Lopinavir/Ritonavir as Single-Drug Therapy for Maintenance of HIV-1 Viral Suppression

48-Week Results of a Randomized, Controlled, Open-Label, Proof-of-Concept Pilot Clinical Trial (OK Study)

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Objective: This study evaluated maintenance with lopinavir/ritonavir monotherapy vs. continuing lopinavir/ritonavir and 2 nucleosides in HIV-infected patients with suppressed HIV replication.

Design: Randomized, controlled, open-label, multicenter, pilot clinical trial.

Methods: Adult patients were eligible if they had no history of virologic failure while receiving a protease inhibitor, were receiving 2 nucleosides + lopinavir/ritonavir (400/100 mg b.i.d.) for >1 month and had maintained serum HIV RNA <50 copies/mL for >6 months prior to enrollment.

Results: Forty-two patients were randomly assigned 1:1 to continue or stop the nucleosides. At baseline there were no significant differences between groups in median CD4 cells/μL (baseline or nadir), pre-HAART (highly active antiretroviral therapy) HIV $\log_{10}$ viremia, or time with HIV RNA <50 copies/mL prior to enrollment. After 48 weeks of follow-up, percentage of patients remaining at <50 HIV RNA copies/mL (intention to treat, M = F) was 81% for the monotherapy group (95% CI: 64% to 98%) vs. 95% for the triple-therapy group (95% CI: 86% to 100%); $P = 0.34$. Patients in whom monotherapy failed had significantly worse adherence than patients who remained virally suppressed on monotherapy. Monotherapy failures did not show primary resistance mutations in the protease gene and were successfully reinduced with prerandomization nucleosides. Mean change in CD4 cells/μL: +70 (monotherapy) and +8 (triple) ($P = 0.27$). Mean serum fasting lipids remained stable in both groups. No serious adverse events were observed.

Conclusion: Most of the patients maintained with lopinavir/ritonavir monotherapy remain with undetectable viral load after 48 weeks. Failures of lopinavir/ritonavir monotherapy were not associated with the development of primary resistance mutations in the protease gene and could be successfully reinduced adding back prior nucleosides.

Key Words: lopinavir/ritonavir, monotherapy, induction maintenance

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At present it is recommended[1] that initial treatment of HIV infection should include 3 antiretroviral drugs, usually 2 reverse transcriptase inhibitors and either a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor (NNRTI).

Despite its dramatic success, triple-drug therapy for HIV infection is not without important complications. Antiretroviral regimens including 3 drugs can produce severe toxic effects such as lipodystrophy,[2] dyslipidemia, and lactic acidosis.[3] With some triple-drug combinations, long-term adherence can be difficult due to a high daily pill burden and complex dosing schedules. Finally, the cost of triple-drug therapy makes it out of reach for the majority of HIV-infected patients.

The concept of induction therapy followed by maintenance (with 1 or 2 drugs) therapy for HIV infection is attractive. Theoretically, single-drug antiretroviral therapy would be less toxic, easier to use, and less costly. In addition, patients treated with single-drug therapy would preserve future treatment options, maximizing the probability of success for rescue therapy.

Three clinical trials[4–6] have explored the concept of induction maintenance therapy for HIV infection. Unfortunately these 3 trials had to be prematurely stopped due to an unacceptable risk of virologic failure in patients maintained with single- or dual-drug regimens. One of the possible

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explanations for these failures is that antiretroviral drugs used for maintenance therapy in those trials were suboptimal due to their limited potency (nelfinavir), low genetic (lamivudine) or pharmacologic barriers to resistance (nelfinavir, indinavir), and complex dosing schedules (indinavir, nelfinavir/saquinavir). Other clinical trials[7,8] have explored the concept of induction with 4 drugs and maintenance with standard, triple-drug highly active antiretroviral therapy (HAART). So far these trials have not shown a clear advantage of an induction period with >3 drugs.

