ORIGINAL RESEARCH

Combined therapy with saquinavir, ritonavir and stavudine in moderately to severely immunosuppressed HIV-infected protease inhibitor-naive patients

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Objective

To assess the short-term and long-term effect of a combination of saquinavir, ritonavir and stavudine in moderately to severely immunosuppressed protease inhibitor-naive patients.

Design

Prospective open-label multicentre study.

Patients and methods

A total of 64 protease inhibitor-naive and stavudine-naive HIV-infected patients with a CD4 count of < 250 cells/μL and > 10 000 HIV-1 RNA copies/mL received saquinavir hard-gelatin capsules, ritonavir and stavudine. Full (drop in viraemia of > 2 $\log_{10}$ and/or < 500 copies/mL) and partial responders (drop to between 500 and 5000 viraemia copies/mL) at week 9 (end of phase I) entered the second phase (additional 12-month period).

Results

Fifty-six patients completed phase I, 45 (70%) full responders and nine (14%) partial responders by intent-to-treat analysis. Thirty-nine patients completed phase II, 33 (52%) full responders and two (3%) partial responders. Six patients had < 50 HIV-1 RNA copies/mL at week 9, and 20 (31%) patients at month 12 of phase II. Mean CD4 cell counts increased significantly in the 56 patients from 89 to 184 cells/μL after 9 weeks and from 100 to 292 cells/μL in the 39 patients treated for another 12 months. Higher baseline viraemia and lower baseline CD4 cell counts were not associated with an unfavourable virological response at week 9 and month 12 of phase II. HIV DNA in peripheral blood monocytes decreased substantially (– 1.5 $\log_{10}$) but was detectable in all except one patient at the end of phase II.

Conclusion

In protease- and stavudine-naive HIV-infected patients with moderate to severe immunosuppression, saquinavir in combination with ritonavir and stavudine caused a substantial long-term decrease in plasma viral load in approximately half the participants and a substantial increase in CD4 cell counts.

Key words: HIV-DNA, protease inhibitor, ritonavir, saquinavir

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Introduction

The introduction of highly active antiretroviral therapy (HAART) has led to a dramatic decline in disease progression in severely immunosuppressed HIV-infected patients [1–3]. Saquinavir hard gel capsule (SQV) compared to other protease inhibitors (PI) has only limited antiviral effect *in vivo* because of the poor bioavailability of the hard-gelatin formula [4,5–7]. However, results from pharmacokinetic studies have shown that co-administration of ritonavir (RTV) boosts plasma SQV levels as a result of drug–drug interactions in the cytochrome metabolic pathway [8].

The safety and efficacy of antiretroviral combination therapies including RTV and SQV has been investigated in several clinical trials. This combination has been studied, including aspects of quality of life, in PI-naive patients [9–11] and in cohorts of mixed PI-naive and PI-experienced patients [12–14], most of whom were not very immunosuppressed. In addition, this combination has also been studied where previous PI therapy has failed [15–18].

In the present study we investigated the antiviral effect, immunological response and safety of a regimen containing SQV and RTV in combination with stavudine (d4T) in PI-naive patients, with moderate to severe immunosuppression and a high viral load. Hence, inclusion criteria were an initial CD4 count of < 250 cells/μL and a > 10 000 HIV-1 RNA copies/mL. As this was one of the first studies using SQV/RTV in combination with d4T, we observed the initial response over a short period of 9 weeks. If patients tolerated the combination well and responded to therapy, we treated them for a longer period of an additional 12 months. Virological response was evaluated, including assessment of syncytium-inducing (SI) and nonsyncytium-inducing (NSI) strains as well as HIV DNA in peripheral blood monocytes (PBMC).

Methods

Patients

Adult HIV-infected patients between 18 and 65 years within the Swiss HIV Cohort Study (Basel, Bern, Geneva, Lausanne, Lugano, St Gallen, Zurich) were included in this prospective open-label study in 1996 and 1997. They were required to be either antiretroviral naive, with prior therapy unchanged for at least 2 months or after a treatment interruption of more than 2 months. All patients were naive to any PI and to d4T. HIV RNA levels had to be above 10 000 copies/mL and the CD4 count < 250 cells/μL twice within 10 days of inclusion (and 3 days apart). Patients receiving antineoplastic agents, immunmodulators, radiotherapy or foscarnet were excluded from the study. Further exclusion criteria were acute opportunistic infections and a history of severe peripheral neuropathy. Written informed consent was obtained from all patients. The study was conducted in compliance with local ethical committee approval and the principles of the declaration of Helsinki.

