

RARE HIV-1 DRUG RESISTANCE MUTATIONS EXERT SUBTLE SYNERGISTIC AND ANTAGONISTIC EFFECTS IN THE CONTEXT OF THE GENETIC BACKGROUND

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OBJECTIVES

Rare resistance-associated mutations (RAMs) are seldom characterized due to inherent difficulty in evaluating their contribution amongst well-characterized RAMs. We used new approaches to identify mutations that influence susceptibility to non-nucleoside reverse transcriptase inhibitors (NNRTIs).

METHODS

- Resistance weight factors (RWF) for each RAM considered in vircoTYPE, the linear-modeling-based quantitative drug-resistance prediction tool, were used to derive an estimated RWF for mutations chemically similar to known RAMs, but not characterized thus far. Two amino acids were identified as chemically similar when they had a positive BLOSUM62 matrix score.
- Additional candidate RAMs were selected from a novel cross-validated procedure for the generation of linear regression models, which led to models with reduced complexity compared to the current vircoTYPE HIV-1.
- Several candidate mutations were identified for in silico analysis. Viral isolates in Virco's database containing a candidate were paired with samples having like patterns of primary (IAS, Stanford or ANRS lists) RAMs without the candidate. Mean measured Antivirogram fold-change (FC) for each drug was calculated. Paired sets of samples that, on average, exhibited a ≥ 3 -fold difference of any NNRTI FC for RAM combinations with and without the candidate mutation, were identified for site-directed mutagenesis (SDM) and the mutant constructs phenotyped (3 replicates) in the Antivirogram assay (AVG2.5.00).

RESULTS AND DISCUSSION

The NNRTI mutations Y181F, K102L, E138Q and T139R were identified for further testing.

- Overall these mutations have a low prevalence as shown in Table 1, where their prevalence over the last 12-year period may be compared with the better known mutations K103N and Y181C.

Table 1. Prevalence (%) of RT mutations Y181F, K102L, E138Q, T139R, K103N and Y181C in the Virco geno database throughout the 12-year period ending 30/9/2009

Mutation	1/10/97 - 30/9/99	1/10/99 - 30/9/01	1/10/01 - 30/9/03
Y181F	none	<0.002	0.01
K102L	none	0.01	0.01
E138Q	0.15	0.22	0.32
T139R	0.26	0.29	0.35
K103N	17.83	23.53	20.68
Y181C	11.65	10.81	9.78
N*	16 161	51 819	73 217

Mutation	1/10/03 - 30/9/05	1/10/05 - 30/9/07	1/10/07 - 30/9/09
Y181F	0.02	0.01	0.01
K102L	0.03	0.01	0.02
E138Q	0.37	0.30	0.28
T139R	0.36	0.32	0.29
K103N	19.23	17.47	14.39
Y181C	9.32	7.46	5.72
N*	78 529	90 569	92 673

* Total number of isolates per time period

The results of the analysis of paired clinical isolates are shown in Table 2.

- The RAM combinations tested were based on the mutational contexts in which the candidate mutations were observed.
- Addition of the mutations K102L, E138Q and T139R to the selected RAM combinations resulted in an increase in the mean FC for EFV and ETR. For NVP, the selected RAM-combinations already caused the FC value to be out of the dilution range tested (censored) in most cases, i.e. the FC values were above the upper limit of the dynamic range of the assay. Therefore, no meaningful comparisons could be made for this drug. There were very few viral isolates with RAM-combinations also harboring mutation Y181F. However, this mutation was chosen for further analysis because the overall results suggested a possible effect of this mutation. In addition, other mutant variants at this position are associated with high-level resistance to one or more NNRTI.

Table 2. Analysis of paired clinical isolates harboring selected RAM-combinations

Genotype	Nevirapine (BCO = 6.0)		Efavirenz (BCO = 3.3)		Etravirine (BCO = 3.2)	
	FC*	N**	FC	N	FC	N
wild-type virus	1.1	3065	1.0	3093	0.9	3981
181F	n.a.†		n.a.		n.a.	
103N	>64.4	2582	25.6	2553	0.8	1103
181F+103N	>125.8	1	84.7	1	0.5	1
102L	n.a.		n.a.		n.a.	
188L	>73.3	664	109.4	641	1	338
102L+188L	>85.1	17	382.5	17	3	17
138Q	2.7	6	1.9	8	3.4	6
100I+103N	>74	922	564.7	922	7.7	513
138Q+100I+103N	>92.4	4	>1455.5	4	31.9	3
103N+181C	>69.3	862	50.3	828	3.6	306
138Q+103N+181C	>86.9	12	168.8	12	7.5	9
139R	2.7	20	0.8	19	1.2	9
103N+181C	>69.6	870	50.8	836	3.6	312
139R+103N+181C	>85.4	5	99.3	5	20.3	4
181C+188L	>75.4	32	541.7	34	23.7	24
139R+181C+188L	>81.3	11	458.2	11	136.4	6

* Mean fold-change values; ** Number of viral isolates with the mutation combination; † Not available

The results of the analysis of paired site-directed mutant constructs are shown in Table 3.