Lopinavir/ritonavir (lopinavir/r) is a boosted protease inhibitor that in clinical trials has shown high efficacy rates both in antiretroviral-naive[9] and antiretroviral-experienced patients.[10] When administered at a dose of 400 mg of lopinavir and 100 mg of ritonavir twice daily, the resulting mean trough lopinavir concentrations are at least 75 times as high as the protein binding–corrected concentration needed to inhibit the replication of wild-type HIV by 50%.[11]

In very short-term studies (2 weeks), lopinavir/r alone has shown antiviral potency similar to lopinavir/r and 2 nucleosides.[11] Resistance to lopinavir/r requires the accumulation of multiple mutations in the protease (PR) gene[12] and is extremely rare in antiretroviral-naive patients.[13–15] These characteristics make lopinavir/r an ideal candidate for single-drug maintenance therapy. Results of preliminary experiences using lopinavir/ritonavir[16–18] or indinavir/ritonavir[19] also support the use of boosted protease inhibitors as single-drug therapy for maintenance of HIV viral suppression.

A definitive study of lopinavir/r single-drug maintenance therapy would require a multicenter investigation with many collaborators and it would be very difficult to recruit them without some preliminary findings that suggest the feasibility of lopinavir/r monotherapy. This is why we have performed a small, carefully monitored, pilot, controlled clinical trial of lopinavir/r single drug vs. lopinavir/r and 2 NRTIs for maintenance of HIV-1 viral suppression.

PATIENTS AND METHODS

Patients

This multicenter, randomized, open-label, investigator-initiated, clinical trial was carried out in 4 centers in Madrid, Spain. Patients were at least 18 years old, had no history of virologic failure while receiving a protease inhibitor, were receiving 2 NRTIs (or tenofovir and 1 nucleoside) and lopinavir/r (400/100 mg b.i.d.) for at least 4 weeks, and had had <50 copies of HIV RNA/mL for at least the prior 6 months. Exclusion criteria were pregnancy, presence of serum hepatitis B surface antigen, need for treatment with agents known to have potential major interactions with lopinavir/r, and major psychiatric disease. The study was approved by the Regional Ethics Committee for Clinical Research of the Community of Madrid and by the Spanish Agency for Medicines and Healthcare Products. All patients gave dated and written informed consent.

Randomization and Treatment

Patients were randomly assigned in a 1:1 ratio to stop or continue the 2 NRTIs (or tenofovir and 1 nucleoside). Randomization was centralized. A random sequence was generated by a computer (Clin Stat v.08.05.96, Department of Public Health Sciences, St. George's Hospital Medical School). Each patient's identification number and treatment group were assigned at the coordinating center after the center had received the randomization form.

After randomization, patients were assessed at baseline, 1, 2, 4, 8, 12, 16, and 24 weeks and every 12 weeks thereafter until week 48. At each visit, clinical data were collected and blood specimens were obtained after an overnight fast. Analyses included a complete blood count; CD4 cell count; measurement of plasma HIV-1 RNA, glucose, triglycerides, cholesterol (total, low-, and high-density lipoprotein); and tests of liver, kidney, and pancreatic function. All determinations of plasma HIV-1 RNA and RNA/DNA HIV genotyping were centrally performed in the Laboratory of Molecular Microbiology at the Hospital Doce de Octubre. All other laboratory determinations were performed at each site throughout the follow-up period.

HIV RNA in Blood Plasma

HIV viral load in blood plasma was performed by automatized RNA extraction in an AmpliPrep instrument (Roche Diagnostics, Mannheim, Germany) followed by quantitation using the COBAS AMPLICOR MONITOR HIV-1 test version 1.5 (Roche Diagnostic Systems, Branchburg, NJ).

Genotypic Resistance Tests

The genotypic pattern of resistance was evaluated in circulating virions by sequencing the PR and RT (1–335 amino acids) genes using ViroSeq HIV-1 Genotyping system (Celera Diagnostics, Alameda, CA). In selected samples a fragment corresponding to PR and RT was amplified by nested polymerase chain reaction (Expand High Fidelity PCR System, Roche) from proviral DNA obtained from peripheral blood mononuclear cells (PBMCs) (QiaAmp DNA blood kit, Qiagen, Hilden, Germany) and sequenced using ViroSeq HIV-1 genotyping reagents.

Viral genotyping was performed on samples from all patients who had >500 copies/mL of HIV RNA at any study visit. In addition, in patients with loss of virologic response, genotyping was performed in stored pre-HAART samples or, if a pre-HAART sample was not available, in baseline HIV proviral DNA.

