

# Single Agent Therapy with Lopinavir/ritonavir Controls HIV-1 Replication in the Central Nervous System

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## Background

- Lopinavir/ritonavir (LPV/r) as single-agent therapy has exhibited virologic suppression comparable to combination HAART when utilized in a variety of treatment strategies.<sup>1-6</sup>
- Inability to control HIV replication in sanctuary sites such as the central nervous system (CNS) may limit success of antiretroviral therapy.<sup>7</sup>
- LPV/r has demonstrated control of HIV replication when used in combination with other antiretroviral therapy (ART). Although LPV concentrations in CSF are lower than plasma, studies have shown concentrations exceed levels that suppress wild-type HIV replication.<sup>8,9,10</sup>
- Previous studies not detecting LPV in the cerebrospinal fluid (CSF) have been limited by insufficient assay sensitivity.<sup>8</sup>
- Previous data describing CNS antiretroviral activity of LPV/r when used as a sole agent was limited by a short duration and was prior to full suppression.<sup>11</sup>

## Methods

IMANI-2 is an ongoing, prospective, open-label investigation of LPV/r in 40 antiretroviral naïve HIV-1 infected subjects. LPV/r is administered as a single-agent, 400/100 mg twice daily.

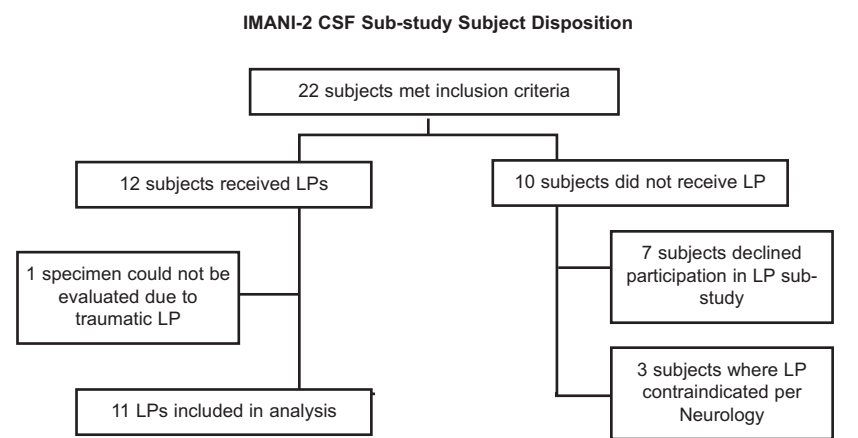
Sub-study was approved by the Institutional Review Board and all participating subjects provided written, informed consent.

### Inclusion criteria:

All subjects enrolled in IMANI-2 who had reached at least week 24 with 2 most recent plasma HIV-1 RNA < 75 copies (Bayer Versant™ HIV RNA 3.0, bDNA) by September 15, 2006, were approached for enrollment in this sub-study. Plasma HIV RNA were performed by LabCorp®.

### Exclusion criteria:

Contraindication to lumbar puncture (LP) (e.g., coagulopathy).



- LPs were performed by a board certified neurologist using standard technique.
- LPs were performed at median week 38 (range 24–48).
- CSF samples from 3 subjects for HIV RNA were obtained 0–6 hours after dosing and the remaining samples were obtained 6–12 hours after dosing. Plasma was also sampled at the time of LP. The median time between CSF and plasma sampling was 37 minutes (range -31–112).
- As there is no validated commercially available bDNA assay for CSF, HIV RNA in CSF was measured via RT-PCR (Roche Amplicor ultrasensitive version 1.5) with limits of detection at < 50 copies/mL.
- LPV was measured by liquid chromatography mass spectrometry (LC-MS) in CSF and high performance liquid chromatography (HPLC) in plasma lower limit of quantification (LLOQ) of 100 ng/mL and an inter-assay variability of < 7.5%.
- LC-MS assay was modified from previously published version.<sup>8</sup> LPV and internal standard (13C-amprenavir) were isolated from matrix by solvent extraction with methy-tert-butyl ether (MTBE), resulting in a LLOQ of 0.5 ng/mL with variability < 10%.
- LPV concentrations in CSF were compared to LPV's 50% inhibitory concentration (IC<sub>50</sub>) derived from a panel of 2381 wild-type HIV-1 isolates by the Monogram Biosciences PhenoSense™ assay [median 3.0 nmol/L (1.9 ng/mL)].<sup>12</sup>

