Poster #064

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Patterns of Resistance to Lopinavir in PI-Experienced Patients Following Viral Rebound During Lopinavir/ritonavir Therapy

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ABSTRACT

Background: Lopinavir, a novel protease inhibitor, displays potent antiviral activity both in treatment-naive and PI-experienced patients. To date, the selection of resistance to lopinavir has not been identified among treatment-naive patients for >72 weeks, apparently due to a high genetic barrier to resistance. In order to characterize mutational patterns associated with resistance to lopinavir, longitudinal rebound isolates from 26 single and multiple PI-experienced patients with HIV RNA >1000 copies/mL while on treatment with lopinavir/ritonavir (lopinavir/r) in two Phase II studies have been analyzed.

Methods: Genotype was analyzed by population sequencing. Phenotype was analyzed using single cycle recombinant assays.

Results: Baseline isolates from the five single and four multiple PI-experienced patients that displayed evolution all had at least four protease mutations associated with reduced susceptibility to lopinavir. The latest rebound isolates displayed 1-6 additional mutations. The most common mutations found in the rebound isolates were at positions 10, 46, 54 and 82 (≥8/9 patients). A new mutation at position 10, 20, 24, 33, 36, 46, 50, 53, 54, 63, 71, 82 and/or 88 was observed in at least one rebound isolate, relative to baseline. New mutations were most frequent (≥3 patients) at positions 10, 33, 46, 54 and 82. The evolution of additional mutations was associated with incrementally decreased susceptibility to lopinavir from a median of 5.2-fold (range 0.7- to 26-fold) at baseline (relative to wt) to 53-fold (range 24- to 252-fold) at time of viral rebound. All nine rebound isolates remained relatively susceptible (<10 fold) to amprenavir when evolution of reduced susceptibility to lopinavir was initially documented. However, amprenavir resistance subsequently evolved in isolates from two patients with ongoing viral replication during continuing lopinavir/r therapy.

Conclusions: Viruses from single or multiple PI-experienced patients with viral rebound during continued lopinavir/r therapy evolve additional mutations, leading to further decreased susceptibility to lopinavir. Mutations at positions 10, 46, 54 and 82 appeared most commonly in the rebound isolates. The additional presence of mutations at positions 20, 24, 33, 50 or 53 appears to contribute to higher levels of phenotypic resistance.

INTRODUCTION

Lopinavir /ritonavir (LPV/r, Kaletra™, formerly known as ABT-378/r) is a novel HIV protease inhibitor (PI) that maintains high plasma trough levels >75-fold above the serum adjusted IC_{95} of LPV for wild-type HIV-1. Therapy with LPV/r has been shown to produce a significant decline in plasma HIV RNA in Phase II and III clinical trials.[1] To date, the selection of resistance to LPV has not been identified among treatment-naive patients for >72 weeks, apparently due to a high genetic barrier to resistance.[2] However, in PI-experienced patients, where the genetic barrier is compromised by the selection of mutants during prior therapy, the evolution of resistance to LPV/r *in vivo* has been observed.[3] In those patients, several additional PI mutations were observed following rebound.[3] This study is a continuation of that analysis, and includes the characterization of the mutational patterns observed in longitudinal rebound isolates from 26 PI-experienced patients with HIV RNA >1000 copies/mL while on treatment with LPV/r in two Phase II studies (M97-765 and M98-957).In addition, phenotypic susceptibility to LPV and other PIs is compared in nine protease inhibitor experienced patients in whom resistance to LPV developed during virologic rebound on LPV/r therapy.

METHODS

- Study M97-765 evaluated the activity of LPV/r plus nevirapine (NVP) and NRTIs in 70 single PI-experienced, NNRTI-naive patients.
- Study M98-957 examined the activity of LPV/r plus efavirenz (EFV) and NRTIs in 57 multiple PI-experienced, NNRTI-naive patients.
- Baseline phenotypes were determined by either Virologic (PhenoSense™) or Virco (Antivirogram™). Genotype was determined by population sequencing.
- Following initial response, plasma samples from patients with plasma HIV RNA >1000 copies/mL without documented treatment interruption were submitted for phenotypic and genotypic analysis.

