Incidence of Resistance in a Double-Blind Study Comparing Lopinavir/Ritonavir Plus Stavudine and Lamivudine to Nelfinavir plus Stavudine and Lamivudine

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Study M98-863 was a double-blind, randomized, phase 3 study that compared lopinavir/ritonavir with nelfinavir, each coadministered with stavudine and lamivudine, in 653 antiretroviral therapy–naive human immunodeficiency virus (HIV) type 1–infected subjects. The incidence of HIV drug resistance was analyzed using baseline and rebound virus isolates from subjects with plasma HIV RNA >400 copies/mL from weeks 24 to 108 of therapy. No evidence of genotypic or phenotypic resistance to lopinavir/ritonavir, defined as any active site or primary mutation in HIV protease, was detected in virus isolates from 51 lopinavir/ritonavir-treated subjects with available genotypes. Primary mutations related to nelfinavir resistance (D30N and/or L90M) were observed in 43 (45%) of 96 nelfinavir-treated subjects. Resistance to lamivudine and stavudine was also significantly higher in nelfinavir-treated versus lopinavir/ritonavir-treated subjects. These differences suggest substantially different genetic and pharmacological barriers to resistance for these 2 protease inhibitors and may have implications for strategies for initiating antiretroviral therapy.

The introduction of protease inhibitor (PI) therapy was accompanied by a substantial decrease in the mortality and morbidity of human immunodeficiency virus (HIV) infection [1, 2]. Nonetheless, if adequate suppression of viral replication is not achieved, a rebound in plasma HIV RNA load is usually observed [3] that is often accompanied by the emergence of drug resistance. Resistance to PIs has been observed both during monotherapy [4, 5] and combination therapy [6, 7], and specific mutations associated with reduced phenotypic susceptibility to various PIs have been characterized [8–12]. However, the incidence of PI resistance during the failure of initial combination therapy has generally not been adequately quantitated [6, 13–15].

Lopinavir/ritonavir (lopinavir/r) is a combination PI regimen wherein the plasma levels of the active component (lopinavir) are “boosted” by virtue of the inhibition of its cytochrome P450-mediated metabolism by ritonavir [16, 17]. Lopinavir/r, in combination with other antiretroviral agents, has demonstrated potent activity in antiretroviral therapy (ART)–naive and PI-experienced patients [18, 19], and evidence suggests that lopinavir/r retains some activity against HIV with up to 40-fold reduced susceptibility and/or 7 mutations in HIV protease that have been associated with reduced susceptibility to lopinavir [20]. This activity against highly mutant HIV suggests the possibility of a significant pharmacological barrier to resistance in ART-naive patients who have wild-type plasma virus. The incidence of resistance in such patients receiving lopinavir/r is therefore of high interest.

Recently, the activity of lopinavir/r, in combination with stavudine (d4T) and lamivudine (3TC), was com-
pared with that of nelfinavir plus d4T/3TC in ART-naive sub-
jects (study M98-863). The proportion of subjects without a loss of
virological response (achieving and maintaining a plasma HIV
RNA level <400 copies/mL) through 48 weeks was 84% for lo-
pinavir/r-treated subjects and 66% for nelfinavir-treated subjects
\( (P < .001) \) [21]. The corresponding proportions through 96
weeks were 79% and 58%, respectively \( (P < .001) \) [22]. To assess
the incidence and cumulative emergence of resistance to initial
therapy with lopinavir/r and nelfinavir-based ART regimens,
we examined the rebound isolates from study M98-863. Our
primary objective was the comparison of the incidence of PI
resistance between the 2 regimens. Secondary objectives in-
cluded the analysis of the appearance of 3TC and d4T resistance
and secondary protease mutations, the estimation of relative
resistance rates through 108 weeks of therapy, the analysis of the
relationship between drug resistance and virus load or med-
ication adherence, and the assessment of the clinical conse-
quences of resistance.

