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Background

Recent studies have analysed in integrase strand transfer inhibitor (INSTI) naïve patients certain specific HIV-1 integrase (IN) mutations associated with resistance to raltegravir. Their assessment by parallel allele-specific sequencing and allele-specific-PCR showed no association with virological response to raltegravir (Liu et al, CROI 2009, Charpentier et al, CROI 2009). We extended the observation to all mutations today associated with resistance to raltegravir, and assessed (by population- and ultra-deep sequencing) their prevalence and evolution among HIV-infected patients starting raltegravir treatment.

Aim

To correlate integrase mutations present at baseline with virological outcome and appearance of mutations at failure, by combining standard Sanger and ultra-deep "454" genotyping and phenotyping assays.

Methods

111 HIV-1 infected treatment-experienced patients with triple-class resistant virus who received raltegravir plus optimized-background-therapy (OBT) were analyzed (Tab.1).

IN genotyping together with plasma HIV-1-RNA were assessed at baseline and during raltegravir treatment.

IN genotyping at baseline was performed on plasma HIV-RNA of all 111 patients by bulk Sanger sequencing, while 454-pyrosequencing was done in 23 (14 failing patients and 9 with virological success).

IN 454 pyrosequencing covered IN region from aa 66 up to aa163; IN haplotypes covered aa130 up to aa163.

IN phenotyping was performed by homologous recombination of IN region with a IN-deleted HXB2-based backbone. Recombinant viruses were titrated and tested for raltegravir and elvitegravir susceptibility.

The durability of antiretroviral and immunological activity was assessed by the following endpoints measured at weeks 24: HIV RNA <50 copies/mL, HIV RNA <400 copies/mL, change from baseline in plasma HIV RNA (log₁₀ copies/mL) and change from baseline in CD4 cell count (cells/mm³).

Stanford raltegravir-resistance mutations (primary: N155H, Q148H/R/K, Y143R/C; and secondary: L74M, E92Q, T97A, F121Y, E138A/K, G140A/S, Y143H, S147G, V151I, N155S, E157Q, G163R, I203M, S230R/N) have been analyzed. Mutations detected at levels ≥0.1% (with ≥10 reads) were considered minor variants.

