Ritonavir and saquinavir combination therapy for the treatment of HIV infection

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Objective: To evaluate the safety and antiretroviral activity of ritonavir (Norvir™) and saquinavir (Invirase™) combination therapy in patients with HIV infection.

Design: A multicenter, randomized, open-label clinical trial.

Setting: Seven HIV research units in the USA and Canada.

Patients: A group of 141 adults with HIV infection, CD4 T lymphocyte counts of 100–500 × 10^6 cells/l, whether treated previously or not with reverse transcriptase inhibitor therapy, but without previous HIV protease inhibitor drug therapy.

Interventions: After discontinuation of prior therapy for 2 weeks, group I patients were randomized to receive either combination (A) ritonavir 400 mg and saquinavir 400 mg twice daily or (B) ritonavir 600 mg and saquinavir 400 mg twice daily. After an initial safety assessment of group I patients, group II patients were randomized to receive either (C) ritonavir 400 mg and saquinavir 400 mg three times daily or (D) ritonavir 600 mg and saquinavir 600 mg twice daily. Investigators were allowed to add up to two reverse transcriptase inhibitors (including at least one with which the patient had not been previously treated) to a patient's regimen after week 12 for failure to achieve or maintain an HIV RNA level ≤ 200 copies/ml documented on two consecutive occasions.

Measurements: Plasma HIV RNA levels and CD4+ T-lymphocyte counts were measured at baseline, every 2 weeks for 2 months, and monthly thereafter. Safety was assessed through the reporting of adverse events, physical examinations, and the monitoring of routine laboratory tests.

Results: The 48 weeks of study treatment was completed by 75% (106/141) of the patients. Over 80% of the patients on treatment at week 48 had an HIV RNA level ≤ 200 copies/ml. In addition, intent-to-treat and on-treatment analyses revealed comparable results. Suppression of plasma HIV RNA levels was similar for all treatment arms (mean areas under the curve minus baseline through 48 weeks were

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–1.9, –2.0, –1.6, –1.8 $\log_{10}$ copies/ml in ritonavir–saquinavir 400–400 mg twice daily, 600–400 mg twice daily, 400–400 mg three times daily, and 600–600 mg twice daily, respectively). Median CD4 T-lymphocyte count rose by 128×10^6 cells/l from baseline, with an interquartile range (IQR) of $82–221 \times 10^6$ cells/l. The most common adverse events were diarrhea, circumoral paresthesia, asthenia, and nausea. Reversible elevation of serum transaminases (> 5 × upper limit of normal) occurred in 10% (14/141) of the patients enrolled in this study and was associated with baseline abnormalities in liver function tests, baseline hepatitis B surface antigen positivity, or hepatitis C antibody positivity (relative risk, 5.0; 95% confidence interval 1.5–16.9). Most moderate or severe elevations in liver function tests occurred in patients treated with ritonavir–saquinavir 600–600 mg twice daily.

Conclusions: Ritonavir 400 mg combined with saquinavir 400 mg twice daily with the selective addition of reverse transcriptase inhibitors was the best-tolerated regimen of four dose-ranging regimens and was equally as active as the higher dose combinations in HIV-positive patients without previous protease inhibitor treatment.

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Introduction

HIV protease inhibitors represent an effective class of antiretroviral agents for the treatment of HIV disease [1–5]. The HIV protease is a virus-encoded enzyme that processes viral proteins essential for the maturation of infectious virions [6–8]. Inhibition of the HIV protease yields immature virions incapable of further infectious cycles. Ritonavir and saquinavir are both licensed HIV protease inhibitors with potent *in vitro* inhibition of HIV [9,10].