Medication

After the baseline assessment, treatment was initiated on day 1, the patients receiving saquinavir mesylate (SQV; Invirase) 400–600 mg twice a day (bid), ritonavir (RTV; Norvir) 400–600 mg bid (dose adjustment for side-effects) and d4T (Zerit) 30–40 mg bid according to body weight. Patients with a body weight of less than 50 kg received 30 mg d4T bid. RTV was given in an escalated manner during the first week (on day 1, 300 mg bid; then a daily increase of 100 mg, up to 600 mg bid). The majority of patients received the higher dosage of each drug. However, the lower dosage was formally instituted in all patients as a protocol amendment following the 20-week results of a study that indicated no additional benefit of the higher dosage [8].

Examinations

After the baseline assessment, patients periodically underwent clinical and laboratory examination on day 1, week 1, week 5 and week 9, which constituted the end of phase I of the study, and in month 6 and month 12 of phase II, which constituted the end of the study. Laboratory tests included CD4 cell counts, CD8 cell counts and HIV RNA (Roche amplicor ultrasensitive assay with a limit of detection of 50 copies/mL; Roche Diagnostic Systems, Basel, Switzerland). Further laboratory examinations included a complete blood count and chemistry (electrolytes, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin, creatinine, pancreatic amylase).

For HIV DNA measurements, two cell preparation tubes (CPT, 8 mL each, Becton Dickinson, Basel, Switzerland) were obtained at baseline, week 9 and the end of phase II. Cells were separated from plasma by centrifugation, 600 ***g*** for 10 min, washed twice with Hank's buffered salt solution. One aliquot containing 5×10^6 cells was stored as a dry cell pellet at – 75°C, 10^7 PBMC were used fresh for qualitative HIV culture and the remaining cells were stored frozen in liquid nitrogen.

Qualitative HIV culture from PBMC and SI/NSI phenotyping was performed using standard procedures described in detail elsewhere [25,26]. Extraction of DNA from PBMCs was performed using the QIAamp blood kit (Qiagen Inc., Santa Clarita, CA, USA) according to the manufacturer's

Table 1 Baseline characteristics of the study population

Number of patients (%)	64 (100)
Mean age, years (SD)	37.9 (9.2)
Male	53
Race	
White	54
Black	4
Hispanic	3
Asian	3
Transmission category	
Men having sex with men	25
Heterosexuals	22
Intravenous drug user	10
Other, unclear	7
Median viral load (log_{10} copies/mL) (IQR)	5.0 (4.6–5.4)
Median CD4 count (cells/μL) (IQR)	87 (25–149)
Median CD8 count (cells/μL) (IQR)	650 (425–958)
Naive to protease inhibitor therapy (%)	64 (100%)
Naive to NRTI therapy (%)	57 (89%)
Mean Karnowsky (range)	94 (70–100)

IQR = interquartile range; NRTI = nucleoside reverse transcriptase inhibitor

instructions. Following this, 1 μg of DNA was used for the polymerase chain reaction (qc-PCR) with five parallel DNA reactions (and one negative control) containing increasing amounts of pSKAN.D18 competitor DNA molecules (4, 20, 100, 500 and 2000 copies). Hot-start PCR conditions were achieved with TaqStart® neutralizing monoclonal antibodies from Clontech (Palo Alto, CA, USA). Then 10 μL each of the amplification products were separated by gel electrophoresis on a 6% polyacrylamide gel followed by ethidium bromide staining. PCR products were visualized by ultraviolet transillumination and images were captured on an Eagle Eye II video system (Stratagene, La Jolla, CA, USA). Densitometric quantification was performed by OneDscan® software (Scanalytics, Billerica, MA, USA) and results were expressed as HIV DNA copies per million CD4 cells.

Genotyping of plasma viral RNA

The entire sequence of the HIV protease gene was sequenced from plasma viral RNA by Professional Genetics Laboratory (Uppsala, Sweden) as described previously [21]. Initial sequencing was carried out to a limit of 600 HIV RNA copies/mL plasma. In the event of failure to detect sequence, a second attempt was made with the limit of detection increased to 60 copies/mL using a proprietary modification of the methodology. Changes from baseline were assessed by the main proteinase residues associated with resistance to SQV or RTV. Forty-two patients were studied at baseline, week 9 and week 61.