Results

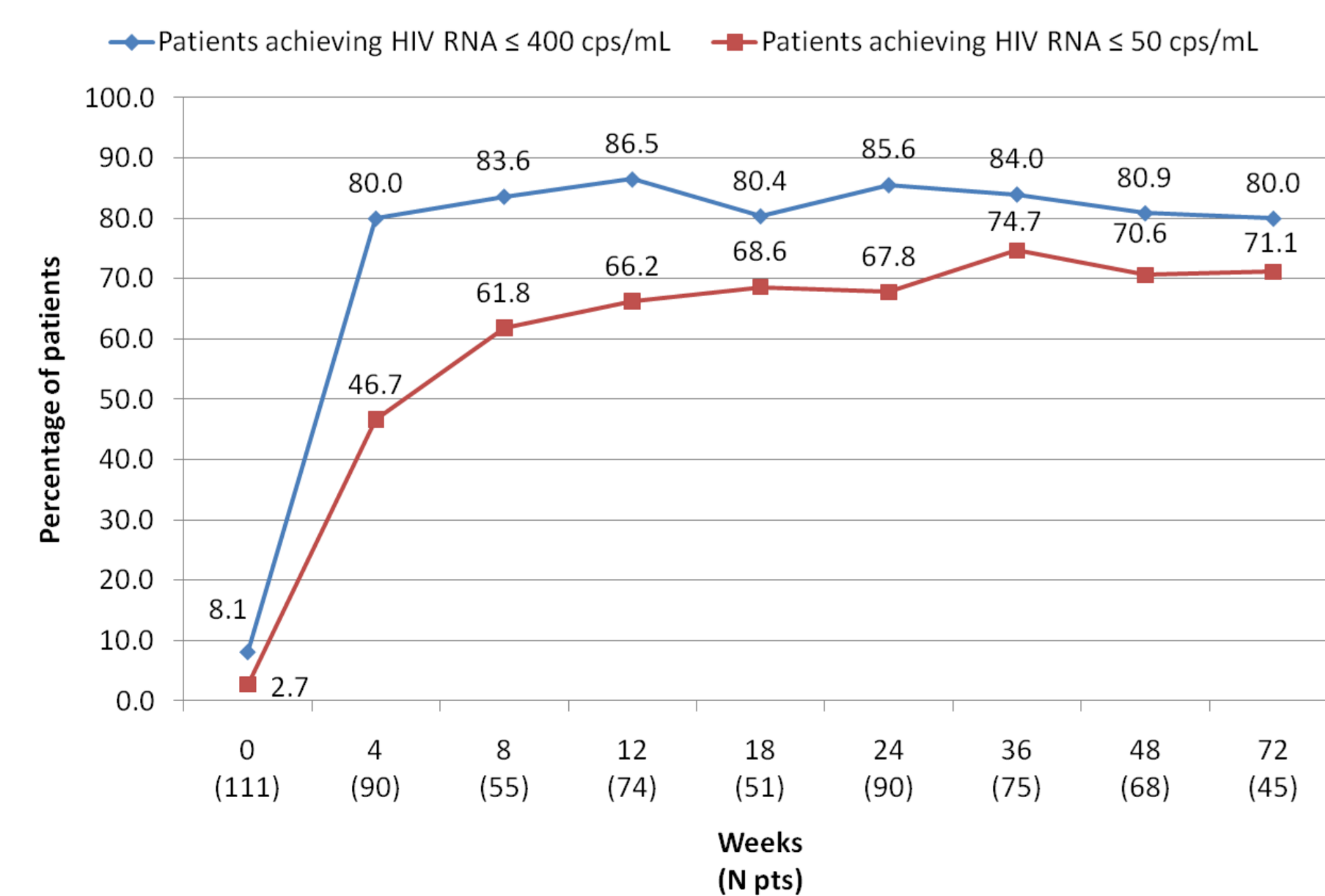
At baseline, Sanger sequences showed only secondary (and not primary) raltegravir-resistance mutations

Mean Age, years (N=111)	45
% Male	69
Median CD4 cell count (IQR) Cells/mm ³	155 (66-264)
Median HIV RNA (IQR) log ₁₀ copies/mL	4.4 (3.5-5.1)
HIV-1 IN Subtype	B=93.7% non-B=6.3%
Mean No. NNRTI Mutations	4.2 ± 0.6
Mean No. NNRTI Mutations	1.9 ± 0.9
Mean No. PI Major Mutations	4.1 ± 1.8

Raltegravir Resistance Associated Mutations	Frequency (% N=111)	Raltegravir Resistance Associated Mutations	Frequency (% N=111)
L74M	0	V151I	0.9 (1)
E92Q	0	N155S	0.9 (1)
T97A	0.9 (1)	N155H	0
E138AK	0	E157Q	0
G140S	0	G163R	0.9 (1)
G140A	0.9 (1)	I203M	1.8 (2)
Y143CRH	0	S230N	12 (10.8)
Q148H/R	0	S230R	0

In bold: Primary raltegravir resistance mutations

Fig.1. Patients achieving HIV RNA <50 or <400 copies/mL during raltegravir treatment

