Poster #3.1/2

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Characterizing Evolution of Protease Inhibitor (PI) Resistance During Lopinavir/ritonavir (LPV/r) Treatment and Study of the Salvage of Lopinavir Resistance (SOKRATES Trials)

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BACKGROUND

Lopinavir/ritonavir (Kaletra™, LPV/r) is a novel protease inhibitor (PI) that achieves lopinavir trough concentrations >75-fold above the protein binding-adjusted IC_{50} of LPV relative to wild type virus when dosed at 400/100 mg BID. This high ratio of LPV trough concentrations to IC_{50} (known as, the inhibitory quotient or IQ) provides a significant pharmacologic barrier to emergence of viral resistance in antiretroviral (ARV)-naïve patients.[1] In over 500 ARV-naïve patients enrolled in Phase II/III trials with LPV/r for a median duration of 97 weeks (range, 0-252 weeks) there has been no evolution of genotypic or phenotypic resistance to LPV[2-5] (Table 1).

Table 1. Clinical Trials Supporting Absence of Resistance to LPV in 508 ARV-Naïve Patients

Study	Age Group	N	Duration	Reference
M97-720 (Phase II)	Adults	100	5 Years[2]	Eron, 2003
M98-863 (Phase III)	Adults	326	96 Weeks[3]	Kempf, 2003
M98-940 (Phase II)	Pediatric	44	72 Weeks[4]	Cahn, 2001
M00-056 (Phase II)	Adults	38	72 Weeks[5]	Feinberg, 2002

LPV/r showed superior efficacy in a randomized, double-blind Phase III clinical trial (Study M98-863) comparing Kaletra to nelfinavir with d4T/3TC in ARV-naïve patients (Figure 1).[3] Through 96 weeks of therapy, no evidence of primary resistance to LPV (defined as any primary or active site mutation) was detected in any of 51 Kaletra-treated patients with detectable viral load for whom genotype was available (Table 2). In contrast, 48% of isolates from nelfinavir-treated patients displayed primary resistance to nelfinavir (emergence of D30N and/or L90M) or displayed substantially reduced (>6.8-fold) susceptibility to nelfinavir in the absence of either primary mutation. Moreover, through 96 weeks, patients on LPV/r demonstrated a significantly lower cumulative probability of resistance in protease and reverse transcriptase (Figure 2).[3,6,7]

Figure 1. Study 863, LPV/r vs. NFV: Time to Loss of Virologic Response

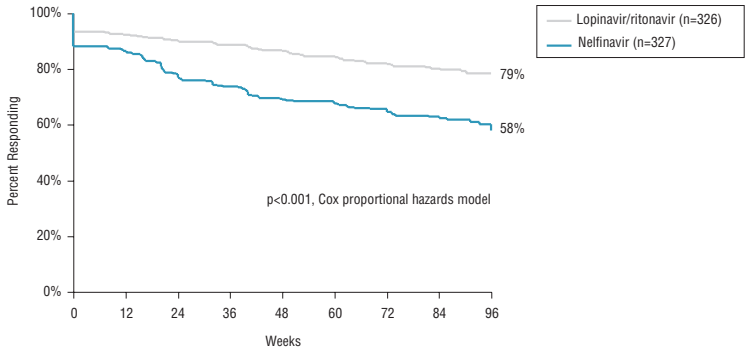


Figure 2. Study 863, LPV/r vs. NFV: Cumulative Probability of Resistance Development

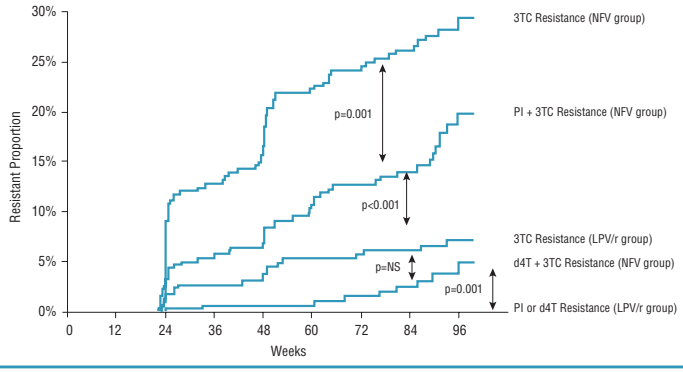


Table 2. Summary of Resistance Analysis of LPV/r vs. Nelfinavir in ARV-Naïve Patients in Study M98-863 Through Week 96[6,7]