## Results

- Of the twelve CSF samples obtained, 11 were evaluable.
- One CSF sample was excluded because the LP was traumatic (RBC 125, WBC 3, 128 HIV RNA copies/mL).
- Included subjects represented diverse racial/ethnic background, age, and gender. (Table 1)

**Table 1: Demographics**

	n = 11	
Age (yrs) – median [range]	39	[20–66]
Gender male:female (n)	6:5	
Ethnicity n (%)		
African American	5	(46)
Caucasian	3	(27)
Hispanic	2	(18)
Asian	1	(9)
Weight (lbs) – median [range]	165	[133–244]
BMI (kg/m <sup>2</sup> ) – median [range]	25	[20.7–36.0]
Pretreatment CD4+ T cells (cells/mm <sup>3</sup> ) – median [range]	264	[200–491]
Pretreatment HIV RNA (bDNA) (log <sub>10</sub> copies/mL) – median [range]	4.28	[3.72–4.78]

With the exception of M41L in 1 subject, baseline genotypic resistance analyses showed only polymorphisms. (Table 2)

**Table 2: Baseline mutations**

Subject	Pre-drug plasma mutations in protease	Pre-drug mutations in reverse transcriptase
003	E35D, M36I, L63P, A71T, V77I, I93L	R211K
004	L63P, V77I, I93L	M41L, R211K, T215D, 223E, I135V, V179E
010	E35D, M36I, L63P, A71T, V77I, I93L	R211K
016	L63P, V77I, I93L	R211K
017	M36I, L63P, I93L	R211K
031	L63P	No mutations detected
032 (Sample 9/06)	M36I	No mutations detected
032 (Sample 1/07)	—	No mutations detected
036	L101, L63P	No mutations detected
037	M36I	No mutations detected
041	No mutations detected	No mutations detected
044	No mutations detected	No mutations detected

- Median LPV/r exposure at the time of LP was 38 weeks (range 24–48)
- Median blood CD4+ cells at LP were 471 cells/mm<sup>3</sup> (range 265–769)
- All subjects were asymptomatic at the time of LP (Table 3)

**Table 3: Plasma viral load, CD4+ and week of LP**

Subject	Week of LPV/r	Pre-treatment plasma CD4+ cells/mm <sup>3</sup>	Plasma CD4+ cells/mm <sup>3</sup> at LP	Plasma copies/mL (bDNA)	CSF HIV RNA copies/mL
003	48	228	449	< 75	< 50
004	48	482	546	< 75	< 50
010	48	204	646	< 75	< 50
016	48	308	471	< 75	< 50
017	48	257	515	< 75	< 50
031	32	530	599	< 75	< 50
032 (Sample 9/06)	36	171	348	< 75	251
032 (Sample 1/07)	48	—	399	< 75	747
036	32	272	458	< 75	< 50
037	32	143	265	< 75	< 50
041	32	316	371	< 75	< 50
044	24	186	769	< 75	< 50

## Results (continued)

- 11/11 subjects had quantifiable LPV plasma concentrations. (Table 4)
- Median plasma LPV concentration was 9679 ng/mL (range 4887–177003).
- Median CSF LPV concentration was 24.3 ng/mL, mean 30.4 ng/mL (range 7.1–85.3).
- The median LPV/IC<sub>50</sub> ratio was 12.8, mean 16.0 (range of 3.7–44.9).
- All subjects had LPV concentrations of at least 3-fold above the median IC<sub>50</sub> for wild-type HIV-1.

**Table 4: Drug concentrations and CSF viral load**

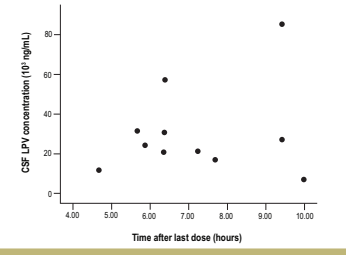
Subject	Plasma LPV ng/mL	Plasma RTV ng/mL	CSF LPV ng/mL	LPV/IC <sub>50</sub> ng/mL	CSF:Plasma LPV ratio
003	10,984	918	24.3	12.8	0.22%
004	4,887	480	7.1	3.7	0.15%
010	17,003	871	27.1	14.3	0.16%
016	13,900	1,045	17.0	8.9	0.12%
017	7,702	410	11.8	6.2	0.15%
031	8,514	575	20.8	10.9	0.24%
032	11,666	616	85.3	44.9	0.73%
036	9,240	1,256	31.5	16.6	0.34%
037	13,753	838	57.3	30.2	0.42%
041	9,679	773	30.7	16.2	0.32%
044	6,888	486	21.3	11.2	0.31%

- LPV median post-dose CSF sampling interval was 6.38 hours (range 4.67–9.98).
- Post-dose plasma sampling interval was 6.0 hours (range 4.08–8.77).
- The median CSF-plasma ratio was 0.24% (range 0.12–0.73). (Table 5)
- The relationship between time after last dose and CSF concentration is displayed in Figure 1.