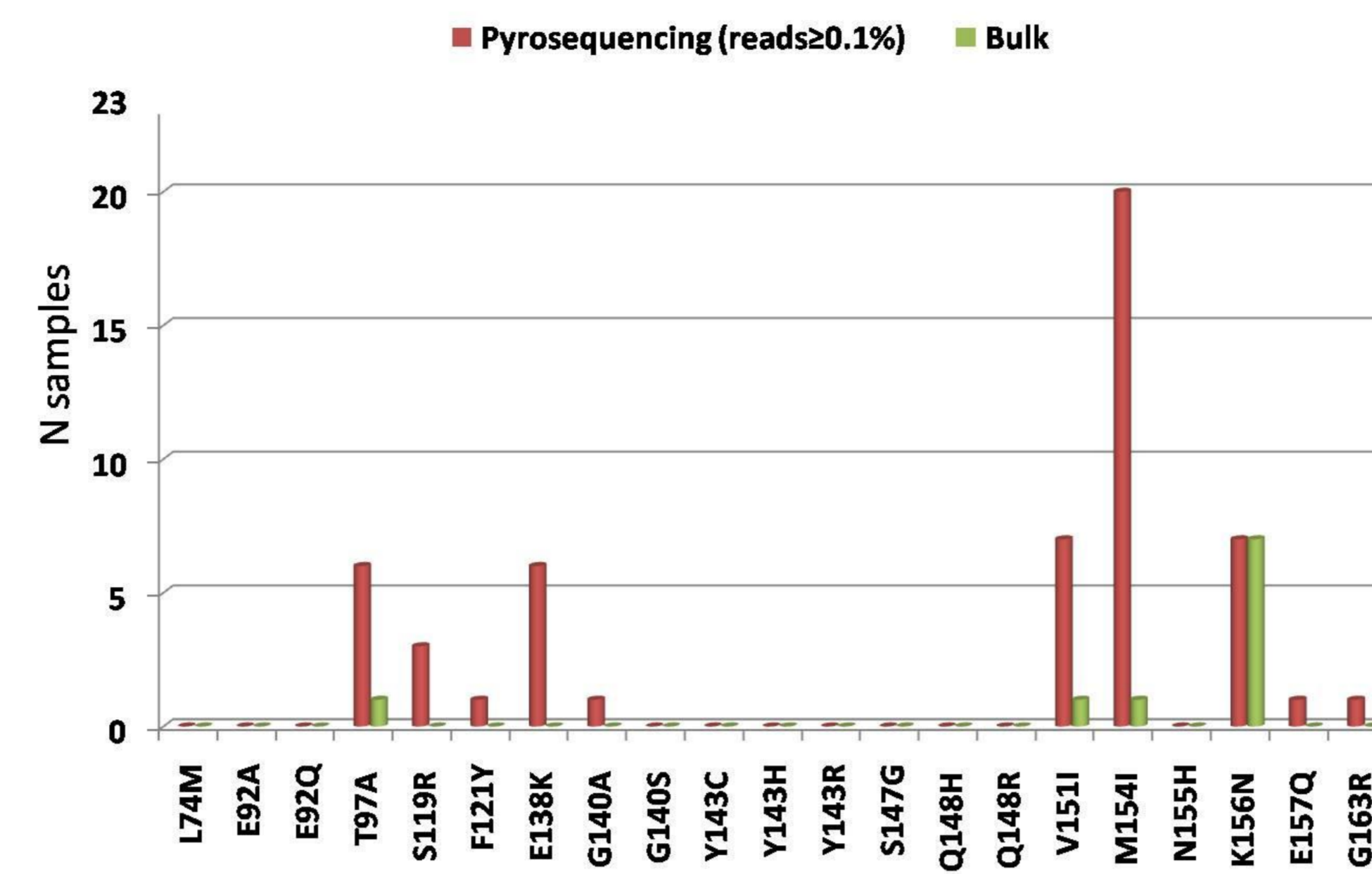


At week 24, 90 patients had available viral load values. During raltegravir treatment, a sharp decrease of viral load was observed in the majority of patients: 61 out 90 (67.8%) of patients achieved HIV RNA <50 copies/mL and 77 (85.6%) achieved HIV RNA <400 copies/mL at 24 week (Fig.1).

At baseline Sanger sequences showed only secondary (and not primary) raltegravir resistance mutations (Tab.2). Overall, only T97A, G140A, N155S, V151I and G163R were present with a prevalence of 0.9% of patients. Patients carrying T97A, V151I and G163R mutations at baseline did not achieve virologic success at 24 weeks.