RESULTS

- Rebound isolates were analyzed from 14 single and 12 multiple PI-experienced patients (Table 1).
- Evolution in the protease gene and a change in phenotypic susceptibility to LPV were observed in 5/14 single PI experienced and 4/12 multiple PI experienced patients, respectively.
- NNRTI resistance was observed in 24/26 patients following rebound. No phenotypic or genotypic changes were observed in either protease or RT in 2/26 subjects

Table 1. Summary of Observation from Subjects with Viral Rebound During LPV/r Therapy

Study Number Treatment Experience	M97-765 Single PI	M98-957 Multiple PI
No. of patients enrolled	70	57
No. of patients analyzed	14	12
No. of patients with development of resistance to LPV	5	4
No. of patients with resistance to the NNRTI of the regimen	12*	12

*1 pt had NNRTI resistance at baseline.

Characterization of Rebound Isolates in Single PI-Experienced Patients

Table 2. Phenotypic and Genotypic Characterization of Isolates from Subjects in Study M97-765 to Protease and RT Inhibitors

Pt. No.	Rx Day	Rx Regimens	Protease Genotype	RT Genotype#	Fold IC_{95} LPV	Fold IC_{95} NVP
S1	BL	IDV	L10IV, I54V, L63A, A71V, V82A	No NNRTI mutation	2.3	1.7
	660	LPV/r + NVP*	L10IV, K20K/T, M36M/I, M46M/I/L, I54V, L63A, A71V, V82A	K103N/K G190G/A	**24.0**	**555.6**
S2	BL	IDV	L10F, V32I, M36I, M46L, A71V, V82A	No NNRTI mutation	**5.2**	**8.9**
	84	LPV/r + NVP*	L10X, L24I, M36I, M46L, I54V, L63P, V82A	K101K/Q, K103K/N, Y181V/C	**20.3**	**>85.0**
	140	LPV/r + NVP*	L10F, L24I, M36I, M46L, I54V, L63P, V82A	K103K/N, Y181C	**33.6**	**>85.0**
S3	BL	IDV	L10F, M36V, I54V, L63P, V82A	No NNRTI mutation	**26.0**	**12.3**
	867	LPV/r + NVP*	L10F, L24I, M36V, M46I, F53F/L, I54V, L63P, V82A	G190A	**59.0**	**182.0**
S4	BL	SQV	L10X, L63P, A71V, I84V, L90M	No NNRTI mutation	2.5	2.4
	341	LPV/r + NVP*	L10F, M46I, L63P, A71V, I84V, L90M	K103N, Y181C	**9.4**	**>24.9**
	763	LPV/r + NVP*	L10F/I, L33L/F, M36M/I, M46I, I54V, L63P, A71V, I84V, L90M	K103N, Y181C	**37.0**	**461.0**
	922	LPV/r + NVP*	L10F, L33F, M46I, I54V, L63P, A71V, I84V, L90M	K103N, Y181C	**67.0**	**>625.0**
S5	BL	NFV	L10M/I, M46I, L63P, A71T, V77I, N88S, L90M	L100X, K101K/Q, K103N, G190G/C	0.7	**>22.7**
	84	LPV/r + NVP*	L10I, M46I, L63P, A71T, V77I, N88G, L90M	K101Q, G190C	**6.5**	**>59.5**
	168	LPV/r + NVP*	L10I, L33F, M46I, I54V/I, L63P, A71L, V77I, N88G, L90M	K101Q, G190C	**23.6**	**>59.5**
	337	LPV/r + NVP*	L10I, L33F, M46I, I54V, L63P, A71L, V77I, N88G, L90M	K101Q, G190C	**46.3**	**>24.9**
	501	LPV/r + NVP*	L10I, L33F, M46I, I54V, L63P, A71L, V82V/A, N88G, L90M	K101Q, G190C	**90.6**	ND

BL, baseline sample; * LPV/r + NVP + 2NRTI. at least one of which was new of physician's choice; ND, not determined; blue text indicates new mutation; bold text indicates ≥4 fold change in IC_{95} compared to wt HIV; # only NNRTI mutations were included; LPV, lopinavir; IDV, indinavir; NVP, nevirapine; NFV, nelfinavir; SQV, saquinavir

