Randomized controlled study demonstrating failure of LPV/r monotherapy in HIV: the role of compartment and CD4-nadir

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Background: Long-term side-effects and cost of HIV treatment motivate the development of simplified maintenance. Monotherapy with ritonavir-boosted lopinavir (LPV/r-MT) is the most widely studied strategy. However, efficacy of LPV/r-MT in compartments remains to be shown.

Methods: Randomized controlled open-label trial comparing LPV/r-MT with continued treatment for 48 weeks in treated patients with fully suppressed viral load. The primary endpoint was treatment failure in the central nervous system [cerebrospinal fluid (CSF)] and/or genital tract. Treatment failure in blood was defined as two consecutive HIV RNA levels more than 400 copies/ml.

Results: The trial was prematurely stopped when six patients on monotherapy (none in continued treatment-arm) demonstrated a viral failure in blood. At study termination, 60 patients were included, 29 randomized to monotherapy and 13 additional patients switched from continued treatment to monotherapy after 48 weeks. All failures occurred in patients with a nadir CD4 cell count below 200/μl and within the first 24 weeks of monotherapy. Among failing patients, all five patients with a lumbar puncture had an elevated HIV RNA load in CSF and four of six had neurological symptoms. Viral load was fully resuppressed in all failing patients after resumption of the original combination therapy. No drug resistant virus was found. The only predictor of failure was low nadir CD4 cell count ($P < 0.02$).

Conclusion: Maintenance of HIV therapy with LPV/r alone should not be recommended as a standard strategy; particularly not in patients with a CD4 cell count nadir less than 200/μl. Further studies are warranted to elucidate the role of the central nervous system compartment in monotherapy-failure.

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Introduction

A combination of three antiretroviral drugs is the recommended treatment for HIV-infected patients [1]. Lifelong HIV-therapy is needed to keep HIV replication under control [2]. Although the benefits of a triple therapy regimen are unquestionable, patients on long-term therapy are at risk of side effects, particularly

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mitochondrial toxicity, metabolic effects and lipodystrophy [3]. Concerns about side effects and cost of therapy have stimulated the search for alternative treatment strategies to limit drug exposure. Treatment simplification using monotherapy is a potential method to reduce toxicity and costs while maintaining full viral suppression. First trials with unboosted protease inhibitors (PIs) were unsuccessful [4]. However, randomized studies with ritonavir-boosted protease inhibitors have demonstrated continued viral load suppression for more than 1 year [5]. Ritonavir-boosted lopinavir (LPV/r) is the most widely studied candidate for monotherapy. However, the population at risk for monotherapy failure is not well described, although predictive factors for failing LPV/r monotherapy have recently been investigated. Concerns remain regarding residual HIV replication in compartments such as the central nervous system (CNS) and the genital tract because of limited drug penetration.

The Monotherapy Switzerland/Thailand study (MOST) evaluated the activity of monotherapy in the central nervous system, in the genital tract and risk factors associated with the loss of virological response on LPV/r monotherapy. The trial was prematurely terminated when the protocol defined stopping criteria were reached.

Patients and methods

Study design and patients

Randomized controlled open label trial comparing LPV/r [400/100 mg twice daily (b.i.d.)] monotherapy with continuation of triple therapy (continued treatment) for 96 weeks, with an optional switch to monotherapy offered to all patients on continued treatment at week 48 (Figure 2, supplemental material, http://links.lww.com/QAD/A69).

The study was conducted in St. Gallen, Zurich, Bern, Geneva and Lausanne within the framework of the Swiss HIV Cohort Study (SHCS). Patients on ART for at least 6 months with suppressed HIV RNA (<50 copies (cp)/ml for at least 3 months) were eligible for the study if they consented to a spinal tab at screening prior to randomization. After 48 weeks patients on continued treatment had the option to switch to monotherapy (delayed switch).

One exclusion criterion was previous history of virologic treatment failure with any drug combination or documented protease inhibitor resistance.

Further exclusion criteria, randomization procedures, neuropsychological tests and the details of premature study termination are described in the online supplement.

