Abstract #41

Replication Capacity and Drug Susceptibility of Lopinavir-Resistant HIV-2

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Background

- 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]
- Lower response rates to LPV/r are observed in HIV-1 infected PI-experienced patients with 10-fold or greater reduced susceptibility to LPV. Similarly, 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.[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]
- Several case 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.

Methods

- The susceptibilities of the wild-type HIV-2 strains (MS, CBL-23, and CDC310319), passaged HIV-2 variants and the mutant molecular clones were determined by the MTT colorimetric assay or an RT assay.
- LPV-resistant HIV-2 variants were generated by passaging the HIV-2 MS strain in the presence of increasing concentrations of LPV.
  - 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 CPE. 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.
- 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.

Results

- The activity of LPV against HIV-2 MS and CBL-23 was similar to that against HIV-1. Activity against HIV-2 CDC310319 was reduced 10-fold compared to HIV-1 (Table 1).
- While SQV and IDV maintained activity against HIV-2 MS and CBL-23 comparable to HIV-1, varying levels of reduced susceptibility were generally observed in these two strains to ATV, RTV, NFV, APV and TMC-114 (Table 1).
- The third HIV-2 strain (CDC310319) was generally less susceptible than HIV-2 MS or CBL-23 to all PIs tested (Table 1).

Table 1. Anti-HIV-2 Activities of Protease Inhibitors In Vitro

	Mean EC_{50} (μM) ± SD			
Inhibitor	**HIV-1 NL4-3**	**HIV-2 MS**	**HIV-2 CBL-23**	**HIV-2 CDC310319**
LPV	0.018	0.015	0.012	0.180
ATV	0.005	0.020	0.039	0.110
SQV	0.012	0.005	0.008	0.068
RTV	0.050	0.349	0.514	0.665
IDV	0.041	0.022	0.033	0.108
NFV	0.032	0.048	0.083	0.389
APV	0.069	0.900	0.939	0.674
TMC-114	0.012	nd	0.083	0.155

- The substitutions 10V, 32I, 36I, 46I, 47V, 71V and 82I, which are associated with PI resistance in HIV-1, were present in all three strains of HIV-2 (Figure 1).
- Compared to HIV-2 MS and HIV-2 CBL-23 strains, HIV-2 CDC310319 had six unique substitutions: 7R, 12K, 64V, 67V, 91N, 92S and 99F (Figure 1).

Figure 1. Sequence Alignment of the HIV Strains With HIV-1 NL4-3 and HIV-2 MS

A: Alignment with HIV-1 NL4-3

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                          10         20         30         40         50         60         70         80         90
                           *          *          *          *          *          *          *          *          *
HIV-1 NL4-3      PQITLWQRPLVTIKIGGQLK 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
HIV-2-CBL23      ..FS.LK..V..AYVE..PV .V........ SIVAGIE.GS NYS..IV... ....NTKE.K NVK..VL.K. VRA.IMT.D. .I..F...I. .AL.MS..L
HIV-2-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....
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B: Alignment with HIV-2 MS

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                          10         20         30         40         50         60         70         80         90
                           *          *          *          *          *          *          *          *          *
HIV2-MS          PQFSLWKRPV VTAHIEDQPV EVLLDTGADD SIVAGIELGD NYTPKIVGGI GGFINTKEYK NVEIKVLNKR VRATIMTGDT PINIFGRNVL TALGMTLNL
HIV-2-CBL23      .....L.... ...YV.G... .......... .........S ..S....... .......... ..K.E..G.K .......... ........I. .....S...
HIV-2-CDC310319 ......R... .K.N..G... .......... .........N .......... .......... ...VE.VG.. .......... ........I. NS......F
HIV-1 NL4-3      ..IT..Q..L ..IK.GG.LK .A........ TVLEEMN.PG RWK..MI... ....KVGQ.D QIL.EICGHK AIG.VLV.P. .V..I...L. .QI.C...F
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- 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 2 and 3 and Table 2).

