Mechanisms of Virologic Failure in Previously Untreated HIV-Infected Patients From a Trial of Induction-Maintenance Therapy

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Context In the Trile`ge trial, following induction with a zidovudine, lamivudine, and indinavir regimen, human immunodeficiency virus (HIV) replication was less suppressed by 2-drug maintenance therapy than by triple-drug therapy.

Objective To identify mechanisms of virologic failure in the 3 arms of the Trile`ge trial.

Design Case-control study conducted from February to October 1998.

Setting Three urban hospitals in Paris, France.

Patients Fifty-eight case patients with virologic failure (HIV RNA rebound to >500 copies/mL in 2 consecutive samples) randomized to 3 therapy groups: triple drug (zidovudine, lamivudine, and indinavir), 8; zidovudine-lamivudine, 29; and zidovudine-indinavir, 21; the case patients were randomly matched with 58 control patients with sustained viral suppression.

Main Outcome Measures At virologic failure (S1 sample) and 6 weeks later (S2 sample), assessment of protease and reverse transcriptase gene mutations, plasma indinavir level, and degree of viral load rebound; pill count during induction and maintenance periods.

Results Only 1 primary resistance mutation, M184V, was detected in S1 plasma samples from 4 of 6 patients in the triple-drug and in all 22 in the zidovudine-lamivudine therapy groups and in S2 plasma samples from 3 of 6 in the triple-drug and 20 of 21 in the zidovudine-lamivudine groups. Of controls, M184V was detected in 11 of 13 S1 plasma samples and in 10 of 11 S2 plasma samples. Indinavir levels were undetectable in all S1 samples but 2 in 7 triple-drug cases tested and in the expected range in 11 of 18 S1 and 5 of 12 S2 zidovudine-indinavir case plasma samples tested. Maintenance adherence rates were lower for cases vs controls for zidovudine (P = .05) and indinavir (P = .05). Low indinavir levels, lower adherence rates for zidovudine (P = .04) and lamivudine (P = .03), and rebound to near-baseline values suggested adherence as cause of early failure for 4 of 8 triple-drug cases. In the zidovudine-lamivudine arm, for which case and control adherence rates did not differ significantly (P = .96), most failures occurred late with low rebound, suggesting suboptimal drug potency. In the zidovudine-indinavir arm, virologic failures may be related to both mechanisms.

Conclusions During the maintenance phase early and late virologic failures appeared to be related more to problems of adherence and antiretroviral treatment potency, respectively, than to selection of resistant mutant viruses.

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See also pp 229 and 250.
patients reached the primary end point (viral rebound >500 copies/mL in 2 consecutive samples). The proportion of patients reaching the primary end point was significantly higher in the zidovudine-lamivudine (29/93, P<.001) and zidovudine-indinavir groups (21/94, P = 01) than in the continued triple-drug maintenance arm (8/92). In multivariate analysis, baseline HIV RNA level was the only factor predictive of virologic failure.

Antiretroviral therapy failure has been attributed to low antiviral potency, selection of drug-resistant variants, poor treatment adherence, and pharmacokinetic interactions. The main aim of this study was to identify mechanisms of virologic failure during the maintenance phase of the trial by screening for genotypic resistance, estimating treatment adherence, and assessing degree of viral rebound.

METHODS

Study Design

When the Trile`ge trial was stopped in December 1997, the 58 patients with virologic failure (2 consecutive HIV RNA levels >500 copies/mL) were individually and randomly matched in a case-control study with 58 patients with sustained reduction in viral load of below 500 copies/mL. Cases and controls were matched for the randomization arm, maintenance therapy duration, baseline CD4+ cell count (±0.10 × 10^9/L), baseline plasma HIV RNA level (±0.8 log copies/mL), and plasma HIV RNA level at the end of the induction phase (month 3; ≤ or >50 copies/mL).

The current study took place in Paris, France, involving 3 urban hospitals (Bichat-Claude Bernard, Pitié-Salpêtrière, and Paul Brousse Hospitals), and was conducted from February to October 1998.

During the maintenance phase, clinical and biologic assessments were performed every 6 weeks from randomization. Virologic and pharmacological studies of cases and controls were done in plasma sample 1 (S1), corresponding to initial virologic failure, and plasma sample 2 (S2), taken 6 weeks after S1 for confirmation of viral rebound. In controls, S1 and S2 samples were collected at times corresponding to those of samples for matched cases. Trial approval was given by institutional review boards of participating centers and patients provided written informed consent.