- Susceptibility of the baseline isolates from the 5 single PI-experienced patients ranged from 0.7- to 26-fold (median 2.5-fold), compared to wt HIV (Table 2).
- Following rebound, the fold IC_{95} of LPV increased to 24- to 91-fold (median 59-fold), compared to wt HIV. The change in susceptibility during therapy (rebound phenotype compared to baseline phenotype) ranged from 2.3- to 130-fold (median 27-fold).
- At baseline, isolates from the 5 single PI-experienced patients contained 5-6 mutations associated with PI resistance. These included mutations at positions 10, 32, 36, 46, 54, 63, 71, 77, 82, 84, 88 and 90.
- New mutations were observed during rebound at the following positions previously associated with PI resistance: 10, 20, 24, 33, 36, 46, 53, 54, 63, 71 and 82. Nine of these mutations were previously identified as associated with reduced susceptibility to LPV *in vitro* and constitute 9/11 of the mutations in the LPV mutation score.[4]

Characterization of Rebound Isolates in Multiple PI-Experienced Patients

Table 3. Phenotypic and Genotypic Characterization of Isolates from Subjects in Study M98-957 to Protease and RT Inhibitors

Pt. No.	Rx Day	Rx Regimens	Protease Genotype	RT Genotype#	Fold IC_{95} LPV	Fold IC_{95} NVP
M1	BL	SQV (previous) SQV/RTV (BL)	L10I, G48V, I54V, L63P, A71V, V77I, V82A, L90M	No NNRTI mutation	**20.0**	**4.7**
	336	LPV/r + EFV*	L10I, M46M/I, G48V, I54V, L63P, A71V, V77I, V82A, L90M	K103N	**43.0**	**>285.7**
M2	BL	IDV (previous), NFV (previous),	L10I, M46I, I54V, L63P, A71V, V77I, V82A	No NNRTI mutation	**17.0**	0.5
	434	LPV/r + EFV*	L10I, L33L/F, M46I, I54V, L63P, A71V, V77I, V82A	K101K/P/Q/T, K103N	**30.0**	**>285.7**
M3	BL	SQV (previous), RTV (previous), IDV (previous), SQV/NFV (BL)	L10I, I54V, L63P, A71V, V77I, V82T, L90M	No NNRTI mutation	**12.0**	0.9
	276	LPV/r + EFV*	L10F, K20M, M36M/I, M46M/I, I54V, L63P, A71V, V77I, V82T/S, L90M	G190S	**23.0**	**104.0**
	506	LPV/r + EFV*	L10F, K20M/I/V, M36I, I54V, L63P, A71V, V77I, V82S, L90M	G190S	**51.0**	**138.0**
M4	BL	SQV (previous), RTV (previous), IDV (previous), SQV/NFV (BL)	L10I,/I, K20R, M36I, I54I/V, L63L/H, L90M	No NNRTI mutation	2.8	0.3
	112	LPV/r + EFV*	L10I,/I, K20R, M36I, I54A/V, A71V, V82A, L90M	L100I,/I, K103N	**99.0**	**>333.3**
	280	LPV/r + EFV*	L10I,/I, K20R, V32I/I, M36I, M46I, I47I/V, I50I/V, I54V, A71V, V82A, L90M	L100I, K103N	**149.0**	**>333.3**
	427	LPV/r + EFV*	L10V, K20R, M36I, M46I, I50V, I54V, A71V, V82A, L90M	L100I, K103N	**252.0**	**>285.7**

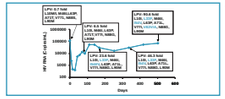
BL, baseline sample; * LPV/r + EFV + NRTIs of physician's choice, blue text indicates new mutation; bold text indicates ≥ 4 fold change in IC compared to wt HIV; # only NNRTI mutations were included; LPV, lopinavir; IDV, indinavir; NFV, nelfinavir; SQV, saquinavir; RTV, ritonavir; EFV, efavirenz

- Susceptibility of the baseline isolates from the 4 multiple PI-experienced patients ranged from 2.8- to 20-fold (median 13-fold), compared to wt HIV (Table 3).
- Following rebound, the fold IC_{95} of LPV increased to 30- to 252-fold, compared to wt HIV. The change in susceptibility during therapy (rebound phenotype compared to baseline phenotype) ranged from 1.8- to 90-fold.
- At baseline, isolates from the 4 multiple PI-experienced patients contained 6-8 mutations associated with PI resistance. These included mutations at positions 10, 20, 36, 46, 48, 54, 63, 71, 77, 82, and 90.
- New mutations (n=1-5) that have previously been associated with PI resistance were observed during rebound at the following positions: 10, 20, 33, 36, 46, 50, 71 and 82. Five of these mutations were previously identified as part of the LPV mutation score.[4] Mutations at positions 33, 36 and 50, which are not part of the LPV mutation score, were observed following rebound in either single or multiple PI-experienced patients. No unique mutation that has not previously been associated with PI resistance was consistently observed.