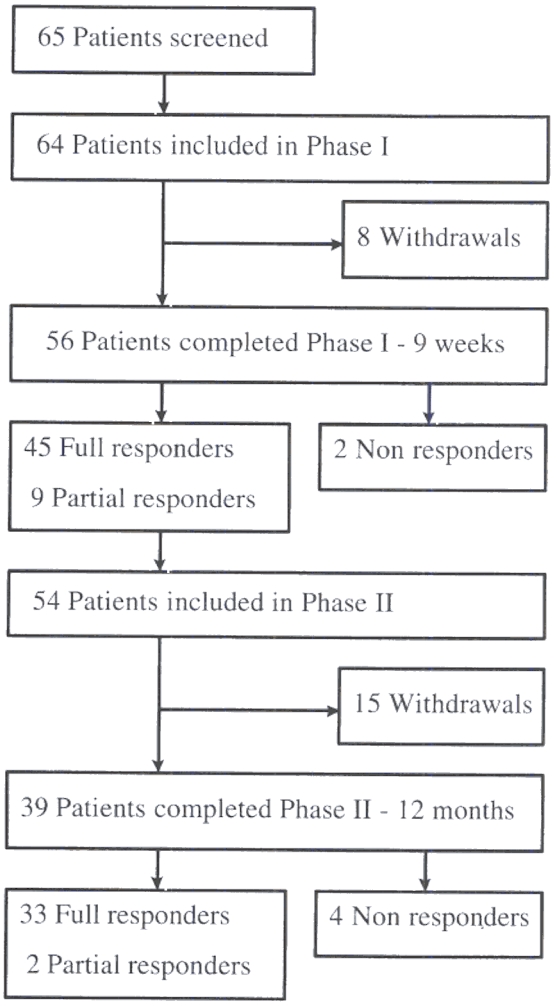

Fig. 1 Study flow diagram. Responders: 2 log_{10} decrease of viraemia or less than 500 HIV RNA copies/mL from any baseline viraemia above 10 000 copies/mL. Partial responders: viraemia 500–5000 HIV RNA copies/mL; nonresponders: < 2 log_{10} decrease of viraemia and > 5000 HIV RNA copies/mL. Phase I lasted 9 weeks, and phase II an additional 12 months.

Statistical analysis

The primary endpoint of the study was the percentage of patients with virological success at week 9 and month 12 of phase II. Patients with a 2 log_{10} decrease in viraemia or less than 500 HIV RNA copies/mL at week 9 were defined as full responders. Patients with > 500 and < 5000 HIV RNA copies/mL were defined as partial responders if the starting viral load at baseline was < 50 000 copies/mL. Patients with < 5000 HIV RNA copies/mL at week 9 were permitted to proceed to phase II of the study. This was the long-term phase, with a further follow-up period of 12 months

Table 2 Drop-outs during phase I and phase II of the study

Drop-out period	Reason for withdrawal	Time of withdrawal	*n*
Phase I (9 weeks)	Adverse events		4
	Asthenia, severe fatigue	Day 4	
	Mycobacterial infection[1]	Week 2	
	Hepatotoxicity (grade III)[2]	Week 2	
	Glomerulonephritis	Week 5	
	Patient request		3
	Lost to follow-up		1
Phase II (12 months)	Adverse events		8
	Nausea	Week 15	
	Hepatotoxicity	Week 16	
	Diarrhoea	Week 17	
	Pancreatic cancer[3]	Week 21	
	Nausea	Week 37	
	Nausea	Week 45	
	Neuropathy	Week 48	
	Neuropathy	Week 52	
	Lost to follow-up		4
	Patient request		2
	Suicide		1

[1] This patient most probably had immune reconstitution mycobacterial disease. [2] This patient with hepatotoxicity (grade III) had a history of prior alcohol abuse, but liver enzymes were not elevated at study entry. [3] After occurrence of pancreatic cancer the history of this patient was investigated and a pancreatic lesion was detected on a computed tomography scan prior to study entry, suggesting prior existence.

(week 61). Additionally, we examined the virological response with a detection limit of below 50 copies/mL.

For comparison of non-continuous variables, the χ^2 test was used, or Fisher's exact test where appropriate. Continuous variables were analysed using the Kruskal–Wallis test. Only if the overall *P* value of more than two groups was significant were group-to-group comparisons performed. These analyses were supplemented with confidence intervals for measurements of plasma HIV-RNA, CD4 cell counts and CD8 cell counts.