At baseline pyrosequencing, no minor variants of primary raltegravir-resistance mutations with a prevalence of ≥0.1% were found

Fig.2. Comparison of mutation detectability with Sanger sequencing and 454 pyrosequencing



Tab.3. Baseline pyrosequencing prevalence of Stanford raltegravir resistance mutations according to virologic response at 24 weeks

Mutation	HIV RNA >50 cps/mL at 24 weeks (N=14)				HIV RNA <50 cps/mL at 24 weeks (N=9)				Overall Frequency (N=23)		Sign.
	Frequency (N=14)		Reads Range (Min-Max)		Frequency (N=9)		Reads Range (Min-Max)		N	%	
	N	%	N	%	N	%	N	%			
L74M	0	0	-	-	0	0	-	-	0	0	-
E92Q	0	0	-	-	0	0	-	-	0	0	-
T97A	4	28.6	29-6941	0.2-99.0	2	22.1	21-54	0.3-0.3	6	26.1	NS
F121Y	1	7.1	75	0.6	0	0	0	0	1	4.3	NS
E138K	2	14.3	15-23	0.3-0.4	1	11.1	16	0.2	3	13.0	NS
G140A	0	0	-	-	0	0	-	-	0	0	-
G140S	0	0	-	-	0	0	-	-	0	0	-
Y143H	0	0	-	-	0	0	-	-	0	0	-
Y143C	0	0	-	-	0	0	-	-	0	0	-
Y143R	0	0	-	-	0	0	-	-	0	0	-
Q148H	0	0	-	-	0	0	-	-	0	0	-
Q148R	0	0	-	-	0	0	-	-	0	0	-
N155H	0	0	-	-	0	0	-	-	0	0	-
N155S	0	0	-	-	0	0	-	-	0	0	-
V151I	4	28.6	17-6106	0.3-98.3	1	11.1	27	0.3	5	21.7	NS
E157Q	0	0	-	-	0	0	-	-	0	0	-
G163R	0	0	-	-	1	11.1	500	8.6	1	4.3	NS