Sample collection and study visits

Patients were assessed at baseline, then every 6 weeks to week 24 and every 8 weeks thereafter. The same follow-up procedure was conducted during the second phase of the study until week 96. At each visit, patient history and symptom directed examination were performed, self-reported adherence was documented. HIV RNA in blood, CD4 cell count and routine hematology and chemistry tests were performed at the local laboratory.

Patients with an increase in blood HIV RNA above 10^2 cp/ml were asked to return for confirmatory testing as soon as possible but not later than 4 weeks after the last visit. All confirmation tests were performed in one central laboratory (Geneva).

At baseline, week 48 and study termination, CSF and genital secretions (semen, cervical swab) were collected for quantitative HIV RNA analysis, and DEXA scan, as well as neuropsychological tests were performed.

Lumbar puncture was performed with an atraumatic needle. A specific protocol for patient preparation (hydration) was used, as in the ATARITMO study [6].

Male patients obtained the semen sample by masturbation at home. Timing of the sample collection was recorded for future reference. Cervical swab samples were obtained by patients themselves by standard protocol described in Kovacs *et al.* [7].

Virologic analysis

Quantification of HIV RNA in blood was done in the local laboratories (Cobas Ampliprep/ COBAS Taqman HIV-1 version 1.0, Roche Diagnostic Systems). HIV RNA measurements in CSF and semen were performed by PCR in a central laboratory in Geneva with the methodology used for blood plasma including a precentrifugation step for seminal plasma (8 min, 10 000×*g*). Cell-associated DNA and RNA levels were quantified as previously described [8,9].

Population-based sequence analysis of the reverse transcriptase and protease gene was performed on an Applied Biosystems (ABI) sequencer as previously described [10] in all samples with treatment failure. Resistance mutations were analyzed according to the IAS-USA mutation list [11].

Procedures for failing patients and premature study termination rule

On the basis of previous data [12], we expected a low frequency of treatment failure in blood HIV-RNA in the monotherapy arm. Nevertheless, we defined study termination criteria in the case of an unexpectedly high degree of treatment failure in blood. Premature study termination was mandated if more than six (20%) of the first 30 patients on monotherapy failed treatment. Failure

was defined as two consecutive plasma HIV RNA levels more than 400 cell/ml. Patients with a monotherapy failure were switched back to the previous triple therapy.

Endpoints

The primary endpoint was treatment failure in the CNS or genital compartment. As expected HIV RNA levels in the compartments are not fully established, compartment failure was defined as an HIV RNA level one log above the respective value at baseline. If baseline values were undetectable, a level of 40 cp/ml was assumed.

However, as the trial was terminated when recruitment reached 60% of plan, the analysis of primary endpoints was not possible. The focus of investigations, therefore, shifted to explaining these failures and looking for predictive factors.

Statistical analysis

The study was designed as a noninferiority trial. The primary hypothesis was that compartment failure rates were noninferior on monotherapy compared with continued treatment in patients having fully suppressed viral load in the blood. According to the hypothesis, only patients who had undetectable blood plasma viremia (<50 cp/ml) were to be considered for compartment evaluation. On the basis of the selected non inferiority-boundary of 12%, an estimated failure rate of less than 3%, a sample size of 74 patients was calculated to reach 80% power with an alpha error of 5% (one-sided). With an estimate of 10% for loss of follow-up and 15% for patients withdrawing consent for spinal tab at study termination the study size was set to 100.

Treatment groups were compared using the Fisher exact test for categorical variables and the Mann–Whitney U-test for continuous variables. Time to event analyses were performed using Kaplan–Meier survival curves and Cox regression was used to evaluate predictors of failure in patients on monotherapy. All calculations were performed using SPPS software, version 11 (SPSS Inc., Chicago, Illinois, USA).

Results

Between May 2007 and September 2008, 60 patients were included in the trial. 31 patients were randomized to continued treatment, and 29 patients were switched to monotherapy at baseline. Thirteen continued treatment patients switched to monotherapy after having reached week 48.

