Abstract 145

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# Analysis of the Emergence of Secondary Mutations with or without Primary PI Resistance in ARV-Naive Subjects with Detectable Viral Load on Nelfinavir or Lopinavir/Ritonavir Therapy

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## INTRODUCTION

The development of protease inhibitor (PI) resistance generally requires the accumulation of both primary and secondary mutations in HIV protease.[1] Secondary protease mutations lie outside of the active site and are thought to contribute to phenotypic resistance to PIs indirectly through subtle changes in the orientation of active site residues and/or by affecting the kinetics of polyprotein processing by protease (and thus modulating viral replication capacity). A number of secondary mutations also occur commonly in viruses from individuals who have not received drug therapy (polymorphisms); thus, the emergence of a secondary mutation during therapy does not necessarily imply drug selection. The comparison of the rates of the emergence of secondary mutations during therapy with different regimens can therefore provide information regarding the relative selective pressure of those regimens.

We have recently reported the comparison of the emergence of primary PI resistance in a randomized, double-blind Phase III clinical trial comparing Kaletra (lopinavir/ritonavir) plus d4T/3TC with nelfinavir plus d4T/3TC (Study 863) in antiretroviral-naive subjects.[2] Through 96 weeks of therapy, no evidence of primary resistance to lopinavir (any primary or active site mutation) was detected in any of 51 Kaletra-treated subjects with detectable viral load for whom genotype was available (Table 1). In contrast >40% of isolates from nelfinavir-treated subjects displayed primary resistance to nelfinavir (emergence of D30N and/or L90M). Resistance to 3TC (emergence of M184V, I or T in reverse transcriptase) was also significantly less common in the lopinavir/r arm.

In order to complete the characterization of the development of PI resistance in Study 863, we analyzed the emergence of secondary mutations in both treatment arms, either concomitant with or in the absence of primary resistance, during viral rebound.

**Table 1. Primary Genotypic Resistance in Study M98-863 Through Week 96**

| | Kaletra | Nelfinavir | p-value |
|---|---|---|---|
| No. of subjects enrolled | 326 | 327 | |
| Subjects with HIV RNA >400 copies/mL | 74 (23%) | 113 (35%) | |
| Genotype available | 51/74 (69%) | 96/113 (85%) | |
| PI resistance | 0/51 (0%) | 41/96 (43%) | <0.001 |
| 3TC resistance | 19/51 (37%) | 79/96 (82%)* | <0.001 |

* Previous results that showed 78/96 with 3TC resistance (Reference 2) did not include one isolate with a M184T mutation that demonstrated >100-fold phenotypic resistance to 3TC.

## METHODS

Samples from all subjects who had at least one HIV RNA >400 copies/mL at Week 24 through Week 96 without a documented treatment interruption greater than 7 days or study discontinuation were submitted for resistance testing. In all, samples from 51 Kaletra-treated subjects and 96 nelfinavir-treated subjects could be amplified for genotype. Genotype (population sequencing) and phenotype (PhenoSense™) were performed at ViroLogic, Inc. Secondary protease mutations were defined as any change at the following codons: position 10, 20, 24, 33, 36, 46, 54, 71, 73, 77, 88.[1]

## Prevalence of Baseline Secondary Mutations

- Baseline polymorphisms at positions also classified as secondary mutations (at positions 10, 20, 24, 33, 36, 46, 54, 71, 73, 77 and 88) are provided in Table 2.
- There was no difference in the prevalence of baseline polymorphisms between study arms.
- Baseline polymorphisms at positions 36 and 77 were most common, followed by those at positions 10, 20 and 71.
- The presence of baseline polymorphisms was not associated with the development of primary resistance to nelfinavir or resistance to 3TC upon rebound (data not shown).

**Table 2. Prevalence of Secondary Mutations at Baseline**

| Study Arm | Kaletra | Nelfinavir |
|---|---|---|
| No. of subjects with baseline samples available | 51 | 96 |
| No. of samples with: | | |
| Any secondary mutation* | 34 (67%) | 64 (68%) |
| 1 secondary mutation | 25 (49%) | 46 (48%) |
| 2 secondary mutations | 8 (16%) | 17 (18%) |
| 3 secondary mutations | 1 (2%) | 2 (2%) |
| No. of samples with mutations at: | | |
| L10 | 4 (8%) | 10 (10%) |
| K20 | 3 (6%) | 6 (6%) |
| L33 | 1 (2%) | 1 (1%) |
| M36 | 13 (25%) | 31 (32%) |
| M46 | 1 (2%) | 0 (0%) |
| A71 | 6 (12%) | 9 (9%) |
| V77 | 16 (31%) | 29 (30%) |

* Any change from the pNL4-3 sequence at positions 10, 20, 24, 33, 36, 46, 54, 71, 73, 77 and 88 in HIV protease.

## Emergence of Secondary Mutations in the Context of Primary PI Resistance

- Only nelfinavir-treated subjects (41/96) demonstrated primary resistance to nelfinavir, defined as the emergence of D30N (28/41), L90M (12/41) or both (1/41).
- New secondary mutations that were not present in the corresponding baseline samples are provided in Table 3.
- At least one new secondary mutation accompanied the D30N or L90M primary mutation in the substantial majority (31/41, 76%) of nelfinavir-treated subjects. Over 50% (21/41) of the subjects demonstrated two or more new secondary mutations.
- New mutations at positions 10, 36, 46, 71, 77 and 88 were most commonly observed.
- New secondary mutations emerged commonly with either primary mutation pattern (21/28 with D30N, 9/12 with L90M, and 1/1 with both D30N and L90M).
- The rebound isolates from these 41 nelfinavir-treated subjects displayed a median (range) of 3 (1-8) total mutations associated with PI resistance.