Mutations conferring resistance to lopinavir were defined according to the 2004 International AIDS Society list (http://www.iasusa.org/resistance_mutations/mutations_figures.pdf).

Pharmacokinetic Analysis

At each study visit, plasma samples were stored frozen at −80°C for determination of lopinavir levels in patients with loss of virologic suppression. Date and time of last lopinavir dose were recorded. Plasma concentrations of lopinavir were determined with a high-performance liquid chromatographic assay.[20]

Adherence

To assess adherence we evaluated both pharmacy services computer databases and the GEEMA adherence questionnaire.[21] Pharmacy services databases record date and

quantity of antiretrovirals dispensed. An adherence score was calculated using the following formula: number of total days of antiretroviral dispensation/number of total days until next dispensation ×100. Using this formula we evaluated adherence throughout the 48 weeks or in patients in whom therapy failed from baseline until the time of failure. The GEEMA questionnaire[21] has 4 qualitative and 2 quantitative questions. We classified patients as adherent or nonadherent when there was a positive response to any of the qualitative questions. In addition we quantified responses to the 2 quantitative questions: "Thinking about the last week, how often have you not taken your medicine?" and "Since the last visit, how many days have you not taken any medicine at all?"

Definitions

Confirmed loss of virologic suppression was defined by 2 consecutive measurements of plasma HIV-1 RNA of >500 copies/mL separated by at least 2 weeks. A 500-copies/mL cutoff was selected to facilitate genotyping in all patients with confirmed loss of virologic response. In addition an analysis using a more stringent 50-copies/mL cutoff was also established.

"Blip" was defined as a plasma HIV RNA >50 copies/mL with subsequent sample <50 copies/mL without change in randomized therapy.

Endpoints

The primary outcome measures for efficacy were the proportion of patients with <500 copies/mL of HIV RNA of plasma at 48 weeks. Secondary efficacy outcomes included the proportion of patients with <50 copies/mL of HIV RNA at week 48, time to loss of virologic suppression through week 48, development of HIV resistance, and changes in the CD4 cell count. To assess safety, the frequency and severity of treatment-related adverse events, the incidence of laboratory abnormalities, and changes from baseline in clinical and laboratory values were compared between the 2 treatment groups.

Statistical Analysis

This was a pilot trial to explore the feasibility of using lopinavir/r as single-drug therapy for maintenance of HIV-1 viral suppression. Given the very large failure rate observed in previous single-drug maintenance trials and the possible risk of development of HIV resistance it was considered that an exploratory, pilot trial with a limited sample size would provide useful information for the design of future, adequately powered trials of single-drug therapy.

The main reason to include a control arm receiving lopinavir/ritonavir and 2 nucleosides was not to compare efficacy but to be able to continuously compare the failure rate in the monotherapy arm with the failure rate in the triple-therapy arm. We arbitrarily decided to include 21 patients per arm and we established that if there were 7 failures more in the lopinavir/r monotherapy arm than in the triple arm, an independent safety board could take a decision about terminating the trial. With 21 patients per arm the study had a statistical power of 80% to detect a 41% difference between treatment arms assuming that in the triple-therapy arm the percentage of patients with <500 copies of HIV RNA/mL at 48 weeks would be 95%.

All randomized patients were included in the analyses. Patients were followed for the entire trial regardless of whether they prematurely discontinued the assigned therapy. The proportion of patients with HIV RNA levels lower than the limit of quantification was summarized using an intent-to-treat analysis, with missing values or changes in randomized therapy considered to be above the limit of quantification.

Treatment groups were compared using the Fisher exact test and χ^2 test for categorical variables and the Wilcoxon 2-sample test for continuous variables. Time-to-event analyses were performed using Kaplan-Meier survival curves and the log-rank test.

RESULTS

Baseline Characteristics

Between May and August 2003, all 42 patients screened were found to be eligible for the study and underwent randomization. Baseline characteristics of the patients included in the study were not significantly different between the groups (Table 1). Patients included in the trial were receiving treatment with lopinavir/r mostly as their 1st or 2nd protease inhibitor. Prior to randomization, the most common nucleoside combinations used along with lopinavir/r were zidovudine–lamivudine and stavudine–lamivudine (Table 1).

