



Characterization of New Mutational Pattern in HIV-1 gp41 Associated with T-20 Treatment

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Abstract

Background: Recently, it has been shown that V38A and Q40H+L45M correlated with a gain and with a loss of CD4 count, respectively, in HIV-infected patients (pts) receiving T-20. The aim of this study is to investigate in a large cohort of T-20 treated pts the long-term association of such mutations with the viro-immunological parameters, and whether selected gp41 mutational clusters correlate with viro-immunological outcome.

Methods: 195 sequences of HIV-1 gp41 and clinical follow-up from 75 T-20 treated pts were analyzed at baseline (BL) and monthly up to week (wk) 48. The association of mutations with T-20 treatment and between mutations at BL and during treatment was assessed by Fisher exact test and linear regression model. Association of mutations with viremia (VL)-CD4 count (c/u) was assessed by Mann-Whitney test. Covariation analysis was based on binomial correlation coefficient (phi) and hierarchical clustering.

Results: Seven mutations (L54M, E119Q, S129D, N126K, N140I, D239H, T268A) are positively associated with T-20 treatment and correlate with known T-20 resistance mutations. In particular, strong correlation ($\phi > 0.30$; $P < 0.01$) is observed for N140I with V38A and for D239H with Q40H and L45M. Cluster analysis reveals the existence of 4 clusters of mutations 1) V38A with N140I, 2) N43D with S138A; 3) G36V with N126K; 4) Q40H, L45M with the L54M, E119Q, S129D, T268A and D239H. Co-presence of N140I with V38A is associated ($P < 0.05$) with a CD4 increase from BL (40c/u) of 1.9-fold (210c/u) at wk24 and 5.2-fold (249c/u) at wk48 compared with V38A alone, without significant changes in VL (from 5.1log at wk24 to 4.5log at wk48, $P = ns$). In contrast, the presence of D239H duplicates CD4 loss from BL (124c/u) to wk48 (35c/u) given by Q40H+L45M ($P = 0.05$), without significant changes in VL. Moreover, specific polymorphisms at BL, correlated ($P < 0.05$) with the on treatment development of T-20 resistance mutations, were identified. In particular, BL presence of P213Q and V321I correlated with development of V38A, while BL presence of R236Q correlated with development of N43D.

Conclusions: Gp41-mutational patterns under T-20 pressure are more complex than currently known, suggesting that an ordered network of mutations, regulated by natural polymorphisms present before T-20 treatment, modulates positively and negatively the HIV ability to damage the immune system. Their knowledge is important for a correct use of T-20 and for innovative therapeutic strategies.

Background

The HIV-1 envelope glycoprotein gp41 is essential for mediating the fusion between virus and host cell membranes (Dettlyn et al., *J Virol* 2001; Jung et al., *Curr Pharm Des* 2002). Moreover, gp41 is a target for neutralizing antibodies and is directly involved in mechanisms that trigger CD4+ T lymphocytes apoptosis thus playing a key role also in HIV-1 immunogenicity and pathogenicity (Kalia et al., *J Virol* 2003; Garg et al., *J Leukoc Biol* 2005; Micoli et al., *Virology* 2006).

T-20, the first fusion inhibitor approved for clinical practice, is a synthetic peptide that binds gp41 within a region encompassing residues 36-45. Recently, the T-20 resistance mutations V38A and Q40H+L45M have been significantly associated with a gain and with a loss of CD4 cell count, respectively (Aquaro et al., *J Antimicrob Chemother* 2006; Melby et al., *XIV International HIV Drug Resistance Workshop* 2006), thus suggesting the ability of T-20 selected mutations to modulate HIV-1 induced damage of the immune system.

Thus, the goal of this study is to investigate in a large cohort of T-20 treated patients the long-term association of such mutations with the viro-immunological parameters, and whether selected gp41 mutational clusters correlate with viro-immunological outcome.

Methods

Plasma-derived HIV-1 gp41 sequences from 75 HIV-1 infected patients were collected and analyzed. T-20 was added as a single active drug to the last failing regimen. Baseline samples from 51 out of 75 patients were also available for the analysis. Plasma-derived gp41 sequences from other 17 patients, not treated with T-20, were included in the analysis of T-20-related mutations.

The association of mutations with T-20 treatment was assessed by using Fisher exact tests, while the association between mutations present at BL and at week 24 of T-20 treatment was assessed by using logistic regression model.

