Resistance mutations to protease inhibitors in proviral DNA of HIV-2 infected patients predict response to treatment

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Background and Aims

- HIV-2 has recently been ranked as the third most important human pathogen in Europe based on the H-index. [1]
- The impact of HIV-2 infection is especially noted in Portugal where this virus accounts for 3.3% of all HIV cases. [2]
- Compared to HIV-1, data on the diversity of the HIV-2 protease (PR) gene and evolution of resistance to protease inhibitors (PIs) is limited. Moreover, the impact of PI resistance mutations in the HIV-2 provirus on treatment outcomes of HIV-2 infected individuals is still unclear. [3,4]
- Hence, the main objectives of this study were to: 1) make the first characterization of protease diversity and PI resistance mutations in HIV-2 proviral DNA archived in peripheral blood mononuclear cells (PBMCs) of PI treated and untreated HIV-2 infected individuals living in Portugal over a period of eight years; 2) evaluate the impact of resistance mutations in treatment outcome eight years post-therapy.

Methods

- The PR gene was amplified from proviral DNA present in PBMCs from 27 HIV-2 infected patients attending a central hospital in Lisbon, Portugal. Fifteen were on treatment and 12 were untreated.
- Amplified products were cloned into pCR4-TOPO® (Invitrogen) and a median of 8 clones per patient were sequenced.
- PR diversity was analyzed by phylogenetic and entropy analysis.
- PIs resistance mutations were identified using EU HIV-2 internet tool-HIV Grade (http://www.hiv-grade.de).
- The treatment outcomes and resistance mutations of all patients were analyzed eight years after enrolment.

Results

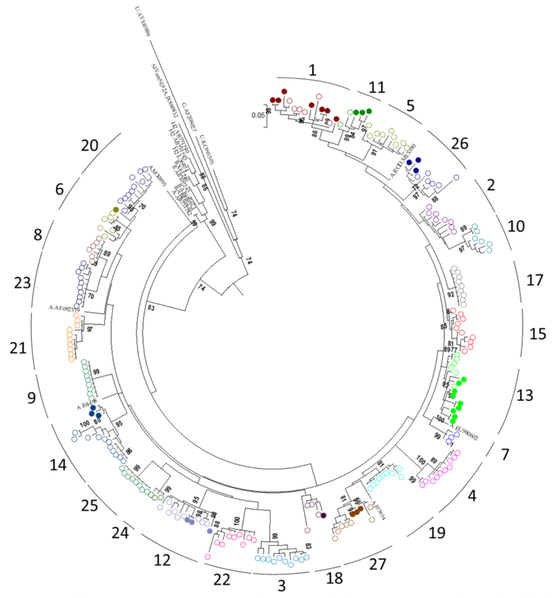
At study entry, median T CD4+ cell count was significantly lower in the treated patients compared to untreated patients (264 cells/mm3±197 vs 553 cells/mm3±442; P=0.0019). Of the 22 patients with viral load data, 5 (19%) had detectable viral load (range: 8841-100.000 copies/ml) and 17 had low or undetectable levels (<200 copies/ml).

Variable	Total (%)	Treated patients (%)	Untreated patients (%)	P value[a]
No. of subjects (%)	27 (100)	15 (56)	12 (44)	
Gender [N (%)]				0.6957*
Female	19 (70)	10 (67)	9 (75)	
Male	8 (30)	5 (33)	3 (25)	
Mean age, years (SD; range)	48 (11.5;27-64)	48 (9.7;29-63)	47 (14;27-64)	0.8261#
Country of origin [N(%)]				
Portugal	8 (30)	5 (33)	3 (25)	0.6957*
Guinea-Bissau	12 (45)	5 (33)	7 (58)	0.2576*
Cape-Verde	3 (11)	3 (20)	0 (0)	0.2308*
Mozambique	2 (7)	2 (14)	0 (0)	0.4872*
Unknown	2 (7)	0 (0)	2 (17)	0.1880*
Ethnicity [N(%)]				
Caucasian	10 (37)	5 (33)	5 (42)	0.7063*
Black	15 (55)	9 (60)	6 (50)	0.7068*
Indian	1 (4)	1(7)	0 (0)	1.0000*
Unknown	1 (4)	0 (0)	1 (8)	0.4444*
Median CD4, cells/mm3 (SD; range)	448 (400;72-1594)	264 (197;72-731)	553 (442;305-1594)	0.0019#
Viral load, cp/ml [N(%)]				
<200	17 (62)	10 (67)	7 (58)	0,7063*
>200 [N(%; range)]	5 (19; 8841-100.000)	3 (20; 8841-100.000)	2 (17; 10425-13627)	1.0000*
Unknown	5 (19)	2 (13)	3 (25)	0,6280*

aP values are based on comparison of treated patients and untreated patients. Values in bold indicates a statistically significant difference (P<0.05);
*Fisher´s exact test;
Mann Whitney test;
SD-standard deviation.

