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Abstract #75

Selection of Protease Inhibitor Resistance Mutations During Virologic Failure of Lopinavir/ritonavir Monotherapy in an Induction-Maintenance Study

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Background

When dosed twice daily, lopinavir/ritonavir (LPV/r) produces C_{trough} >70-fold the concentration required to inhibit wild type HIV-1.[1]

LPV/r used as part of a 3-drug regimen in antiretroviral-naïve patients is highly effective in durably suppressing plasma HIV-1 RNA to <50 copies/mL.[2,3]

In antiretroviral-naïve patients, combination therapy with LPV/r only rarely selects for protease inhibitor (PI) resistance.

- In a comparative clinical trial of LPV/r versus nelfinavir (N=653 followed up to 108 weeks), each in combination with stavudine and lamivudine, LPV/r therapy resulted in no PI or stavudine resistance and in significantly lower rates of lamivudine resistance compared to nelfinavir therapy.[4]
- In a smaller but longer study of LPV/r in combination with stavudine and lamivudine (N=100 followed up to 7 years), no PI resistance developed at times of confirmed virologic failure or isolated virologic rebound.[3]
- A single case of development of PI resistance during therapy with a LPV/r-based 3-drug regimen has been reported in the literature.[5]

LPV/r monotherapy has shown promising short-term efficacy in small studies of relatively short duration.[6,7,8]

Little is known about the propensity of LPV/r monotherapy to select drug resistance. A few case reports of evolution of PI resistance during periods of LPV/r monotherapy have been presented.[9,10,11,12]

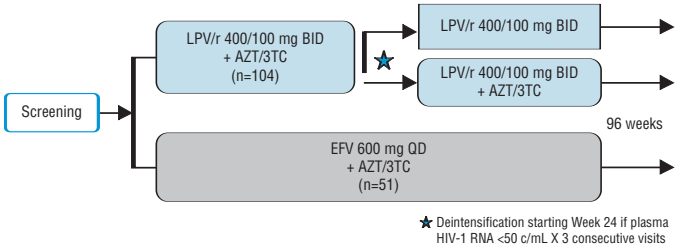
We describe the emergence of drug resistance in subjects simplifying to LPV/r monotherapy in a controlled randomized trial examining the safety and efficacy of an induction-maintenance strategy with LPV/r.

Methods

Study Design

Study M03-613 is a controlled, randomized, open-label 96-week study in antiretroviral-naïve HIV-1-infected subjects comparing a 3-drug regimen of efavirenz (EFV) plus zidovudine/lamivudine (AZT/3TC) with an induction-maintenance strategy of LPV/r plus AZT/3TC induction for 24 weeks followed by deintensification to LPV/r monotherapy after 3 consecutive monthly plasma HIV-1 RNA <50 copies/mL (Figure 1).

Figure 1. M03-613 Study Design



Inclusion criteria included:

- Antiretroviral naïve
- Plasma HIV-1 RNA >1000 copies/mL
- Any CD4+ T-cell count
- No evidence of resistance to study drugs on screening HIV-1 drug resistance genotype (TRUGENE HIV-1™, Bayer Health Care, New York). Resistance to study drugs was defined as follows:
  - EFV, AZT, 3TC: Resistance report (TRUGENE HIV-1™ interpretation guidelines v. 8.0) indicates resistance or possible resistance
  - LPV/r: Presence of any mutation in the protease gene leading to an amino acid substitution at the following loci: 8, 30, 32, 46, 47, 48, 50, 54, 82, 84, or 90; or four or more mutations at the following loci: 10, 20, 24, 36, 53, 63, or 71.

Subjects were randomized to either the LPV/r induction/maintenance regimen (n=104) or the EFV-based regimen (n=51) in a 2:1 ratio.

Subjects were monitored with plasma HIV-1 RNA levels every 4 weeks until Week 72 then every 8 weeks.

Subjects randomized to the LPV/r arm who achieved plasma HIV-1 RNA values <50 copies/mL on 3 consecutive study visits between Weeks 12 and 44 (inclusive) were permitted to discontinue AZT/3TC at their next study visit and remain on LPV/r twice daily monotherapy through study completion or discontinuation.

Resistance, Phylogenetic, and Antibody-Profile Western Blot Analysis

HIV-1 drug resistance genotype testing (TRUGENE™) was performed at screening to determine study eligibility, and at time of virologic failure (defined as 1st of 2 consecutive plasma HIV-1 RNA values >500 copies/mL).