	Kaletra	Nelfinavir	p-value
Number of patients enrolled	326	327	
Patients with HIV RNA >400 copies/mL	74 (23%)	113 (35%)	
Genotype available	51/74 (69%)	96/113 (85%)	
PI resistance[a]	0/51 (0%)	46/96 (48%)	<0.001
3TC resistance	19/51 (37%)	79/96 (82%)[b]	<0.001

[a] LPV resistance defined as the emergence of any primary or active site mutation in protease (amino acids 8, 30, 32, 46, 47, 48, 50, 82, 84, or 90) and confirmed by phenotypic analysis. NFV resistance defined as the emergence of the D30N or L90M mutation in protease, or the emergence of the M46I/L mutation in protease with confirmed reduced phenotypic susceptibility to NFV.

[b] Previous results[7] that reported 78 of 96 patients with 3TC resistance did not include one isolate with a M184T mutation that demonstrated >100-fold phenotypic resistance to 3TC.

METHODS

The SOKRATES studies are prospective, open-label, studies designed to further define patterns of PI resistance in patients receiving LPV/r as first (SOKRATES I) or second (SOKRATES II) PI-based therapy and to evaluate potential salvage regimens in patients with LPV resistance on LPV/r therapy. 114 sites in Argentina, Brazil, Canada, France, Germany (SOKRATES I only), Italy, Poland, Puerto Rico, Spain, the United Kingdom and the United States are participating in the SOKRATES studies.

Eligibility Criteria for SOKRATES I

- Confirmed HIV RNA rebound to >1000 copies/mL after 24 weeks of treatment or lack of suppression below 1000 copies/mL while receiving LPV/r after at least 16 weeks of treatment.
- Patient screening genotypes demonstrating at least one primary protease mutation plus at least two other protease mutations, primary or secondary (Table 3).
- Reduced phenotypic susceptibility to LPV (defined as >10-fold increase in IC_{50} relative to wild type HIV).
- No protease mutations associated with saquinavir or amprenavir and phenotypic susceptibility to at least one of the salvage regimens (Table 4).

Table 3. Primary and Secondary Protease Mutations

Primary Mutations	Secondary Mutations	
V32I	L10F or I or R or V	F53L
G48V	K20M or R	I54V or L or T
I50V	L24I	A71V or T
V82A or F or T or S	L33F	G73S or A or T
I84V	M36I	V77I
L90M	M46I or L	N88D
	I47V	

Table 4. Criteria for the Selection of Treatment Regimens

Regimen	Requirements for Selection (genotypic and phenotypic requirements must be met)	
SQV/RTV Arm	Phenotype:	≤4-fold loss of susceptibility to SQV
	Genotype:	Absence of L90M* and G48V* in protease
APV/RTV Arm	Phenotype:	≤8-fold loss of susceptibility to APV
	Genotype:	Absence of I50V* in protease
EFV Arm	Genotype:	Absence of K103N, Y188L, and G190S/E in reverse transcriptase and patient must be NNRTI naïve

* Presence of L90M, G48V and I50V mutations are associated with resistance to saquinavir and amprenavir, respectively. The presence of these mutations will therefore exclude use of the respective regimen regardless of phenotypic susceptibility.

Once LPV resistance is identified, patients are switched to efavirenz (600 mg QD), saquinavir/ritonavir (400 mg/400 mg BID or 800 mg/200 mg BID), or amprenavir/ritonavir (1200 mg/200 mg BID or 750 mg/300 mg BID), based on phenotypic susceptibility, along with two nucleoside analogues (Figure 3).