In a placebo-controlled clinical trial, ritonavir with continued reverse transcriptase inhibitor (RTI) therapy led to a 50% decrease in disease progression and death among patients with advanced HIV disease after a median follow-up of 29 weeks [3]. While saquinavir is a highly potent protease inhibitor *in vitro*, its clinical antiviral activity is limited by low oral bioavailability, 2–9% [11]. Saquinavir has extensive first-pass metabolic clearance, mediated primarily by the intestinal and hepatic cytochrome P450 pathways. Saquinavir 600 mg three times daily in combination with zalcitabine (ddC) was found to decrease mortality and delay the progression of HIV disease compared with that using either agent alone after a median follow-up of 17 months [12]. When given at daily doses of 3600 and 7200 mg, higher plasma levels are achieved that result in greater clinical antiviral activity [13]. A new formulation of saquinavir (Fortovase™) has improved bioavailability and achieves an approximately threefold increase in saquinavir exposure compared with the hard gel formulation (Invirase™) [14].

The combination of ritonavir and saquinavir for the treatment of HIV disease is based on rational pharmacokinetic and virologic principles. First, ritonavir is a potent inhibitor of the cytochrome P450 metabolic pathway and when coadministered with other HIV protease inhibitors, including saquinavir, substantially increases the bioavailability of those agents [15]. In humans, plasma saquinavir levels are increased by more than 20-fold when ritonavir and saquinavir are coadministered; this degree of increased saquinavir exposure is unprecedented [16,17]. This effect on first-pass metabolism improves the pharmacokinetic profile not only by increasing bioavailability but also by allowing the reduction of the dose and dosing frequency of saquinavir. Thus, a thrice daily (or every 8 h) regimen can potentially be reduced to a twice daily regimen. Second, the mutation patterns within the HIV protease gene following monotherapy with either ritonavir or saquinavir suggest that these drugs select for non-overlapping initial mutations [18,19]. Therefore, the use of both drugs simultaneously may decrease the emergence of drug-resistant strains by maintaining suppressive plasma levels of both protease inhibitors. The purpose of this study was to assess the antiretroviral activity, durability, and safety of ritonavir and saquinavir combination therapy at four different dose regimens.

Methods

Study design

Our study was a multicenter, randomized, open-label clinical trial. A randomization schedule with a fixed block size of four was used for each group. Patients were required to discontinue all other antiretroviral medications at least 14 days before initiating the study drug. During the initial enrollment period (April 1996), the protocol was amended to include a pharmacoki-

netic substudy at study week 4 and to increase the sample size to ensure the inclusion of at least 30 patients in each treatment arm with a baseline plasma HIV RNA level > 5000 copies/ml. The protocol was further amended in October 1996 to require patients who were positive for the hepatitis B (HBV) surface antigen or the hepatitis C (HCV) antibody at baseline to reduce their dose to ritonavir 400 mg plus saquinavir 400 mg twice daily. The institutional review board at each institution approved the study and all patients gave written consent.

Study population

To be eligible for participation, patients had to meet all of the following criteria: documented HIV infection, a CD4+ cell count between 100 and 500×10^6 cells/l, Karnofsky score ≥ 70, age ≥ 12 years, exhibit no evidence of an acute illness as documented by vital signs, physical examination, fundoscopic examination, electrocardiogram, and laboratory assessments. They also had to agree not to take any of the medications contraindicated with the study medications during the course of the study. In addition, female patients were required to have a negative pregnancy test and agree to practice a barrier birth control method.

Exclusion criteria included previous treatment with any licensed or investigational HIV protease inhibitor, or an investigational drug within 30 days prior to study drug initiation, pregnant or lactating women, a history of significant drug hypersensitivity, any condition or situation that would preclude compliance with the protocol, active substance abuse, history of acute or chronic pancreatitis, hemoglobin < 8.5 g/dl (85 g/l), absolute neutrophil count $< 1 \times 10^9$ cells/l, platelet count $< 50 \times 10^9$ cells/l, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2.5 times upper limit of normal (ULN), creatinine > 1.5 times ULN, pancreatic amylase above the ULN, or fasting triglyceride > 400 mg/dl (4 g/l).

