



Detection of Low-frequency Mutations Associated with Drug Resistance to Raltegravir before ART

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Abstract

Background: Raltegravir (RAL) is highly efficacious in the treatment of HIV infection. The prevalence and impact on virologic outcome of low frequency mutations in HIV-1 integrase among HIV-infected persons not previously treated with RAL is not known.

Methods: Using a parallel allele-specific sequencing (PASS) method, pre-treatment samples available from 32 persons in the MK-0518-019 study who experienced virologic failure per study protocol while on RAL were tested for minority viral populations containing mutations associated with decreased susceptibility to RAL (N155H, Q148K/R/H, Y143R/C/H, L74M, E92Q, T97A and G140S/A). Logistic regression (SAS v9.1) was performed to compare treatment outcomes between groups with or without these mutations.

Results: Drug-resistance associated mutations (Q148K/R/H, Y143C/H, L74M, T97A, and G140S) were detected in 14 of 32 individuals. Nearly all mutations were present in low frequency (<0.5% of genomes) except L74M (2.22%). Pre-existing mutations in the principal resistance pathways for RAL (N155H, Q148H/K/R and Y143R/C) were uncommon and the frequencies of such mutations were low (0.01% for Q148K/R and Y143C, 0.16% for Q148H). Secondary mutations (L74M, T97A, G140S, and Y143H) were more common, detected in 6, 3, 8 and 2 patients, respectively. Two or more mutations were found in 3 patients but none were linked in the same viral genomes. Of 8 persons, 3 with the pre-treatment G140S mutation experienced viral rebound with this mutation by population sequencing. Among them, only one had the mutation detected in first failure sample. No statistically significant differences in drug-resistance mutation frequencies were observed between treatment failure and treatment success groups, perhaps in part related to sample size.

Conclusions: Low frequency integrase mutations associated with RAL drug resistance are uncommon in patients naive to RAL. Additional studies in larger populations are needed to fully characterize the importance and clinical significance of these mutations.

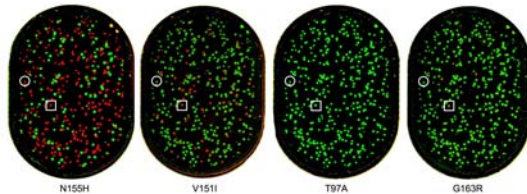


Fig 1. Detection of drug-resistant mutations by PASS in patient M010 after treatment failure

Table 1. Linkage analysis of drug-resistant mutations in patient M010

Species	N155H	V151I	T97A	G163R	No.	Percentage (%)
1	-	-	-	-	370	33.9
2	-	-	-	+	1	0.1
3	+	-	-	-	598	54.8
4	+	-	-	+	1	0.1
5	+	-	+	-	1	0.1
6	+	+	-	-	113	10.4
7	+	+	-	+	7	0.6
Total	720	120	1	9	1091	100.0

Table 2. Detection of low frequency drug-resistant mutations at baseline in patients from Merck P019 study