**MATERIALS AND METHODS**

**Clinical isolates.** Isolates for the assessment of resistance
were collected from study M98-863, which was a randomized,
double-blind, placebo-controlled phase 3 study that compared
therapy that consisted of lopinavir/r plus d4T/3TC with nel-
finavir plus d4T/3TC in 653 ART-naive subjects. Subjects were
enrolled at 93 centers in 13 countries and were centrally ran-
domized in a 1:1 fashion to receive lopinavir/r (400/100 mg 2
times/day) plus nelfinavir placebo 3 times/day or nelfinavir (750
mg 3 times/day) plus lopinavir/r placebo 2 times/day. All sub-
jects received standard doses of d4T and 3TC 2 times/day.
Subjects were allowed to switch to twice-daily dosing of nel-
finavir or nelfinavir placebo after Food and Drug Administra-
tion approval of the nelfinavir 1250 mg 2 times/day. Subjects
were evaluated at baseline, every 4 weeks through week 24, every 8 weeks through week 48, and every 12 weeks thereafter. The study was approved by the institutional
review board or ethics committee at each center, and all patients
provided written informed consent.

**Determination of genotype and phenotype.** Samples for
viral phenotype and genotype were collected at each study visit
and archived for analysis according to specifications supplied
by ViroLogic. Samples from all subjects who had at least 1 HIV
RNA value >400 copies/mL at week 24, 32, 40, 48, 60, 72, 84,
96, or 108 without a documented treatment interruption of >7
days were submitted for resistance testing. Initially, samples
from the week 24, 48, and 60 study visits were sequentially
analyzed. If HIV RNA was <400 copies/mL at week 48 but >400
copies/mL at week 32 and/or 40, the latter of these 2 samples
was submitted for analysis. Finally, samples were submitted
from the latest time point with HIV RNA >400 copies/mL
during the study. Baseline samples were retrospectively sub-
mitted from each subject for whom a rebound genotype was
available. Plasma samples were amplified by reverse-transcrip-
tion polymerase chain reaction and incorporated into resistance
test vectors for genotypic and phenotypic analysis. Genotype
was determined at ViroLogic by population sequencing and
was compared with the pNL4-3 laboratory strain of HIV-1.
Viral phenotypes of all rebound samples from lopinavir/r-
treated subjects were determined using the PhenoSense HIV
assay [23]. Phenotypic analysis was not routinely conducted on
samples from nelfinavir-treated subjects; however, phenotypes
were obtained on selected samples that did not qualify as re-
sistant to nelfinavir on the basis of the genotypic definition
below but were suspected to be resistant by virtue of a mutation
at position 46.

**Definition of genotypic resistance.** We defined genotypic
resistance according to consensus algorithms [24, 25]. Resis-
tance to nelfinavir was defined as the presence of either the
D30N and/or L90M mutation in the protease gene [7, 26]. Because
the patterns of resistance to lopinavir/r in ART-naive
patients have not been defined, resistance to lopinavir was de-
fined as any primary mutation in the protease gene associated
with PI resistance or a mutation at any other amino acid in
the HIV protease active site (aa 8, 30, 32, 46, 47, 48, 50, 54,
82, 84, and 90). Secondary mutations in protease were defined
as those appearing at positions 10, 20, 24, 33, 36, 53, 71, 73,
77, and 88 [24, 25]. For samples from nelfinavir-treated sub-
jects, mutations at positions 46 and 54 were also considered to
be secondary mutations. Genotypic resistance to 3TC was de-
defined as the presence of the M184I, V, or T mutation in the
reverse transcriptase (RT) gene. Genotypic resistance to d4T
was defined as the presence of ≥1 thymidine-associated mu-
in RT. Comparisons of the incidence of genotypic resistance
between treatment arms were done using Fisher’s exact test.
Comparisons of difference in phenotypes between lopinavir/r-
treated subjects with and without new secondary mutations
were done using a 1-way analysis of variance.