Treatment regimens

Patients were randomly assigned to receive one of two treatment regimens in group I: 400 mg ritonavir twice daily plus 400 mg saquinavir (hard gel formulation) twice daily (arm A) or 600 mg ritonavir twice daily plus 400 mg saquinavir twice daily (arm B). After group I enrollment was completed and 50% of the patients were treated for ≥ 2 weeks with an acceptable safety profile, a second group of patients was randomly assigned to receive one of two treatment regimens in group II: 400 mg ritonavir three times daily plus 400 mg saquinavir three times daily (arm C) or 600 mg ritonavir twice daily plus 600 mg saquinavir twice daily (arm D).

Ritonavir was supplied as 100 mg semisolid capsules (Norvir™; Abbott Laboratories, Abbott Park, Illinois, USA). Saquinavir was supplied as 200 mg hard gel capsules (Invirase™; Hoffman-LaRoche, Nutley, New Jersey, USA). Study medications were initiated at reduced doses with escalation to the assigned doses by day 14.

Treatment intensification with reverse transcriptase inhibitors

Treating physicians were allowed to add up to two RTI (including at least one with which the patient had not been previously treated) to a patient's regimen after week 12 for failure to achieve or maintain an HIV RNA level ≤ 200 copies/ml documented on two consecutive occasions.

Management of patients with intolerance or adverse events

Adverse events and abnormal laboratory results were graded according to a toxicity rating scale developed by the National Institute of Health-supported AIDS Clinical Trials Group [20]. Patients experiencing clinically significant drug-associated toxicity were required to interrupt study medications until satisfactory resolution. Patients assigned to arms B, C, and D who experienced moderately severe toxicity (defined as an adverse event that causes the patient discomfort and interrupts the patient's usual activities) were permitted to have the ritonavir and/or saquinavir doses reduced by the clinical investigators in a stepwise manner to minimum doses of 400 mg twice daily of both drugs.

Study assessments

Each patient was evaluated at a screening visit, a baseline visit (within 72 h before study drugs were started) and at study weeks 2, 4, 6, and 8, and every 4 weeks thereafter. A 2-week washout period was required before study drugs were begun for patients who were currently taking antiretroviral drugs. Assessments of antiretroviral activity were based on plasma HIV RNA measured using a polymerase chain reaction (PCR)-based assay with a lower limit of quantitation of 200 copies/ml and performed at the Laboratory Corporation of America, Triangle Park, North Carolina, USA [21]. HIV RNA values as low as 0 copies/ml (PCR signal below background) were reported to the clinical investigators. The Roche Ultrasensitive HIV RNA assay with a lower quantitation limit of 50 copies/ml was retrospectively performed at the week 48 visit on samples for which sufficient stored plasma was available. CD4+ and CD8+ T lymphocyte cell counts were also measured at the same time points. Safety of the study regimens was evaluated through medical history and physical examinations, clinical laboratory tests, and the reporting of adverse events. The database was screened for specific medical terms that would indicate signs or symptoms of lipodystrophy or Cushing's syndrome, including lump, hump, moon, adipose, fatty, lipoma, and Cushing's. All

clinical laboratory studies with the exception of viral load assays were performed by Covance Laboratories, Indianapolis, Indiana, USA. Study investigators reviewed and interpreted the results.

Pharmacokinetic substudy

A 12-hour pharmacokinetic profile was performed after the morning dose at week 4 in a subset of patients for each arm. The plasma samples were stored frozen until analyzed using a validated high performance liquid chromatography (HPLC) method with ultraviolet detection at 210 nm [22,16]. The pharmacokinetic parameters of ritonavir and saquinavir were determined using non-compartmental methods. The peak plasma concentration (C_{max}), the minimum plasma concentration (C_{min}) and the time to reach C_{max} (t_{max}) for ritonavir and saquinavir were obtained directly from the observed data. The area under the plasma concentration–time profile (AUC) for a dose interval (AUC_τ) was calculated using the linear trapezoidal rule. The degree of fluctuation (DFL) was calculated as $(C_{max} - C_{min})/(AUC_\tau/\tau)$. Analyses of variance (ANOVA) or covariance (ANCOVA) were performed as appropriate on the ritonavir and saquinavir pharmacokinetic variables with effects for regimen and site. To investigate whether there were differences in ritonavir and saquinavir AUC among the twice daily and three times daily regimens, the observed AUC_τ was normalized to a daily AUC (AUC_{24}) by multiplying the observed AUC_τ with the appropriate dosing frequency.