In red Raltegravir primary resistance mutations

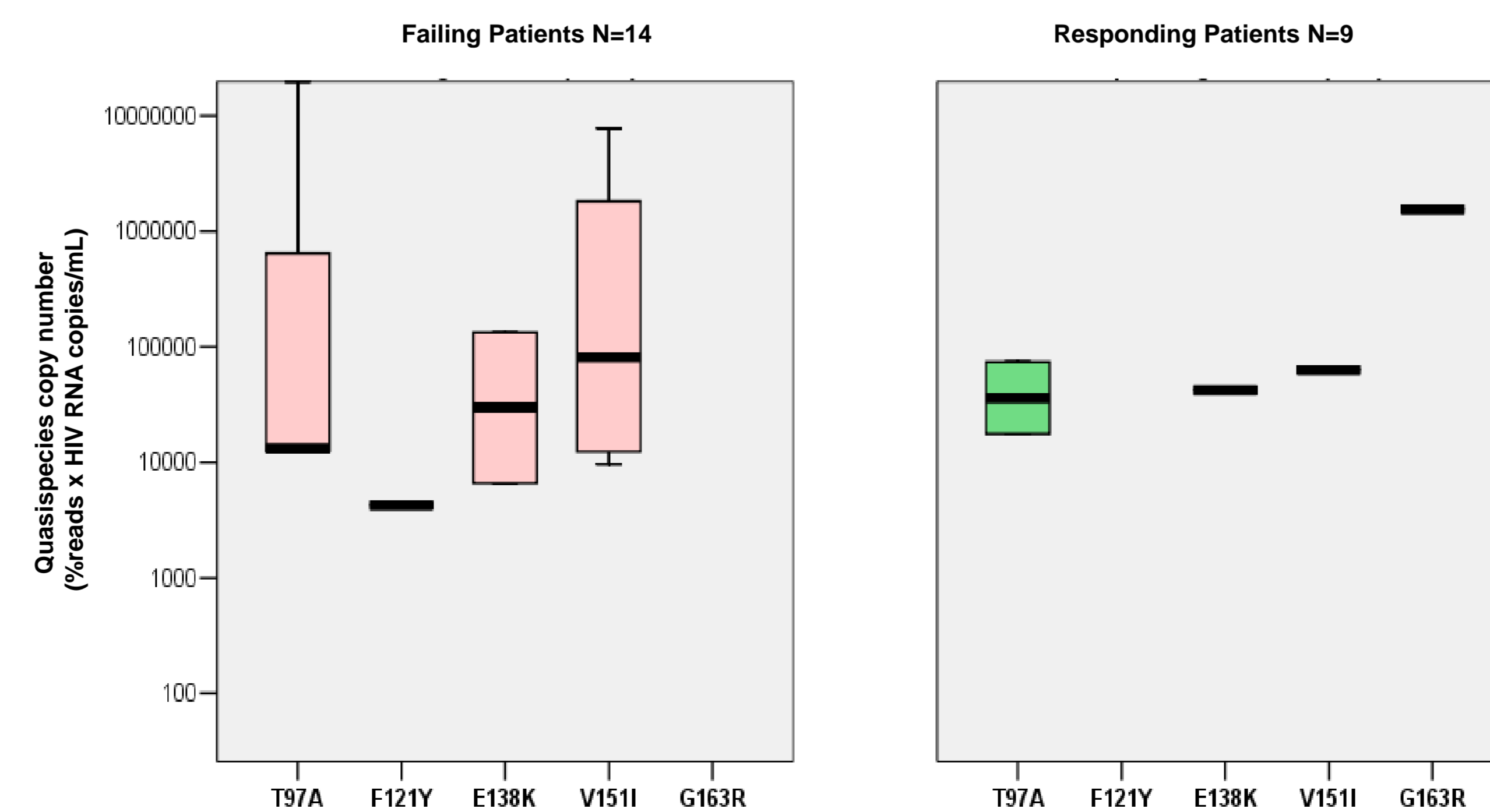
At Baseline for 23 patients (14 failing and 9 responding to raltegravir treatment) pyrosequencing was performed and among >200,000 IN sequences analyzed, no minor variants of primary raltegravir mutations with a reads prevalence ≥0.1% were found (Fig.2, Tab.3).

Secondary F121Y mutation was found at baseline with a low frequency (0.6% of sequences) in 1 failing patient. T97A, E138K and V151I mutations were also found at baseline in both failing- and responding-patients (6, 3 and 5 patients overall, respectively) with a frequency ranging from 0.25 to 98% of viral species (Fig.2, Tab.3).

Independently of the sequencing method, the presence of secondary-resistant species at baseline was not associated, at failure, with evolution at the same amino acid position or to specific primary raltegravir resistance mutations.

Baseline secondary raltegravir resistance minor quasispecies were not associated with virologic response at 24 weeks

Fig.3. Quasispecies copy number in failing and responding patients



Recently, the presence of NNRTI resistance K103N minor quasispecies, detected by AS-PCR, with copy number >2000 (% reads x HIV RNA copies/mL) was associated with virologic failure to first line Efavirenz containing regimen (Goodman et al. Antivir. Ther. 14 Suppl 1:A43).

In Raltegravir naïve patients, the detected secondary mutations by pyrosequencing with reads ≥0.1% and copy number >2000 were found in both responding and not responding patients (Fig.3).

Primary raltegravir resistance mutations not detected at baseline were selected at failure together with known secondary mutations and the novel T112A