Baseline characteristics

Baseline characteristics were similar in both study arms (see Table 1). No patient was lost from follow up. One patient in the monotherapy arm developed severe diarrhea on LPV/r and was switched back to his previous treatment on week 6. For the analysis of risk factors, he remained in the assigned treatment arm. Median follow up of all patients was 48 weeks. Delayed switch patients were on monotherapy for a median duration of 18 weeks. One patient was observed until week 96. Prior to randomization the most common nucleoside reverse transcriptase inhibitors used were zidovudine plus lamivudine (3TC) ($n=23$, 38%), tenofovir (TDF) plus emtricitabine (FTC) ($n=15$, 25%), abacavir plus 3TC ($n=14$, 24%) and TDF plus 3TC ($n=8$, 13%).

Description of failing patients

Six patients reached HIV-RNA failing criteria in plasma after a median of 12 weeks (range: 6–24). Three were female and all were on monotherapy (five in mono-therapy, one in delayed switch group). All six patients reported an optimal adherence to monotherapy and none of them had documented HIV-RNA values more than 50 cp/ml during the conventional therapy prior to study enrollment. The median duration of uninterrupted full

Table 1. Baseline characteristics.

		Continuous therapy (CT) $n=31$ (52%)	Monotherapy (MT) $n=29$ (48%)
Sex	Male	24 (77%)	19 (66%)
Age mean	Years	46 ± 11	42 ± 7
Pretreatment (%)	PI based	23 (74%)	21 (73%)
	NNRTI based	7 (23%)	7 (24%)
	Triple N based	1 (3%)	1 (3%)
HIV RNA Set-point mean	Log_{10} cp/ml	4.7 ± 0.9	4.9 ± 0.9
CD4 baseline median (IQR)	G/l lymphocytes	465 (356–625)	498 (360–670)
CD4 nadir median (IQR)	G/l lymphocytes	160 (126–211)	160 (37–246)
Duration of previous ART mean	Years	4.4 ± 3.3	5.4 ± 3.7
CDC stage	A	14 (45%)	14 (49%)
	B	10 (32%)	5 (17%)
	C	7 (23%)	10 (34%)
Cholesterol mean	mmo/l	5.3 ± 1.1	5.0 ± 1.0
Cell-associated HIV RNA	Log_{10} cp/10^6 PBMC	1.6 ± 0.8	1.4 ± 0.7
Cell-associated HIV-DNA	Log_{10} cp/10^6 PBMC	2.3 ± 0.5	2.2 ± 0.5

IQR, interquartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor; PBMC, peripheral blood mononuclear cells; PI, protease inhibitor.

Table 2. Summary of all patients with treatment failure in blood or detection of elevated HIV-RNA in CSF at any time during the study.

Patient group and ID[1]	Sex	Pre-treatment	CD4 nadir (/μl)	Treatment arm[2]	Week on study/MT	log RNA blood	log RNA CSF	WBC count CSF (/μl)	Protein CSF (g/l)	CD4 cell at term[5]	Symptoms of acute HIV infection
Blood failure											
101	Male	ATV/r, TDF, 3TC	57	MT	12	4.3	5.1	124	0.6	680	Yes
108	Female	LPV/r, ZDV, 3TC	5	DS	60/12	4.2	3.1	10	0.4	361	Yes
126	Female	LPV/r, ABC, 3TC	149	MT	12	4.1	5.0	67	0.9	380	No
302	Male	EFV, ZDV, 3TC	7	MT	24	3.0	4.1	10	0.4	130	Yes
303	Male	LPV/r, TDF, 3TC	54	MT	6	5.0	nd[3]	nd	nd	250	No
713	Female	EFV, TDF, 3TC	160	MT	24	3.0	3.7	29	0.4	710	Yes
CNS +RNA MT											
107	Male	LPV/r, TDF, FTC	211	DS	96/48	<1.6	2.9	3	nd	nd	No
703	Male	ATV/r, TDF, 3TC	370	DS	66/18	2.2	3.4	56	0.7	1030	No
704	Female	LPV/r, ABC, 3TC	100	MT	63	2.3	4.3	47	0.7	380	Yes
707	Male	TDF, 3TC, ZDV, EFV	130	DS	68/20	2.1	3.4	15	0.4	780	No
702	Male	LPV/r, 3TC, ZDV	120	DS	72/24	<1.6	2.1	2	0.4	1050	No
709	Male	LPV/r, TDF, FTC	20	MT	37	<1.6	2.4	1	0.2	410	No
714	Female	ABC, ZDV, 3TC, LPV/r	220	MT	48	1.9	2.5	22	0.4	680	No
124	Female	LPV/r, ZDV, 3TC	17	MT	44	<1.6	1.9	2	0.2	474	No
CNS +RNA CT											
709	Male	LPV/r, TDF, FTC	20	BL	0	<1.6	1.6	1	0.5	360	No
309	Male	ATV/r, TDF, FTC	126	BL	0	<1.6	1.7	1	0.4	333	No
110	Male	TDF, 3TC, EFV	185	BL	0	<1.6	1.9	2	0.5	447	No
703	Male	ATV/r, TDF, 3TC	370	DS	48/0	<1.6	1.6	2	0.9	1010	No