Cerebrospinal fluid substudy

All patients who had plasma HIV RNA levels below the limit of detection on two consecutive visits after approximately 1 year of ritonavir–saquinavir therapy and did not receive any RTI during the study were eligible to enroll in the CSF HIV RNA substudy. Samples of cerebrospinal fluid (CSF) were frozen and shipped on dry ice to a central laboratory where the Roche Amplicor HIV RNA assay was performed (quantitation limit of 400 copies/ml). The CSF samples were stored frozen until analyzed using a validated HPLC method with UV detection at 210 nm [22,16].

Statistical analysis

The number of patients with plasma HIV RNA levels below the limit of quantitation (i.e., 200 copies/ml) was used as the primary measure of antiretroviral response during the 48 weeks of study therapy. Similarly, the number of patients that experienced adverse events was used as the primary measure of the safety and tolerability of the treatment combination during the study period. Since patients were randomized separately in groups I and II, any between-group comparisons should be considered exploratory in nature. Assuming a type I error of 5% for a two-tailed test, the sample size in this study allows for the detection of a 40% difference between treatment arms with 80% power.

Baseline demographic and clinical characteristics were compared using Fisher's exact test or a one-way ANOVA model to perform within-group comparisons. In addition, Fisher's exact test was used to perform within-group comparisons for the safety and tolerability profiles of the treatment arms, as well as to perform within-group comparisons of the proportion of patients with plasma HIV RNA measurements below 200 copies/ml after 48 weeks of study therapy. The following plasma HIV RNA measurements were analyzed using a one-way ANOVA model to assess within-group differences: baseline, change from baseline, and the time-adjusted AUC minus baseline (AUCMB; computed using the trapezoidal rule). Similar measurements for the CD4 cell count and CD4 : CD8 ratio were analyzed using the Wilcoxon rank–sum test. All statistical (two-sided) tests were performed at the 0.05 level of significance. Activity analyses have excluded measurements after patients discontinued the study. (Note: data have been analyzed as reported for an individual patient. No attempt has been made to estimate missing data.) Activity data were analyzed using an on-treatment approach unless otherwise specified. Safety and tolerability data were analyzed on an intent-to-treat basis.

Results

Patient selection

In seven centers, a total of 213 patients were screened after informed consent was obtained and 141 patients were enrolled into the study from April to August 1996 (Fig. 1). Of the 72 patients that were not enrolled, 49 did not meet the entry criteria and 16 withdrew consent. The remaining seven patients were not enrolled for other reasons. Of the 141 patients enrolled, 71 were enrolled in group I (35 in arm A and 36 in arm B). After an initial safety assessment of group I patients, 70 additional patients were enrolled in group II. During group II enrollment, arm C had noticeably higher intolerance compared with the other treatment arms. Therefore, the final four patients enrolled were assigned to arm D. The final enrollment in group II was 33 in arm C and 37 in arm D.

Baseline demographic and clinical characteristics

Baseline demographic and clinical characteristics were similar within randomization groups (Table 1). Prior treatment with RTI drugs was common (78%), with a median total prior use of two nucleoside analog medications and a median total duration of prior therapy of 28 months. Zidovudine was the most commonly previously used nucleoside drug (n = 104, 74%), followed by didanosine (n = 68, 48%), lamivudine (n = 60, 43%), zalcitabine (n = 44, 31%), and stavudine (n = 29,