Patient Disposition

At 48 weeks, 20 patients in each arm were still in the study. One patient in the monotherapy arm was lost to follow-up and one patient in the triple-therapy arm stopped treatment at week 24 due to hyperlipidemia not responding to lipid-lowering drugs. In 3 patients in the monotherapy arm, nucleosides were added back after confirmed loss of virologic suppression and are still actively followed (Table 2).

At 72 weeks another patient in the triple-therapy arm had to discontinue therapy due to hyperlipidemia not responding to lipid-lowering drugs. Therefore, at 72 weeks 20 patients in the monotherapy arm and 19 patients in the triple-therapy arm are still followed.

Antiviral Activity

In an intent-to-treat analysis, with missing HIV RNA level values or change in randomized therapy considered to be >500 copies/mL, 81% (17/21, 95% CI: 64% to 98%) of the patients in the monotherapy group and 95% (20/21, 95% CI: 86% to 100%) of the patients in the triple-therapy group maintained an HIV RNA level of <500 copies/mL at week 48 ($P = 0.34$; Fisher exact test) (Fig. 1). All patients who had an HIV RNA level of <500 copies/mL at week 48 were also below detection limit using the <50-copies/mL cutoff. The 95% CI for the difference in response rates at week 48 was −33.4% to 4.9%. At 72 weeks, percent of patients <50 copies/mL (intention to treat) were 81% (monotherapy arm) and 90.5% (triple-therapy arm). The 95% CI for this difference in response rates at week 72 was −30.5% to 11.4%.

TABLE 1. Base-Line Characteristics of the Patients

	Monotherapy n = 21	Triple Therapy n = 21
Age, y		
Median	42	42
Range	25–54	31–48
Male sex, n (%)	17 (81)	18 (86)
Route of HIV infection, n (%)		
Injection drug use	8 (38)	6 (29)
Male homosexual sex	5 (24)	8 (38)
Heterosexual sex	8 (38)	7 (33)
AIDS, n (%)	15 (71)	14 (67)
CD4 cells per μL, median (IQR)		
Baseline	662 (446–740)	585 (331–721)
Nadir	139 (53–248)	90 (29–261)
Serum HIV RNA $\log_{10}$ copies/mL (pre-HAART)		
Median	5.11	4.93
IQR	(4.7–5.5)	(4.5–5.6)
Time with HIV RNA <50 copies/mL (mo)*		
Median	28.6	15.7
IQR	11.3–44.9	8.6–27.5
Serum lipids, median (IQR), mg/dL		
Total cholesterol	176 (131–211)	193 (173–236)
LDL cholesterol	84 (56–121)	104 (84–126)
HDL cholesterol	44 (31–53)	43 (37–49)
Triglycerides	186 (121–244)	208 (160–310)
HCV coinfection, n (%)	10 (48%)	10 (48%)
Months on lopinavir/r, median (IQR)	13 (10–20)	13 (8–21)
Number of PIs prior to lopinavir/r, n (%)		
None	7 (33%)	6 (29%)
1	14 (67%)	10 (47%)
2	0 (0%)	5 (24%)
PIs prior to lopinavir/r, n (%)		
Nelfinavir	3 (14%)	7 (33%)
Indinavir	4 (19%)	9 (43%)
Ritonavir	5 (24%)	3 (14%)
Saquinavir/r	2 (10%)	1 (5%)
Nucleosides prior to randomization, n (%)		
Zidovudine + lamivudine	7 (33)	9 (43)
Stavudine + lamivudine	8 (38)	6 (29)
Other	6 (29)	6 (29)

*$P = 0.09$.

HCV indicates hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PI, protease inhibitor.

There were 4 blips (viral loads 61–385 copies/mL) in the monotherapy group and 1 in the triple-therapy group (viral load 1020 copies/mL).

Immunologic Changes

No significant change in CD4 cell count was seen in any group from baseline to week 48. The mean increase from baseline in CD4 cell counts at week 48 was 70 cells/μL for the monotherapy group and 8 cells/μL for the triple-therapy group ($P = 0.36$; Mann–Whitney U test).