ABC, abacavir; ATV/r, atazanavir, ritonavir-boosted; EFV, efavirenz; FTC, emtricitabine; LPV/r, lopinavir, ritonavir boosted; TDF, tenofovir; ZDV, zidovudine; 3TC, lamivudine. [1]Patient: ID Number (first digit for study center), Blood failure: HIV-RNA in blood plasma >400 cp/ml, CNS +RNA MT or CT: Patients with detectable HIV-RNA in CNS either in MT or CT arm, respectively. [2]Treatment arm: MT, Monotherapy; CT, continuation therapy; DS, delayed switch; BL, triple therapy at baseline. [3]nd: not done. [4]HIV-RNA values are given as $\log_{10}$ cp/ml, values shown in bold are above the predefined failing criteria (2.6 $\log_{10}$, i.e. 400 cp/ml). [5]term: termination visit.

HIV-RNA suppression was 50 months (range: 9–63). Previous history of virological failure under any combination antiretroviral treatment was a predefined exclusion criterion.

Key characteristics of the failing patients are summarized in Table 2. With a median of 4.2 $\log_{10}$ cp/ml, CSF HIV-RNA in the five failures who consented to lumbar puncture was higher than the respective level in blood plasma (median 3.4 $\log_{10}$ cp/ml, $P = 0.15$). Elevation of HIV RNA in CSF was associated with elevated white blood cell counts in CSF (Table 2). The median WBC count in CSF when HIV RNA in CSF was more than 40 cp/ml was 15 cells/μl, (1–124) as compared with 1 cell/μl (0–6) when HIV RNA in CSF was less than 40 cp/ml ($P < 0.001$).

Five of the six failing patients presented with clinical symptoms at the time of failure: one patient had sialadenitis, four had neurological symptoms such as headache, dizziness, visual disturbance, deficit in concentration and ataxic gait. There was no history of previous neurological symptoms in all four failing patients. None of the other patients during the trial presented with signs or symptoms of acute neurological discomfort. In all failing patients, viral RNA was completely resuppressed after switching to previous triple therapy.

The median duration of uninterrupted complete HIV RNA suppression before study enrollment was 50 month (range 9–63) in monotherapy and 25 month (range 6–121) in continued treatment group. Genotypic resistance testing performed in CSF and in plasma of the failing patients did not reveal any mutation associated with drug either in the protease or in the reverse transcriptase region. All clinical findings, especially CNS symptoms, resolved completely after treatment switch.

HIV RNA in cerebrospinal fluid, isolated cerebrospinal fluid failures

Cerebrospinal fluid was examined in all 60 patients at baseline and in 45 patients at study termination (25 monotherapy with blood viral load <400, five failing monotherapy, 15 continued treatment patients with blood viral load <50). At baseline, three patients had low-level HIV-RNA in CSF (82, 56, and 43 cp/ml). Two of the three were randomized to continuous therapy [efavirenz + TDF + 3TC and TDF + FTC + atazanavir, ritonavir-boosted (ATV/r)] and both had undetectable HIV-RNA in CSF and blood at study termination. The third patient with 1.6 $\log_{10}$ (43) cp/ml, was randomized to monotherapy. At week 37, when the study was prematurely terminated, his viral load in CSF was 2.4 $\log_{10}$ (250) cp/ml, whereas blood viral load was undetectable (# 709, Table 2).