**Estimation of resistance rates.** The cumulative rate of re-
sistance development was assessed by Kaplan-Meier analysis
of all enrolled subjects (lopinavir/r arm, \( n = 326 \); nelfinavir arm,
\( n = 327 \)), to account for varying durations of treatment and
data censoring occurring as a result of dropouts. The 3 subjects
with documented nelfinavir resistance with the M46I/L mutation
but without D30N or L90M (see Results) were considered to be
nelfinavir resistant, and subjects for whom genotype was not
obtained were assumed to have wild-type virus. Rates of resis-
tance during periods of detectable virus load (HIV RNA >400
copies/mL) were estimated in the subset of subjects with available

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RESULTS

The disposition of the 74 (23%) of 326 lopinavir/r-treated and 123 (38%) of 327 nelfinavir-treated subjects in study M98-863 who had at least 1 HIV RNA value >400 copies/mL while receiving treatment at any time point from week 24 to 108 is detailed in figure 1. Samples from 51 (69%) lopinavir/r-treated and 96 (78%) nelfinavir-treated subjects could be amplified for resistance testing. Genotypes were not obtained on samples from the remaining 50 subjects, generally because of low plasma HIV RNA copy numbers (median, 819 copies/mL). Genotype was available at the initial virus load rebound time point in approximately one-half (64/126) of subjects who experienced initial suppression to <400 copies/mL followed by a rebound. The me-
Table 2. New polymorphisms/secondary mutations in lopinavir/ritonavir-treated subjects.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Baseline protease sequence</th>
<th>Rebound protease sequence</th>
<th>Rebound lopinavir, $\eta$-fold IC$_{50}$</th>
<th>Rebound RT resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T12T/S</td>
<td>L19L/V, M36M/I</td>
<td>0.8</td>
<td>M184V</td>
</tr>
<tr>
<td>3</td>
<td>E35D/N, N37D, L63P, K70R, V77V</td>
<td>E35D/N, M36M/I, N37D, L63P, K70R, V77V</td>
<td>0.6</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>R41K, D60E, I64V</td>
<td>M36M/I, R41K, D60E, I64V</td>
<td>1.2</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>R41R/K, Q61E, L63P, V77I</td>
<td>M36M/I, R41R/K, Q61E, L63P, V77I</td>
<td>0.7</td>
<td>M184M/V</td>
</tr>
<tr>
<td>6</td>
<td>L33V, E235D, M36M/I, N37A/T, R41K, I64V</td>
<td>L10L/F, L33V, E235D, M36M/I, L33N/A/D, D7T, R41K, I64V, I72I/V, I93L</td>
<td>0.4</td>
<td>None</td>
</tr>
</tbody>
</table>

NOTE. New secondary mutations associated with protease inhibitor resistance are indicated by bold type.

* The M36M/I mutation was no longer present in a later sequence sample from subject 3.
* The L10L/F mutation was no longer present in later sequence samples from subject 6.
* The rebound sequence from subject 7 is likely to have been an archival virus, given the reverse-transcriptase sequence.

Comparison of the incidence of resistance between treatment arms. Through 108 weeks, the incidence of resistance to lopinavir/r and nelfinavir differed markedly. No primary or active site mutations were observed in the rebound samples from any of the 51 lopinavir/r-treated subjects (table 1). The absence of detectable resistance to lopinavir was confirmed by phenotypic analysis. Thus, all 51 rebound samples displayed susceptibility to lopinavir within 2.5-fold of the susceptibility of the wild-type viral standard. In contrast, the D30N and/or L90M mutation emerged in 43 (45%) of 96 samples from nelfinavir-treated subjects ($P<.001$ for comparison of PI resistance between lopinavir/r- and nelfinavir-treated subjects), including 28 subjects with D30N, 14 with L90M, and 1 with both D30N and L90M. The emergence of nelfinavir resistance was confirmed by genotypic analysis of the corresponding baseline isolates, none of which displayed evidence of genotypic resistance to nelfinavir.