TABLE 2. Patient Disposition

n	Monotherapy	Triple
Discontinuation due to noncompliance	1	0
Discontinuation due to adverse event	0	1
Loss of virologic suppression	3	0
Still on study at 48 weeks	20	20

Adherence

Median adherence by drug refill score was 96% (interquartile range [IQR] 84–100) in the triple-therapy and 86% (IQR 75–98) in the monotherapy group ($P = 0.09$).

Using the GEEMA adherence questionnaire, more patients were classified as adherent in the triple-therapy (57%) than in the monotherapy group (33%) ($P = 0.12$). The median accumulated number of missed doses in the week prior to each visit was 0 (IQR 0–1) in the triple-therapy group and 0 (IQR 0–3) in the monotherapy group ($P = 0.14$). The median number of total days without medication was 0 (IQR 0–0) in the triple-therapy group and 0 (IQR 0–2) in the monotherapy group ($P = 0.1$).

Patients With Loss of Virologic Suppression

Of the 21 patients randomly assigned to the monotherapy arm, 3 had loss of virologic suppression while on therapy and 1 patient discontinued treatment and was lost to follow-up. In all cases, loss of virologic suppression started after week 12 (weeks 14, 16, 25, and 29; Fig. 2). A detailed analysis of each of these rebounds in the monotherapy arm is shown in Figure 3.

Patient DO-17 (Fig. 3A) had 59% compliance by drug refill from baseline to week 12. Plasma lopinavir troughs decreased to subtherapeutic levels at the time of 1st virologic rebound. This period of nonadherence occurred along with a relapse of illicit drug use after the trial initiation. Two genotypes performed 4 weeks apart after 1st rebound showed the presence of 63P in the PR gene. The same mutation was present in a stored plasma sample obtained prior to any HAART initiation. This patient was lost to follow-up after week 16 of follow-up.

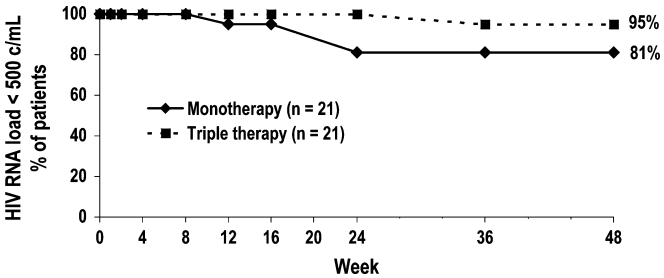

FIGURE 1. Percentage of patients with plasma HIV RNA levels <500 copies/mL, by treatment group (intent-to-treat analysis, missing data or change in randomized therapy = failure).

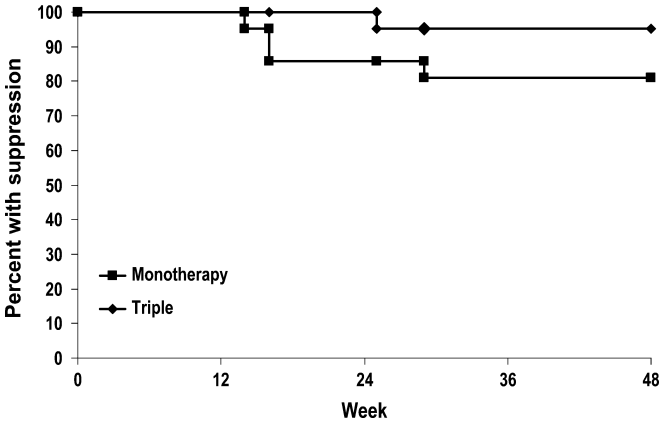

FIGURE 2. Time to loss of virologic suppression.

Patient DO-14 (Fig. 3B) had 79% compliance by drug refill from baseline to week 24. At the time of 1st virologic rebound, plasma lopinavir trough was within therapeutic range. Although per protocol loss of virologic response had to be confirmed 2 weeks after the 1st viral load of >500 copies/mL, the patient could not be reached and kept taking lopinavir/r monotherapy from week 24 to week 32. Two HIV-RNA genotypes performed 8 weeks apart showed the presence of the 77I mutation. Genotyping performed in a stored plasma sample obtained prior to any HAART showed the presence of 10I and 77I. At week 32, the patient was successfully reinduced with the same nucleosides that he was receiving before randomization and he has maintained virologic suppression for 12 weeks since reinduction.