**Table 3. Analysis of Secondary Mutations in Nelfinavir-Treated Subjects with Primary PI Resistance**

| | |
|---|---|
| No. of Nelfinavir-Treated Subjects with Both Baseline and Rebound Samples Available | 41 |
| No. of samples with: | |
| Any new secondary mutation | 31 |
| 1 new secondary mutation | 10 |
| 2 new secondary mutations | 14 |
| 3 new secondary mutations | 4 |
| 4 new secondary mutations | 2 |
| 5 new secondary mutations | 1 |
| No. of samples with mutations at: | |
| L10 | 7 |
| K20 | 4 |
| L33 | 1 |
| M36 | 11 |
| M46 | 7 |
| A71 | 13 |
| G73 | 3 |
| V77 | 6 |
| N88 | 11 |

## Emergence of Secondary Mutations in the Absence of Primary PI Resistance

- New secondary mutations in the rebound isolates from subjects who did not display primary PI resistance are provided in Table 4.
- A single new secondary mutation that was not present at baseline emerged in 7/51 (14%) Kaletra-treated subjects. These mutations were restricted to sites (positions 10, 36 or 71) at which polymorphisms are commonly observed in untreated subjects (see above).
- The accumulation of a secondary mutation in the above 7 rebound isolates had no detectable effect on the phenotypic susceptibility to lopinavir (data not shown).
- In contrast, new secondary mutations were observed at rebound in 17/55 nelfinavir-treated subjects in the absence of a primary D30N or L90M mutation (31% vs. 14%, p=0.039, nelfinavir vs. Kaletra, respectively).
- New secondary mutations appeared at a greater diversity of amino acid positions in nelfinavir-treated subjects (positions 10, 20, 33, 36, 46, 54, 71, 73, 77 and 88) than in Kaletra-treated subjects.

**Table 4. Analysis of Secondary Mutations in the Absence of Primary PI Resistance**

| Study Arm | Kaletra | Nelfinavir |
|---|---|---|
| No. of subjects with both baseline and rebound samples available | 51 | 55 |
| No. of samples with: | | |
| Any new secondary mutation | 7 | 17 |
| 1 new secondary mutation | 7 | 12 |
| 2 new secondary mutations | 0 | 3 |
| 3 new secondary mutations | 0 | 2 |
| No. of samples with mutations at: | | |
| L10 | 1 | 1 |
| K20 | 0 | 6 |
| M36 | 5 | 3 |
| M46 | 0 | 5 |
| I54 | 0 | 2 |
| A71 | 1 | 2 |
| V77 | 0 | 2 |
| N88 | 0 | 3 |

## RESULTS

# Identification of a Novel Pathway to Nelfinavir Resistance

- The emergence of a M46I/L mutation, which is not a common polymorphism in the absence of selective pressure, was observed in 5 nelfinavir-treated subjects in the absence of D30N or L90M.
- Baseline and rebound genotypes for these five subjects are provided in Table 5.
- Unexpectedly, 3 of these 5 isolates displayed substantially reduced (6.8- to 8.7-fold) susceptibility to NFV, even in the absence of a primary mutation at position 30 or 90.
- These three isolates also displayed a significant (>2.5-fold) change in susceptibility to indinavir, compared to wild-type virus.
- Including the above three subjects, the total incidence of resistance to nelfinavir in subjects with detectable viral load in Study 863 was 44/96 (46%).

**Table 5. Selection of Nelfinavir Resistance in the Absence of D30N or L90M**

| | Protease Genotype* | | Fold $EC_{50}$ (Rebound) | |
|---|---|---|---|---|
| Subject | Baseline | Rebound | NFV | IDV |
| A | M36I, V82V/I | K20M, M36I, M46L, N88S | 8.7 | 3.1 |
| B | L10V | L10V, M46M/I, N88S | 6.8 | 3.0 |
| C | A71V | K20I, M46M/I, A71V, V77V/I | 7.6 | 2.7 |
| D | V82I | M46M/I, V82I | 1.7 | 0.5 |
| E | | M36M/V, M46M/I | 0.5 | 0.5 |

* Primary and secondary mutations (see Methods).

## CONCLUSIONS

- In addition to the large difference in the emergence of primary resistance to lopinavir vs. nelfinavir in a randomized, blinded, Phase III trial, the emergence of secondary mutations during rebound on nelfinavir-based combination therapy is significantly more frequent than during Kaletra-based therapy.
- The infrequent appearance of new secondary mutations during Kaletra therapy only at positions that are commonly polymorphic suggests minimal selective pressure by Kaletra during periods of low adherence that produce detectable viral replication.[2]
- Rebound during nelfinavir-based therapy can produce a previously unrecognized mutation pattern, including M46I/L and other secondary mutations but lacking D30N or L90M, in a minority of patients, producing both phenotypic resistance to nelfinavir and cross-resistance to other PIs.

## REFERENCES


1. Hirsch MS et al. Antiretroviral drug resistance testing in adults with HIV infection: implications for clinical management. International AIDS Society – USA Panel. *JAMA* 1998;279:1984-91.

2. 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 to week 96. 41st ICAAC, Chicago, IL, December 2001. Abstract I-1768.