Pol secondary mutations in HIV-1 subtype B and non-B isolates coming from acute infected patients followed prior and after initiation of HAART

Poster #26

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Background

Current knowledge concerning HIV resistant mutations to HAART has been mainly obtained from patients harboring subtype B strains and at chronic phases of infection. Therefore little information regarding the combination of HAART and immune-based selective pressures in acute infected patients is available.

Objective

To follow-up HAART related mutations and polymorphisms among acute infected patients harboring B and non-B HIV strains.

Patients and methods

Viral RNA isolates from HIV-1 acute infected individuals enrolled in an ongoing clinical trial (HAART + CSA) were amplified and sequenced to obtain fragments of *gag* 460 bp and *pol* 1496 bp (1570-2034; 2253-3749 relative positions in HXB2). Subtype assignment was perform by phylogenetic analysis and bootscanning[1] of *gag* and *pol* partial sequences. HAART related mutations were screened by using the Stanford University guidelines[2]. In addition, HLA genotyping was available as a study parameter.

Results I

Thirteen patients (12 men and 1 woman) were studied, among which MSM (9/12) was the main transmission category. Beyond B and F subtypes different "CRFs" circulating recombinant forms related-viruses were present. Particularly, strains related to CRF02, CRF11 and CRF15 were identified when running *pol* and *gag* bootscanning (Fig. 1a-b). Patients harboring mosaic genomes CRF15 (1010) and D/CRF11 (1030) were presumably infected abroad at Thailand and Congo, respectively. Second time-point assessment (2-4 weeks after HAART initiation) of *pol* sequences in 9 cases, allowed the analysis of intrapatient sequence phylogeny (Fig.2) and Pol mutation evolution.

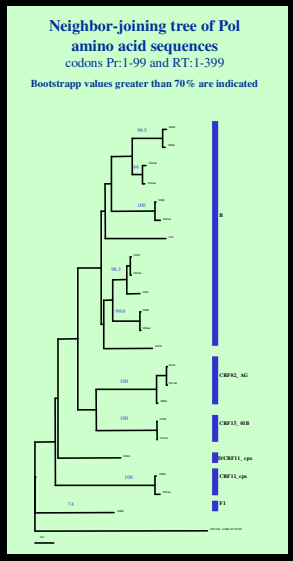
Table 1

HAART related mutations and *gag/pol* genotyping results

Viral isolate	Viral genotype	RTIs related mutations after starting tx: emergence	RTIs related mutations after starting tx: reversion	PIs related mutations
1010	CRF15_AE/B	E138EK, K219KT	--	I13V, E35D, M36I, N37D, R41K, H69K, I93M
1021	CRF02_AG	--	--	K20I, T12Q, I13V, K14R, I15V, E35D, F53L, M36I, L63P, R41K, H69K, L89M
1025	CRF11_AE/G/J	P245LP	I293IV	T12A, I15V,G16A, E35D, M36I, N37D, D60E, L63T, Q64D, I72VL, H69K, L72I, V77I, R83L, L89M
1028	CRF02_AG	ND	ND	T12Q, I13V, K14R, K20I, E35D, M36I, R41K, H69K, L72I, V77I, L89M
1029	F1	ND	ND	L10V, I13V, G16E, E35D, M36I, F53Y, R41K, H69K, D60E, G63N, H69V, L72I, V77I, L89M
1030	D/CRF11	ND	ND	I15V, G16A, E35D, M36I, N37D, D60E, G63E, L63I D63E, H69K, L89M, I135V, I84V
1013	B	ND	ND	K14R, N37S, R41K, L63H, E65D, K70R
1022	B	Q34EK, V24KQ, E312EK	--	K14R, N37S, R41K, L63P, E65D, K70R
1023	B	Q24EK	--	K14R, N37S, R41K, L63P, E65D, K70R
1024	B	I293IR	--	T12P, K14R, L63P, A71T, R83L
1026	B	--	--	E35D, R41K, I72V
1027	B	--	--	K14R, V7I
1031	B	--	--	M36I, L63P, T45I, I15V, M43T, R2A

RTIs= Reverse transcriptase inhibitors
PIs= Protease inhibitors
ND = not determined

Figure 2



Results II

Mutations to Protease Inhibitors remained stable after HAART (table 1). By contrast, mutations to Reverse transcriptase Inhibitors (RTIs) did show changes after treatment intervention (table 1). No major resistance mutations were seen. Additionally, NNRTIs minor mutation K103N was present in patient 1029 harboring a clade F strain.