Genotypic Resistance Studies

Genotypic studies were done on masked plasma samples from cases and controls. The reverse transcriptase gene was analyzed using the line-probe reverse-transcriptase assay, or by sequencing when the line-probe assay failed to give a signal after hybridization. The protease gene was analyzed by sequencing. Reverse transcriptase and protease genes were analyzed at baseline and at the time of S1 and S2 in cases.

The reverse transcriptase gene M184V mutation confers high-level resistance to lamivudine and appears rapidly when viral suppression is not maintained in patients receiving lamivudine-containing regimens. The M184V mutation was screened for by a restriction fragment digestion assay in samples obtained at the end of the induction from 50 of the 58 cases, and in S1 and S2 samples from zidovudine-lamivudine controls. This method amplified a short reverse transcriptase gene fragment and has higher sensitivity than sequencing when applied to plasma containing fewer than 500 copies/mL.

Adherence Assessment

Trile`ge trial protocol included treatment adherence assessment via pill count and plasma drug measurement. The number of tablets dispensed at each visit (weeks 2, 4, 8, and 12, then every 6 weeks) was recorded as was the number returned at the following visit; average between-visit adherence rate was calculated for each patient and each drug. During induction, patients received 100% of the pills corresponding to the interval before the next scheduled visit. During maintenance, they received 133% of prescribed tablets at the first of 2 visits, and 67% at the second visit. For example, if a subject was prescribed 2 pills of indinavir 3 times a day for 6 weeks (252 pills), 336 pills were dispensed at the first visit. If 100 pills were returned, the adherence rate was [(336-100)/252] × 100 = 93.7%. Thus, ranges may exceed 100%.

Indinavir concentrations were measured in masked plasma S1 and S2 samples from cases and controls in both indinavir-containing regimens, using high-performance liquid chromatography with a quantification limit of 5 ng/mL of plasma. Interval between last drug ingestion and sampling was recorded. Measured indinavir concentrations were interpreted relative to expected concentrations derived from mean 24-hour pharmacokinetic profiles in 24 healthy volunteers (area under the curve, 330.24 ng/mL per hour; C_{\text{max}} = 1.463 ng/mL; half-life = 1.85 hours). Because the coefficient of variation at various sampling times was close to 40% in the volunteers, a ratio of 0.6 between the study sample concentrations and expected indinavir concentrations was taken as the lowest value compatible with expected level.

Analysis of Viral Load Rebound

Plasma HIV RNA levels were determined using the Amplicor HIV assay (Roche Laboratories, Alameda, Calif) (detection limit, 200 copies/mL). Median viral load in S1 and S2 samples from patients with virologic failure was compared with baseline value to assess degree of rebound.

Statistical Analysis

We used an intent-to-treat approach. All the case-control study observations were included in the analysis, even when the study treatments were discontinued prematurely. Wilcoxon rank-sum test was used to compare groups regarding continuous variables (ie, pill count, plasma indinavir level, and degree of viral load rebound).

Plasma indinavir ratios in cases and controls were compared separately at S1 and S2. Pill count data were censored at the time of the S2 sample. Adherence rates were calculated for the induction (enrollment to month 3), maintenance (month 3 to sample S2), and entire study
periods. Case and control adherence rates were compared by prescribed drug, study period, treatment group, and time to virologic failure. In cases, early and late failures during the maintenance phase were respectively defined as those occurring at month 4.5 (patients were evaluated at 6-week intervals) and at month 6 or later. Degree of viral rebound in the two 2-drug arms was analyzed by pairwise comparisons against the triple-drug arm. Correlation analysis was used to determine strength of association between plasma indinavir ratio and corresponding degree of viral rebound, individually in S1 and S2 samples. P values were adjusted for multiple comparisons using the Hochberg method. All reported P values are 2-sided. Analyses were performed using SAS software (Version 6.12; SAS Institute Inc, Cary, NC).

We included all cases available in December 1997. The choice of 1 control per case was dictated more for feasibility and study duration reasons than for power of comparisons. However, this case-control study is based on a relatively large number of patients, enabling us to reliably check our assumptions.

