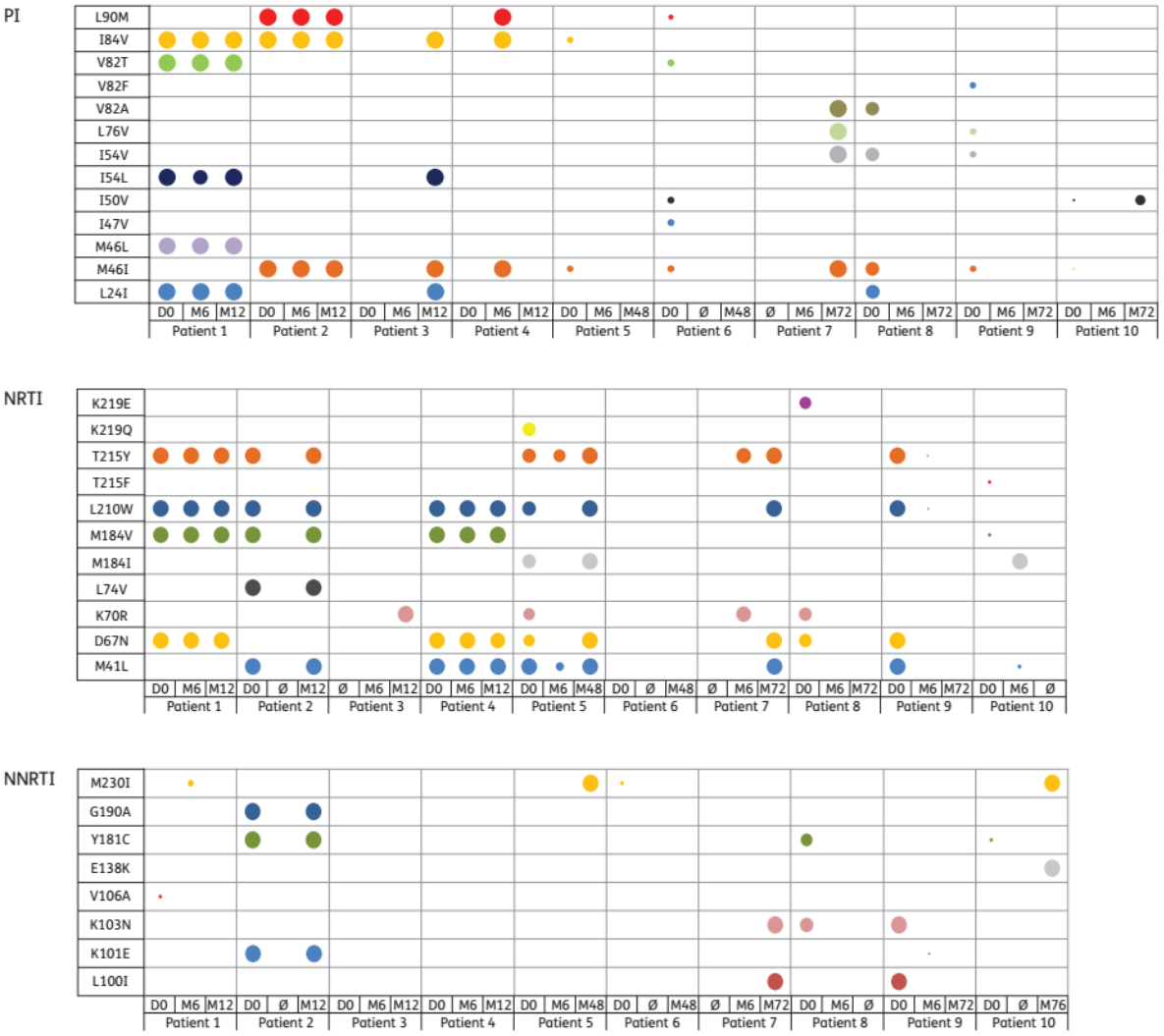


RAMs: K65R, L100I, K101E/P, 138A/G/K/R/Q, V179L, Y181C/I/V, M184V/I, Y188L, H221Y, F227C, M230I/L

- ✓ 130 patients without previous virological failure (**non-VF**) and 114 patients with at least one previous virological failure (**VF**)
- ✓ DNA at 1 year following VL suppression compared with a prior available RNA (46 months earlier in non-VF and 37 months earlier in VF)
- ✓ At least one mutation for TDF or FTC or RPV
  - ✓ Comparable in non-VF, 8% RNA vs. 9% DNA
  - ✓ Different in VF, 60% RNA vs. 45% DNA
- ✓ **DNA at later time point does not recapitulate RNA drug resistance info if there were previous failures**