One additional patient on triple therapy had a detectable viral load in CSF of 1.6 $\log_{10}$ (45) cp/ml at week 48, whereas plasma viral load was undetectable. At this time, he was switched from TDF + FTC + ATV/r to

monotherapy. Eighteen weeks later, at the termination visit, viral load in CSF was 3.4 $\log_{10}$ (2300) cp/ml, whereas viral load in plasma was 2.2 $\log_{10}$ (170) cp/ml (#703, Table 2).

Low level HIV-RNA in cerebrospinal fluid ('blips')

Among all non-failing patients (viral load <400) at study termination, none of the 15 patients still under continued treatment had an HIV-RNA value in CSF more than 1.6 $\log_{10}$ (40) cp/ml, as opposed to eight of 25 monotherapy patients (32%, $P = 0.01$, Fisher's exact). Only four of the eight did reach the predefined CSF-failing criteria (>2.6 $\log_{10}$ cp/ml). Interestingly, three of the four CSF-failures had a plasma HIV-RNA value between 1.6 and 2.6 $\log_{10}$ (40–400) cp/ml (Table 2, CNS + RNA monotherapy). In all four patients, HIV RNA was more than one log higher in CSF than in blood. Mean CD4 nadir in cases with isolated CSF failures was not significantly different than in the monotherapy patients who had undetectable HIV-RNA in CSF at termination; 171/μl (IQR 123–251) vs. 211/μl (IQR 168–272), $P = 0.28$.

Analysis of risk factors

Only patients on monotherapy (≥6 weeks, $n = 42$) were included in the analysis of risk factors for treatment failure ($n = 6$). In univariate analysis, the following parameters were not associated with treatment failure in blood: age, sex, therapy prior to baseline and duration of HIV-RNA suppression less than 50 cp/ml, CDC classification, RNA set point, hepatitis C virus coinfection, length of therapy, peripheral blood mononuclear cell-associated HIV-DNA and RNA, hemoglobin and platelets. Cholesterol showed a trend for lower baseline cholesterol (*t*-test; $P = 0.053$), with failures having lower baseline cholesterol levels compared with nonfailures (4.5 ± 0.7 vs. 5.3 ± 1.1). Median nadir CD4 cell count in failing patients was 56/μl (IQR 19-126) vs. 194/μl (IQR 99-257) in nonfailing patients ($P = 0.026$; Mann–Whitney-U). Similarly, median baseline CD4 cell count was 335/μl (IQR 301–373) vs. 554/μl (IQR 413–720, $P = 0.019$; Mann–Whitney-U).

Kaplan–Meyer analysis (Fig. 1) demonstrates that all failures occurred within the first 24 weeks after switch to monotherapy. Cox regression analysis revealed a significant difference between the number of failures in patients with low (<200/μl) and high CD4 nadir ($P < 0.01$). No monotherapy failure occurred in patients with nadir CD4 cell count more than 200 cells/μl.

Low level replication and CD4 cell count

Evaluation of frequency of blips as a proxy for decreased potency of monotherapy showed that low level rebound (40–400 cp/ml) was significantly more frequent in the monotherapy arm (8 vs. 2% with HIV RNA 40–400 cp/ml under monotherapy vs. continued treatment among 191 vs. 210 RNA determinations per group; $P < 0.01$,

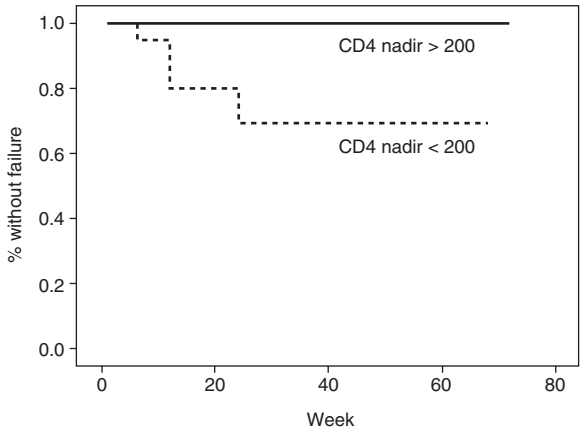

Fig. 1. Kaplan–Meier analysis of failure by nadir CD4 cell count (including all 42 patients who started monotherapy).