RESULTS

Genotypic Resistance

No mutations associated with reverse transcriptase inhibitor resistance were found at baseline. At the end of induction, the M184V mutation was not detected in any of the 22 successfully amplified case samples (of 50 samples available). In S1 and S2 case samples, the only primary resistance mutation detected in the reverse transcriptase gene was the M184V substitution, present in most patients receiving a lamivudine-containing maintenance regimen (Table 1). The key codon 215 zidovudine resistance mutation was not detected. Two patients developed minor codon 41 or 70 zidovudine resistance mutations.

In comparison with baseline data, the protease genotype in S1 and S2 samples showed only secondary indinavir-associated mutations, and in only 7 patients, 4 of whom were in the zidovudine-lamivudine maintenance arm. A set of 4 secondary mutations (codons 20, 36, 71, and 77) was found in sample S2 from 1 case in the triple-drug maintenance arm; these can be selected before the primary mutations at codon 46 or 82, conferring resistance to indinavir.

There were 22 S1 and 21 S2 samples available from zidovudine-lamivudine controls for M184V testing; of the successfully amplified specimens, the M184V mutation was found in 11 of 13 S1 and in 10 of 11 S2 samples.

Adherence

Plasma indinavir levels in S1 and S2 samples from cases and controls with an indinavir-containing regimen are shown in Figure 1. Controls had indinavir levels compatible with expected values, except for 1 patient in the zidovudine-indinavir arm. Indinavir concentrations in S1 and S2 samples were paired for controls and 3 cases. In the zidovudine-indinavir group, no statistical difference between cases and controls was seen at S1 (P = .35) but was observed at S2 (P = .04). The S1 sample corresponds to initial virologic failure. The S2 sample was obtained 6 weeks after S1 for confirmation of viral rebound. The gray line indicates an indinavir ratio of 0.6, which corresponds to the lowest value of expected indinavir level, taking into account intersubject variability (see “Adherence Assessment” section). Samples for S1 and S2 were not paired for some cases and controls.

Table 1. Mutations in the Reverse Transcriptase (RT) and Protease Genes in Patients With Virologic Failure

<table>
<thead>
<tr>
<th>Treatment Group*</th>
<th>Gene</th>
<th>Sample 1†</th>
<th></th>
<th>Sample 2‡</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of Amplified</td>
<td>No. of Samples</td>
<td>No. of Amplified</td>
<td>No. of Samples</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Samples</td>
<td>With Mutations</td>
<td>Samples</td>
<td>With Mutations</td>
</tr>
<tr>
<td>Triple drug</td>
<td>RT</td>
<td>6</td>
<td>M184V: 4</td>
<td>6</td>
<td>M184V: 3</td>
</tr>
<tr>
<td></td>
<td>Protease</td>
<td>6</td>
<td>No mutation</td>
<td>7</td>
<td>K20R, M36I, A71V, V77I: 1</td>
</tr>
<tr>
<td>Zidovudine-lamivudine</td>
<td>RT</td>
<td>22</td>
<td>M41L: 1</td>
<td>21</td>
<td>M41L: 1</td>
</tr>
<tr>
<td></td>
<td>Protease</td>
<td>28</td>
<td>M36I: 2</td>
<td>25</td>
<td>K20K/M: 1</td>
</tr>
<tr>
<td>Zidovudine-indinavir</td>
<td>RT</td>
<td>16</td>
<td>No mutation</td>
<td>14</td>
<td>K70R: 1</td>
</tr>
<tr>
<td></td>
<td>Protease</td>
<td>18</td>
<td>No mutation</td>
<td>14</td>
<td>L63L/A: 1</td>
</tr>
</tbody>
</table>

*Plasma samples were available from 7 of 8 patients taking the triple-drug regimen (zidovudine, lamivudine, and indinavir), 28 of 29 taking zidovudine-lamivudine, and 19 of 21 taking zidovudine-indinavir.
†First sample corresponding to initial virologic failure.
‡Second sample, 6 weeks after first sample, for confirmation of the viral rebound.
Triple-drug regimen includes zidovudine, lamivudine, and indinavir.

Table 2 compares adherence rates based on pill counts in cases and controls for each drug throughout the study period. Globally, adherence to zidovudine (P = .02) and indinavir (P = .02) was significantly lower in cases than controls, with no statistical differences for lamivudine (P = .16). During induction, there was no significant difference in adherence rates between cases and controls. During the maintenance period, adherence to zidovudine (P = .05) and indinavir (P = .05) regimens was significantly lower in cases than controls.