The incidence of resistance to the accompanying nucleoside analogues also differed between treatment arms. 3TC resistance emerged in 79 (82%) of 96 nelfinavir-treated subjects but in only 19 (37%) of 51 lopinavir/r-treated subjects ($P<.001$). Isolates from all nelfinavir-treated subjects with either the D30N or L90M protease mutation also exhibited resistance to 3TC. Even in subjects without primary PI mutations, the incidence of 3TC resistance in lopinavir/r-treated subjects (19/51 [37%]) was significantly lower than that in nelfinavir-treated subjects (36/53 [68%]; $P = .003$). The emergence of resistance to d4T occurred in rebound samples from 9 (9%) of 96 nelfinavir-treated subjects, all of which also displayed 3TC resistance (resistance to nelfinavir was also observed in 8/9 of the above samples). In contrast, the emergence of de novo d4T resistance was not documented in lopinavir/r-treated subjects. One rebound sample from a lopinavir/r-treated subject displayed the simultaneous appearance of 4 thymidine-associated mutations (positions 67, 70, 215, and 219 in RT) at the first time point with virus load >400 copies/mL (see table 2 below). However, these mutations appeared to represent archival virus from prob-
able transmission of a drug-resistant strain, as evidenced by a mixture of T/A/I/V at position 215 [27], and were not judged to be the result of de novo selection during lopinavir/r plus d4T/3TC therapy.

Secondary mutations associated with PI resistance [24, 25] also emerged significantly more frequently in nelfinavir-treated subjects (51/96 [53%]) than in lopinavir/r-treated subjects (7/51 [14%]; \( P < .001 \)). The distribution and identity of new secondary mutations are described in table 3. In lopinavir/r-treated subjects, new secondary mutations emerged only at amino-acid positions (10, 36, or 71) that are highly polymorphic in untreated patients (the incidence of these 3 mutations at baseline in all subjects with genotype was 10%, 30%, and 10%, respectively). Details of the above 7 subjects are provided in table 2. The presence of a new secondary mutation had no detectable effect on the phenotypic susceptibility of the isolates to lopinavir (the mean fold lopinavir IC\(_{50}\) values for the 7 isolates with a new secondary mutation and the 44 isolates with no new secondary mutation were both 0.8-fold; \( P = .652 \)). Finally, 3TC resistance was evident in only 4 (57%) of 7 isolates with a new secondary protease mutation. Other polymorphisms that are generally not classified as secondary mutations also appeared in a subset of sequences from lopinavir/r-treated subjects (data not shown); however, these polymorphisms were not con-

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**Figure 2.** Kaplan-Meier analysis of time to resistance, starting from the initiation of therapy. A, Nelfinavir-treated subjects \( (n = 327) \). B, Lopinavir/ritonavir-treated subjects \( (n = 326) \).
Figure 3. Kaplan-Meier analysis of time to resistance after human immunodeficiency virus (HIV) RNA rebound from <400 copies/mL. Time 0 represents the last determination of human immunodeficiency virus RNA load <400 copies/mL. A, Nelfinavir-treated subjects (n = 80) through 48 weeks. B, Lamivudine resistance through 26 weeks by study arm (nelfinavir, n = 80; lopinavir/ritonavir, n = 46).

Persistently selected, and none appeared to produce any detectable loss of phenotypic susceptibility to nelfinavir.

In the absence of a primary D30N or L90M mutation, secondary mutations were observed in the rebound sequences of 16 (30%) of 53 nelfinavir-treated subjects (table 3). New secondary mutations appeared at a greater diversity of amino-acid positions in these subjects than in lopinavir/r-treated subjects, and, with one exception, were always accompanied by 3TC resistance. In particular, the emergence of the M46I/L mutation, which is not a common polymorphism, in 4 nelfinavir-treated subjects, suggests substantial selective pressure by nelfinavir. The phenotypic susceptibilities of these 4 isolates were therefore determined. Three of the isolates (protease genotypes K20M, M36I, M46L, N88S; L10V, M46M/I, L63L/P, N88S; and K20I, M46M/I, L63V, A71V, V77V/I) displayed reduced phenotypic susceptibility to nelfinavir (6.8–8.7-fold). Finally, as anticipated, the incidence of new secondary mutations in rebound isolates from nelfinavir-treated subjects that also exhibited a D30N or
L90M mutation was significantly greater (35/43 [81%]) than in those without a primary mutation (P < .001). New secondary mutations were common with either primary mutation pattern (23/28 [82%] isolates that contained the D30N mutation, 11/14 [79%] isolates that contained the L90M mutation, and 1/1 isolate that contained both D30N and L90M).