Selection of the reverse transcriptase inhibitors (RTIs) are guided by the patient's past antiretroviral therapy history, resistance testing and Department of Health and Human Services (DHHS) guidelines. When possible, two nucleoside reverse transcriptase inhibitors (NRTIs) or one non-nucleoside reverse transcriptase inhibitor (NNRTI) and one NRTI are to be administered.

Twenty-four patients were planned to be enrolled and followed for 48 weeks.

Eligibility Criteria for SOKRATES II

- Results of a pre-Kaletra genotype within two months prior to the start of Kaletra therapy.
- Confirmed HIV RNA rebound to >1000 copies/mL while receiving LPV/r after at least 16 weeks of treatment with LPV/r as the patient's second PI-based therapy.
- Patient screening genotype demonstrating the presence of either:
  1. A new primary protease mutations not present in the pre-LPV/r genotype

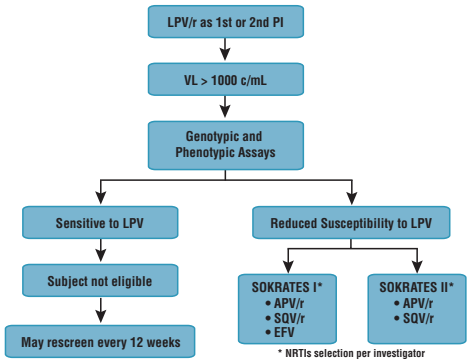
  or

  2. Two new secondary protease mutations and a primary protease mutation that may have been present on the pre-LPV/r genotype (Table 3).
- Reduced phenotypic susceptibility to LPV (defined as >10-fold increase in IC_{50} relative to wild type HIV).
- No protease mutations associated with saquinavir or amprenavir and phenotypic susceptibility to at least one of the salvage regimens (Table 4).

Once LPV resistance is identified, patients are switched to saquinavir/ritonavir (400 mg/400 mg BID or 800 mg/200 mg BID), or amprenavir/ritonavir (1200 mg/200 mg BID or 750 mg/300 mg BID), based on phenotypic susceptibility, along with 2 nucleoside analogues (Figure 3). Selection of the RTIs are guided by the patient's past antiretroviral therapy history, resistance testing and DHHS guidelines. When possible, two NRTIs or one NNRTI and one NRTI are to be administered.

Sixteen patients were planned to be enrolled and followed for 48 weeks.

Figure 3. SOKRATES I AND II Study Design



As an adjunct to these two studies, patients who are receiving LPV/r as either a first or second PI containing regimen were tracked at each of the participating sites to characterize the incidence of resistance to LPV.

RESULTS

Sites have been actively screening for patients for a median of 15 months (range 4-29).

SOKRATES I

Seven patients were screened for SOKRATES I after having met HIV RNA entry criteria. No viral isolates from patients screened for SOKRATES I demonstrated any new primary or active site mutations in protease, consistent with phase II/III LPV/r clinical trial data listed in Tables 1 and 2.

SOKRATES II

Twenty-two patients were screened for SOKRATES II after having met HIV RNA entry criteria. Viral isolates from eight of these patients demonstrated evolution of resistance to LPV. The protease mutations for these patients are listed in Table 5.

Only one patient (50241) met entry criteria for salvage therapy based on inclusion/exclusion criteria and phenotypic susceptibility to saquinavir. This patient is currently receiving saquinavir/ritonavir (800 mg/200 mg BID) in conjunction with lamivudine and tenofovir. The patient's HIV RNA decreased from 17,936 copies/mL to <50 copies/mL after 20 weeks and had low level rebound at week 32 with HIV RNA of 225 copies/mL at week 40. The regimen has been well tolerated.