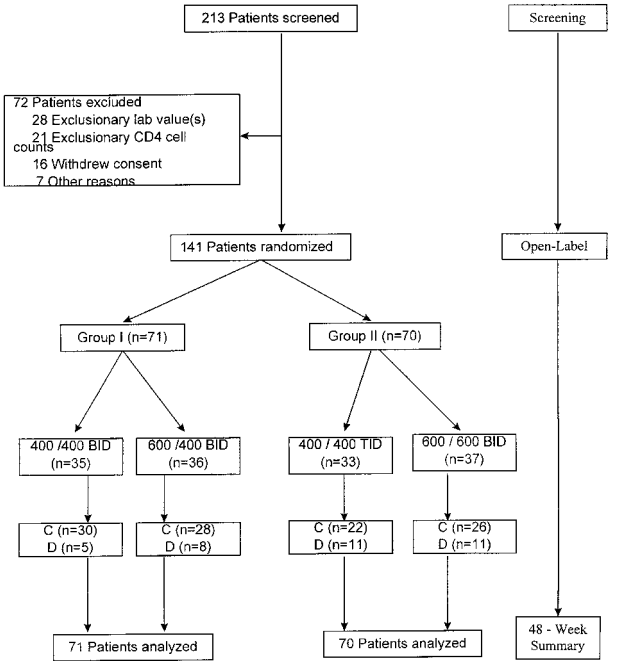

Fig. 1. Study flow diagram. C, continuing study; D, discontinued study.

21%). Baseline serologies were positive for HBV surface antigen or HCV antibody in 17/141 (12%) patients (n = 3, 3, 6, and 5 for arms A, B, C, and D, respectively). No patient was positive for both HBV surface antigen and HCV antibody. The median plasma HIV RNA was 4.63 $\log_{10}$ copies/ml [interquartile range (IQR) 4.1–5.1 $\log_{10}$ copies/ml] and the CD4+ T-lymphocyte count was 273×10^6 cells/l (IQR $200–403 \times 10^6$ cells/l). Two patients in arm B had non-subtype B HIV strains (HIV RNA 0 copies/ml) at baseline; since HIV RNA levels could not be quantitated by the assays used, they were excluded from all plasma HIV RNA analyses.

Patient disposition

Of the 141 patients originally assigned to treatment, 106 (75%) received study therapy for 48 weeks. Twenty-two (16%) of the patients withdrew because of an adverse event, five (4%) withdrew for personal reasons, five (4%) were lost to follow-up, and three (2%) withdrew for other reasons (Table 1). The most common cause of discontinuation for adverse events was related to gastrointestinal symptoms. Of the patients that discontinued because of an adverse event, the lowest frequency (1 of 35) was reported in arm A. In addition, patients in arm C prematurely discontinued study therapy more frequently than patients in arm D, although patients in both arms were to have received the same total daily dosages of the study medication.

Investigators were allowed to reduce the ritonavir and/or saquinavir doses for intolerance. Thirty-two

Table 1. Baseline characteristics and patient disposition by treatment assignment.

	Group I		Group II	
Characteristic	A	B	C	D
Baseline characteristics				
Mean age (years)	39	39	38	39
Men (%)	77	92	91	84
Race or ethnicity (%)				
White	94	81	76	78
Black	3	8	9	14
Hispanic	3	8	12	8
Asian	0	3	3	0
Median duration of previous antiretroviral therapy (months)	27	24	24	33
Median number of previous antiretroviral drugs	2	2	2	2
Baseline median plasma HIV RNA ($\log_{10}$ copies/ml)	4.58	4.72	4.47	4.63
Baseline median CD4+ lymphocyte count ($\times 10^6$ cells/l)	277	264	300	266
Baseline median CD8+ lymphocyte count ($\times 10^6$ cells/l)	802	915	910	901
Baseline median CD4/CD8 ratio	0.29	0.29	0.37	0.30
Patient disposition				
Continuing study	30	28	22	26
On randomized dose	28	17	12	13
On reduced dose*	0	10 (8)	10 (9)	12 (7)
Other†	2	1	0	1
Patients discontinuing therapy	5	8	11	11
For adverse event	1	6	9	6
For personal reasons	3	0	1	1
Lost to follow-up	0	1	1	3
Other	1‡	1§	0	1§
Treatment intensification with reverse transcriptase inhibitors after week 12	8‖	4	5‖**	11‖