Kaplan-Meier estimates of the cumulative incidence of resistance in all enrolled subjects through 108 weeks of therapy for the 2 treatment arms are shown in figure 2. In this analysis, the 3 subjects with documented nelfinavir resistance via the M46I/L pathway were considered to be nelfinavir resistant, and patients without genotype data were considered to have wild-type virus. After 2 years, the following relative resistance rates were observed: 3TC resistance in nelfinavir-treated subjects (29%) >* nelfinavir + 3TC resistance in nelfinavir-treated subjects (20%) >* 3TC resistance in lopinavir/r-treated subjects (7%) ~ d4T + 3TC resistance in nelfinavir-treated subjects (5%) >* lopinavir or d4T resistance in lopinavir/r-treated subjects (0%, P < .001 for each pairwise comparison signified with an asterisk). The onset of resistance to d4T in nelfinavir-treated subjects was less rapid than 3TC resistance in lopinavir/r-treated subjects, but the incidence to both was similar after 108 weeks. Estimated rates of resistance during periods of continuously detectable virus load in nelfinavir-treated subjects are shown in figure 3A. After 6 months, the rates of 3TC, nelfinavir, and d4T resistance were 86%, 43%, and 7%, respectively. Corresponding rates after 1 year of replicating HIV were 100%, 74%, and 15%, respectively. A comparison of 3TC resistance emergence between treatment arms is provided in figure 3B. 3TC resistance emerged significantly more rapidly during periods of detectable virus load on nelfinavir-based therapy (P = .002), and after 6 months, the 3TC resistance rates were 86% and 48% for nelfinavir- and lopinavir/r-treated subjects, respectively.

Resuppression of virus load after resistance analysis. Resuppression of HIV RNA was assessed in the 27 lopinavir/r- and 53 nelfinavir-treated subjects with data available subsequent to the determination of genotype. A total of 25 (93%) of 27 lopinavir/r-treated subjects experienced virus load resuppression, including 9 (82%) of 11 subjects whose isolates demonstrated 3TC resistance. Of note, none of the above 27 subjects altered their originally randomized antiretroviral regimen during the time of assessment. In contrast, resuppression was observed in only 20 (38%) of 53 nelfinavir-treated subjects, including 0 of 20 subjects with nelfinavir resistance, 9 (45%) of 20 subjects with 3TC resistance but no nelfinavir resistance, and 11 (85%) of 13 with neither nelfinavir or 3TC resistance. Of the 18 subjects in either arm who demonstrated virus load resuppression after the detection of 3TC resistance alone, 8 subjects in each treatment group had virus load data available after the date resuppression was first observed. Two of 8 lopinavir/r-treated subjects and 6 of 8 nelfinavir-treated subjects had a subsequent repeat rebound of virus load, whereupon samples from 4 of 6 nelfinavir-treated subjects but neither of the lopinavir/r-treated subjects demonstrated new primary PI mutations.