Figure 2. In Vitro Selection and Genotype of the Mutant Variants Observed During Passage of HIV-2 MS in the Presence of LPV

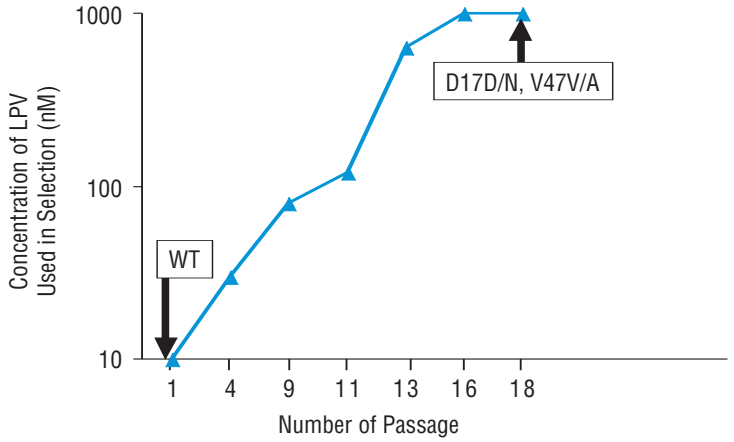

Figure 3. Sequence Alignment of the In Vitro Passaged HIV-2 Variants with HIV-2 MS

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                    10         20         30         40         50         60         70         80         90           Fraction
                     *          *          *          *          *          *          *          *          *           of clones
HIV2-MS  PQFSLWKRPV VTAHIEDQPV EVLLDTGADD SIVAGIELGD NYTPKIVGGI GGFINTKEYK NVEIKVLNKR VRATIMTGDT PINIFGRNVL TALGMTLNL
P11      .......... .......... .......... .......... .......... .......... .......... .......... .......... .........     12/23
P11      .......... ......N... .......... .......... .......... .......... .......... .......... .......... .........      7/23
P11      .......... .......... .......... .......... ......A... .......... .......... .......... .......... .........      4/23

P18      .......... .......... .......... .......... .......... .......... .......... .......... .......... .........      5/22
P18      .......... ......N... .......... .......... .......... .......... .......... .......... .......... .........      5/22
P18      .......... .......... .......... .......... ......A... .......... .......... .......... .......... .........      1/22
P18      .......... ......N... .......... .......... ......A... .......... .......... .......... .......... .........     11/22
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Table 2. Susceptibility of HIV-2 MS Passage Variants to LPV and Other PIs

Passage	Conc. of LPV Used in Selection (μM)	Sensitivity to LPV	
		IC_{50} (μM)	Fold Change in IC_{50}
Wild-type	NA	0.015	1
P18	1.0	0.515	34

- Recombinant single mutant V47A and double mutant G17N/V47A exhibited approximately 10-fold reduced susceptibility to LPV (Table 3).
- In contrast, these two mutants retained wild-type susceptibility to TMC-114 and the other PIs tested.
- In addition, the single mutants displayed 5- to 10-fold hypersusceptibility to ATV and SQV, compared to HIV-2 ROD.

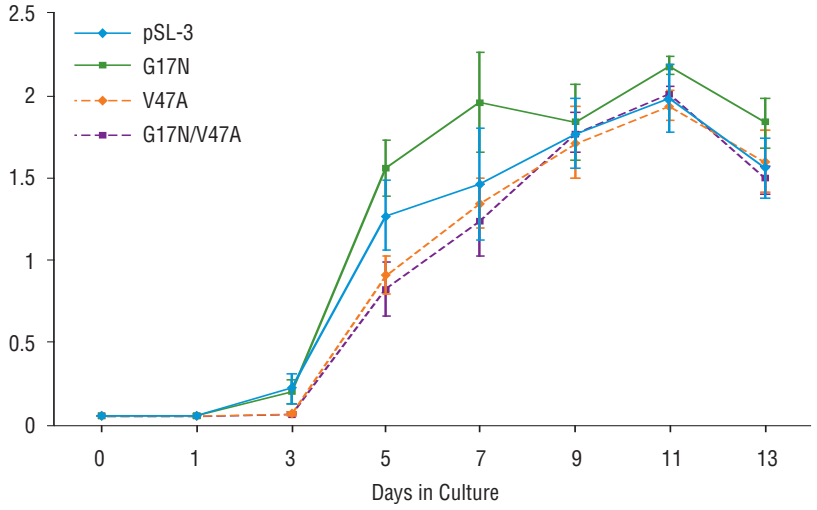
Table 3. Susceptibility of the Molecular Mutant Clones to LPV and Other PIs