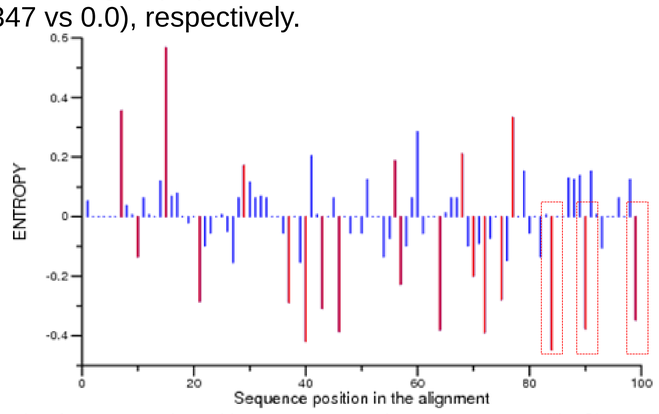
Eight years after enrolment median total T CD4+ cell count was 625 cells/mm3 ±316 (range: 163-1369). Of the 13 patients with viral load data, 9 (69.2%) had undetectable viral load (<40 copies/ml) and the remaining 4 (30.7%) had detectable viral levels (range: 67- 6637 copies/ml).

Genetic distance is similar between treated and untreated patients. Mean genetic distance within patients was 0.020±0.012 substitutions per site and did not differ significantly between treated and untreated patients (0.02379 vs 0.01658, p= 0.1611, respectively)*. In total, 91 clonal sequences were generated from treated patients and 96 from untreated patients (median: 8 clones/patient; min-max: 1-11)



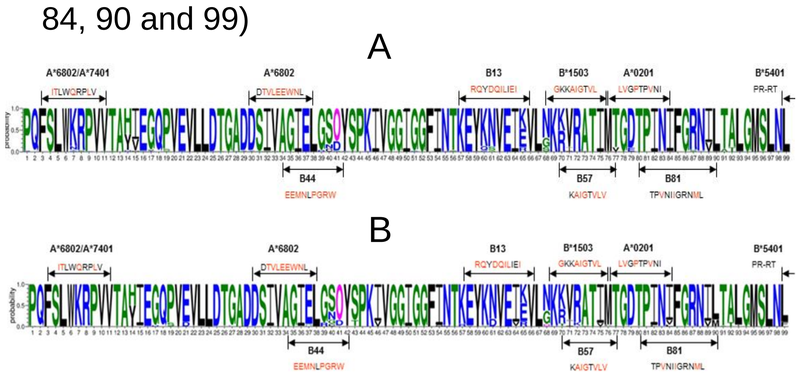
*Clonal sequences from HIV-2 patients are shown with the patients numbers highlighted next to the respective cluster. Only bootstrap values above 70% are shown. Filled markers correspond to 2015 protease sequences; non-filled markers correspond to protease sequences at study entry.

Significant entropy variation between PR sequences from treated and untreated patients⁺. Amino acids at positions 84, 90 and 99 well associated with HIV-2 resistance to PIs presented higher entropy in treated group compared with untreated group (0.51 vs 0.063; 0.44 vs 0.063 and 0.347 vs 0.0), respectively.



⁺Diversity of protease amino acid sequences at study entry as assessed by Shannon's entropy. Entropy difference between protease sequences from treated and untreated patients. Sites with significant differences in entropy (with P≤0.05) are shown in red in the plot.

Interestingly, 13 out of the 20 (65%) amino acids showing high entropy were located in CTL epitopes described for HIV-1 PR (positions: 7, 37, 40, 57, 64, 68, 70, 72, 75, 77, 84, 90 and 99)



Diversity of protease amino acid sequences at study entry as shown in LOGO plots. (A) untreated patients; (B) treated patients. The colors of amino acids correspond to their hydrophobicity: hydrophilic amino acids are blue, neutral are green and hydrophobic are black. N-linked glycosylation sites are marked as "O" in pink color. The overall height of each letter or a stack of letters indicates the sequence conservation at that position (measured in probability. LOGO plots were generated using AnalyzeAlign. The location and sequence of HLA restriction elements of CTL epitopes present in HIV-1 protease are indicated on the amino acid sequence. The CTL epitopes were obtained from http://www.hiv.lanl.gov/content/immunology. Red letters indicate residues that differ from HIV-1 sequence for which the epitope was defined.

