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Variant L at codon 214 of the HIV-1 reverse transcriptase is antagonist to thymidine analogue mutations type1 profiles and is associated with better virological response to thymidine analogue-contaning cART regimens than variant 214F if TAM type1 profiles are concomitantly detected

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BACKGROUND

Previous reports have shown that polymorphism 214L is found in around 20% of patients' virus populations with similar frequency of detection in antiretroviral-naïve and treated individuals.

However, it was observed a positive association between polymorphism 214L and mutations of the thymidine analogue mutations (TAM) type 2 profile (e.g. 67N, 70R, 219E/Q), and, viceversa, a negative association between polymorphism 214L and mutations of the TAM1 profile (e.g 41L, 210W, 215Y) in several studies [Sturmer et al., AAC 2003;47:54-61; , AG Marcelin et al. Abs 23, Antivir Ther 2005;10: S25, Ceccherini-Silberstein et al., Abs 96, Antivir Ther 2005;10:S106, Geretti AM et al, Abs 94, Antivir Ther 2005;10:S104].

One possible explanation for this finding can be found in the structural vicinity of position 214 to the mutations L210W and T215Y/F (Figure 1). Indeed, a background of 214F (instead of L), because of the interaction of the aromatic side chains, favours the presence of TAM1 mutations (and, therefore, 214F may direct the course of HIV evolution along the TAM1 pathway) or, viceversa, TAM1 mutations may favour the presence of 214F.

This interaction of the aromatic side chains is reported to increase the stability of the three-dimensional structure of the HIV enzyme so that it is expected that virus populations carrying a TAM1 profile are less susceptible to zidovudine/stavudine if there is F at position 214 compared with if there is L [Sturmer et al., AAC 2003;47:54-61].

In order to test this hypothesis, we evaluated the week 24 virological response to thymidine analogue-containing cART regimens according to a number of mutations patterns including TAMs and the variants F or L at codon 214 of RT.

STUDY DESIGN - METHODS

Patients included were those of EuroSIDA who started zidovudine or stavudine for the first time as part of potent combination therapy (cART -^{3}3 drugs) and who have been tested for genotypic resistance over the previous 6 months. Patients could have been tested for genotypic resistance on more than one occasion before this most recent test. Aminoacids at each position of RT and PR regions of HIV are available in the EuroSIDA resistance database.
TAM1 and TAM2 profiles were defined as in previous analyses [Cozzi Lepri A et al. Antivir Ther. 2005;10(7):791-802].

OBJECTIVES

- To evaluate the prevalence of polymorphism 214L in our study populations and its association with other resistance mutations / patters of mutations in the RT region.
- To study the virological response to zidovudine/stavudine-containing cART regimens according to different patterns of mutations detected before therapy initiation.

Assumption: all mutations detected 6 months before treatment initiation or at any other genotypic test performed at earlier dates were counted as present in individuals' virus populations.

STATISTICAL ANALYSIS

Association between mutations was tested using chi-square test and Fisher exact test.

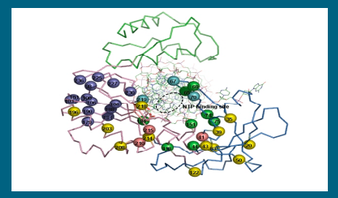
The Kaplan-Meier approach was used to estimate the overall week 24 viral load change from pre-therapy levels and the viral load reductions in sub-groups.

The effect of specific mutations / set of mutations on the week 24 viral load change was evaluated using a liner regression model accounting for the fact that some of the changes could be censored, due to the lower limit of the viral load assay.

Potential confounders included in the final multivariable model were: pre-cART viral load, exact number of weeks between the date of genotypic test and the date of starting cART, exact number of weeks between the date of starting cART and the week 24 viral load, whether a patient was starting cART from ART-naïve, whether a patient started a zidovudine or stavudine-containing cART, number of drugs besides zidovudine/stavudine to which virus was susceptible (according to IS Rega v 6.3) and other RT mutations (those listed in Table 2).

Differences in virological responses associated to 214F or 214L according to the presence of other mutations / TAM profiles were formally tested including an interaction term in the regression model.