Treatment groups divided into four arms: A (n = 35), ritonavir 400 mg twice daily, saquinavir 400 mg twice daily; B (n = 36), ritonavir 600 mg twice daily, saquinavir 400 mg twice daily; C (n = 33), ritonavir 400 mg three times daily, saquinavir 400 mg three times daily; D (n = 37), ritonavir 600 mg twice daily, saquinavir 600 mg twice daily. *Numbers in parentheses are those switching to ritonavir 400 mg twice daily, saquinavir 400 mg twice daily. †Arm A: dose interruption (1), increased ritonavir (1); arm B: for tolerability reasons decreased ritonavir and increased saquinavir; arm D: dose interruption. ‡Protocol violation. §Non-compliance. ‖One patient added reverse transcriptase inhibitor (RTI) in violation of protocol. **One patient added a third RTI because of increasing viral load after initial RTI intensification.

patients who remained on study treatment had dose reductions. The majority of these patients (24/32) had their dose reduced to ritonavir 400 mg twice daily and saquinavir 400 mg twice daily (n = 8, 9, and 7 in arms B, C, and D, respectively). At 48 weeks, 49% (52/106) of the patients continuing on study were taking the 400–400 mg twice daily regimen.

Four patients in arm A were excluded from efficacy analyses after June 1996 because of protocol violations (ritonavir dose increased from 400 mg twice daily to 600 mg twice daily).

Antiretroviral activity

We assessed the antiretroviral activity of each dose combination by measuring changes in $\log_{10}$ plasma HIV RNA levels over time. Profiles for the median HIV RNA at each visit are presented in Figure 2. Results indicate that there was a dramatic decrease from baseline in the median viral load in all arms. The average change from baseline in plasma HIV RNA was evaluated using the time-adjusted AUCMB. The 48-week mean (standard error) AUCMB values were –1.91 (0.112), –2.03 (0.114), –1.57 (0.143), and –1.84 (0.135) $\log_{10}$ copies/ml for arms A, B, C, and D, respectively. No significant difference was detected between arms in either group ($P > 0.05$, ANOVA).

The proportion of patients that experienced a virologic response (plasma HIV RNA < 200 copies/ml) during the 48 weeks of study therapy is illustrated in Figure 3. Overall, a steady increase in the proportion of patients with plasma viremia levels < 200 copies/ml was observed during this time period. At week 48, the proportion of patients with plasma HIV RNA below the limit of quantitation (200 copies/ml) was 89% (25/28) for arm A, 88% (23/26) for arm B, 82% (18/22) for arm C, and 96% (26/27) for arm D. There was no statistically significant difference observed between treatment arms within either group at each study visit ($P > 0.05$, Fisher's exact test).

Most patients with viral RNA levels below the limit of quantitation (< 200 copies/ml) had zero copies/ml (PCR signal below background) HIV RNA; 83% (86/103) of the patients on study at week 48 had viral RNA below background. All patients that were on study at week 48 and with sufficient stored plasma available had their HIV RNA reassayed using the Roche Ultrasensitive assay. Of these patients, 84% (66/79) with HIV RNA levels below the limit of quantitation using the standard assay (< 200 copies/ml) also had HIV RNA levels below the limit of quantitation using the Roche Ultrasensitive assay (< 50 copies/ml).

Using an intent-to-treat analysis in which all patients who discontinued study therapy for any reason were treated as virologic failures, the proportion of patients with plasma HIV RNA below 200 copies/ml at week 48 was 74% (26/35) for arm A, 68% (23/34) for arm B, 55% (18/33) for arm C, and 70% (26/37) for arm D.