At study entry 42.8% of the patients treated with PIs harbored at least one proviral DNA clone with resistance mutations. The most common resistance mutations in PIs treated patients were L90M (n=3, 21.4%) and I84V (n=2, 14.2%). Genotypic characterization of resistance mutations was done at year 8 for 10/16 treated patients. Of these, two had resistance mutations at baseline that were not detected eight years later **(patients 1 and 14)**, one presented more resistance mutations than those initially detected **(patient 11)**; two presented resistance mutations only at the last genotypic analysis **(patients 8 and 12)** and two had no detectable resistance mutations at baseline or eight years after study entry **(patients 6 and 13).**

Patient	2007 VL cp/ml	2007 CD4 count cells/mm³	2007 ART[a]	2007 Resistance mutations	2007 Resistance Profile [ab]	2015 VL cp/ml	2015 CD4 count cells/mm³	2015 ART	2015 Resistance mutations	2015 Resistance Profile [ab]
1	<200	600	AZT,3TC, LPV/r	I84V (1/6)	DRV,LPV, SQV/r	<40	813	AZT/3TC, LPV/r	None (0/7)	-
2	<200	313	3TC,AZT, SQV	-	-	NA	385	3TC/AZT, RAL	-	-
3	>100000	72	3TC,d4T, SQV/r	I84V (10/10), L90M (10/10)	DRV,LPV, SQV	NA	NA	NA	-	-
4	75571	159	AZT,3TC, LPV/r	I54M (2/2), I82F (2/2), L90M (2/2)	DRV,LPV, SQV	NA	NA	NA	-	-
5	<200	403	AZT,3TC, LPV/r	G48R* (1/9)	?	NA	946	TDF/FTC, DRV/r	-	-
6	<200	484	AZT,3TC, SQV/r	-	-	125	295	TDF,3TC, DRV/r	None (0/1)	-
7	<200	409	AZT,3TC, LPV/r	-	-	NA	969	ABC/3TC, DRV/r	-	-
8	<200	87	AZT,3TC, IDV/r	-	-	NA	413	TDF/FTC, SQV/r	I84V(1/1); L90M (1/1)	DRV, LPV, SQV
9	<200	448	AZT,3TC	I50T* (2/9)	?	NA	NA	NA	-	-
10	<200	264	TDF/FTC, LPV/r	-	-	NA	NA	NA	-	-
11	NA	161	TDF/FTC, LPV/r	I54M (1/2), I82F (1/2)	DRV,LPV	<40	420	RAL, DRV/r	V47A (1/3); I54M (2/3);I82F (3/3); I84L* (2/3); L90M (2/3)	DRV, LPV, SQV
12	NA	190	ABC,3TC, SQV/r	-	-	<40	163	ABC,RAL DRV/r	I54M (1/3); I84V (1/3)	DRV, LPV; SQV/r
13	<200	99	AZT/3TC, LPV/r	-	-	<40	866	AZT/3TC, SQV/r	None (0/9)	-
14	<200	731	3TC,d4T, SQV/r	I84L* (4/11), L90M (4/11)	DRV,LPV/r SQV	<40	1369	DRV/r, RAL	None (0/3)	-
15	NA	1182	Untreated	-	-	NA	NA	NA	-	-
16	<200	1202	Untreated	-	-	NA	NA	NA+	-	-
17	<200	305	Untreated	-	-	<40	433	Untreated	-	-
18	<200	548	Untreated	-	-	67	428	TDF/FTC, RAL	None (0/1)	-
19	NA	1594	Untreated	-	-	NA	NA	NA+	-	-
20	NA	557	Untreated	-	-	NA	NA	NA	-	-
21	10425	453	Untreated	-	-	NA	NA	NA	-	-
22	<200	722	Untreated	-	-	100	507	TDF/FTC, DRV/r	-	-
23	<200	462	Untreated	I84V (1/10)	DRV,LPV, SQV/r	<40	766	ABC/3TC, SQV/r	-	-
24	<200	546	Untreated	-	-	NA	NA	NA+	-	-
25	<200	1409	Untreated	L90M (1/9)	DRV,LPV/r ,SQV	<40	754	TDF/FTC, LPV/r	-	-
26	13627	385	Untreated	-	-	6637	299	AZT/3TC, RAL	None (0/3)	-
27	8841	254	ABC,3TC, SQV/r	-	-	<40	803	ABC/3TC, SQV/r	None (0/3)	-

[a]Although included in HIV-Grade, IDV exhibits at least partial resistance in vivo and/or in vitro to HIV-2 and is not recommended for clinical use in HIV-2 infected patients; NA - not available; Patient 9 was on non-PI-based ART; [ab] According to HIV-Grade; *G48R, I50T and I84L are rare mutations not yet scored by HIV-Grade.

Conclusions

- **Our results suggest that proviral DNA is a good alternative to genomic RNA for testing for drug resistance mutations in HIV-2 infected patients.**
- **These results also indicate that early resistance analysis of the viruses archived in PBMCs predict treatment response particularly at low or undetectable viral loads.**

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