Analysis of the relationship of drug resistance to virus load exposure and medication adherence. To probe whether differences in exposure to viral replication or medication adherence between the 2 study arms could account for the above differences in resistance development, the relationships between virus load, adherence and resistance were examined. All lopinavir/r-treated subjects and all but 1 nelfinavir-treated subject with genotype experienced a ≥ 1.9 log_{10} copies/mL decline in HIV RNA from baseline and/or HIV RNA <400 copies/mL. Furthermore, no statistically significant differences between the treatment groups were observed in a variety of measures (see Materials and Methods) of exposure to viral replication (data not shown). Likewise, no difference between treatment groups in mean overall adherence was observed, either in all treated subjects (93% for lopinavir/r vs. 94% for nelfinavir; P = .600) or in only those subjects with genotype data (89% for lopinavir/r vs. 90% for nelfinavir; P = .55). However, a significant association between adherence and virological response was noted. Thus, subjects who remained in the study for at least 24 weeks and who never demonstrated HIV RNA >400 copies/mL after that point had higher mean adherence than did patients with at least one HIV RNA value >400 copies/mL at or after week 24 (95% vs. 91%; P < .001). Finally, there were no apparent differences in adherence to any of the individual study medications among subjects with available genotype (correlation coefficients, lopinavir:3TC, 0.94; lopinavir:d4T, 0.93; nelfinavir:3TC, 0.91; and nelfinavir:d4T, 0.90) that accounted for differences in resistance development.

DISCUSSION

In the course of this analysis, we quantitated the emergence of resistance over the course of 108 weeks in a randomized, double-blind, phase 3 study that compared lopinavir/r plus d4T/3TC with nelfinavir plus d4T/3TC in ART-naive subjects. Genotypic resistance to nelfinavir [7, 26] was observed in 43 (45%) of 96 subjects with HIV RNA loads >400 copies/mL after 24 weeks of therapy and for whom plasma HIV RNA could be sufficiently amplified for population sequencing. In contrast, no active site or primary mutations were observed in any of the samples from the 51 lopinavir/r-treated subjects with genotype available, despite the presence of replicating virus as judged by plasma HIV RNA >400 copies/mL. The absence of resistance in the lopinavir/r-treated subjects was confirmed by phenotypic analysis of the 51 rebound samples, none of which demonstrated a detectable change in susceptibility, compared with the wild-type standard. New secondary PI mutations also emerged more frequently in nelfinavir-treated subjects than in...
lopinavir/r-treated subjects. The 3 secondary mutations observed in samples from 7 subjects during lopinavir/r therapy (L10F, M36I/L, and A71T) all commonly occur as polymorphisms and were observed at a relatively high prevalence prior to therapy. Thus, although the appearance of some of these secondary mutations is likely to be a consequence of selective pressure by lopinavir, their appearance may also reflect the natural variation of HIV caused by ongoing replication. An accompanying M184V mutation in RT was observed in only 4 of 7 of the above samples, which suggests that selective drug pressure in these subjects was low. In contrast, the new secondary mutations observed in nelfinavir-treated subjects (either with or without D30N or L90M) were nearly always accompanied by 3TC resistance and included amino-acid changes not only at highly polymorphic positions but also at residues that are generally not polymorphic, such as positions 33, 46, 54, and 88. The appearance of these mutations suggests substantial selection by nelfinavir beyond the appearance of the canonical primary mutations for this drug, and the loss of phenotypic susceptibility to nelfinavir by 3 isolates that contained M46I/L without D30N or L90M indicates an alternate, previously unrecognized pathway toward resistance to this drug.

Although subjects in both arms of the study received the same nucleoside combination (d4T/3TC), the incidence of resistance to both nucleoside analogues was significantly higher in nelfinavir-treated subjects than in lopinavir/r-treated subjects. Indeed, the highest estimated rate of resistance in lopinavir/r-treated subjects (3TC resistance, 7% overall in the Kaplan-Meier estimate) was similar to the lowest rate (d4T, 5% overall) in nelfinavir-treated subjects. Notably, nearly all of these nelfinavir-treated subjects demonstrated resistance to all 3 drugs in their regimen. The comparisons available in this controlled, double-blind study clearly demonstrate that a single drug within a combination antiretroviral regimen can profoundly affect the likelihood of resistance development to all of the drugs in the regimen.