Tab.4. Prevalence of raltegravir resistance mutations using 454-Pyrosequencing

Patient	Time (Months)	HIV RNA (log ₁₀ copies/mL)	L74M	E92A	E92Q	T97A	F121Y	E138K	G140A	G140S	Y143C	Y143H	Y143R	Q148H	Q148R	V151I	N155H	N155S	E157Q	G163R	
12	0	4.9																			
	1	5.0				1.9	99.0														
	3	5.2					99.6	0.5													
	7	5.1	1.7				99.4	31.0	0.6											0.5	0.3
	9	4.8	3.3				99.4	40.1	0.4											1.1	0.4
141	0	4.9																			
	6	4.3																			
	10	3.2			0.5	96.5														98.3	37.2
	14	3.2																		99.8	2.0
	15	3.2																		99.8	0.4
145	0	4.7																			
	7	5.7	15.0																	10.2	98.0
	9	5.7	15.1																	10.6	98.3
	11	4.1	0.3																	10.3	10.0
	18	8	2.6	2.6	70.4			0.2	2.8							17.0				60.6	93.5
69	0	4.4					0.5	0.1												6.5	98.1
	5	3.8			4.1		0.4													41.6	97.4
	7	3.6			21.2																4.3
	8	5.3																			
	10	3.5							0.6												
81	0	4.9																			
	3	5.1																			
	10	3.5							0.1												
	15	5.9																			
	16	4.6																			
84	0	5.7																			
	1	3.2																			
	2	2.7																			
	3	4.7																			
	4	5.4																			
229	0	4.9																			
	3	5.5																			
	5	5.1																			
	7	5.5																			
	9	5.8																			

In red raltegravir primary resistance mutations; *T112A is a novel mutation analyzed; ** Months after raltegravir interruption

At baseline, primary mutations N155H and Y143R were completely absent (0 reads among > 200,000 IN sequences). Primary mutations Q148H/R and Y143C/H detected only below the limits of the assay reliability (reads <0.1%), were found in both responding and not responding patients. The presence of these mutations at baseline was not associated with development of the same mutation at failure.

Pre-existing secondary resistance mutations at low frequency (<0.4%) did not emerge at failure as major variants.

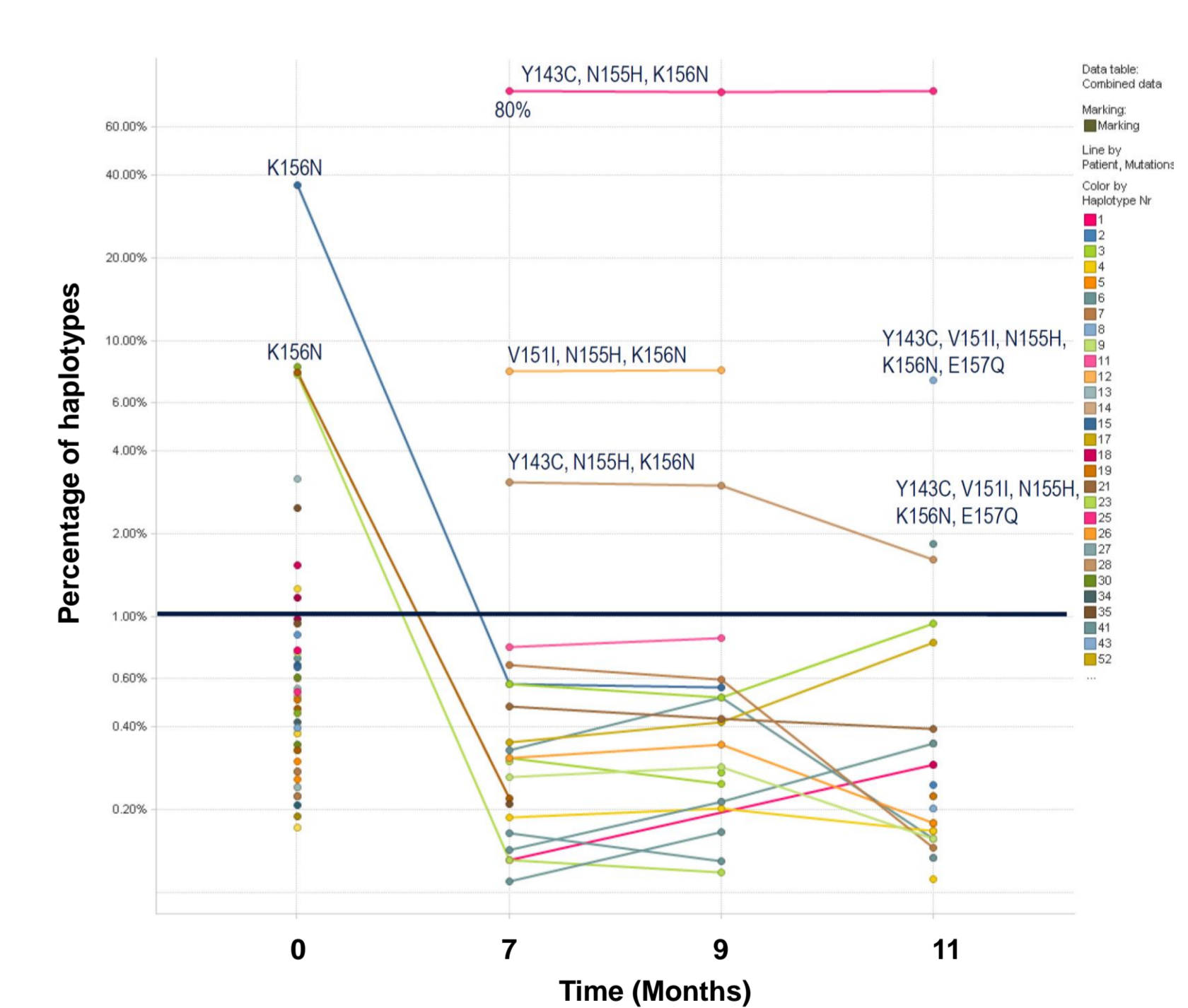
The combination of N155H and Y143C at failure was associated with high phenotypic resistance to raltegravir