Results

Patients

A total of 64 patients were enrolled between November 1996 and November 1997. The demographic characteristics of these patients are shown in Table 1. Only 11% of the patients were not naive to nucleoside reverse-transcriptase inhibitors (NRTI). Fifty-six patients completed week 9 of the study (Fig. 1). During phase I, eight patients discontinued therapy (Table 2). Figure 1 depicts the further follow-up. Fifty-five (86%) patients experienced one or more adverse events, the most frequent being rash, paraesthesia and diarrhoea. Four adverse events occurring during phase I of the study led to study withdrawal (Table 2). Adverse events were also the most frequent reason for study withdrawals during phase II of the study (Table 2). Five AIDS-defining events were reported during the study in five patients; disseminated mycobacteriosis, Kaposi's sarcoma, oropharyngeal candidiasis, extrapulmonary cryptococcosis and esophageal candidiasis. These adverse events could not be correlated to different SQV or RTV dosages.

Virological and immunological response

Forty-five patients were full responders at week 9, and nine patients were partial responders. The percentage of complete and partial responders was thus 70% and 14%, respectively (intent-to-treat analysis). The percentage of complete and partial responders at month 12 (of phase II) was 51% and 3%, respectively. There was a statistically significant reduction in HIV RNA concentrations at week 9 and month 12 (Fig. 2). In the 56 patients who reached week 9 of the study, the median plasma viral load decreased from 5.0 $\log_{10}$ copies/mL (interquartile range, IQR: 4.6–5.4) at baseline to 2.3 $\log_{10}$ copies/mL (IQR: 2.0–2.9). In the 39 patients who completed month 12 of phase II, the median plasma viral load decreased from 5.0 $\log_{10}$ copies/mL (IQR: 4.6–5.4) at baseline to 1.7 $\log_{10}$ copies/mL (IQR: 1.3–2.7). Thirty-six of 56 patients who completed phase I had < 500 HIV-1 RNA copies/mL. Among these, six individuals had an HIV RNA concentration below 50 copies/mL. After completion of phase II, 29 patients (45% of 64 patients) had an HIV RNA below 500 copies/mL and 20 individuals (31%) had an HIV RNA below 50 copies/mL. In addition, four patients were considered as full responders because they had more than a 2 $\log_{10}$ drop in viraemia. Baseline viraemia was statistically not associated with virological response at week 9 and month 12.

There was a statistically significant increase in CD4 cell counts at week 9 and month 12 of phase II, and in CD8 cell counts at month 12, as shown in Fig. 2. In the 56 patients who completed week 9 of phase I, the median CD4 count increased from 89 cells/μL (IQR: 25–158) at baseline to 184 cells/μL (IQR: 103–281). In the 39 patients who completed month 12 of phase II, the median CD4 count increased from 100 cells/μL (IQR: 40–151) at baseline to 292 cells/μL (IQR: 209–440). No association between baseline CD4 cell counts and virological outcomes at month 12 was observed at any time.

We examined the possibility whether pretreatment with NRTI therapy affected outcome. However, seven patients, all treated with zidovudine and lamivudine combination therapy, had a comparable response rate, i.e. three patients had a viral load < 50 copies/mL and one additional patient < 500 copies/mL. Hence, pretreatment did not impact on the response rate as a whole.