The substantial difference in the rate of resistance emergence between lopinavir/r- and nelfinavir-treated subjects does not appear to result from differences in overall viral replication or from differences in adherence to the treatment regimen. Plasma HIV RNA >400 copies/mL was associated with lower adherence. However, most subjects with genotype data experienced either a ≥1.9 log10 copies/mL decline in HIV RNA from baseline and/or HIV RNA <400 copies/mL, which suggests that nonadherence to the regimen was periodic. This assumption is further supported by the high rate of virus load resuppression in the lopinavir/r arm, even in the context of 3TC resistance, which suggests that, with improved adherence, the combination of lopinavir/r and d4T was sufficient to keep viral replication in check. In contrast, the overall rate of resuppression among nelfinavir-treated subjects was modest and was largely accounted for by 11 of 13 subjects whose isolates displayed resistance to neither nelfinavir nor 3TC. Resuppression did not occur after the emergence of resistance to nelfinavir, and 17 of 20 nelfinavir-treated subjects whose isolates displayed 3TC resistance but not nelfinavir resistance either did not experience resuppression at all or had a “repeat rebound” after a brief period with HIV RNA loads <400 copies/mL. Taken together, these results suggest that the consequences of periodic low adherence rates for the development of resistance are very different for lopinavir/r- and nelfinavir-based regimens and that the development of 3TC resistance substantially compromises the success of nelfinavir-based therapy but may only minimally affect the activity of lopinavir/r. Finally, the virological failure of nelfinavir-based therapy is much more likely to require salvage therapy than lopinavir/r-based therapy in ART-naive patients.

The marked difference in the rates of emergence of resistance to lopinavir/r, compared with nelfinavir, suggests large differences in the genetic barrier to resistance (the number of mutations required to overcome drug activity) between the 2 regimens. In vitro studies, using mutant viruses selected by other PIs as well as mutant viruses selected by passaging lopinavir in vitro, have indicated that single mutations in protease confer, at the most, 2–3-fold reduced susceptibility to lopinavir [28, 29]. Moreover, no previously unknown mutations conferring reduced susceptibility to lopinavir were identified in the present study. Consequently, the concentration “zone of high selective pressure” between the IC50 of wild-type and mutant viral strains (i.e., where the replication of wild-type virus is >50% inhibited but replication of mutant virus is <50% inhibited) is expected to be relatively small for lopinavir/r. Lopinavir/r dosed as 400/100 mg twice daily produces a mean trough concentration (Ctrough) (predose concentration) of lopinavir (5.5 μg/mL) that is 75-fold above the mean IC50 (0.07 μg/mL) assessed in the presence of human serum (inhibitory quotient >75) [28, 30]. Virus strains with a sufficient number of mutations to overcome lopinavir drug concentrations are not likely to preexist in ART-naive patients who have not received drug-resistant HIV through transmission. On the basis of its pharmacokinetic profile, lopinavir/r is therefore expected to exert a high genetic barrier to resistance in this patient population. In contrast, the mean Ctrough of nelfinavir [31] is only 2–4-fold above its IC50 in the presence of human serum (0.52 μg/mL) [28], and single mutants that contain D30N or L90M display 5–6-fold reduced susceptibility to nelfinavir [7, 26]. Mutants that compromise the activity of nelfinavir are therefore likely to preexist. The low genetic barrier for nelfinavir implies a greater need for the activity of accompanying antiretroviral agents, consistent with the low rate of viral resuppression in nelfinavir-treated subjects who have 3TC resistance.