The binomial correlation coefficient (ϕ) was calculated for any pair of mutations as a measure of the strength of their association. Significantly correlated pairs of mutations were assessed by using Fisher exact tests. Benjamini-Hochberg method was used to correct for multiple hypothesis testing, false discovery rate (FDR) of 0.05. A pairwise distance matrix was constructed to obtain dendrograms by using average linkage hierarchical clustering.

Wilcoxon tests were used to identify statistically significant differences in viremia and CD4 cell count between different time-points.

Mann-Whitney tests were used to compare the median fold change of viremia and CD4 cell count between baseline and different T-20 time-points in patients harboring HIV-1 strains with and without specific gp41 mutational clusters.

The X-ray crystallographic coordinates of HIV-1 gp41 deposited in the Protein Data Bank (PDB, <http://www.rcsb.org/PDB/>) with code 1f33 were used as template to construct the three-dimensional structure of the gp41 ectodomain trimers by using the Maestro software.

Results

Patients' characteristics at baseline						
N°	Male	Median Age years (IQR)	Sexual	Risk Factor N (%/75)	Drug addict	Other
75	65	43 (38-48)	26 (34.6)	7 (9.3)	46 (61.3)	43 (57.3)

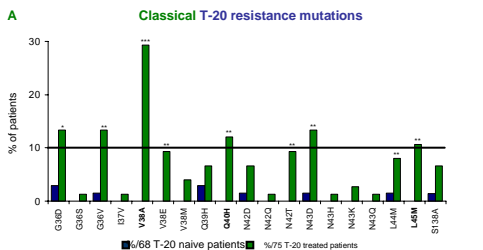
All patients were heavily drug-experienced with resistance to multiple NRTIs, NNRTIs, and PI

	NRTI	NNRTI	PI
Median number (IQR) of previously received drugs	5 (4-6)	1 (1-2)	

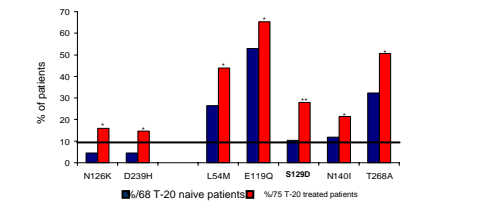
T-20 co-administered drugs N(%/75)	75 (100) ^a	11 (14.6) ^b	70 (93.3) ^c
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Median duration of resistance mutations 5 (3-8)
^a 8 out of 11 patients were receiving EFV.
^b 2 (2/2) 70 patients were receiving PI boosted with 2 (2/2)

Gp41 mutations associated with T-20 treatment

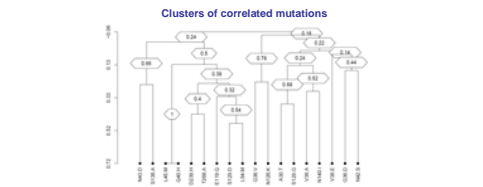
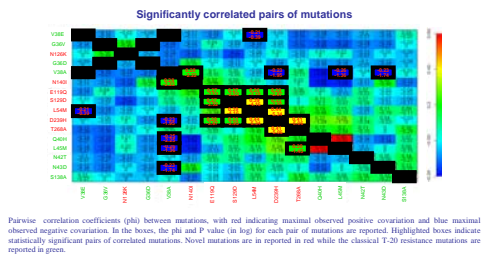


7 Novel gp41 mutations are associated with T-20 treatment



Frequency of the classical (A) and the novel (B) gp41 mutations in isolates from 68 T-20 naive patients and 75 T-20 treated patients. Statistically significant differences were assessed by Fisher exact tests. Mutations whose increase in frequency remained significant at a false discovery rate of 0.05 following correction for multiple testing are in boldface. * indicates $P < 0.05$; ** $P < 0.01$. In A, we reported all mutations known to be associated with T-20 resistance (Johnson et al., 2006; Mink et al., 2002; Greenberg et al., 2004; Miller et al., 2004).