Treatment	PID	Viral load (copies/ml)	No. of genomes analyzed	N155 pathway				Q148 pathway				Y143 pathway			Genotype	
				N155H	L74M	E92Q	T97A	G163R	Q148K	Q148R	Q148H	G140S	G140A	Y143R		Y143C
	M010	105,000	3,640	0	0	0	0	1 (0.03)	0	0	1 (0.03)	0	0	0	0	N155H, V151I, E157Q
	M011	172,000	3,347	0	3 (0.09)	0	1 (0.03)	4 (0.12)	0	0	1 (0.03)	0	0	0	0	E92Q, T977A, Y143YH, N155NH
	M017	160,000	2,018	0	1 (0.05)	0	3 (0.15)	0	0	0	0	0	0	0	0	N155H, T97A, L74M, S230R, Y143C
	M019	8,430	408	0	0	0	0	1 (0.25)	0	0	0	0	0	0	0	Q148H, G140S
	M020	34,300	836	0	0	0	0	0	0	0	0	0	0	0	0	N155NH, Q148Q/R/H, V151V/I, G140S
	M023	36,800	525	0	0	0	0	1 (0.19)	0	0	0	0	0	0	0	N155H, Q148H, G140S
Failure	M027	34,700	531	0	0	0	0	1 (0.19)	0	0	0	0	0	0	0	Q148R, G140S, D167D/N
	M031	27,700	621	0	0	0	0	0	0	0	1 (0.16)	0	0	0	0	Q148R/H, G140S
	M032	12,200	45	0	0	0	0	1 (2.22)	0	0	0	0	0	0	0	N155NH, Q148Q/R, E138E/K
	M034	414,000	1,644	0	0	0	0	0	0	0	1 (0.06)	0	0	0	0	Q148H, G140S
	M035	18,900	349	0	0	0	0	0	0	0	0	0	0	0	0	N155NH, Q148Q/R
	M036	132,000	705	0	0	0	0	0	0	0	1 (0.14)	0	0	0	0	N155H, Q148H, G140S
	M038	41,500	2,529	0	0	0	0	0	0	0	3 (0.12)	0	0	0	0	N155H, G163R
	M039	109,000	1,019	0	0	0	0	0	0	0	1 (0.10)	0	0	0	0	N155NH, E92E/Q
	M001	152,000	1,691	0	0	0	0	0	0	0	2 (0.12)	0	0	0	1 (0.06)	Not done
	M002	5,710	74	0	0	0	0	0	0	0	0	0	0	0	0	Not done
	M003	4,090	6	0	0	0	0	0	0	0	0	0	0	0	0	Not done
	M004	8,160	144	0	0	0	0	0	0	0	0	0	0	0	0	Not done
	M006	59,900	572	0	0	0	0	0	0	0	1 (0.17)	0	0	0	0	Not done
	M007	134,000	1,000	0	0	0	0	0	0	0	1 (0.10)	0	0	0	0	Not done
	M009	30,400	630	0	0	0	0	0	0	0	1 (0.16)	0	0	0	0	Not done
	M012	750,000	12,015	0	1 (0.01)	0	2 (0.02)	13 (0.11)	1 (0.01)	1 (0.01)	1 (0.01)	0	0	1 (0.01)	1 (0.01)	Not done
	M014	241,000	976	0	0	0	0	3 (0.31)	0	0	1 (0.10)	0	0	0	0	Not done
Success	M021	2,880	26	0	0	0	0	0	0	0	0	0	0	0	0	Not done
	M022	55,000	2,402	0	2 (0.08)	0	0	0	0	0	0	0	0	0	0	Not done
	M024	5,430	3	0	0	0	0	0	0	0	0	0	0	0	0	Not done
	M025	99,100	1,551	0	1 (0.06)	0	1 (0.06)	0	0	0	1 (0.06)	0	0	0	0	Not done
	M028	5,910	107	0	0	0	0	0	0	0	0	0	0	0	0	Not done
	M029	5,490	87	0	0	0	0	0	0	0	0	0	0	0	0	Not done
	M030	5,630	10	0	0	0	0	0	0	0	0	0	0	0	0	Not done
	M033	66,000	492	0	0	0	0	0	0	0	0	0	0	0	0	Not done
	M037	111,000	14	0	0	0	0	0	0	0	0	0	0	0	0	Not done

Table 3. Number of individuals infected with viruses carrying drug-resistant mutations at baseline

Pathway	Primary mutation			Secondary mutation		
	Mutation	No. of patients	Percentage (%)	Mutation	No. of patients	Percentage (%)
Q148	Q148K	1	3.1	G140S	8	25.0
	Q148R	1	3.1	G140A	0	0.0
	Q148H	1	3.1			
N155	N155H	0	0.0	L74M	6	18.8
				E92Q	0	0.0
				T97A	3	9.4
Y143	Y143C	1	3.1	G163R	13	40.6
	Y143R	0	0.0			
				Y143H	2	6.3

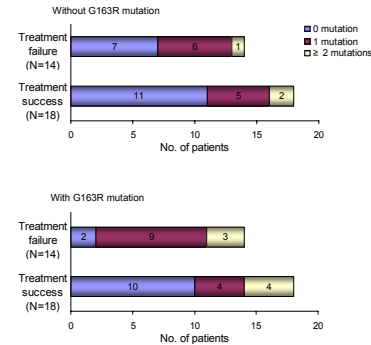


Fig 2. Number of individuals with drug-resistant mutations

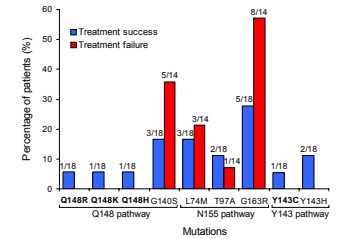


Fig 3. Frequency of individuals with drug-resistant mutations

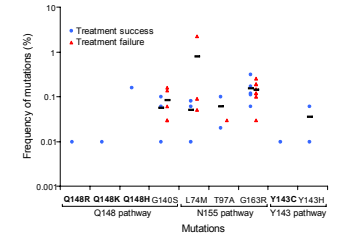


Fig 4. Frequency of minority drug-resistant viruses in each patient

Summary

- Primary RAL resistant mutations are rare in treatment naive patients, although secondary RAL resistant mutations (L74M, G140S, G163R) are detected more frequently; overall <0.5% of the viral population.
- G140S and G163R are detected more often in treatment failure patients than in treatment success patients.
- In some patients, pre-existing minority variants were detected by population sequencing after treatment failure, suggesting expansion of these viruses in response to drug selection pressure.