The results of the present study suggest that in vivo selective pressure by lopinavir/r is minimal, even in the context of ongoing
viral replication (as evidenced by HIV RNA >400 copies/mL) that might be expected to ultimately produce resistant strains of virus. Because selection requires the simultaneous presence of both drug and viral replication, the cumulative in vivo selective pressure of a drug is likely to be a function of (1) the probability that drug concentrations enter the zone of high selective pressure and (2) the time that drug concentrations remain in the zone before either declining further or increasing after a new dose. At concentrations significantly above the selective pressure zone, replication rates of both wild-type and mutant viruses will be low because of drug activity. As concentrations decline below the zone, the replication advantage of the mutants diminishes and ultimately disappears, particularly if the mutants have reduced replicative capacity in the absence of drug [32, 33]. During periods of good adherence, the decline of lopinavir plasma concentrations to levels that allow significant replication of wild-type virus is likely to be rare. Modeling the in vivo selective pressure of lopinavir/r thus requires an examination of its pharmacokinetics after missed doses. Although the terminal pharmacokinetics of lopinavir/r at steady state have not been described, the results of single-dose studies [34] have suggested that the clearance of lopinavir begins to increase substantially ≥20 h after dosing as its plasma concentration approaches the zone of high selective pressure. This accelerated terminal decay is caused by the simultaneous decline of ritonavir levels below the in vivo $K_c$ for inhibition of the metabolism of lopinavir. Under conditions of rapid terminal clearance, lopinavir concentrations are expected to be highly selective only briefly before declining further to concentrations in which the replication of wild-type virus outcompetes that of mutant strains. Over extended times of periodic and/or low adherence, during which virus load might become detectable, the cumulative time over which lopinavir concentrations reside within the zone of high selective pressure may be relatively short. Under such conditions, the population of preexisting mutants within the quasi species is not likely to expand sufficiently to allow the accumulation of the multiple mutations required for resistance to lopinavir/r [8, 20]. This model of a pharmacological barrier to resistance may also account for the significant differences in 3TC resistance seen between treatment arms in the present study. Adherence to 3TC was highly correlated with PI adherence. If the onset of viral replication after a missed dose of lopinavir/r is delayed compared with nelfinavir, intracellular 3TC triphosphate concentrations would be expected to decline to lower levels before replication begins, potentially exerting lower selective pressure for 3TC resistance.

Some limitations of the study should be noted. The genotype was obtained at the point of initial virus load rebound in only a subset of subjects; consequently, the temporal emergence of resistance quantified in the Kaplan-Meier analysis is conservative (i.e., resistance undoubtedly occurred earlier in some subjects than indicated). Indeed, the large incremental increases in resistance observed at weeks 24 and 48 in figure 2 are likely a result of the timing of sample selection, because those samples were submitted for genotype first (see Materials and Methods). Assessing medication adherence through pill count also presents a limitation. The adherence values used in the present analysis are conservatively high. Actual adherence is likely to be lower in most individuals but is likely to be highly correlated with the pill count–based measurements of adherence used in the present study [35, 36].

The assessment of relative probabilities of resistance development for individual drugs within the context of combination therapy may have implications for HIV treatment strategies. Under many current paradigms, initial regimen choices are made on the basis of preserving future therapeutic options once the inevitable resistance emerges. With increasing quantitative data on the incidence of resistance development, paradigms that recognize substantial differences in the probability of resistance among ARTs of the same or different drug class may become feasible. Such probabilistic paradigms consider drug resistance in the same manner as other adverse effects of therapy and may allow for the quantitative assessment of the risk/benefit of various antiretroviral therapeutic regimens.

In summary, a marked difference in the emergence of PI resistance has been noted between subjects receiving lopinavir/r plus d4T/3TC, compared with those receiving nelfinavir plus d4T/3TC, in a double-blind, randomized clinical trial. Significant differences in the rates of emergence of 3TC and d4T resistance were also observed. The present study provides, to our knowledge, the first quantitative estimate of differences in PI resistance in such a large controlled setting. Because no significant differences between treatment regimens were observed in adherence or overall viral replication among subjects with genotype data, it is likely that pharmacological factors, including differences in the genetic barrier and differences in the probabilities that drug levels reside in the concentration zone of high selective pressure, account for the observed differences in resistance rates. These differences are likely to also be manifested, and potentially exacerbated, under less controlled circumstances (outside of a clinical study), where adherence may be lower. The low rate of resistance development observed with lopinavir/r may provide the opportunity for the use of patient counseling or other adherence optimization tools to maximize the durability of initial antiretroviral therapy.

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