Lambert-Niclot, JAC 2016

Dynamics of HIV DNA quasispecies in blood cells of pretreated patients who achieved sustained virological suppression



n=10, 29 DNA samples

① Viral quasispecies with the same DRAMs at the same level as at the time of virological failure (salvage ART exerting the same selective pressure as previous failing regimens) (#1, 2, 4, 5)

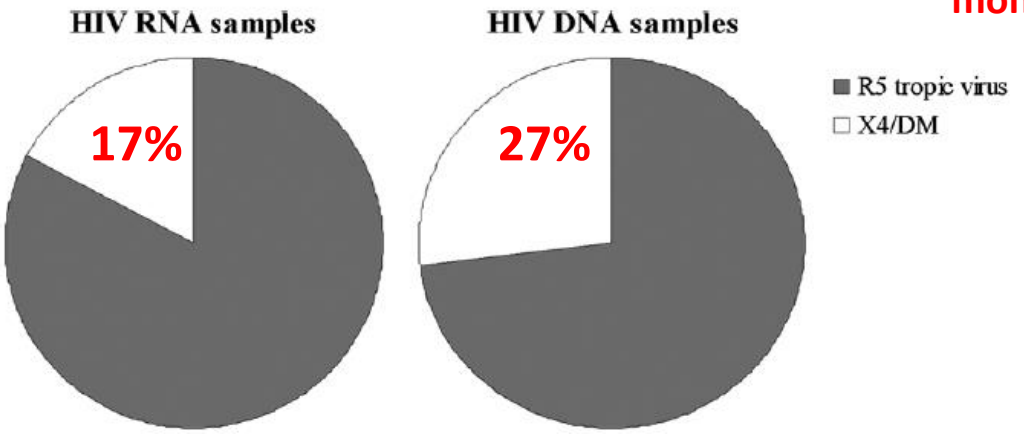
② Some DRAMs no longer detected or resistance profiles distinct from those of previous failing regimens (salvage ART with new drug class) (#3, 6, 8, 9, 10)

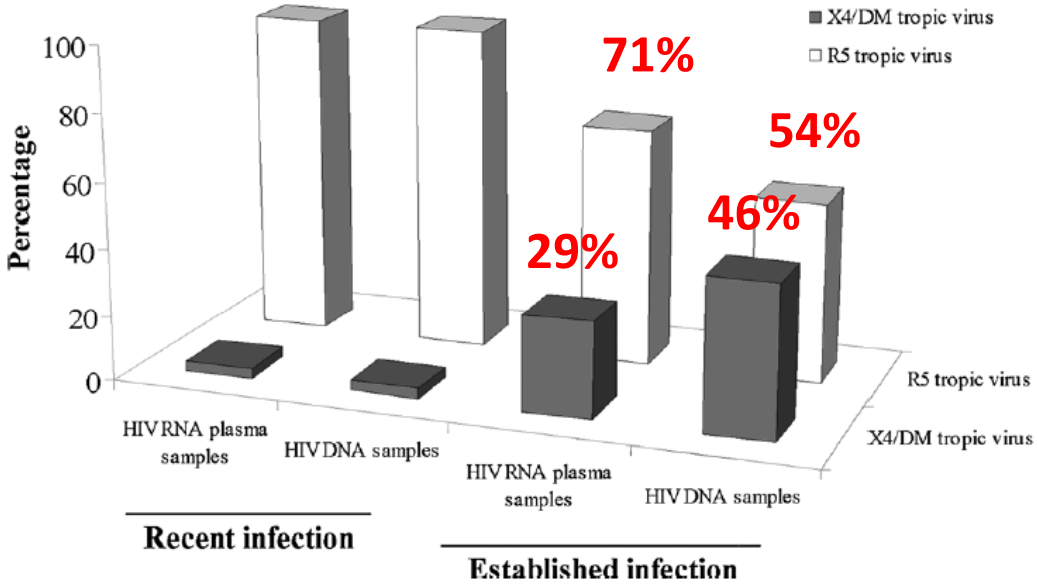
① Emerging DRAMs associated with salvage ART (#3, 5, 7, 10)