Additional HIV-1 drug resistance genotype testing was performed with the ViroSeq™ HIV-1 Genotyping System v2.0 (Celera Diagnostics, Alameda, CA) on the 2 subjects described in this analysis. A 1.6 kB region of *pol* encompassing protease (amino acids 1–99) and residues 1-335 of reverse transcriptase (RT) gene was evaluated with the ViroSeq™ HIV-1 Genotyping System software v2.6.

For phylogenetic analysis, nucleic acid sequences were aligned with HIV-1 group M subtype reference sequences (http://www.hiv.lanl.gov/content/hiv-db/SUBTYPE_REF/Table1.html) using the CLUSTAL W method (MegAlign, Lasergene, DNASTAR Inc., Madison, WI) and manually edited. Alignments were gapstripped using BioEdit Sequence Alignment Editor (version 5.0.9, Tom Hall, North Carolina State University, Raleigh, North Carolina) yielding a consensus length of 630 bp. Phylogenetic analysis was performed with the PHYLIP version 3.572 (http://evolution.genetics.washington.edu/phylip.html). Evolutionary distances were estimated with Dnadist (Kimura two-parameter method) and phylogenetic relationships were determined by Neighbor (neighbor-joining method). Branch reproducibility was evaluated using Seqboot (100 replicates). Consensus trees were displayed with TreeExplorer.

Specific plasma samples were also tested with an antibody-profile western blot assay (Viral and Rickettsial Disease Laboratory, California Department of Health Services, Berkeley, CA) that distinguishes specimens from different persons in order to investigate the possibility of specimen misidentification.[13]

Results

- In the LPV/r induction maintenance arm, 92 of the 104 randomized subjects (88%) deintensified to LPV/r monotherapy.
- Subjects who deintensified to LPV/r monotherapy have been followed for a median (IQR) of 56 (47–64) weeks.

At the time of the present analysis, 9 subjects (9/92, 10%) who were receiving LPV/r monotherapy had confirmed plasma HIV-1 RNA rebound >500 copies/mL and had genotype testing performed.

- Testing failed from 1 subject.
- Results were available from 8 subjects.
  - 2 subjects (subject A and B, described below) developed PI resistance mutations on virologic rebound.
  - 6 subjects showed no evidence of PI resistance mutations on virologic rebound.

Subject A (Figure 2)

The screening sample from Subject A showed RT K219Q (a thymidine analog resistance mutation) and protease M36I and L63P (secondary PI resistance mutations frequently noted as natural polymorphisms in wild type HIV-1). The subject was enrolled into the study since he did not meet criteria for exclusion based on the screening drug resistance genotype.

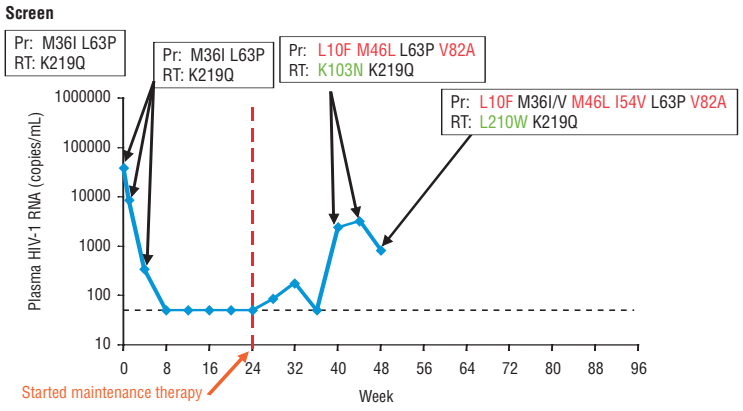
Subject A deintensified to LPV/r monotherapy at Week 24 after 5 consecutive monthly plasma HIV-1 RNA <50 copies/mL. The subject started experiencing low-level viremia at Week 28 and had confirmed rebound >500 copies/mL starting at Week 40.

Drug resistance genotype at Week 40 showed the following drug-resistance mutations:

- RT K219Q persisted from Baseline.
- RT K103N (a non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutation) was noted even though subject was reportedly antiretroviral-naïve and did not receive NNRTIs.
- Protease L10F, M46L, L63P, and V82A were noted with the addition of I54V in protease at Week 48.