**Table 5: LPV in CSF-plasma pairs**

	< 6 h after dose n=3	≥ 6 h after dose n=8	Overall
CSF LPV (ng/mL)	24.3	24.2	24.3
median (range)	(11.8–31.5)	(7.1–85.3)	(7.1–85.3)
Plasma LPV (ng/mL)	9,240	10,672	9,679
median (range)	(7,702–10,984)	(4,887–17,003)	(4,887–17,003)
CSF-plasma ratio	0.22%	0.28%	0.24%
median (range)	(0.15–0.34)	(0.12–0.73)	(0.12–0.73)
Time after dose (h)	5.67	7.46	6.38
median (range)	(4.67–5.87)	(6.35–9.98)	(4.67–9.98)

**Figure 1: Lopinavir concentrations in CSF by post-dose time**



### Subject 032

The subject had quantifiable HIV RNA in 2 CSF specimens (251 and 747 copies/mL). On first LP, LPV CSF concentrations were not obtained. Second LP found a LPV CSF concentration of 85.0 ng/mL. This value exceeded 3 times the median LPV CSF concentration for the cohort. This value was the highest CSF LPV concentration found in our trial. Genotypic resistance testing was performed on HIV derived from CSF revealing no primary mutations in protease.

### Detailed Clinical Summary of Subject 032

Subject 032 is a 36 year old Asian male born in Vietnam, residing in the U.S. since 1989, who was referred from a rheumatology clinic after evaluation in August 2005 for left-sided headache, decreased vision, pruritic maculopapular rash, joint pain, and right lower quadrant pain. He had unremarkable social history, no known HIV risk factors, was not receiving any medications and had no known allergies. Laboratory results were as follows:

Hemoglobin	10.8 gm/dL
Hematocrit	32.0%
Leukocytes	3.0 x 10 <sup>3</sup> /µL
Erythrocyte sedimentation rate	125 mm/h (normal ≤ 15)
Antinuclear antibody screen	positive
Antinuclear antibody pattern	homogenous
Antinuclear antibodies	1:640 (> 1:80 elevated antibody level)

## Results (continued)

He was diagnosed with optic neuritis and suspected systemic lupus erythematosus, treated with intravenous methylprednisolone and prednisone tapered over 3 weeks. When he did not respond, he was referred to our clinic where he was found to have:

Laboratory results	HIV-1 ELISA	positive
	Western blot	positive
	CMV PCR (retinitis primarily in left eye)	1760 copies/mL
	CD4 count	118 (9.1%)
	HIV RNA Roche Amplicor	577,000 copies/mL
	Hepatitis B surface antibody	> 2000 mIU/mL
	Hepatitis B surface antigen	non-reactive
	Hepatitis B core antibody	non-reactive
	Hepatitis C antibody	non-reactive
	Hepatitis A antibodies	+ IgG

Other infectious disease work-up was negative. CMV retinitis was treated with intravenous ganciclovir for 4 weeks and maintenance therapy with oral valganciclovir prior to screening for IMANI-2.

### Clinical course during IMANI-2 Trial

Week of Therapy	CD4 count (cells/mm <sup>3</sup> )	HIV viral load (copies/mL)*	Event
8/2005	118	577,000*	Genotype M36I, Phenotype to LPV 1.36 FC, RC=191%
11/2005	194	117,532	IMANI-2 screen
12/2005 (Week 0)	171	185,176	Start LPV/r
Week 4	218	783	
Week 6		94	Herpes zoster over left T-3 dermatome – resolved with oral famciclovir, hydrocodone/acetaminophen after 2 weeks
Week 8	358	142	RLQ pain, nausea, vomiting resolved with IV ketorolac, promethazine, and saline bolus
Week 12	362	< 75	
Week 24	386	< 75	
Week 32	364	170	
Week 36	348	< 75	1st lumbar puncture – CSF HIV RNA* - 251 copies/mL
Week 48	399	< 75	2nd lumbar puncture – CSF HIV RNA* - 747 copies/mL

\* HIV RNA PCR (Roche Amplicor), other values obtained with Bayer Versant™ HIV-1 RNA 3.0, bDNA.