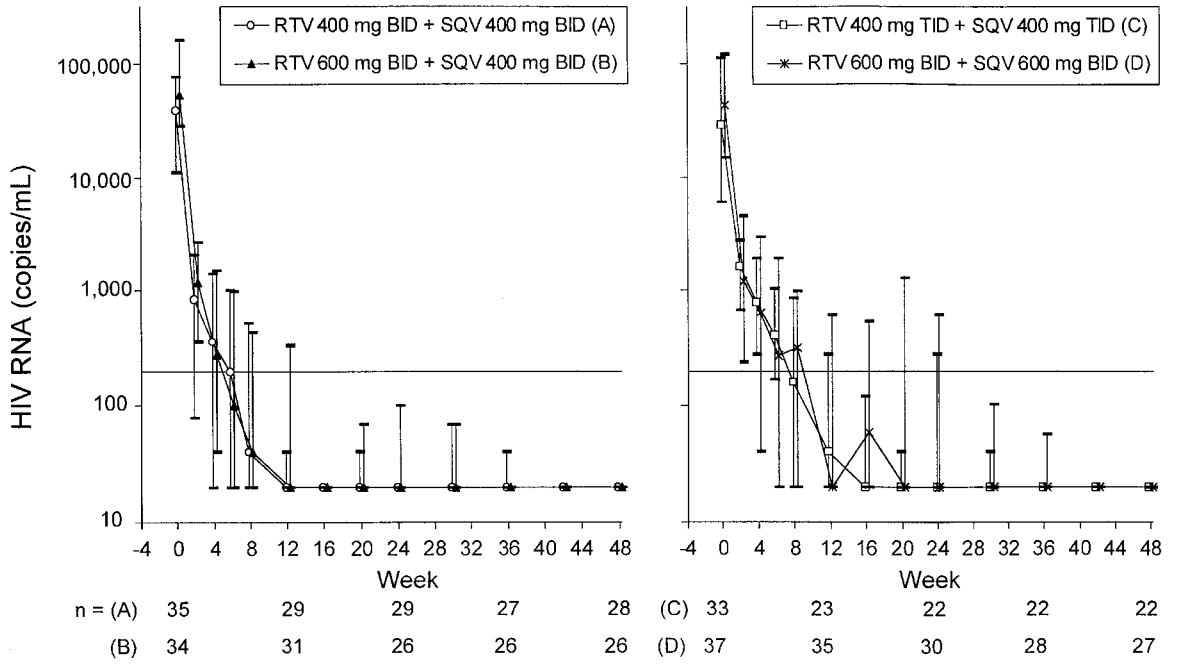

Fig. 2. Median HIV RNA (copies/ml) ± interquartile range by week of study. The horizontal line corresponds to the lower quantitation limit of the assay (200 copies/ml). For descriptive purposes, all HIV RNA measurements were used as reported with the exception of the undetectable values, which were converted to 20 copies/ml (i.e., the lowest possible non-zero value for the assay). BID, twice daily; TID, three times daily; RTV, ritonavir; SQV, saquinavir.