Table 3 provides P values for differences in adherence rates between cases and controls in each maintenance arm. In the triple-drug maintenance arm, there was no difference during both induction and maintenance phase between patients with and without subsequent virologic failure. However, sample sizes in this group were small. In the zidovudine-lamivudine group, there was no significant difference in adherence rate for either drug during induction or maintenance periods. In the zidovudine-indinavir arm, cases had a lower adherence rate than controls for both drugs during the total period, and for the induction phase, it was significantly different (P = .04). Regarding whether possible poor indinavir adherence in the triple-drug arm (Figure 1) was associated with poor zidovudine-lamivudine adherence, a comparison between triple-drug and zidovudine-lamivudine cases showed the former had lower adherence to zidovudine (P = .04) and lamivudine (P = .03).

The significantly lower adherence rate for indinavir (P = .04) during induction in cases randomized to zidovudine-indinavir maintenance was associated with a large number of virologic failures at month 4.5 (early failure). Failures occurred early in 22 of the 58 cases (4 receiving triple-drug maintenance; 6, zidovudine-lamivudine; and 12, zidovudine-indinavir) and later (after month 6) in 36 patients (4 receiving triple-drug maintenance; 23, zidovudine-lamivudine; and 9, zidovudine-indinavir). Adherence rates in cases and controls are shown in Table 4 according to time of virologic failure. Globally, there was a significant difference

**Table 2.** Comparison of Adherence Rates of Cases vs Controls by Drugs and Trial Phase*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cases (n = 58)</th>
<th>Controls (n = 58)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zidovudine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction</td>
<td>99 (89-103)</td>
<td>99 (95-103)</td>
<td>.26</td>
</tr>
<tr>
<td>Maintenance</td>
<td>96 (83-102)</td>
<td>100 (96-107)</td>
<td>.05</td>
</tr>
<tr>
<td>Total</td>
<td>96 (87-100)</td>
<td>100 (97-103)</td>
<td>.02</td>
</tr>
<tr>
<td>Lamivudine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction</td>
<td>99 (90-102)</td>
<td>99 (95-103)</td>
<td>.26</td>
</tr>
<tr>
<td>Maintenance</td>
<td>96 (85-104)</td>
<td>100 (97-108)</td>
<td>.16</td>
</tr>
<tr>
<td>Total</td>
<td>97 (92-100)</td>
<td>100 (96-105)</td>
<td>.16</td>
</tr>
<tr>
<td>Indinavir</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction</td>
<td>97 (82-101)</td>
<td>99 (93-103)</td>
<td>.24</td>
</tr>
<tr>
<td>Maintenance</td>
<td>83 (68-103)</td>
<td>99 (95-109)</td>
<td>.05</td>
</tr>
<tr>
<td>Total</td>
<td>86 (75-99)</td>
<td>99 (95-103)</td>
<td>.02</td>
</tr>
</tbody>
</table>

*Values are median adherence rate (interquartile range) and are expressed as percentages. The adherence assessment method based on pill count used herein allows ranges to exceed 100%.

**Table 3.** Comparison of Adherence Rates of Cases vs Controls by Drugs, Trial Phase, and Maintenance Regimen*

<table>
<thead>
<tr>
<th></th>
<th>Triple-Drug†</th>
<th>Zidovudine- Lamivudine</th>
<th>Zidovudine-Indinavir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases Controls</td>
<td>P Value</td>
<td>Cases Controls</td>
</tr>
<tr>
<td>Zidovudine Induction</td>
<td>92 (79-101) 97 (89-100)</td>
<td>.83</td>
<td>100 (98-106) 99 (96-103)</td>
</tr>
<tr>
<td>Maintenance</td>
<td>81 (67-98) 99 (94-114)</td>
<td>.14</td>
<td>98 (91-103) 101 (95-104)</td>
</tr>
<tr>
<td>Total</td>
<td>88 (74-95) 99 (95-104)</td>
<td>.14</td>
<td>99 (96-104) 100 (96-105)</td>
</tr>
<tr>
<td>Lamivudine Induction</td>
<td>93 (79-101) 97 (89-101)</td>
<td>.83</td>
<td>99 (95-105) 99 (97-103)</td>
</tr>
<tr>
<td>Maintenance</td>
<td>81 (67-98) 99 (94-114)</td>
<td>.14</td>
<td>98 (91-105) 101 (97-106)</td>
</tr>
<tr>
<td>Total</td>
<td>88 (74-95) 99 (95-104)</td>
<td>.14</td>
<td>99 (96-102) 100 (96-105)</td>
</tr>
<tr>
<td>Indinavir Induction</td>
<td>98 (80-104) 97 (84-100)</td>
<td>.83</td>
<td>98 (82-103) 99 (94-102)</td>
</tr>
<tr>
<td>Maintenance</td>
<td>74 (46-97) 94 (90-107)</td>
<td>.50</td>
<td>...</td>
</tr>
<tr>
<td>Total</td>
<td>83 (53-97) 97 (91-99)</td>
<td>.83</td>
<td>...</td>
</tr>
</tbody>
</table>