Figure 1



Localization of polymorphism 214F/L in the structure of HIV-1 reverse transcriptase (RT). The structure of HIV-1 RT in the region near the polymerase active site is shown. The subdomains are colour coded (palm, magenta; thumb, green; fingers, blue). TAMs 1 are in salmon; TAMs 2 in cyan; NNRTI resistance mutations in blue; novel mutations in yellow; other RT inhibitors resistance mutations in green.

Table 1

Patients characteristics (n=500)

Age, median (IQR)	39 (21-57)
Female, n(%)	99 (19.8%)
Homosexual contacts, n(%)	235 (47.0%)
Heterosexual contacts, n(%)	125 (25.0%)
IDUs, n(%)	97 (19.4%)
Year of test, median (range)	Apr 98 (Nov 95-Feb 04)
Months between test and initiation of cART	1 (0-6)
zidovudine-containing regimens, n(%)	249 (49.8%)
ART-naive, n(%)	224 (44.8%)
214L, n(%)	99 (19.8%)

Table 2

Association between 214L and other resistance mutations (all patients)

	Variant at codon 214 of RT		p-value
	F (n=401)	L (n=99)	
41L	113 (28.2%)	27 (27.3%)	0.85
210W	87 (21.7%)	24 (24.2%)	0.59
215Y	131 (32.7%)	20 (20.2%)	0.02
67N	91 (22.7%)	39 (39.4%)	0.0007
70R	66 (16.5%)	37 (37.4%)	0.0001
215F	131 (32.7%)	20 (20.2%)	0.02
219E	18 (4.5%)	13 (13.1%)	0.001
219Q	30 (7.5%)	29 (29.3%)	0.0001
65R	8 (2.0%)	15 (15.2%)	0.0001
74V	16 (4.0%)	16 (16.2%)	0.0001
118I	37 (9.2%)	33 (33.3%)	0.0001
184V	127 (31.7%)	41 (41.4%)	0.07
No TAMs	234 (68.8%)	47 (65.3%)	
TAM1 profile	84 (24.7%)	6 (8.3%)	0.0001
TAM2 profile	22 (6.5%)	19 (26.4%)	

RESULTS

Table 1 shows the main characteristics of the study population (n=500).

The number (%) of patients in whom RT resistance mutations were detected at any time before starting cART were the following: 41L (n=140, 28%), 44E (n=9, 2%), 67N (n=130, 26%), 70R (n=103, 21%), 118I (n=70, 14%), 210W (n=111, 22%), 215Y (n=151, 30%), 215F (n=42, 8%), 219Q (n=59, 12%), 219E (n=31, 6%), 65R (n=23, 5%), 74V (n=32, 6%), 184V (n=168, 34%), TAM1 profile (n=90, 22% of classifiable), TAM2 profile (n=41, 10% of classifiable).

In pre-treated patients, the median number of antiretrovirals previously used was 4 (range:1-11). These included: zidovudine (96%), stavudine (39%), lamivudine (75%), didanosine (60%), abacavir (11%), zalcitabine (37%), tenofovir (3%), nevirapine (16%), efavirenz (11%), delarvidine (1%), saquinavir (27%), indinavir (39%), ritonavir (29%), nelfinavir (17%), saquinavir SG (3%), amprenavir (3%), lopinavir/r (4%) and fuzeon (0.5%). The type of cART started were: 3 NRTIs (6%), 2NRTI+PI (40%), 2NRTI+NNRTI (14%), 2NRTI+rtv boosted PI (14%). other >3 antiretrovirals (26%).

Table 2 confirms previous observations that polymorphisms 214L is more likely to be detected if a mutation of the TAM2 profile is also concomitantly detected; viceversa it is less likely to be detected if a mutation of the TAM1 profile is detected. An agonistic interaction between polymorphism 214L and mutations 65R, 74V and 118I was also found (Table 2). Results were similar when we repeated the analysis using only patients who had been exposed to ART before starting the thymidine analogue-containing regimen (Table 3).