Patient LP12 (Fig. 3C) had 60% compliance by drug refill from baseline to week 32. At the time of 1st virologic rebound, plasma lopinavir trough was within therapeutic range. An HIV RNA genotype showed the presence of the 63P and 77I mutations. Genotyping was also performed in HIV-1 proviral DNA from a baseline sample, which showed the presence of 36I, 71V, and 77I. At week 32, the patient was successfully reinduced with the same nucleosides that he was receiving before randomization and he has maintained virologic suppression for 36 weeks after reinduction.

Patient DO-10 (Fig. 3D) had a 100% compliance by drug refill from baseline to week 16. At the time of 1st virologic rebound, plasma lopinavir trough was within therapeutic range. Two RNA genotypes performed 8 weeks apart after the 1st virologic rebound showed the presence of wild-type virus. Genotyping was also performed in a stored pre-HAART sample and in PBMC-associated HIV-1 proviral DNA from a baseline sample. Both showed the presence of wild-type HIV. The patient was successfully reinduced with the same nucleosides (zidovudine and lamivudine) that he was receiving before randomization and has maintained virologic suppression for 44 weeks after reinduction.

The analysis of adherence measures restricted to the monotherapy group showed that patients with confirmed loss of virologic suppression had a statistically significant higher number of total days without medication and total missed doses in the week prior to each visit. In addition, patients with confirmed loss of virologic suppression had shorter time with suppressed viral replication before randomization (Table 3).

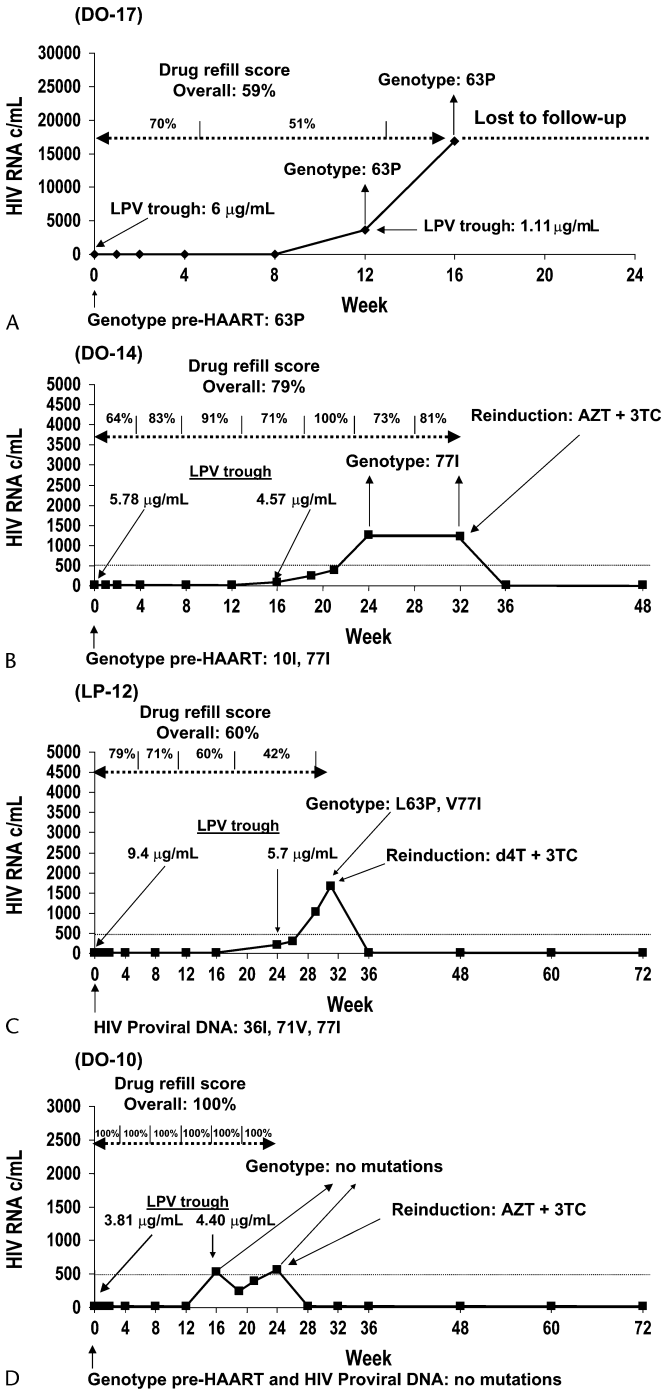

FIGURE 3. Patients with loss of virologic suppression in the lopinavir/r monotherapy group. Parts A, B, C and depict the evolution of each patient with virological rebound. Adherence is evaluated by drug refill from baseline to the time of 1st virologic rebound (overall) and between each pharmacy visits (percents between bars).

