Characterization of HIV-2 Variants in Response to *in vitro* Passage with Lopinavir

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Backgroun

- Human immunodeficiency virus type 2 (HIV-2) infection is endemic in Western Africa and has spread in the last decade to India and Europe. HIV-2 infection is associated with significant morbidity and mortality.
- Lopinavir/ritonavir (LPV/r) has demonstrated durable antiviral activity in HIV-1 infected antiretroviral-naïve and protease inhibitor (PI)-experienced patients.[1,2] Response rates are diminished in those with a greater than 10-fold reduced baseline susceptibility to LPV.
- The presence of 6 or more LPV-associated resistance mutations in the protease gene (L10F/I/R/V, K20M/R, L24I, M46I/L, F53L, I54L/T/V, L63P, A71I/L/T/V, V82A/F/T, I84V, AND L90M) is also associated with lower virologic response rates in HIV-1 infected patients.[3] The emergence of the I47A mutation (a two step mutation of I47→I47V→I47A) together with V32I has recently been described *in vitro* and in two HIV-1-infected patients during LPV/r therapy. Emergence of this mutation was associated with significant reductions in LPV activity.[4]
- Case reports have suggested antiviral activity of LPV against HIV-2.[5], but limited information is available on LPV antiviral activity and mechanisms of resistance in HIV-2. Several reports describe the failure of LPV in HIV-2 infected patients in association with a single mutation of 47V to 47A.[6]
- Information on LPV activity against HIV-2, patterns of HIV-2 resistance mutations to LPV, or cross-resistance to other PIs of HIV-2 isolates with reduced LPV sensitivity is limited.

Method

- The activity of different PIs including LPV against various wild-type HIV-2 strains were compared in relation to the wild type HIV-1 strain pNL4-3.
- HIV-2 strain MS was chosen for the *in vitro* resistance selection study.
- Mutations were introduced into an HIV-2 ROD molecular clone by using the QuikChange II XL site-directed mutagenesis kit (Stratagene).
- The replication capacity of the mutants was determined using the MT4/RT assay.
- Generation of resistant variants:

MT4 cells were infected with HIV-2 MS strain at an MOI of 0.003 for 2 hours, washed, then cultured in increasing concentrations of LPV beginning with 10 nM. Concentrations were increased by 2- to 3-fold every passage for over one month.

  - HIV-2 levels were monitored by determination of p27 antigen levels and observed cytopathic effects. When p27 levels were positive, supernatant was harvested and virus was serially passaged using one aliquot of viral supernatant to infect fresh MT4 cells.
  - The protease coding region was amplified from the passaged variants and underwent automated sequencing (ABI-373)

Result

- LPV demonstrated activity similar to that observed against HIV-1 virus in two strains of HIV-2 (MS and CBL-23) with EC_{50} values of 12-15 nM in MS, compared to 18 nM in HIV-1 pNL4-3. (Table 1). However, HIV-2 strain CDC310319 had increased LPV EC_{50} values of 180 nM.
- The substitutions 10V, 32I, 36I, 46I, 47V, 71V and 82I, which are associated with PI resistance in HIV-1, were present in all HIV-2 MS strain (Figure 1).
- Compared to HIV-2 strains MS and CBL-23, HIV-2 CDC310319 had six unique substitutions: 7R, 12K, 64V, 67V, 91N, 92S and 99F (Figure 1).
- Passage of HIV-2 MS with LPV selected a viral strain with two mutations (D17N and V47A) which displayed 34-fold reduced susceptibility to LPV (Figures 2A & 2B).
- Recombinant single mutant V47A and double mutant G17N/V47A exhibited approximately 10-fold reduced susceptibility to LPV (Table 2).
- The single mutant V47A and double mutant G17N/V47A displayed apparent 5- to 10-fold hypersusceptibility to ATV and SQV, compared to HIV-2 ROD but retained wild-type susceptibility to DRV and the other PIs tested. (Table 2).
- Both V47A and G17N/V47A mutants grew slightly more slowly than wild-type HIV-2 (Figure 3).

Table 1 Anti-HIV-2 activities of Protease Inhibitors

Inhibitor	Mean EC_{50} (nM)					
	HIV-1 NL4-3	HIV-2 MS	HIV-2 CBL23	HIV-2 CDC310319	HIV-2 ROD*	
LPV	18	15	12	180	35	
ATV	5	20	39	110	34	
SQV	12	5	8	68	33	
RTV	50	349	514	665	421	
IDV	41	22	33	108	65	
NFV	32	48	83	389	281	
APV	69	900	939	674	855	
DRV	12	nd	83	155	9	

* ROD is the moleuclar clone used to test individual mutations

Figure 1 Alignment of MS with HIV-1 NL4-3 and LPV-derived mutants.