*Values are presented as median adherence rate (interquartile range) and are expressed as percentages. The adherence assessment method based on pill count used herein allows ranges to exceed 100%.

†Triple-drug regimen includes zidovudine, lamivudine, and indinavir.
in adherence rates of zidovudine, lamivudine, and indinavir during induction between cases and controls with early failure. We emphasize that for these cases, the difference in adherence rates during maintenance could not be reliably estimated because of the short follow-up. In late virologic failure, zidovudine, lamivudine, and indinavir adherence rates did not differ between cases and controls during induction or maintenance.

Viral Load Rebound
Baseline median viral load was 53,000 copies/mL in the 58 cases, with no statistical difference among the maintenance arms. At the time of virologic failure, the median viral load for each arm was 21,000 copies/mL for triple-drug; 1100 copies/mL for zidovudine-lamivudine; and 1800 copies/mL for zidovudine-indinavir. Median time to virologic failure after maintenance therapy initiation was 3 months. Viral rebound at S1 and S2 in the maintenance arms is shown in Figure 2. Patients randomized to the zidovudine-indinavir arm were divided in 2 subgroups according to plasma indinavir levels: patients having at least 1 indinavir ratio below 0.6 were classified as subgroup indinavir-0 with the others classified as subgroup indinavir-1.

In the triple-drug and zidovudine-lamivudine maintenance arms, median viral load at S1 was 0.35 and 1.56 log lower than at baseline, respectively. In the zidovudine-indinavir arm, it was 0.73 and 1.66 log lower, respectively, than at baseline in the cases with indinavir levels below and in keeping with expected values. In S2 samples, the corresponding values were −0.39, −1.38, −0.31, and −0.31 log.

Correlation of degree of viral rebound with indinavir levels at S1 was $r = 0.44$ ($P = .03$) and at S2, $r = 0.40$ ($P = .09$) in patients randomized to triple-drug and zidovudine-indinavir maintenance arms.

**COMMENT**
This study shows that virologic failure during the Trilége trial maintenance phase was not associated with key zidovudine or indinavir resistance mutations. No such mutations were found at viral rebound or baseline, consistent with the patients’ antiretroviral-naive status. In the 2 lamivudine-containing maintenance arms, the M184V mutation in the reverse transcriptase gene was seen in most successfully amplified samples from cases at viral rebound and from matched zidovudine-lamivudine controls (about 50% of which could be evaluated). Similar results have been seen in patients receiving lamivudine as part of a 2-nucleoside analog regimen. However, as the M184V mutation is associated with persistent low-level viral replication, its significance may be minimal in patients with low viral load (<300 copies/mL). Our data suggest that this mutation does not play a ma-

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**Table 4. Comparison of Adherence Rates of Cases vs Controls by Drugs, Trial Phase, and Time of Virologic Failure**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Early Failure†</th>
<th>Late Failure‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>Zidovudine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction</td>
<td>90 (77-108)</td>
<td>100 (95-105)</td>
</tr>
<tr>
<td>Maintenance</td>
<td>88 (71-101)</td>
<td>99 (97-109)</td>
</tr>
<tr>
<td>Total</td>
<td>88 (74-98)</td>
<td>101 (98-103)</td>
</tr>
<tr>
<td>Lamivudine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction</td>
<td>91 (77-99)</td>
<td>100 (95-105)</td>
</tr>
<tr>
<td>Maintenance</td>
<td>93 (80-104)</td>
<td>99 (92-106)</td>
</tr>
<tr>
<td>Total</td>
<td>96 (69-98)</td>
<td>101 (98-107)</td>
</tr>
<tr>
<td>Indinavir</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction</td>
<td>86 (65-99)</td>
<td>100 (93-105)</td>
</tr>
<tr>
<td>Maintenance</td>
<td>74 (62-102)</td>
<td>99 (95-115)</td>
</tr>
<tr>
<td>Total</td>
<td>78 (62-97)</td>
<td>99 (98-103)</td>
</tr>
</tbody>
</table>