Adverse Events

One patient in the triple-therapy group discontinued therapy due to hyperlipidemia associated with multiple cardiovascular risk factors despite treatment with a lipid-lowering

TABLE 3. Comparison of Disease and Treatment Characteristics of Patients Treated With Monotherapy With and Without Confirmed Loss of Virological Suppression

Characteristic	Virologic Suppression Maintained	Virologic Suppression Lost	*P* Value
n	17	4	
AIDS, n (%)	7 (41)	2 (50)	0.99
Serum HIV RNA $\log_{10}$ copies/mL pre-HAART (mean, range)	5.34 (2.7–5.7)	4.68 (4.44–4.78)	0.13
Weeks with HIV-RNA <50 copies/mL prior to monotherapy (median, range)	132 (40–331)	40 (30–84)	0.02
CD4 Cells/μL, (mean, range)			
Baseline	658 (196–1037)	437 (293–722)	0.21
Nadir	158 (6–416)	95 (8–252)	0.37
Months on Lopinavir/r prior to monotherapy (mean, range)	17 (2.6–48)	16 (10.8–27.9)	1
Lopinavir/r 1st PI, n (%)	4 (23.5)	2 (50)	0.54
Adherence by drug refill score (median, range)	94 (71–100)	70 (59–100)	0.14
GEEMA adherence questionnaire			
Classified as "adherent," n (%)	7 (41)	0 (0)	0.25
Total missed doses in prior week (median, range)	0 (0–4)	3 (2–10)	0.013
Total days without medication (median, range)	0 (0–31)	3 (1–65)	0.008

PI indicates protease inhibitor.

drug. At week 24, his fasting total cholesterol was 282 mg/dL, low-density lipoprotein cholesterol 164 mg/dL, and serum triglycerides were 421 mg/dL. No other discontinuations related to adverse events were observed during follow-up until 48 weeks.

During follow-up, grade 3 hypertriglyceridemia was seen in 1 patient in the triple-therapy arm and in none in the monotherapy arm. One patient in each arm had grade 3 hypercholesterolemia. No other grade 3 or 4 clinical or laboratory adverse events were seen during follow-up in any patient.

Compared with baseline, at week 48 there were nonsignificant differences between the monotherapy and the triple-therapy arm with regard to mean increases of fasting serum triglycerides (−3 vs. −6 mg/dL), total cholesterol (+17 vs. + 7 mg/dL), low-density lipoprotein cholesterol (+18 vs. +11 mg/dL), or high-density lipoprotein cholesterol (+2 vs. −3 mg/dL).

DISCUSSION

Our pilot clinical trial provides preliminary evidence suggesting that lopinavir/r alone could maintain HIV-1 viral suppression in a large proportion of HIV-1–infected patients. In our study, 81% of patients randomly allocated to maintenance with lopinavir/r monotherapy had plasma HIV RNA levels <50 copies/mL after 72 weeks by intention to treat. Importantly, in patients with loss of virologic suppression after starting lopinavir/r monotherapy, development of primary or active site mutations in the protease was not detected by standard genotyping. Maintenance failures that occurred in the monotherapy group were successfully reinduced by adding back prior nucleosides.

None of the rebound isolates in the lopinavir/r monotherapy group had primary or active site mutations in the PR gene by standard genotyping. In all but 1 patient, lack of adherence to treatment might explain loss of virologic suppression in the absence of new resistance mutations (Figs. 3A–C). Interestingly, 2 of these patients had adequate lopinavir through levels at the time of 1st virologic rebound (Figs. 3B and D) despite drug refill scores of only 71% and 79%, suggesting better adherence shortly before study visit. Paradoxically, adherence rates were worse in the monotherapy group than in the triple-therapy group. Due to our small sample size, it is difficult to speculate whether this finding was due just to chance or to other reasons such as a negative impact of discontinuing nucleosides on patients' perceptions on the importance of high adherence to antiretroviral treatment. It would be interesting to investigate whether monotherapy with other potent boosted protease inhibitors with a lower daily pill burden (lopinavir/r tablets, atazanavir/r, saquinavir/r, or fosamprenavir/r) can lead to better adherence rates while maintaining efficacy outcomes. At present, outside of the family of boosted protease inhibitors, no other drugs seem to be appropriate candidates for single-drug maintenance.