Gantner, JAC 2016

Use of HIV DNA for genotypic tropism testing

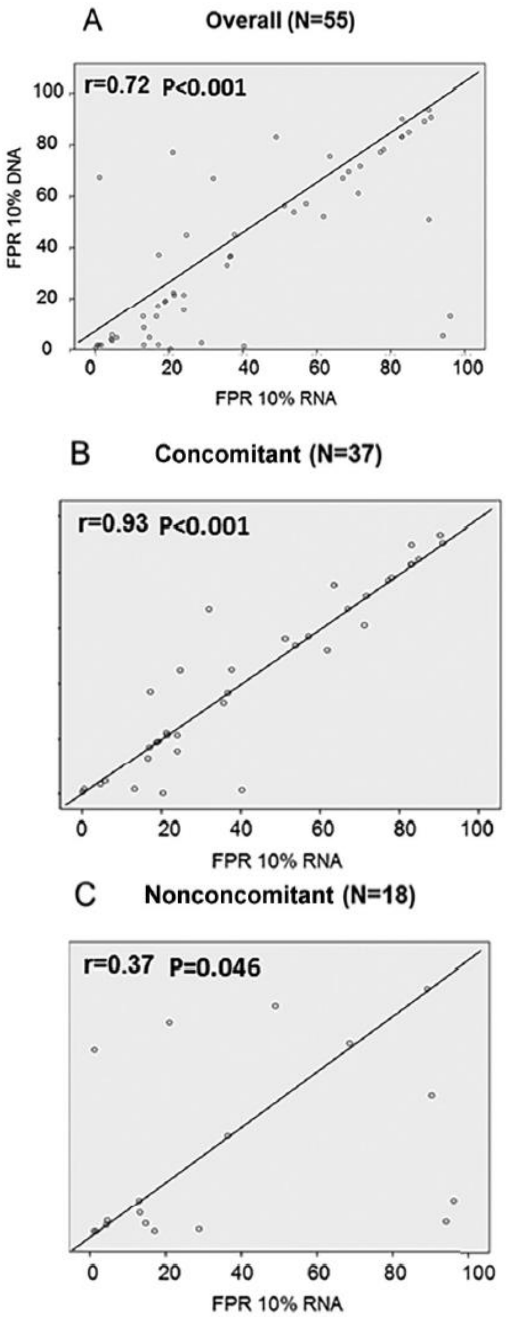
n=75





Bon et al., J Med Virol 2015

n=55, 37 pt with concomitant paired samples, 18 with paired samples collected within 1-11 months



Fabeni et al., JCM 2015

Correlates

- Viral loads >1000 copies/ml are an indicator of proviral drug resistance mutations in PBMCs (Sen et al.,2007).
- Resistance detected in PBMCs, together with low nadir CD4 count (<100 cell/mm^3) and previous short virological control are associated with a higher probability of virological failure in virologically suppressed patients (Armenia et al. 2018).

Conclusions

- Viruses in the reservoir are diverse and reflect, in patients who have received extensive treatment, mutations selected by previous therapy.
- Whether genetic changes are a consequence of preexisting minority variants in plasma or recirculation from other compartments of cells by various strains is unknown.
- The probability of finding a resistant variant within the reservoirs depends, at least in part, on the period over which this variant is able to replicate; thus a delay in changing a failed therapeutic regimen might favor drug-resistant mutants entering in the reservoir.
- Although plasma HIV-1 RNA remains the material of choice for drug resistance testing, several studies conducted in untreated or failing patients, have underlined the value of proviral DNA as additional source of information.
- DNA sequencing cannot be proposed as a substitute for plasma RNA sequencing, however, where plasma HIV-1 RNA cannot be sequenced, proviral DNA may represent an alternative and easy source for resistant testing.


THE END