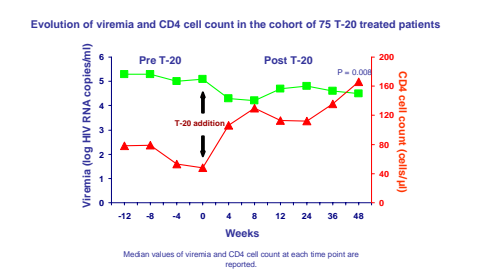
Novel gp41 mutations are strongly correlated in pairs and in clusters with the classical T-20 resistance mutations



Dendrogram obtained from average linkage hierarchical agglomerative clustering, showing clusters of known T-20 resistance mutations and novel mutations. The length of branches reflects distances between mutations in the original distance matrix. Bootstrap values, indicating the significance of clusters, are reported at the boxes.

By evaluating the entire gp41 sequences, we identified 7 novel mutations (L54M, E119Q, N126K, S129D, N140I, D239H, T268A) significantly associated with T-20 treatment. These novel mutations were significantly correlated, both in pairs and in cluster, with the classical T-20 resistance mutations. In particular, the novel N140I was correlated with the classical V38A, while the novel L54M, E119Q, S129D, D239H, T268A were correlated with the classical Q40H and L45M.

Association of gp41 mutations with the viro-immunological outcome



At virological failure, the presence of N140I reinforces the gain of CD4 cell count given by V38A

Pattern of mutations	Frequency ^a N (%)	Median Viremia (IQR)		Ratio ^b P value ^c	Median CD4 cells/u (IQR)		Ratio ^b P value ^c
		Baseline	week 24		Baseline	week 24	
38V	53 (70.6)	4.9 (4.3-5.1)	4.4 (4.0-4.8)		71 (36-174)	46 (28-197)	
38A	15 (20)	4.8 (4.4-5.2)	3.8 (3.3-5.3)	0.9 0.95	64 (26-135)	151 (98-249)	0.06
38A + 140I	7 (9.3)	5.1 (5.0-5.2)	4.5 (4.3-4.8)	0.9 0.96	40 (10-64)	210 (200-342)	0.02

Pattern of mutations	Frequency ^a N (%)	Median Viremia (IQR)		Ratio ^b P value ^c	Median CD4 cells/u (IQR)		Ratio ^b P value ^c
		Baseline	week 48		Baseline	week 48	
38V	53 (70.6)	4.9 (4.3-5.1)	4.8 (4.2-5.3)		71 (36-174)	23 (19-26)	
38A	15 (20)	4.8 (4.4-5.2)	4.3 (3.5-4.3)	0.9 0.94	64 (26-135)	104 (47-175)	0.05
38A + 140I	7 (9.3)	5.1 (5.0-5.2)	4.8 (4.2-5.3)	1.0 0.97	40 (10-64)	249 (196-260)	0.01

^a The frequency was calculated in isolates from 75 T-20 treated patients.
^b The ratio was calculated between the median fold change of viremia and CD4 cell count from baseline to week 24 and week 48 in the presence of 38A or 38A+140I versus the median fold change of viremia and CD4 cell count from baseline to week 24 and week 48 in presence of 38V.
^c P value was determined by Mann-Whitney test.
In presence of V38A+N140I, the increase of CD4 cell count from BL to week 24 and to week 48 is 1.9-fold and 5.2 fold-higher than that observed in presence of V38A alone (P=0.05).

The presence of D239H + T268A reinforces the loss of CD4 cell count given by Q40H+L45M

Pattern of mutations	Frequency ^a N (%)	Median Viremia (IQR)		Ratio ^b P value ^c	Median CD4 cells/u (IQR)		Ratio ^b P value ^c
		Baseline	week 48		Baseline	week 48	
40Q + 45L	68 (90.6)	5.2 (5.0-5.5)	4.7 (4.5-5.3)		48 (33-102)	166(200-200)	
40H + 45M	3 (4)	5.1 (4.6-5.7)	4.9 (4.5-5.4)	1.0 0.96	37 (19-54)	21 (14-29)	0.04
40H + 45M + 239H + 268A	4 (5.3)	5.4 (5.2-5.5)	4.7 (4.6-5.4)	1.0 0.97	124 (74-264)	35 (35-52)	0.04

^a The frequency was calculated in 75 T-20 treated patients.
^b The ratio was calculated between the median fold change of viremia and CD4 cell count from baseline to week 48 in the presence of 40H+45M or 40H+45M+239H+268A versus the median fold change of viremia and CD4 cell count from baseline to week 48 in presence of 40Q+45L.
^c P value was determined by Mann-Whitney test.
In presence of 40H+45M+239H+268A, the increase of CD4 cell count from BL to week 48 is 5.5-fold higher than that observed in presence of 40H+45M.