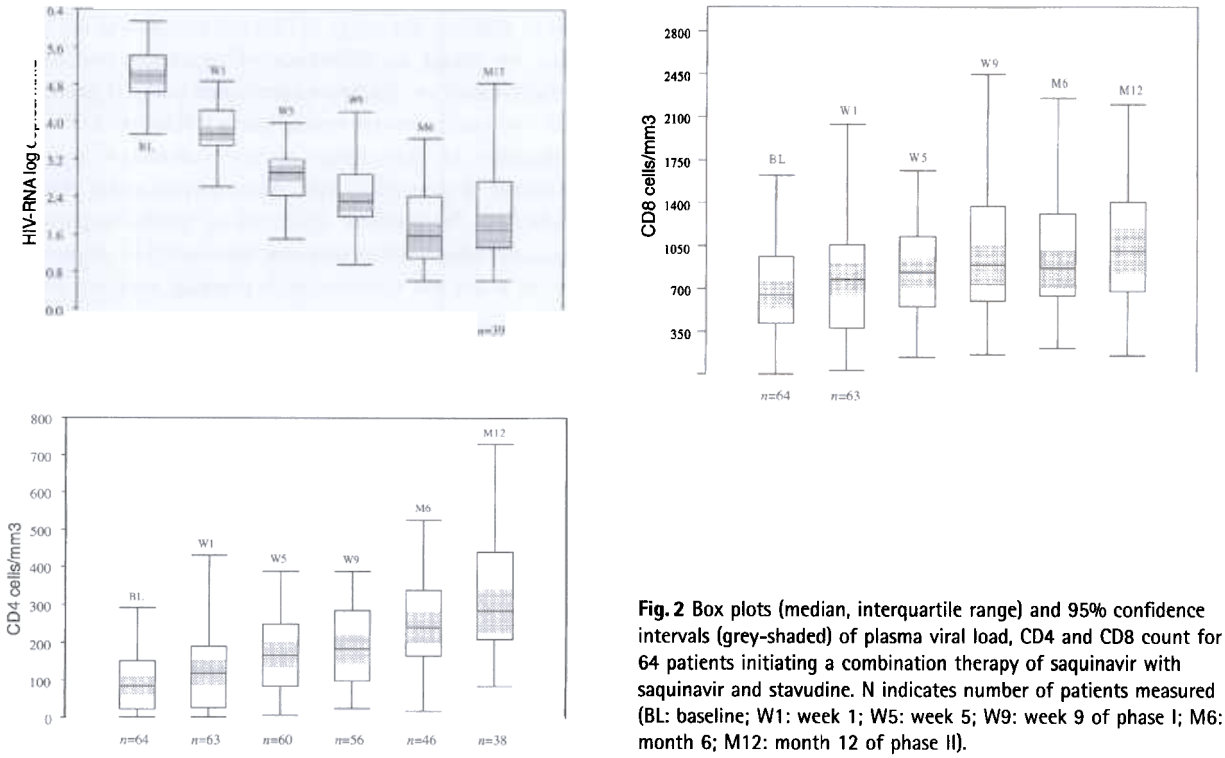

Fig. 2 Box plots (median, interquartile range) and 95% confidence intervals (grey-shaded) of plasma viral load, CD4 and CD8 count for 64 patients initiating a combination therapy of saquinavir with saquinavir and stavudine. N indicates number of patients measured (BL: baseline; W1: week 1; W5: week 5; W9: week 9 of phase I; M6: month 6; M12: month 12 of phase II).

SI/NSI phenotyping and HIV DNA in PBMCs

Syncytium formation was present in 29 patients (45%). The mean DNA concentration tested in 42 patients at baseline was 4.1 $\log_{10}$ copies/million CD4 cells and did not differ by SI/NSI phenotype ($P = 0.2$, t-test). HIV culture from PBMCs was positive at baseline in all 64 patients' cultures (100%). Samples were obtained for HIV culture from 56 and 39 patients who were still on treatment at week 9 and month 12 of phase II (week 61), respectively. Sixteen in 56 (29%) and 31 in 39 (79%) patients had a negative HIV culture at the two time points. Neither RNA or DNA concentration at baseline, nor CD4 count or SI phenotype were predictive for culture negativity during follow-up. A fall in the HIV DNA concentration in CD4 cells was found in 40 and 39 patients at weeks 9 and 61, respectively. HIV DNA was not detectable in PBMCs from only one sample at week 61. The mean drop in the proviral HIV DNA concentration was 0.52 and 1.41 $\log_{10}$ copies per 10^6 CD4 cells, respectively ($P < 0.001$, paired t-test). By multiple linear regression, none of the baseline characteristics (HIV RNA, HIV DNA, CD4 count, SI/NSI phenotype) nor the early response at week 9 (drop in RNA or DNA, culture negativity) was predictive of a viral load response (RNA below 50 or 500 copies/mL) at week 61.

Sequences were successfully obtained from 41 out of 42 plasma samples at baseline (one patient failed to provide a sequence at any time point), from 32/42 samples at week 9 and from 17 out of 42 patient samples at the end of the study (week 61). No mutations were found at baseline. Changes were not observed at the key resistance residues 48 (Gly), 82 (Val) and 90 (Leu) associated with resistance to SQV or RTV. The most significant finding was in one patient with a virus load at week 61 of 655 copies/mL, who developed substitutions I54L, A71V, and I84V, with a sustained baseline polymorphism 63P, after approximately 1 year. This combination of mutations is likely to give rise to resistance to SQV and other PIs. Other less notable changes from baseline were observed in another five patients, in whom polymorphisms previously associated with reduced protease susceptibility in a secondary fashion were observed, particularly at residues 63, 71, 77 and 93.