Table 5. Genotypes of SOKRATES Patients with Phenotypic Resistance to LPV/r

Patient	Protease Sequence at Screening[a]	Fold-Change in PI Susceptibility LPV/APV/SQV[b,c]
50111	L10I T12K I15V M36L S37D M46I F53L I54V I62V L63P C67F H69R A71V I72T V82A N88D L90M Q92K	**70**/3.5/**55**[d]
50114	I15I/V L19I V32I/M/V M36I S37N R41K M46I/M L63P/L	0.8/0.9/0.6
50223	V32I M36I S37N R41K M46I I47A/V L63P K70K/E A71V V82A/V L90M/L I93L	**38**/**14**/1.4[e]
50241	L10I T12I I15V L19V K20R V32I M36I S37N M46I I47V I54M I62V L63P A71T I72E V82T I85V	**98**/**27**/1.7[f]
50281	L10F L24I L33F S37N P39Q R41K K43T M46L I54V D60E I62V L63P A71V V77I/V V82A	**79**/**9.3**/**6.0**
50411	L10V I15V K20R E35D M36I S37E/D M46M/L G48V I50I/V I54A/V Q58Q/E I62V L63P C67S/C A71T/I/A/V V82T/A I84I/V I93L	**202**/**9.9**/**545**
50412	L10I V11I T12P I13V K14R I15V K20I M36I S37N R41K M46I K55R D60E/D L63P C67E H69K/R K70K/Y A71V V82T I84V L89I T91T/S I93I/C G94N	**143**/**38**/3[g]
50521	I13V L33V S37C M46M/L I47A R57K L63P I64V I84V	Unknown[h]

[a] Amino acid changes from the pNL4-3 wild type sequence.

[b] Fold resistances in boldface indicate phenotypic resistance to that drug.

[c] Patients were deemed susceptible to LPV/r, APV, and SQV if the fold-change in PI susceptibility was less than 10, 8, and 4, respectively and the absence of the L90M, G48V and I50V mutations was confirmed on genotype.

[d] Patient 50111 was eligible to enroll, but was hospitalized for an opportunistic infection and died before enrollment.

[e] Patient 50223 demonstrated phenotypic susceptibility to SQV but had the L90M mutation on genotype.

[f] Patient 50241 enrolled in the study and is receiving saquinavir/ritonavir (800 mg/200 mg BID) based on resistance testing.

[g] Patient 50412 switched treatment regimen prior to enrollment.

[h] Laboratory was unable to result phenotype for Patient 50521.

Tracking of Patient Population at SOKRATES Sites

737 patients receiving LPV/r as their first PI and 626 patients receiving LPV/r as their second PI were reported by the 89 study sites tracking patients who could become eligible for SOKRATES screening (Table 6).

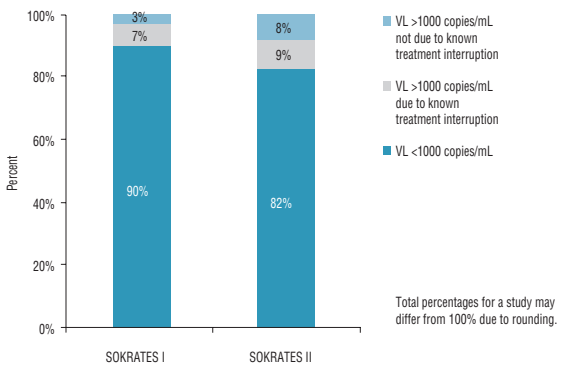
Table 6. LPV/r Exposure in Patients Followed at SOKRATES Sites

	SOKRATES I (LPV/r as first PI)	SOKRATES II (LPV/r as second PI)
Number of sites[a]	46	43
Number of patients	737	626
Median time on LPV/r (weeks)	60	67
Range of time on LPV/r (weeks)	17-167	16-234

[a] Not all 114 sites participating in screening for SOKRATES participated in tracking of LPV/r exposure.

- At the most recent report, 661 (90%) and 511 (82%) patients had HIV RNA <1000 copies/mL in SOKRATES I and II, respectively (Figure 4).
- Investigators reported that the reasons patients with HIV RNA >1000 copies/mL were not screened for enrollment into the SOKRATES studies included non-compliance as assessed by the investigator or the patients were not on therapy at the time of viral load measurement. Other reasons why patients were not screened for enrollment included concurrent illness making patients too ill to enroll, loss to follow-up, or not meeting other inclusion criteria (such as lack of pre-LPV/r genotype) for the SOKRATES studies.