Retrospective genotypic resistance testing of the subject's plasma samples at Baseline, Week 1, and Week 4 showed a genotype identical to the Screening genotype.

Figure 2. Development of PI Resistance in Subject A



Week	Fold Change in LPV/r IC_{50}
Baseline	0.83
40	4.33
48	22

Subject B (Figure 3)

The screening sample from Subject B showed protease L63P (secondary PI resistance mutation frequently noted as natural polymorphism in wild type HIV-1). Subject B was enrolled into the study since he did not meet criteria for exclusion based on the screening drug resistance genotype.

Subject B deintensified to LPV/r monotherapy at Week 40 after 4 consecutive monthly VL <50 copies/mL. The subject had confirmed rebound >500 copies/mL starting at Week 44.

Drug resistance genotype at Week 44 showed the following drug-resistance mutations:

- RT M41L, D67N, V118I, L210W, and T215Y (thymidine analog and nucleoside resistance mutations).
- RT Y188L (a NNRTI resistance mutation).
- Protease M36I, L63P, A71V, G73S, and L90M with addition of protease I54V at Week 56 and L33F and V82A at Week 76.

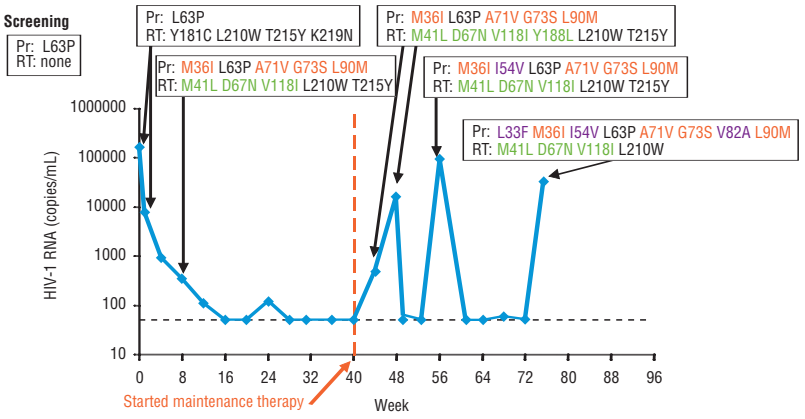
Retrospective genotypic resistance testing of the subject's plasma sample at Baseline showed that the subject had pre-existing NNRTI (Y181C) and thymidine analog (L210W T215Y K219N) resistance mutations, in contrast to the Screening genotype.

- Based on the Baseline genotype, the subject would have been excluded from the study.

Retrospective genotypic resistance testing of the subject's plasma sample at Week 8 showed that the protease resistance mutation M36I, L63P, A71V, G73S, and L90M were present before achieving virologic suppression during the induction phase (LPV/r + AZT/3TC).

- This is consistent with rapid re-emergence of preexisting protease inhibitor resistance mutations rather than *de novo* evolution of resistance.

Figure 3. Development of PI Resistance in Subject B



Week	Fold Change in LPV/r IC_{50}
1	0.82
8	0.87
48	2.24
56	10
76	23

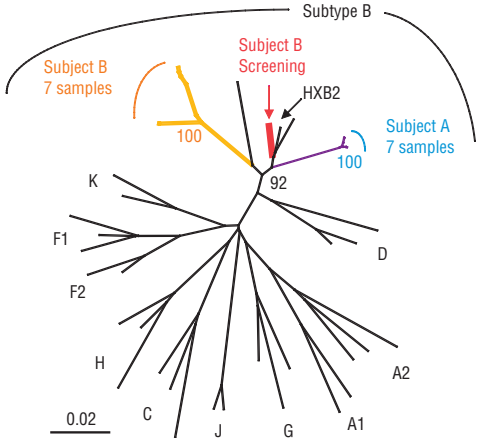
Phylogenetic and Antibody-Profile Western Blot Analysis

In order to understand the discrepancies between the Screening and Baseline drug resistance genotypes in Subject B, a phylogenetic analysis was performed that included the following sequences:

- All 8 genotypes available from Subject B (Screening, Baseline, Week 1, Week 8, Week 44, Week 48, Week 56, Week 76).
- All 7 genotypes available from Subject A (Screening, Baseline, Week 1, Week 4, Week 40, Week 44, Week 48).
- 3 reference sequences from patients with HIV-1 subtype B.
- 26 reference sequences from patients with HIV-1 non-B subtype.