*Values are median adherence rate (interquartile range) and are expressed as percentages. The adherence assessment method based on pill count used herein allows ranges to exceed 100%.
†Virologic failure occurring at month 4.5. For these cases, assessment of differences in adherence rates during maintenance could not be reliably estimated because of the short follow-up.
‡Virologic failure occurring at month 6 or later.

**Figure 2. Viral Load Rebound of First Plasma Sample (S1) and Second Plasma Sample (S2)**

Viral load rebound defined as plasma human immunodeficiency virus (HIV) RNA level in the S1 or S2 specimen after subtraction of the baseline level by treatment group (the zidovudine-indinavir group is split according to the plasma indinavir ratio) and by S1 or S2. Indinavir-0 patients have at least 1 indinavir ratio below 0.6; indinavir-1 patients have individual level within normal range. The line at 0 indicates that viral rebound is exactly equal to the baseline value. Horizontal bar indicates median; box, interquartile range; and error bars, 95% confidence interval.
MECHANISM OF VIROLOGIC FAILURE IN HIV-INFECTED PATIENTS

Major role, at least short-term, in virologic failure in patients with a zidovudine-lamivudine regimen. Virologic failure in the Trile`ge trial was not associated with resistant-mutant selection. Absence of resistance mutations, except for M184V, in this study may partly be explained by the fact that we assessed samples obtained shortly after virologic failure. These results prompted the hypothesis that lamivudine-resistant variants may arise more rapidly than indinavir-resistant variants since the M184V mutation confers greater selective advantage (in the presence of the drug) than any single protease mutation.

We used 2 complementary methods to assess adherence. We investigated adherence rates in cases and controls by drug, treatment arm, and phases (induction and maintenance) of the trial despite the small number of virologic failures (especially in the triple-drug arm). When the drug is present, plasma indinavir measurements provide unequivocal evidence of ingestion but only of the last dose. A potential bias is that patients might comply better than usual just prior to visits, while a good adherer missing a dose before the visit may be misclassified. Assays at 2 different visits, as in this study, may attenuate these shortcomings. A low plasma-indinavir concentration despite claimed ingestion within the prior 8 hours might indicate difficulties with adherence and poor digestive absorption, or both. Use of enzyme-inducing drugs, causing pharmacokinetic interactions, was prohibited by the protocol. Malabsorption was unlikely, because HIV RNA levels were reduced to below 500 copies/mL after the induction period. Pill counts are potentially a more objective way of estimating mid-term to long-term adherence and are relatively inexpensive. Pill counts allow estimation of medication quantity probably taken but not dosing frequency, which is important for drugs with a fixed dosing interval such as indinavir. Most important, the number of returned tablets routinely underestimates the extent of poor or partial adherence. Despite these shortcomings, comparison of cases and controls strongly suggested that poor adherence was associated with virologic failure in patients in the triple-drug maintenance arm and in some assigned to zidovudine-indinavir. Among the 8 cases in the triple-drug maintenance arm, 2 abruptly discontinued the study treatment just before virologic failure (due to pregnancy and a personal decision). Cases assigned to the triple-drug arm, who all had low or undetectable plasma indinavir levels both at time of virologic failure and 6 weeks later, were first thought to have discontinued indinavir only. But adherence rate comparison between triple-drug and zidovudine-lamivudine cases showed the former also had low adherence to zidovudine (P = .04) and lamivudine (P = .03). In the triple-drug arm estimated adherence and the fact that viral load rebounded to near-baseline values suggest that patients with virologic failure had low adherence throughout the maintenance period. In this treatment subgroup particularly, a possible limitation may be the likely low statistical power due to a relatively small number of events and subjects. In contrast, adherence rates were not different between cases and controls assigned to zidovudine-lamivudine maintenance. In this group, 79% of virologic failures occurred late (>6 months), with low viral rebound levels, as described with these 2 drugs.17 Our findings suggest that these failures are mainly related to lack of drug potency. Cases randomized to zidovudine-indinavir fell into 2 subgroups according to plasma indinavir levels and degree of rebound. Cases with low indinavir levels and strong rebound were comparable to cases assigned to triple-drug maintenance, low adherence being the main cause of virologic failure. In contrast, in cases with plasma indinavir concentrations in the range of expected values and weak rebound, virologic failure was probably related to suboptimal antiviral activity.