Figure 4. Most Recent HIV RNA Values for SOKRATES I AND II

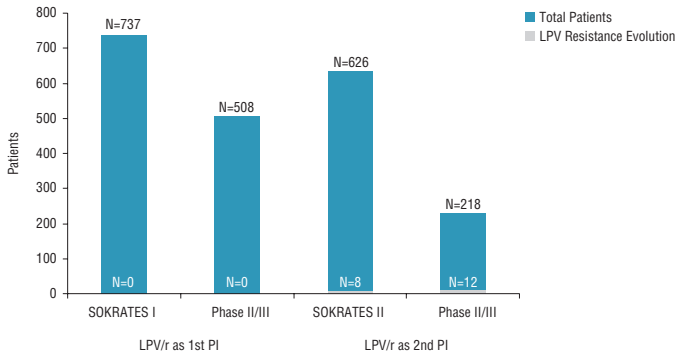


RESULTS *continued*

Observed Rates of Resistance

Based on the number of patients receiving LPV/r-based regimens who were screened for SOKRATES enrollment at participating sites, the observed rate of LPV resistance was 0/737 (0%) patients (99% CI, 0% to 0.6%) in patients receiving LPV/r as a first PI (SOKRATES I) and 8/626 (1.3%) patients (99% CI, 0.4% to 2.9%) when LPV/r is used as a second PI (SOKRATES II). These rates were at least as low as those from Phase II/III clinical trials of LPV/r, in which 0/508 patients using LPV/r as a first PI (0%, 99% CI, 0 to 0.9%) and 12/218 patients using LPV/r as a second PI (5.5%, 99% CI, 2.3% to 10.8%) demonstrated evolution of LPV resistance (Figure 5).

Figure 5. Rate of LPV Resistance Evolution



CONCLUSIONS

- Development of protease inhibitor resistance when LPV/r is used as initial PI-based therapy has not been observed in patients screened for SOKRATES I to date and is likely a rare event based on the sample size available for evaluation.
- Among patients using LPV/r as their second PI-based regimen (SOKRATES II), evolution of resistance to LPV was relatively low in patients screened for the study. In the one patient enrolled, HIV RNA is <400 copies/mL through 40 weeks of treatment with a saquinavir/ritonavir-based salvage regimen.
- Viral rebound above 1000 copies/mL was relatively uncommon in this population of patients using LPV/r as part of their first or second PI-based regimen.

REFERENCES

1. Brun S, et al. The pharmacologic barrier to resistance: Differential patterns of viral evolution in protease inhibitor naïve and experienced patients during viral load rebound on Kaletra (lopinavir/r) therapy. 8th European Conference on Clinical Aspects and Treatment of HIV-Infection, Athens 2001, Abstract 7.
2. Eron J, et al. Lopinavir/ritonavir in antiretroviral naive HIV + patients: 5-year follow-up. 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 2003, Poster #H-844.
3. Kempf D, et al. Comparative incidence and temporal accumulation of PI and NRTI resistance in HIV-infected subjects receiving lopinavir/ritonavir or nelfinavir as initial therapy. 10th Conference on Retroviruses and Opportunistic Infections, Boston, 2003, Abstract 600.
4. Cahn P, et al. Kaletra (ABT-378/ritonavir) in HIV-infected children at 72 weeks. 1st IAS Conference on HIV Pathogenesis and Treatment, Buenos Aires, 2001, Abstract # 779.
5. Feinberg J, et al. Once-daily vs. twice-daily Kaletra (lopinavir/ritonavir) in antiretroviral-naïve HIV+ patients: 72-week follow up. XIV International AIDS Conference, Barcelona, 2002, Abstract # 4445.
6. Bernstein B, et al. Comparison of the emergence of resistance in a blinded phase III study with Kaletra (lopinavir/ritonavir) or nelfinavir plus d4T/3TC from week 24 through week 96. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 2001, Abstract 1768.
7. Kempf D, et al. Analysis of the emergence of secondary mutations with or without primary PI resistance in ARV-naïve subjects with detectable viral load on nelfinavir or lopinavir/ritonavir therapy. *Antiviral Therapy.* 2002; 7: S119.

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