Inhibitor	IC_{50} in μM (Fold Change in IC_{50})			
	HIV-2 ROD	G17N	V47A	G17N/V47A
LPV	0.035	0.028 (1)	0.34 (10)	0.26 (8)
ATV	0.034	0.020 (0.6)	0.005 (0.15)	0.003 (0.1)
SQV	0.0036	0.0023 (0.7)	0.0004 (0.1)	0.0004 (0.1)
RTV	0.421	0.310 (0.7)	0.115 (0.3)	0.084 (0.2)
IDV	0.065	0.052 (0.8)	0.034 (0.5)	0.034 (0.5)
NFV	0.281	0.199 (0.7)	0.177 (0.6)	0.100 (0.4)
APV	0.855	0.583 (0.7)	0.630 (0.7)	0.404 (0.5)
TMC-114	0.009	0.006 (0.7)	0.01 (1)	0.008 (1)

Results (cont.)

- Both V47A and G17N/V47A mutants grew slightly more slowly than wild-type HIV-2 (Figure 4).

Figure 4. Replication Capacity of the Molecular Mutant Clones



Conclusions

- LPV demonstrated substantial antiviral activity against select HIV-2 strains. This observation is consistent with previous reports of antiviral activity of LPV/r in HIV-2 infected patients.
- LPV activity was inconsistent across the three strains tested with HIV-2 CDC310319 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 TMC-114 and were 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.

References

1. Hicks, C., M. S. King, R. M. Gulick, A. C. White, Jr., J. J. Eron, Jr., H. A. Kessler, C. Benson, K. R. King, R. L. Murphy, and S. C. Brun. 2004. Long-term safety and durable antiretroviral activity of lopinavir/ritonavir in treatment-naive patients: 4 year follow-up study. *AIDS* 18:775-9.
2. Benson, C. A., S. G. Deeks, S. C. Brun, R. M. Gulick, J. J. Eron, H. A. Kessler, R. L. Murphy, C. Hicks, M. King, D. Wheeler, J. Feinberg, R. Stryker, P. E. Sax, S. Riddler, M. Thompson, K. Real, A. Hsu, D. Kempf, A. J. Japour, and E. Sun. 2002. Safety and antiviral activity at 48 weeks of lopinavir/ritonavir plus nevirapine and 2 nucleoside reverse-transcriptase inhibitors in human immunodeficiency virus type 1-infected protease inhibitor-experienced patients. *J Infect Dis* 185:599-607.
3. Kaletra™, U.S. prescribing information
4. Kagan, R. M., M. D. Shenderovich, P. N. Heseltine, and K. Ramnarayan. 2005. Structural analysis of an HIV-1 protease I47A mutant resistant to the protease inhibitor lopinavir. *Protein Sci* 14:1870-8.
5. Mullins, C., G. Eisen, S. Popper, A. Dieng Sarr, J. L. Sankale, J. J. Berger, S. B. Wright, H. R. Chang, G. Coste, T. P. Cooley, P. Rice, P. R. Skolnik, M. Sullivan, and P. J. Kanki. 2004. Highly active antiretroviral therapy and viral response in HIV type 2 infection. *Clin Infect Dis* 38:1771-9.
6. Rodes, B., C. Toro, J. A. Sheldon, V. Jimenez, K. Mansinho, and V. Soriano. 2006. High rate of proV47A selection in HIV-2 patients failing lopinavir-based HAART. *AIDS* 20:127-9.