## Discussion

This study demonstrated that single therapy with LPV/r suppressed CSF viral loads in all but one subject. Concentrations of LPV in the CSF while significantly lower compared to blood plasma, are likely adequate for suppression given the relatively protein free environment of CSF. In addition all CSF LPV concentrations did exceed the Monogram Biosciences median IC<sub>50</sub> and 99th percentile IC<sub>90</sub> [8.1 nmol/L (5.1 ng/mL)] for wild-type HIV-1.

- Persistent HIV replication can occur in the plasma during treatment with boosted protease inhibitors in the absence of identifiable resistance.<sup>13</sup> The reasons for this are not completely understood but this phenomenon might also occur in other compartments, such as the CNS. Detectable HIV RNA levels in CSF have been previously reported during otherwise successful therapy with triple agent HAART.<sup>14,15</sup>
- HIV replication in CSF could persist if LPV concentrations were sub-therapeutic. The LPV concentration in the CSF of this individual, however, was the highest in the group and exceeded the Monogram Biosciences median IC<sub>50</sub> and 99th percentile IC<sub>90</sub> by approximately 45- and 17-fold, respectively.
- HIV with reduced susceptibility to LPV could continue to replicate in the presence of higher levels of LPV. However, genotype resistance testing of HIV derived from the CSF of this individual demonstrated the absence of primary protease resistance mutations.
- Protease inhibitors inhibit production of infectious virions but not transcription of viral DNA or translation of viral polyproteins. In the absence of reverse transcriptase inhibition, viral RNA and polyproteins can accumulate in infected cells. This non-infectious viral RNA can be measured by RT-PCR assays, especially if the infected cells lyse during processing. Leukocytes were present in both CSF specimens from this individual and, according to the clinical lab, all leukocytes lysed in the second specimen before they could be counted. Since this subject had therapeutic levels of LPV in CSF and no evidence of reduced susceptibility, the production of intact virions is less likely. Instead, the measured RNA may have reflected release of intracellular HIV RNA from lysed lymphocytes.

## Discussion (continued)

- If the HIV RNA in this CSF specimen derived from lysis of trafficking replication competent lymphocytes, greater trafficking should lead to higher HIV RNA levels. We hypothesized that this individual might have high MCP-1/CCL2 levels in CSF because this potent chemokine induces trafficking of monocytes and lymphocytes into the CNS.<sup>16,17</sup> The MCP-1/CCL2 concentration in this specimen was 934 pg/mL, a level that is at the ~90th percentile of an independent cohort of 119 HAART-treated individuals and is ~4 times more likely to come from an individual who has an A-to-G polymorphism in the promoter region of the gene encoding MCP-1/CCL2.<sup>18,19</sup> Notably, MCP-1 has also been implicated in the neurologic complications of HIV<sup>20</sup> and systemic lupus erythematosus.<sup>21</sup>
- Another possible explanation for the finding depends on the differences between CSF and brain tissue. Since CSF provides an imperfect window into brain events, HIV and LPV characteristics in CSF may not accurately reflect those in the brain. For example, the persistent HIV RNA in CSF could derive from compartmentalized replication in microglia or brain macrophages,<sup>22,23</sup> which is more common in those with CD4 nadirs under 200/µL, and sub-therapeutic LPV concentrations in brain tissue despite having therapeutic concentrations in CSF. At present, this theory cannot be proven without obtaining brain tissue.

## Conclusion

This study is the first to examine CSF viral load in a diverse cohort of naïve subjects treated with LPV/r single-agent therapy for at least 24 weeks. Subjects were sampled throughout the dosing interval and 10 of 11 achieved suppression of CSF viral load < 50 copies/mL.

The LPV CSF median concentration of 24.3 ng/mL (range 7.1–85.3) in single agent therapy was above the previously reported median of 17.0.<sup>7</sup> The median LPV/IC<sub>50</sub> ratio in this study was 12.8 (mean 16.0) with a range of 3.7 to 44.9. All individual subject LPV concentrations exceeded the reference population median IC<sub>50</sub> by at least 3-fold, and the mean CSF LPV concentration exceeded the reference population median IC<sub>90</sub> by 16-fold. Therefore LPV/r delivers adequate LPV concentrations that reliably exceed the reference population median IC<sub>90</sub> for wild-type virus.

In 10 of 11 subjects LPV/r given as single-agent therapy effectively controlled viral replication in the CSF compartment.

The implications of potential CNS viral replication during ARV in the absence of resistance, whether using single agent therapy or triple agent HAART, warrants further study.

## Acknowledgements

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