Examination of Individual Patient Rebound Patterns

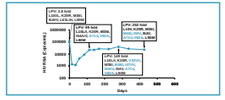
The HIV RNA, phenotypic and genotypic patterns of individual patients varied upon rebound as additional mutations accumulated.

Figure 1. Evolution of Genotype and Phenotype in HIV Isolates from Patient S5



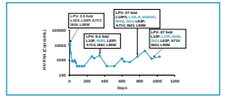
- Patient S5 (Prior PI: NFV) entered LPV/r therapy with >10^ RNA copies/mL and mutations at positions 10, 46, 63, 71, 77, 88 and 90 in place from prior failure on NFV (>54-fold change in NFV IC_{95}). Although the baseline isolate was susceptible to LPV, viral rebound occurred by day 42 following a brief period of initial decline. By day 112, viral rebound had risen to 52,000 copies/mL and remained within 0.5 log copies/mL below baseline at the majority of the subsequent time points. By day 168, additional mutations at positions 33 and 54 had appeared, along with changes of amino acid identity at positions 71 and 88. At day 501, an additional mutation at position 82 had evolved.

Figure 2. Evolution of Genotype and Phenotype in HIV Isolates from Patient M4



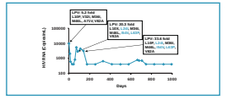
- Patient M4 [Prior PIs: SQV, RTV and IDV (previous) and SQV/NFV (baseline)] also entered LPV/r therapy with >10^ RNA copies/mL. Mutations from prior therapy were evident at positions 10, 20, 36, 54, 63 and 90. Complete response was never achieved, and by day 112, rebound to 21,000 copies/mL had occurred along with the accumulation of additional mutations at positions 54, 71 and 82. Susceptibility to LPV declined from 2.8-fold at baseline to 99-fold during this period. The investigator continued LPV/r therapy in this subject with subsequent viral load values remaining between 20,000 to 40,000 copies/mL. Consequently, the rebound viral isolate tested on day 280 demonstrated 19-fold (Table 4) reduced susceptibility to APV relative to wt type HIV and the development of an I50V mutation together with mutations at positions 32, 46, and 47 in protease. By day 427, the mutations at positions 32 and 47 were no longer detectable by population sequencing, but a new mutation at position 10 appeared.

Figure 3. Evolution of Genotype and Phenotype in HIV Isolates from Patient S4



- Patient S4 (Prior PI: SQV) entered therapy with 13,000 copies/mL and mutations at positions 10, 63, 71, 84 and 90. Following initial response, rebound occurred by day 253. Plasma HIV RNA did not appear to reach a steady state through day 1009 in spite of the accumulation of mutations at positions 33, 46 and 54. Nonetheless, at most time points, HIV RNA was ≥1 log copies/mL lower than baseline.

Figure 4. Evolution of Genotype and Phenotype in HIV Isolates from Patient S2



- Patient S2 (Prior PI: IDV) also entered therapy with >10,000 copies/mL, and mutations at positions 10, 32, 36, 46, 71 and 82. Following a brief response, viral rebound occurred. Coincident with rebound, new mutations at positions 24, 54 and 63 appeared, but mutations at positions 32, 71 were no longer evident in the population. Following this evolution, viral load declined to <1000 copies/mL and remained substantially (>1 log copies/mL) suppressed compared to baseline despite 34-fold reduced susceptibility to LPV and high-level resistance to NVP.