Few studies have simultaneously examined the roles of resistance and adherence in anti-HIV therapeutic failure. It has been stated that not all treatment failure was due to viral resistance, in either naive or heavily pretreated patients.18,19 In the Trile`ge trial, in which the only factor predictive of virologic failure was the baseline HIV RNA level, failure seemed to be related more to low adherence and low antiretroviral potency, or both, than to selection of resistant mutants. In ACTG 343, a similar trial, escaping viruses were also wild-type, except at the reverse transcriptase gene codon 184.20 The possibility that resistance assays used in the 2 trials failed to detect minor resistant species (representing <20% of the total viral population) cannot be dismissed. In the Trile`ge trial, 58 (20%) of 279 patients failed within 1 year, but this rate cannot be compared with rates obtained in other trials, because subjects with high adherence rates were selected during the 3-month induction period.

These results have a major practical implication for antiretroviral drug management: treatment adherence must be thoroughly investigated in virologic failure before switching therapy. When an initial antiretroviral regimen fails, compounds posing adherence problems must be identified and replaced by simpler alternatives. These results raise the hypothesis that compounds that do not pose adherence problems and for which no resistance mutations are detected might be pursued to maintain the largest possible therapeutic potential. However, if minority resistant virus populations are present, continuing with some part of the regimen may result in viral escape; thus, this strategy cannot be recommended today without supporting data from controlled trials.

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Endoscopic Visualization of Breast Tumors

To the Editor: A number of ductal cytologic sampling techniques have been proposed as possible screening techniques for breast cancer.1 We attempted to assess the feasibility of direct intraoperative breast endoscopy in women undergoing partial mastectomy for diagnostic and therapeutic purposes using new endoscopes measuring 1.2 and 0.9 mm in external diameter (DOFI Technologies, Los Angeles, Calif). Until recently, direct visualization techniques have been subject to the technical limitations of endoscopic technology and the excessive cost of the smallest instruments (<5 mm in diameter).2,3

Method. We attempted to examine 55 women with ductal hyperplasia, ductal carcinoma in situ, or invasive breast cancer identified by current breast screening practices. All lesions were confirmed preoperatively by pathologic analysis of a histologic specimen (core biopsy or incisional surgical breast biopsy). At the time of surgical lumpectomy, the target ductal orifice was identified by the presence of discharge after massage in the quadrant containing the target lesion. The orifice was then successfully cannulated and dilated in all but 8 patients. An endoscope was passed into the lactiferous sinus while using local anesthetic and saline distension. The endoscope was advanced into all ductal segments that could be distended. All endoscopic lesions identified were catalogued and, using surgical excision of the lesion at the endoscope tip, were included within the lumpectomy specimen for histologic correlation.

Results. No lesion could be identified endoscopically in 6 of the 47 remaining patients who underwent endoscopy. Of the 41 remaining patients (75% of original sample), the target lesion could be identified by direct cannulation of the suspected ductal orifice on the nipple papilla and navigation of the ducts under saline distention. In 21 patients (38% of original sample), endoscopy revealed more extensive intraluminal disease that required a wider breast resection than previously anticipated. Although limited to examination of the ducts on only the nipple side of the cancer or premalignant lesion, endoscopy reduced need for reexcision by guiding appropriately extensive excisions on the side of the specimen where the ducts were visualized. Following endoscopic guidance, microscopic assessment did not reveal any positive margins within 5 mm in the nippleward boundary of the excised tissue in any of these patients. The Figure shows the spectrum of endoscopic findings in this initial series.

Comment. The ability to identify malignant and premalignant breast diseases endoscopically may allow better direction of surgical therapeutic and diagnostic procedures and also development of new ductal-based screening procedures in women at high risk of breast cancer.

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Figure. Findings in Direct Intraoperative Breast Endoscopy

DCIS indicates ductal carcinoma in situ; ADH, atypical ductal hyperplasia.

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