With the exception of the Screening sample of Subject B, all of Subject B's samples clustered closely together and all of Subject A's samples clustered closely together with high confidence (bootstrap value = 100%). The screening sample of Subject B branched within the subtype B lineage but segregated independently from the Subject B cluster, suggesting that this sequence did not originate from the same subject.

Figure 4. Phylogenetic Analysis of Samples from Subjects A and B

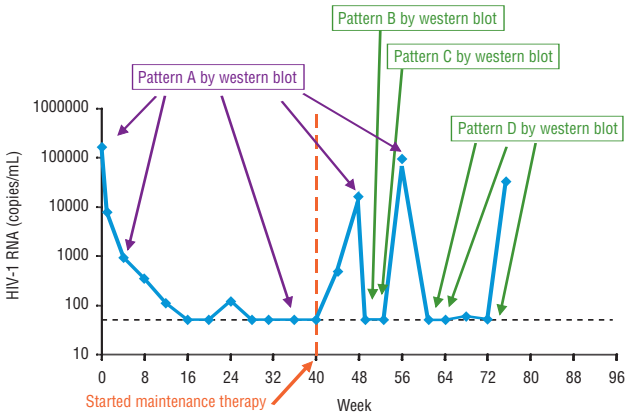


In order to understand the wide variability of the plasma HIV-1 RNA levels after deintensification in Subject B, selected plasma samples from Subject B were also tested with an antibody-profile western blot assay that distinguishes specimens from different persons:

- All specimens tested before initial virologic rebound as well as samples with viremia after the initial rebound (Baseline, Week 4, Week 36, Week 48 and Week 56) had an identical pattern (Pattern A).
- All specimens tested after initial virologic rebound (Week 44) with undetectable plasma HIV-1 RNA (Weeks 48, 52, 60–72) had different patterns distinguishable from Pattern A.

Antibody-profile western blot analysis of samples of Subject B after initial virologic rebound suggested that samples with HIV-1 RNA <50 copies/mL did not originate from the same subject.

Figure 5. Antibody-Profile Western Blot Analysis of Samples from Subject B



Conclusions

After a median follow-up of 56 weeks on LPV/r monotherapy, only 9 out of 92 (10%) subjects met criteria for resistance testing (2 confirmed viral loads > 500 copies/mL).

- The risk of virologic failure with LPV/r monotherapy appears to be much lower than previous historic experience with antiretroviral monotherapy.

Among the 8 subjects with results available from resistance testing while receiving LPV/r monotherapy, 6 did not develop PI resistance mutations. For the 2 cases that developed PI resistance, both demonstrated presence of drug-selected (though not PI-specific) resistance mutations at baseline and may have harbored PI resistance at baseline at levels undetectable by population sequencing.

- In Subject A, the presence of RT K219Q at Baseline is suggestive of prior virus exposure to thymidine analogs.
- In Subject B, the presence of RT Y181C, L210W, T215Y, and K219N at Baseline is suggestive of prior virus exposure to NNRTIs and thymidine analogs. The rapid detection of protease M36I, A71V, G73S, and L90M at Week 8 before virologic suppression is also suggestive of prior virus exposure to PIs (resistance pattern most consistent with saquinavir or nelfinavir exposure).

Despite the presence of drug resistance mutations at baseline in Subjects A and B, virologic suppression was maintained with a 3-drug LPV/r-containing regimen, but breakthrough viremia occurred rapidly after deintensification to monotherapy, followed by progressive evolution of PI resistance.

- The detection of any drug-selected mutations at baseline may be indicative of the presence of other drug-selected (possibly PI resistance) mutations undetectable by population sequencing.
- The use of ritonavir-boosted PI monotherapy in patients with any drug-selected resistance mutations at baseline should be undertaken with caution, especially in populations at increased risk of primary infection with a PI-resistant virus.

Use of phylogenetic analysis and antibody-profile western blot analysis allowed a more complete understanding of discrepant findings in a clinical study.

- These analyses allowed a determination that several specimens attributed to Subject B (including the Screening sample and several samples after deintensification) were misidentified.

In summary, although the risk of PI resistance with failure of LPV/r monotherapy appears to be low, the risk may be higher than with a LPV/r-based 3-drug regimen, stressing the need for appropriate selection of patients for this strategy.

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