It has been repeatedly shown that antiretroviral-naive patients who do not adhere properly to a lopinavir/r-based regimen do not develop primary mutations in the PR gene.[13,14] The rapid terminal clearance of lopinavir after a dose is missed has been proposed as the main reason for the low selective pressure of lopinavir during periods of nonadherence.[13] It is important to emphasize that in the monotherapy group,

patients could maintain virologic suppression despite having adherence rates that are generally considered suboptimal. Half of the patients treated with lopinavir/r monotherapy who did not have treatment failure had adherence rates between 71% and 94%.

Loss of virologic suppression in another patient in the monotherapy group (DO-10, Fig. 3D) does not appear to be caused by poor compliance. This patient had optimal adherence by self-report and drug refill, therapeutic plasma lopinavir trough levels, and serum viral loads after rebound that did not exceed 564 copies/mL. One possible explanation for this failure is development of minority species of HIV with resistance to lopinavir/r that were not detected with the standard genotyping used in our study. This hypothesis has been proposed before to explain viral rebounds with wild-type virus in patients in whom indinavir monotherapy failed in the ACTG 343 trial.[22] However, in contrast to unboosted indinavir, resistance to a boosted protease inhibitor such as lopinavir/r requires the accumulation of multiple mutations in the PR gene. Patients included in our study did not have previous virologic failure while receiving a protease inhibitor. Consequently, virus strains with a sufficient number of mutations to cause lopinavir/r resistance were not likely to preexist before randomization. Indeed, genotyping performed in a pre-HAART sample and in HIV proviral DNA at baseline did not show the presence of primary or active site mutations in this patient (Fig. 3D).

In patients treated with lopinavir/r monotherapy who lost virologic suppression, mutations detected during virologic rebounds are not known to be associated with clinical resistance to lopinavir. All mutations except 1 were present in premonotherapy samples. The only new mutation was 63P (patient LP12), which is found in 45% of protease inhibitor–naive patients and 90% of pretreated patients.[23] The 63P mutation in the absence of other primary mutations does not increase significantly the inhibitory concentration of 50% for lopinavir[12] and consequently does not justify the failure of monotherapy.

We recognize that the presence of either minority populations of resistant viruses or mutations outside the PR gene[24] after loss of virologic suppression in the monotherapy arm has not been ruled out in our trial. However, the fact that after reinduction with nucleosides patients have remained virologically suppressed for up to 44 weeks argues against the existence of mutations associated with resistance to protease inhibitors.

The most important limitation of our study is obviously the small sample size, characteristic of a pilot clinical trial. As expected, no meaningful conclusion can be drawn regarding the comparative efficacy of monotherapy vs. triple therapy. We can only be confident that the failure rate of lopinavir/r monotherapy is not $>36\%$. In addition, with the limited number of patients included in the trial it is possible that imbalances at baseline in several important characteristics (period of viral suppression prior to randomization, adherence rates, and nadir and baseline CD4 cell counts) might have impacted the overall results.

Despite its limitations, we believe that our pilot study provides information that would be useful for the design of future trials. The observed 48-week efficacy rate of the lopinavir/r monotherapy arm in our trial (81%), although with a wide 95% CI (64% to 98%), could be within the range of accepted simplification strategies.[25] However, in contrast with simple triple-drug regimens, our study suggests that patients who lose viral suppression after lopinavir/r monotherapy could be safely reinduced with prior nucleosides. Our results serve as proof of concept that it is possible to use a boosted protease inhibitor alone as maintenance of viral suppression. Given these results and the obvious benefits of single-drug treatment of HIV infection, we believe that an adequately powered trial of lopinavir/r monotherapy is warranted. Such a trial is currently recruiting patients in 30 centers in Spain.

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