- When present alone, the mutations have little or no effect on NNRTI FC as shown in the Table where the mean FC value of wild-type virus is provided for comparison. Only mutation T139R causes very low-level resistance to NVP, bringing the FC value to just above the biological test cutoff.
- In the RAM-combinations investigated, each of the mutations increased or decreased the FC for one or more NNRTI:
 - Y181F:**
 - Combining Y181F with K103N decreases EFV FC 3-fold and ETR FC 3.5-fold compared to K103N present as a single mutation
 - K102L:**
 - Combining K102L with Y188L increases EFV FC 3-fold compared to Y188L present as a single mutation
 - E138Q:**
 - Combining E138Q to L100I+K103N increases ETR FC 4-fold compared to L100I+K103N
 - Combining E138Q with K103N+Y181C increases EFV FC 3-fold and ETR FC 3.5-fold compared to K103N+Y181C
 - T139R:**
 - Combining T139R with K103N+Y181C increases EFV FC 3-fold and ETR FC 3.5-fold compared to K103N+Y181C
 - Combining T139R with Y181C+Y188L increases EFV FC 3-fold and ETR FC 5-fold compared to Y181C+Y188L
- Mutations K103N, Y181C and Y188L have a strong impact on NVP resistance, causing the FC values for NVP to be censored. Therefore, the effect on NVP resistance of adding the rare mutations discussed here could not be assessed, but they are unlikely to be critical with respect to resistance to NVP.
- The FC values for EFV, when K103N, Y181C and Y188L are present, are also high and greater than the BCO (3.3), although they are not censored. For this drug the effect on the FC values of adding the rare mutations is observable, but there is no change in the already "resistant" call.
- In ETR the K103N+Y181C combination itself does not cause resistance, but addition of E138Q or T139R causes the FC to increase to slightly above the BCO (3.2).
- Since the mutational background of the clinical isolates analyzed is likely to be quite diverse (wild-type HIV-1 is known to be highly polymorphic), the average magnitude of the effect of the rare mutations in these isolates is not necessarily expected to be precisely the same as the effect of those mutations tested in a homogeneous background such as a site-directed mutant. Clinical isolates are likely to harbor multiple mutations that modulate drug susceptibility in a subtle way as a response to drug pressure.
- The current study shows, that for EFV and ETR, adding the rare mutations to a K103N, Y181C and Y188L background does alter resistance in a number of cases.

Table 3. Analysis of site-directed mutants harboring selected RAM-combinations

Genotype	Nevirapine (BCO = 6.0)	Efavirenz (BCO = 3.3)	Etravirine (BCO = 3.2)	Comments
wild-type virus	1.1 (3065)*	1 (3093)	0.9 (3981)	
181F	1.5	0.5	0.5	
103N	>28	16.8	0.7	
181F+103N	>23.5	5.9	0.2	Combining Y181F with K103N decreases EFV FC 3-fold and ETR FC 3.5-fold compared to K103N present as a single mutation
102L	2.7	0.9	0.3	
188L	>22.7	86.8 (2)	1.1	
102L+188L	>22.7	275.1	2.7	Combining K102L with Y188L increases EFV FC 3-fold compared to Y188L present as a single mutation, while the ETR FC remains below the BCO
138Q	3.8	2.6	2.5	
100I+103N	>53.2 (6)	>2157 (6)	3.5 (5)	
138Q+100I+103N	>59.1 (2)	3562.3	14.6 (2)	Combining E138Q with L100I+K103N increases ETR FC 4-fold compared to L100I+K103N
103N+181C	>22.7 (6)	19.8 (6)	1.8(6)	
138Q+103N+181C	>31.2	11.9	8.8	Combining E138Q with K103N+Y181C increases EFV FC 3-fold and ETR FC 3.5-fold compared to K103N+Y181C
139R	7	2.1	1.1	T139R present as a single mutation raises NVP FC above the BCO of 6.0
103N+181C	>22.7 (6)	19.8 (6)	1.8(6)	
139R+103N+181C	>22.7	65.7	6.6	Combining T139R with K103N+Y181C increases EFV FC 3-fold and ETR FC 3.5-fold compared to K103N+Y181C
181C+188L	>22.7	144.5	5.4	
139R+181C+188L	>22.7	420.1 (2)	26.6	Combining T139R with Y181C+Y188L increases EFV FC 3-fold and ETR FC 5-fold compared to Y181C+Y188L

* FC values based on 3 observations, except where indicated within round brackets

- This study also illustrates a principle that for some rare mutations, an evaluation of their contribution to resistance may be dependent on the genetic context.
- The question remains whether these rare mutations simply represent polymorphisms or whether they are associated with RAMs. Table 4 indicates that the latter might be true, even though the relative frequency of the mutations remains very low, even among isolates harboring RAMs. A comparison of the prevalence data of the mutations among therapy-naïve and therapy-experienced patients in the Stanford database shows that, depending on the subtype, between 0.0% and 0.2% of RTI-naïve and between 0.0% and 1.2% of NNRTI-treated patients are carrying these mutations.

Table 4. Prevalence (%) of Y181F, K102L, E138Q and T139R in the Virco Database (N = 403 186) among the isolates with and without evidence of drug exposure*

Mutation	Evidence of Drug Exposure	
	No (N = 13 232)	Yes (N = 389 954)
Y181F	none	0.01
K102L	none	0.02
E138Q	0.05	0.31
T139R	0.008	0.33

*WHO 2009 Drug Resistance Surveillance List [Bennett et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One. 2009; 4(3)].

CONCLUSIONS

- Our analysis successfully identified rare mutations influencing NNRTI-resistance.
- Their evaluation in combinations with well-known RAMs demonstrated that these rare mutations may act to either increase resistance or re-sensitize HIV-1 to specific drugs.