Figure 3, supplemental material, http://links.lww.com/QAD/A69). No significant difference in changes in CD4 cell count was detectable between the monotherapy and continued treatment arms.

Genital compartment and neuropsychological performance (see online supplement for details)

Results of HIV-RNA determination in the genital tract showed no marked elevation of HIV-RNA in the genital secretions. Neuropsychological tests demonstrated no significant changes.

Discussion

This study aimed to evaluate efficacy and safety of LPV/r monotherapy, with a special focus on two compartments: the central nervous system and the genital tract. The primary endpoint, which determined the sample size estimate was failure of viral suppression in the selected compartments in patients with a sustained fully suppressed plasma viremia. In addition, the study aimed to define predictors for failures of LPV/r monotherapy.

The study was terminated prematurely because of six failures in patients on monotherapy (6/42) as opposed to none in the continued treatment arm. The failure rate was higher than observed in our first pilot monotherapy study with ritonavir-boosted indinavir (0/12) [13] and significantly different from two similar studies using boosted ATV/r (no failure in a total of 58 patients on monotreatment [6,14]. The inclusion criteria for this study were not less stringent than the criteria used by other monotherapy trials. The stopping criteria for our study were influenced by the results of the OK4 trial [15]. In that randomized study on LPV/r-mono-maintenance compared with continuation with combination therapy, 3 vs. 6% patients (monotherapy vs. continued treatment) failed treatment during the 48 weeks of observation. Thus, the failure rate found in the present study was

2.4 times higher (14.3%, 6/42 vs. 6%, 6/100) than the documented rates in the study by Pulido *et al.* [15]. The difference might have occurred by chance ($P = 0.07$, Fisher's exact test). None of the monotherapy-failing patients had previous virologic failure under conventional triple therapy, duration of full viral suppression prior to randomization was longer in the monotherapy-arm and no resistance was detectable at the time of failure. Thus, the unexpected high failure rate was unlikely to be a result of preexisting low-level resistance or selection of poorly adherent patients.

Our relatively frequent detection of clinical signs of acute HIV infection in patients failing on monotherapy has not been described previously and is an additional cause for concern. In four of six patients, blood viral load failure was associated with neurological symptoms. In addition, virological failure in blood was associated with elevated HIV RNA and an increase in white cell count in CSF. These clinical and laboratory findings resemble the situation of primary HIV infection with aseptic meningitis [16]. None of the remaining patients had complained of neurological signs or symptoms during the study.

A main focus of the study was to explore the compartments CNS and genital tract.

Detectable HIV-RNA in the spinal fluid has been shown to be associated with deficits in neuropsychological functioning [17]. Despite limited power, our results lend support to our theoretical concern about lack of CSF activity of monotherapy. In all five failing patients who could be tested, HIV-RNA was detectable in CSF and the RNA concentration was higher than in blood in four of five patients. This finding is further supported by the demonstration of detectable HIV-RNA in the CSF in an additional eight patients among 25 who consented to a spinal tap at study termination. All these patients were on monotherapy at the time of study termination whereas none of 15 consenting patient in the continued treatment arm had detectable HIV-RNA in the spinal fluid. Interestingly, in three of the four patients whose HIV-RNA in CSF was above 400 cp/ml, HIV-RNA in blood was also detectable in blood but at levels below the failure criteria in blood (<400 cp/ml, Table 2) and the HIV-RNA level in CSF was 1.2 $\log_{10}$ cp/ml higher than in blood. At study termination, the fraction of patients with detectable HIV-RNA in the CSF was significantly higher in patients on monotherapy than on continued treatment.