Discussion

The present study demonstrates that the combination of SQV, RTV and d4T is effective in PI- and d4T-naive, moderately to severely immunosuppressed HIV-infected individuals over the analysed period of 14 months. On an intent-to-treat basis, 70% of participants showed a viral load reduction of at least 2 $\log_{10}$ copies/mL or below 500 copies/mL at 9 weeks, and over half of the patients initially enrolled (52%) had a viral load reduction of at least 2 $\log_{10}$ copies/mL or were below 500 copies/mL at the

end of the entire study period of 14 months. However, only 20 patients (31% intent-to-treat) had a marked reduction in viraemia of below 50 copies/mL.

The combination of SQV and RTV has been studied in naive, PI-naive and PI-experienced patients. Recent studies demonstrate that this combination is highly effective with or without the addition of NRTIs [9–10]. This PI combination was effective in cohorts of mixed PI-naive and PI-experienced patients [12–14]. Conflicting data have been reported on the SQV/RTV combination when given as salvage therapy in PI-experienced patients [15–18]. However, few prospective data exist on PI-naive patients with severe immunosuppression and high viral load, such as examined in this patient population with a median CD4 cell count of 87 cells/μL and a median viral load of 100 000 copies/mL.

The marked decrease in viraemia and the significant increase of CD4 cell counts in our study confirm the results of other studies using a combination of SQV and RTV in PI-naive patients. Kirk *et al.* [22] reported that 89% of antiretroviral drug-naive study participants achieved a viral load below 200 copies/mL while the corresponding figure in drug-experienced patients was 77% at 24 weeks when SQV and RTV 400 mg twice daily with two NRTIs were prescribed. Cameron *et al.* [9] demonstrated that over 80% of PI-naive patients completing the 48 week study had a viral load below 200 copies/mL when an antiretroviral drug regimen including SQV (400–600 mg) and RTV (400–600 mg) was used. The percentage of patients with HIV RNA levels below 500 copies/mL was lower in our study. However, based on CD4 count and HIV RNA levels at baseline, our population was in more immunosuppressed stages of HIV-infection. Indeed, patients in other studies were less immunosuppressed than patients enrolled in our study who had an initial mean CD4 count of less than 100 cells/μL compared to more than 250 cells/μL [9,10]. The mean baseline viral load was also lower in these studies. The response rate measured in an intent-to-treat analysis may be comparable with that of other studies investigating patients with similar characteristics, e.g. the treatment response and durability of a double PI therapy including SQV and RTV were also analysed in an observational cohort and in a community-based study [23,24]. In both studies approximately half of the participants demonstrated a response to therapy, with the plasma viral load decreasing to below the limit of quantification, although CD4 cell counts were significantly higher than in our study. We found no correlation between complete or partial response and baseline CD4 count or baseline viraemia in this study. This lack of correlation may be explained by the fact that not only all patients, by definition, had a CD4 count lower than 250 cells/μL, but that in addition, the range of CD4 cell counts was not large. Also, we found no difference of treatment response in therapy-naive vs. therapy-experienced patients pretreated with nucleoside reverse transcriptase inhibitors. One of the limitations of this study is that adherence was not measured in a specific way. Also, several years into the HAART era the protocol definition of 'partial response' as measured after 9 weeks may not necessarily be of practical use, as it did not correlate with eventual outcome in this study.

SI/NSI phenotype at baseline had no significant effect on the course of the viral load. This is in contrast to a study of Ercoli *et al.* [25] who found a correlation of SI phenotype and poor prognosis in patients receiving antiviral treatment. However, the same authors found no relationship between the development of drug resistance and the progression rate of disease in patients carrying SI strains. HIV DNA in PBMC decreased substantially, confirming the recently obtained results by Andreoni *et al.* [26] who investigated cellular proviral HIV DNA in the very early phase of HIV infection. However, HIV DNA was detectable in all patients but one at week 61.

The significance of the genotypic changes found, except those in one patient, is uncertain with regard to phenotypic alteration in susceptibility to any PI. Differential effects of drugs on the different viral strains making up the quasi-species might drive such changes. Also, these changes demonstrate that even low replication results in genotypic changes in some patients. However, the efficacy of this combination was shown by the fact that even with a low limit of detection between 60 and 600 HIV-RNA copies/mL plasma for sequencing, genotyping was not possible for the majority of 42 patients at the end of the study (week 61).

In conclusion, our study shows that in PI- and d4T-naive HIV-infected patients with moderate to severe immunosuppression, SQV in combination with RTV and d4T results in a substantial long-term decrease of plasma viral load in over half of the participants. As this study was not comparative we cannot conclude that the combination used has advantages over other medications, but our findings support the possible use of this combination as a first-line therapy in advanced HIV infection without prior PI exposure.

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