Specific polymorphisms at BL correlated with the development of T-20 resistance mutations

The baseline presence of V321I and P213Q correlated with the on treatment development of V38A

Amino acid	at Baseline of T-20 treatment		V38A at week 24 N(%/N _{BL})	O.R.	P value
	Frequency	N _{BL} (%/51)			
I	17 (33.3)		7 (41.2)		
V _{WT}	34 (66.7)		4 (11.8)	5.25	0.02
Q	18 (35.3)		8 (44.4)		
P _{WT}	33 (64.7)		6 (18.2)	3.60	0.04

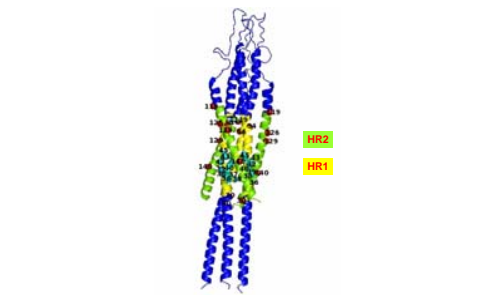
51 T-20 treated patients having two genotypes, one at beginning of the T-20 treatment and one at week 24 of T-20 treatment were selected. N_{BL} indicates the number of patients with the reported mutation at baseline. The odds ratio (O.R.) and P value were calculated by logistic regression analysis.

The baseline presence of R236Q correlated with the on treatment development of N43D

Amino acid	at Baseline of T-20 treatment		N43D at week 24 N(%/N _{BL})	O.R.	P value
	Frequency	N _{BL} (%/51)			
Q	7 (13.7)		3 (42.8)		
R _{WT}	44 (86.3)		1 (2.9)	53	0.0007

51 T-20 treated patients having two genotypes, one at beginning of the T-20 treatment and one at the virological failure of T-20 were selected. N_{BL} indicates the number of patients with the reported mutation at baseline. The odds ratio (O.R.) and the P value were calculated by logistic regression analysis.

Most novel mutations are localized in the HR2 domain of gp41 with the exception of L54M, D239H and T268A



Structure of the HIV-1 gp41 ectodomain trimer. The gp41 domains are color coded (HR1, yellow; HR2, green). T-20 resistance mutations are in cyan; novel mutations in red. Most novel residues (119, 126, 129, 140) are localized in the HR2 domain of HIV-1 gp41, with the exception of residue 54, that is localized in the HR1 domain and residues 239 and 268 that are localized in the gp41 ectodomain.

Results' Summary

Our study shows that other gp41 mutations (L54M, E119Q, N126K, S129D, N140I, D239H, T268A), beyond the currently known T-20 resistance mutations, are positively associated with T-20 treatment.

These novel mutations strongly correlated, both in pairs and in clusters, with the classical T-20-resistance mutations. In particular, the novel N140I was correlated with the classical V38A, while the novel L54M, E119Q, S129D, D239H, T268A were correlated with the classical Q40H and L45M.

Specific patterns of T-20 selected mutations significantly correlated with the virological outcome of T-20 treated patients. In particular:

- The co-presence of N140I with V38A is significantly associated with an increase of CD4 cell count from BL to week 24 and to week 48. In particular, the increase of CD4 cell count in presence of V38A+N140I from BL to week 48 is 20.6-fold higher than that observed in the absence of mutations at position 38.

- In contrast, the co-presence of D239H+T268A with Q40H+L45M is significantly associated with a remarkable loss of CD4 cell count from BL to week 48.

In addition, the presence of specific polymorphisms at baseline (before T-20 treatment) significantly correlated with the on treatment development of T-20 resistance mutations. In particular, the baseline presence of P213Q and V321I correlated with the development of V38A, while BL presence of R236Q correlated with development of N43D.

Conclusions

Specific patterns of mutations associated with T-20 treatment may modulate positively and negatively the HIV-1 ability to damage the immune system. In particular, the cluster V38A+N140I is associated with a gain of CD4 cell count, while the cluster Q40H+L45M+D239H+T268A is associated with a loss of CD4 cell count. In addition, the presence of some polymorphisms at baseline may be specifically associated with the on treatment development of the classical T-20 resistance mutations. Such polymorphisms may represent crucial determinants not only for the course of resistance evolution, but also for the pathogenetic progression of HIV-1 infection.