Tab.5. Phenotypic effects of integrase mutations

Patient	Time (months)	HIV RNA (log ₁₀ copies/mL)	Mutations	Fold Change Elvitegravir	Fold Change Raltegravir
12	0	4.9	T97A	1.22	1.2
	1	5.0	T97A, Y143R	5.2	33.1
	3	5.2	T97A, Y143R	4.2	30.9
	7	5.1	T97A, Y143R, T112A/T, S119S/T	6.7	43.7
	9	4.8	T97A, Y143R, T112A/T	7.5	96.1
69	0	4.4	No resistance mutations	0.8	1.2
	2	3.8	E92A, N155H	45.32	10.42
	4	3.8	E92E/A, N155H	27.65	7.82
	5	3.8	E92A, N155H, D323D/N	117.5	31.6
	7	3.6	E92E/Q/A/P, N155H, E138E/A, V151I/V	7.9	5.3
78	9	4.3	G163R	4.0	3.6
	11	4.1	No resistance mutations	1.05	0.77
81	3	5.1	N155H	29.49	4.52
	10	3.6	No resistance mutations	0.46	0.38
27	0	3.6	G140S, Q148H	456	248.02
	-3	5.9	No resistance mutations	1.13	0.66
84	0	5.7	No resistance mutations	0.59	0.89
	3	3.2	Q148Q/R	0.16	0.4
	4	2.7	G140S, Q148R	50.6	34.5
	5	4.7	Q148Q/R	1.1	1.3
	-3	5.4	No resistance mutations	0.5	0.5
229	0	4.7	No resistance mutations	0.6	0.5
	7	4.1	No resistance mutations	0.8	1.2
	11	4.1	N155H, Y143C	114.4	493.2

In red bold raltegravir primary resistance mutations; in black bold raltegravir secondary resistance mutations

Fig.4. Haplotypes detected over time in patient 229



At failure, all patients carrying the primary mutations N155H, Q148H/R or Y143R, with other secondary mutations (E92A/Q, G140S, T97A), showed a Fold Change >30-100 for raltegravir (Tab.5).

In patient 229, we found the combination of two primary mutations, Y143C and N155H, by both Sanger-sequencing and 454-pyrosequencing. Y143C and N155H mutations were highly prevalent and both mutations appeared for more than 80% on same haplotypes (Fig.4; Tab.5). The combination of these mutations showed a very high phenotypic resistance