Cross-Resistance of Isolates Selected *In Vivo* by LPV/r

Table 4. Cross Resistance of the Viruses Selected by LPV to Other Protease Inhibitors

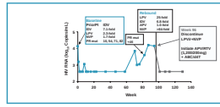
Patient	Day	ABT-378	RTV	SQV	IDV	NFV	APV
S1	BL	2.3	**21.6**	0.9	**7.1**	**7.2**	**ND**
	660	**24.0**	**25.0**	2.0	**11.0**	**13.0**	2.3
S2	BL	**5.2**	**12.1**	1.5	**10.2**	**7.4**	1.0
	84	**20.3**	**101.9**	1.9	**15.6**	**17.8**	**4.6**
	140	**33.6**	**37.9**	2.4	**9.3**	**27.4**	2.5
S3	BL	**26.0**	**47.0**	**4.6**	**26.0**	**49.7**	ND
	867	**59.0**	**64.0**	**6.7**	**24.0**	**36.0**	**4.2**
S4	BL	2.5	**22.6**	**32.4**	**12.6**	**33.5**	3.3
	341	**9.4**	**19.5**	**>29.8**	**10.6**	**30.1**	**5.4**
	763	**37.0**	**54.0**	**81.0**	**20.0**	**91.0**	**11.0**
	922	**67.0**	**63.0**	**96.0**	**29.0**	**101.0**	**41.0**
S5	BL	0.7	**4.5**	3.4	**15.7**	**>54.2**	0.6
	84	**6.5**	**20.1**	2.7	**31.9**	**36.6**	ND
	168	**23.6**	**16.2**	0.6	**22.4**	3.4	ND
	337	**46.3**	**16.2**	0.6	**8.7**	1.6	2.9
	501	**90.6**	**33.9**	0.6	**10.8**	**17.5**	**16.2**
M1	BL	**20.0**	**74.0**	**181.0**	**26.0**	**34.0**	1.3
	336	**43.0**	**76.0**	**200.0**	**62.0**	**68.0**	2.4
M2	BL	**17.0**	**45.0**	1.8	**19.0**	**19.0**	1.6
	434	**30.0**	**54.0**	2.2	**18.0**	**18.0**	3.0
M3	BL	**12.0**	**112.0**	**7.4**	**11.0**	**30.0**	2.7
	276	**23.0**	**57.0**	**4.1**	**14.0**	**19.0**	2.2
	506	**51.0**	**175.0**	**9.0**	**33.0**	**68.0**	3.0
M4	BL	2.8	**5.0**	1.8	1.8	2.3	1.3
	112	**99.0**	**260.9**	**52.0**	**156.0**	**205.0**	**8.5**
	280	**149.0**	**>184.6**	**12.0**	**18.0**	**39.0**	**19.0**
	427	**252.0**	**>176.5**	**25.0**	**20.0**	**35.0**	**37.0**

BL, baseline sample; ND, not determined; bold text indicates ≥ 4 fold change in IC_{95} compared to wt HIV; LPV, lopinavir; IDV, indinavir; NFV, nelfinavir; SQV, saquinavir; RTV, ritonavir; APV, amprenavir

- Among the rebound viruses available at the latest time point on study from these nine subjects, the IC_{95} of LPV ranged from 24- to 252-fold (median 59-fold) above wild-type IC_{95}.
- The rebound viruses from each of the 9 subjects either remained (if cross-resistant at baseline) or developed cross-resistance to RTV, IDV, and NFV (with the exception of the intermediate rebound virus (day 337) from patient S5).
- Five of the nine subjects (patients # S1, S2, S3, S5 and M2) who demonstrated evolution of resistance to LPV were naive to SQV. No significant change in susceptibility to SQV was noted in any of these subjects during rebound. Four of the SQV-naive subjects displayed 2.4-fold or less reduction in susceptibility to SQV. The remaining patient displayed 4.6-fold and 6.7-fold reduced susceptibility to SQV, compared to wt HIV, at baseline and rebound, respectively.
- All 9 subjects were naive to APV. During rebound six of these subjects, susceptibility or modestly reduced susceptibility to APV was observed (<5-fold). A substantial decrease in APV susceptibility was observed in the remaining three subjects (S4, S5 and M4).