Conclusions and discussion

Nearly half of the strains were HIV-1 recombinant genomes of which two have not been yet reported in the local epidemic[3]. Resistance transmission to NNRTIs was verified in one case. Emergence and reversion of RTIs polymorphisms were observed shortly after treatment intervention. Nevertheless, no relation with *pol* gene encoding known CTL epitopes that overlap RT resistance mutation sites[4] were found when analyzing both, autologous sequences and HLA types. Substitution N37I in isolate 1028 has been previously reported among treated patients, here we identified it naturally occurring in a CRF02 virus. Mutations at Pr 34-42 can affect viral antigenicity of CTL epitope EEMNLPGRW in individuals expressing HLA-B44. Two patients harboring mutations at Pr 32-42 were HLA-B44.

Acknowledgments

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References

1. NCBI Genotyping tool. http://www.ncbi.nih.gov/projects/genotyping/
2. Stanford University HIV Resistance Database. http://hivdb6.stanford.edu/
3. HIV database, Los Alamos. Subtype distribution in Switzerland. http://www.hiv.lanl.gov/components/hiv-db/new_geography/
4. Mason et al., 2004. J. Immunol. 172: 7212-7219.

Figure 1a *pol* Bootscanning

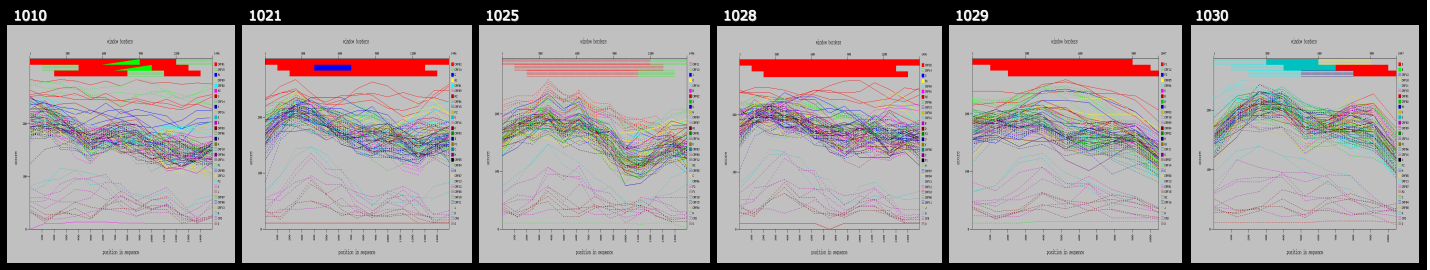
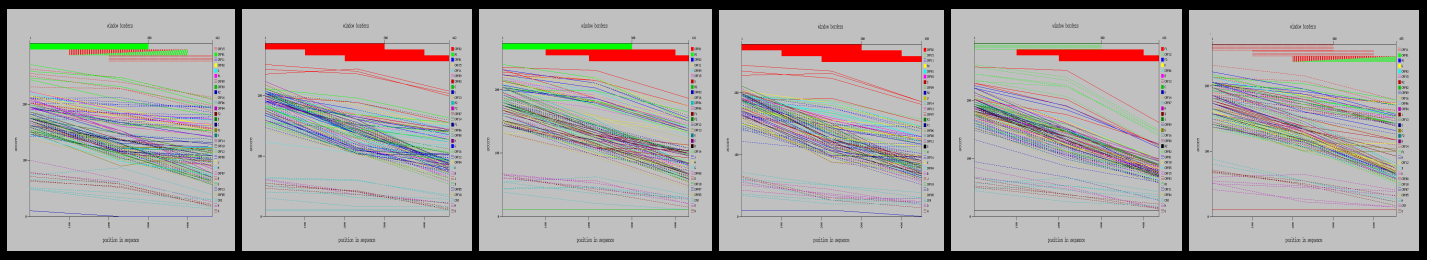
Figure 1b *gag* Bootscanning