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                  10        20        30        40        50        60        70        80        90
                   *         *         *         *         *         *         *         *         *
HIV1-NL4-3   PQITLWQRPL VTIKIGGQLK EALLDTGADD TVLEEMNLPG RWKPKMIGGI GGFIKVGQYD QILIEICGHK AIGTVLVGPT PVNIIGRNLL TQIGCTLNF
HIV2-MS      ..FS..K..V ..AH.ED.PV .V........ SIVAGIE.GD NYT..IV... ....NTKE.K NVE.KVLNKR VRA.IMT.D. .I...F...V. .AL.M...L
HIV2-CBL-23  ..FS.LK..V ..AYVE..PV .V........ SIVAGIE.GS NYS..IV... ....NTKE.K NVK..VL.K. VRA.IMT.D. .I...F...I. .AL.MS...L
HIV2-CDC310319 ..FS..R..V .KAN.E..PV .V........ SIVAGIE.GN NYT..IV... ....NTKE.K NVEV.VV.KR VRA.IMT.D. .I...F...I. NSL.M.....
HIV2-ROD     ..FS..K..V V.AY.E..PV .V........ SIVAGIE.GN NYS..IV... ....NTKE.K NVE..VL.K. VRA.IMT.D. .I...F...I. .AL.MS..L
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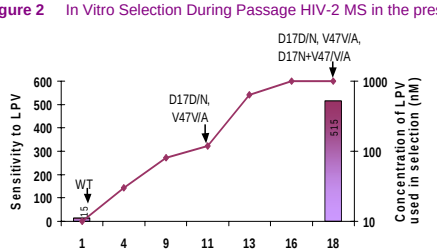
Figure 2 In Vitro Selection During Passage HIV-2 MS in the presence of LPV: Genotype & Phenotype

A.



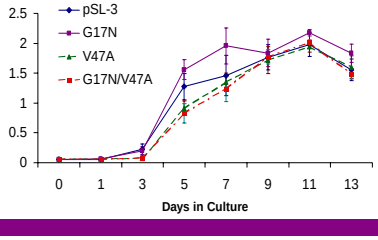
B.

Passage #	1	11	18
Conc. Of LPV [nM]	10	120	1000
Genotype	(# clones/total clones)		
WT	8/8	12/23	5/22
D17N	0/8	7/23	5/22
V47A	0/8	4/23	1/22
D17N/V47A	0/8	0/23	11/22
IC50 [nM]	15		515
Fold Change	1		34

Table 2 Susceptibility of Molecular Mutant Clones to PIs

Inhibitors	EC_{50} in nM (Fold Change in EC_{50})			
	HIV-2 ROD	G17N	V47A	G17N/V47A
LPV	35	28 (1)	340 (10)	260 (8)
ATV	34	20 (0.6)	5 (0.15)	3 (0.1)
SQV	33	22 (0.7)	2 (0.1)	2 (0.1)
RTV	421	310 (0.7)	115 (0.3)	84 (0.2)
IDV	65	52 (0.8)	34 (0.5)	34 (0.5)
NFV	281	199 (0.7)	177 (0.6)	100 (0.4)
APV	855	583 (0.7)	630 (0.7)	404 (0.5)
DRV	9	6 (0.7)	10 (1)	8 (1)

Figure 3 Replication Capacity of the Molecular Mutant Clones



Conclusions

- LPV demonstrated substantial antiviral activity against some HIV-2 strains. This observation is consistent with previous reports of antiviral activity of LPV/r in HIV-2 infected patients.
- LPV activity was not consistent across the three HIV-2 strains tested. The strain CDC310319 appeared approximately 10-fold less susceptible to LPV than HIV-2 MS or CBL-23.
- Emergence of a single mutation (V47A) is associated with significantly reduced LPV activity against HIV-2, suggesting that the presence of V47 in wild-type HIV-2 can impact the genetic barrier noted with LPV/r treatment of HIV-1 infection.
- HIV-2 isolates containing the V47A mutation maintained susceptibility to DRV and were apparently hypersusceptible to SQV and ATV, suggesting that these protease inhibitors may be effective options for infected patients who have HIV-2 with a V47A mutation.

Reference

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