Response to APV/RTV Based Therapy Following Viral Rebound and Development of Lopinavir Resistance During LPV/r Therapy

Figure 5. Initial Salvage of LPV/r Failure with APV/RTV + ABC/d4T in Patient S1



- Preliminary data on the virologic response of one of the above patients (S1) to a subsequent antiretroviral regimen after study discontinuation has been provided by the investigator.
- Patient S1 entered Study M97-765 on an antiviral regimen consisting of IDV, AZT and 3TC (combivir). At baseline, the IC_{95} of LPV was 2.3-fold above wild-type susceptibility and the following four mutations associated with PI resistance were observed: L10I, I54V, A71V, V82A.
- On study day ~1, IDV was replaced with LPV/r at a dose of 400/100 BID. On study day 15, NVP was started at a dose of 200 mg QD and was increased to 200 mg BID on study day 29. Additionally, the NRTIs were altered on study day 15 such that the subject was receiving ddI in conjunction with LPV/r and NVP.
- Viral load declined to <400 copies/mL by day 15. On day 492, rebound of plasma HIV RNA to >400 copies/mL was noted. Rebound was confirmed on continued study therapy at a subsequent visit. A phenotypic susceptibility assay obtained on day 660 demonstrated 25-fold reduced susceptibility to LPV with wt susceptibility to APV.
- Subsequently, the patient was discontinued from Study M97-765 study medications and offered "best available therapy." A regimen of APV 1200 mg BID, RTV 200 mg BID, d4T 40 mg BID, and abacavir 300 mg BID was reportedly started on study day 676. After 32 days on the new regimen, viral load had declined to <400 copies/mL. Viral load suppression was maintained at <400 copies/mL through 228 days of treatment on the new regimen, the latest available time of follow-up.

CONCLUSIONS

- Evolution of resistance during viral rebound on LPV/r therapy has been observed in PI-experienced patients whose prior PI was NFV, SQV or IDV.
- The following mutations have emerged at least once during failure on LPV/r therapy in PI-experienced patients:
  - Previously identified as associated with reduced susceptibility (LPV mutation score): L10F/I/V, K20M, L24I, M46I/L, F53L, I54V, L63P, A71L/V, V82A
  - Not identified previously as contributing to LPV resistance: V32I, L33F, M36I/V, I47V, I50V, V82S
- Mutations at positions 10, 46, 54 and 82 appeared most commonly in the rebound isolates. The additional presence of mutations at positions 20, 24, 33, 50 or 53 may contribute to higher levels of phenotypic resistance.
- Viruses from SQV-naive patients displayed continued susceptibility to SQV following LPV/r failure. Isolates from the majority of patients retained susceptibility to APV. Extended LPV/r therapy following rebound produced the emergence of substantially reduced susceptibility to APV in three patients The I50V mutation, along with new mutations at positions 46, 71 and 82, was noted in one of these patients (patient M4). The rebound isolate from the second patient (S4) contained mutations at positions 84 and 90 together with multiple other mutations. The rebound isolate from third patient (S5) contained mutations 33, 54, 82, 88 and 90 together with multiple other mutations. APV sensitivity was maintained in all of these patients at initial rebound and LPV/r therapy was continued for extended period of time prior to APV resistance development.
- Clinical investigation of APV/RTV and SQV/RTV based regimens in patients who fail LPV/r therapy with virus resistant to LPV is underway. Viral re-suppression to < 400 copies/mL has been observed through 32 weeks to date in a PI-experienced patient treated with a APV/RTV based regimen following viral rebound on LPV/r therapy with development of 25-fold reduced susceptibility to LPV but no reduction in APV susceptibility.

REFERENCES

1. Murphy RL, Brun S, Hicks C, et al. ABT-378/ritonavir plus stavudine and lamivudine for the treatment of antiretroviral-naïve adults with HIV-1 infection: 48-week results. AIDS 2001; 15(1). F1-F9.

2. Stryker R, Brun S, King M, et al. ABT-378/ritonavir (ABT-378/r) in antiretroviral naïve HIV+ patients: follow up beyond two years and viral load suppression below 3 copies/mL. Poster at the Fifth International Congress on Drug Therapy in HIV Infection, Glasgow Scotland (October 2000).

3. Brun S, Kempf D, Isaacson J, et al. Patterns of protease inhibitor cross resistance in viral isolates with reduced susceptibility to ABT-378. Poster at the 8th Conference on Retroviruses and Opportunistic Infections, Chicago (February 2001).

4. Kempf D, Isaacson J, King M et al. Genotypic correlates of reduced *in vitro* susceptibility to ABT-378 in HIV isolates from patients failing protease inhibitor therapy. 4th International Workshop on Drug Resistance and Treatment Strategies, Sitges, Spain (June, 2000).