In fact, follow-up of patients who opted to continue on LPV/r monotherapy when the study was terminated revealed another two patients with detectable HIV-RNA in CSF after 1 year on therapy. An unpublished study had evaluated CSF in 11 patients on LPV/r-MT for at least 24 weeks. In that study, HIV-RNA was detectable in one patient (750 cp/ml) in CSF whereas plasma viral load was undetectable. This small study with 1 CSF failure among 11 patients is not in contradiction to our results where 4/25 had more than 400 HIV-RNA cp/ml CSF [18].

The proportion of patients with detectable HIV-RNA in CSF was not only significantly higher on monotherapy than on continued treatment (32 vs. 0%, $P = 0.01$), but the difference appears to be biologically relevant. Elevation of HIV RNA in CSF was associated with elevated white blood cell counts in CSF indicating an association of CSF failure with inflammation in the central nervous system. Together with the finding of a higher HIV-RNA in CSF than blood in the patients reaching more than 400 cp/ml HIV-RNA in blood, the observation raises a concern that the elevated HIV-RNA in CSF might be a precursor of blood failure. More troublesome is the theoretical concern, that an elevated HIV-RNA concentration in the CSF could remain undetected for a long period in patients under monotherapy if CSF testing is not performed. It remains to be shown, whether LPV/r monotherapy is inferior to other strategies using ritonavir-boosted protease inhibitors owing to differences in CNS penetration.

Low level of HIV-RNA in semen was detectable in a few patients with fully suppressed HIV RNA in blood (<40 cp/ml) but RNA detection rates in semen or CVS were comparable in samples obtained during monotherapy or continued treatment. The most likely explanation for this low-level detection of HIV-RNA in semen even under fully suppressive therapy might be a result of contamination with cell-associated HIV-RNA originating from white blood cells in semen which sometimes can be difficult to separate from the viscous seminal plasma. Undetectable HIV-RNA in semen (<2.3 $\log_{10}$ cp/ml) was also found in a subset of 10 patients enrolled in the MONARK monotherapy trial [19].

As previously described by Arribas *et al.* [12] episodes of low-level viremia were more frequent in the monotherapy group. Differences in antiviral potency but also differences in forgiveness of the regimen for suboptimal adherence might explain the observed increase in low-level viremia.

All patients who experienced virological rebound were successfully resuppressed after reintroducing baseline NRT. This finding is in agreement with the observation by Pulido *et al.* [15] studies evaluating monotherapy with a boosted protease inhibitor as a maintenance strategy.

A limitation of our study is the lack of a standardized adherence questionnaire but adherence was discussed and documented at each visit. All failing patients had a history of successful uninterrupted long-term viral load suppression (<50 cp/ml) for a mean period of 50 months and all of them asserted having not missed a single dose prior to failure. However, two of the six failing patients had

low-lopinavir blood levels at the time of failure, indicating adherence as a potential cofactor of failure in some patients. A manuscript discussing full pharmacologic data of this study is in preparation (Decostered *et al.*). For patients receiving monotherapy an optimal adherence level might be even more important than for patients receiving triple therapy.

Since LPV/r has a short half-life, it is reasonable to hypothesize that patients missing one dose have a greater risk of virological rebound than patients receiving additional nucleosides with a long intracellular half-life. On the contrary the shorter half-life may reduce the risk for resistance mutations during periods of poor adherence [20].

Similar to previous reports [21], nadir CD4 cell count was a significant predictor of failure in this study. No patient with a CD4 nadir above 200/μl failed on monotherapy within the observation period. Low nadir CD4 might imply an irreversible impairment of the immune system. To maintain viral suppression, a therapy with three different substances might be required, particularly in patients having started treatment with advanced disease.

Treatment simplification has the potential to reduce side-effects and costs. This may be of great relevance in countries with limited economic resources, but with LPV/r MT, suboptimal virologic suppression in the central nervous system remains a concern. At least in patients with low HIV RNA level replication in blood, and in patients with a low CD4 nadir, lumbar puncture to confirm virologic suppression in CSF needs to be considered. Further studies with long-term follow up and evaluation of monotherapy efficacy in CNS are needed before monotherapy generally can be considered as an option for HIV therapy.

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