The overall week 24 viral load average reduction from pre-cART levels was 2.14 $\log_{10}$ copies/mL (95% CI: 1.92-2.39). This virological response was similar in patients in whom 214F: 2.07 (95% CI: 1.80-2.33) or 214L was detected: 2.43 (95% CI: 2.06-3.00).
Table 4 shows the average reductions according to the presence of variants 214L or 214F and the concomitant detection of other RT mutations. As expected, the best virological response was observed in patients carrying virus populations in which TAM mutations were not detected. In these patients the concomitant presence of variant 214F or 214L did not seem to make a large difference to the virological response. Results were similar if mutations of the TAM2 profile had been detected. In contrast, if mutations of the TAM1 profile had been detected (in particular, mutations 210W and 215Y) the virological response in people with variant L at position 214 was markedly better than that observed in people with variant F (p-value for interaction =0.0001 – NB also patients with a single TAM1/TAM2 were used in this analysis).
These results were confirmed in the multivariable linear regression adjusted for the potential confounders described in the methods (including the number of active drugs in the background treatment, Table 5).

CONCLUSIONS

Our study confirms that polymorphism 214L tends to cluster with mutations of TAM2 profile while it is rarely detected in concomitance with mutations of the TAM1 profile. A stabilization mechanism explained by the structural vicinity of position 214 to the thymidine-associated mutations L210W and T215Y/F is thought to drive this clustering phenomenon.
The minority of patients carrying a virus with mutations of the TAM1 profile and polymorphism 214L seem to have a better virological response to thymidine analogues-containing regimens compared to those who carry mutations of the TAM1 profile and 214F. This could be due to the fact that the enzyme of virus populations carrying TAM1 changes is more stable in the presence of polymorphism 214F than in the presence of 214L, resulting in decreased susceptibility to zidovudine/stavudine.
The exact molecular mechanism responsible for the action of polymorphism 214L needs to be further investigated.

Table 3

Association between 214L and other resistance mutations (pre-treated patients)

	Variant at codon 214 of RT		
	F (n=214)	L (n=62)	
41L	109 (50.9%)	18 (29.0%)	0.002
210W	85 (39.7%)	15 (24.2%)	0.03
215Y	130 (60.8%)	11 (17.7%)	0.0001
67N	87 (40.7%)	28 (45.2%)	0.53
70R	65 (30.4%)	28 (45.2%)	0.03
215F	21 (9.8%)	12 (19.4%)	0.04
219E	18 (8.4%)	5 (8.1%)	0.93
219Q	28 (13.1%)	19 (30.7%)	0.001
65R	8 (3.7%)	7 (11.3%)	0.02
74V	16 (7.5%)	8 (12.9%)	0.18
118I	36 (16.9%)	18 (29.0%)	0.03
184V	124 (57.9%)	31 (50.05)	0.27
No TAMs	54 (35.3%)	21 (47.7%)	
TAM1 profile	80 (52.3%)	5 (11.4%)	0.0001
TAM2 profile	19 (12.4%)	18 (40.9%)	

Table 4

Kaplan-Meier estimates of week 24 viral load reductions (all patients)

	Variant at codon 214 of RT	
	F	L
No TAMs	2.64 (2.37-2.83)	2.73 (2.06-3.64)
TAM1 profile	1.02 (0.54-1.80)	2.39 (1.14-NE)
TAM2 profile	1.79 (1.45-2.72)	1.45 (1.08-2.20)
41L	1.25 (1.62-1.80)	2.76 (1.63-NE)
210W	0.82 (0.35-1.66)	NE
215Y	1.07 (0.61-1.66)	2.77 (1.36-NE)
67N	1.60 (1.83-2.13)	2.20 (1.28-NE)
70R	1.51 (0.45-1.82)	2.78 (1.38-NE)
215F	1.51 (-0.08--2.09)	2.78 (1.28-NE)
219E	1.13 (-0.10-1.82)	NE
219Q	1.57 (0.61-1.45)	2.78 (1.28-NE)

NE = not possible to estimate due to small numbers

Table 5

Adjusted difference between viral load reductions in people with variant 214L and 214F from fitting a regression model

	All patients		Pre-treated patients	
	Mean	95% CI	Mean	95% CI
No TAMs	+0.38	-0.13; 0.91	+0.29	-0.48; +1.05
TAM1 profile	+1.25	-0.04;+2.54	+1.84	+0.29; +3.39
TAM2 profile	-0.63	-1.73; +0.47	-0.57	-1.84; +0.70
210W	+1.07	+0.11; +2.03	+1.39	+0.36; +2.41
215Y	+0.63	-0.36; +1.62	+0.84	-0.18; +1.87

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