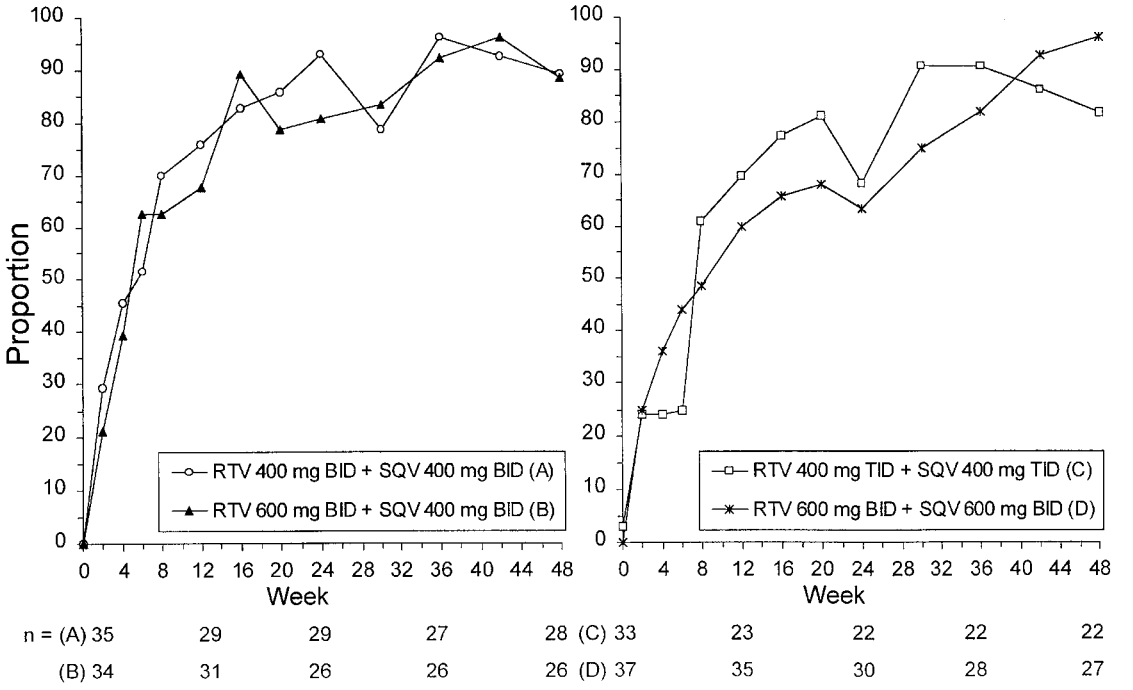

Fig. 3. Proportion of patients with plasma HIV RNA levels below the limit of quantitation by week of study. The number of patients in the study in each treatment group is presented below the time points. BID, twice daily; TID, three times daily; RTV, ritonavir; SQV, saquinavir.

Treatment intensification with reverse transcriptase inhibitors

Patients were allowed to have up to two RTI added to their dual protease regimen by their treating physician after week 12 if they met the criteria for treatment intensification. This happened for 28 of 141 patients (20%) (Table 1). For most of these patients, both RTI were new drugs. Stavudine and lamivudine were the most common pair of RTI added to the regimens (21 of 28). One patient had lamivudine added for the treatment of hepatitis B. One patient had stavudine added, and two patients had nevirapine added to their regimens in violation of the protocol. The median time for RTI addition in the four arms was 26 weeks after the start of the study and the median follow-up after RTI addition was 26 weeks. Two of these patients prematurely discontinued the study prior to the week 48 visit. Of the remaining patients that added an RTI, 22/26 (85%) had viral RNA levels below the limit of quantitation (200 copies/ml) after treatment intensification and continued to have plasma HIV RNA below this limit at week 48. In a supplemental analysis in which patients that added an RTI were considered treatment failures, 71% (20/28), 73% (19/26), 64% (14/22), and 63% (17/27) in arms A, B, C, and D, respectively, had viral RNA < 200 copies/ml at week 48.

Immunologic activity

Immunologic response to each treatment regimen was assessed by measuring changes in T-lymphocyte counts over time. The response of CD4+ T-lymphocyte counts for the 48 weeks of observation is illustrated in Figure 4A. Overall, a steady increase in CD4+ T-lymphocyte count was observed during the study period. In particular, a median increase of 128×10^6 cells/l (IQR 82–221×10^6 cells/l) was observed at the end of the 48-week treatment period. No statistically significant within-group difference was detected at any visit ($P > 0.05$, Wilcoxon rank–sum test) in the CD4+ T-lymphocyte counts except for the comparison of arms A and B at week 20. CD8+ T-lymphocytes increased at week 2, after which counts began to return toward baseline (Fig. 4B). No statistically significant difference in CD8+ T-lymphocytes was found at any time point within group I or II.

Safety

The majority of patients (n = 102, 72%) in this study received more than 336 days (48 weeks) of drug exposure. There was no within-group difference in the duration of drug exposure to ritonavir or saquinavir. The incidence of the most frequently reported adverse events that were of at least moderate severity and of possible, probable, or unknown relationship to study medications is shown in Table 2. A lower incidence of adverse events was generally observed in arm A compared with the other arms; this is reflected in the number of patients discontinuing study medications for adverse events (1, 6, 9, and 6 for arms A, B, C, and D, respectively).

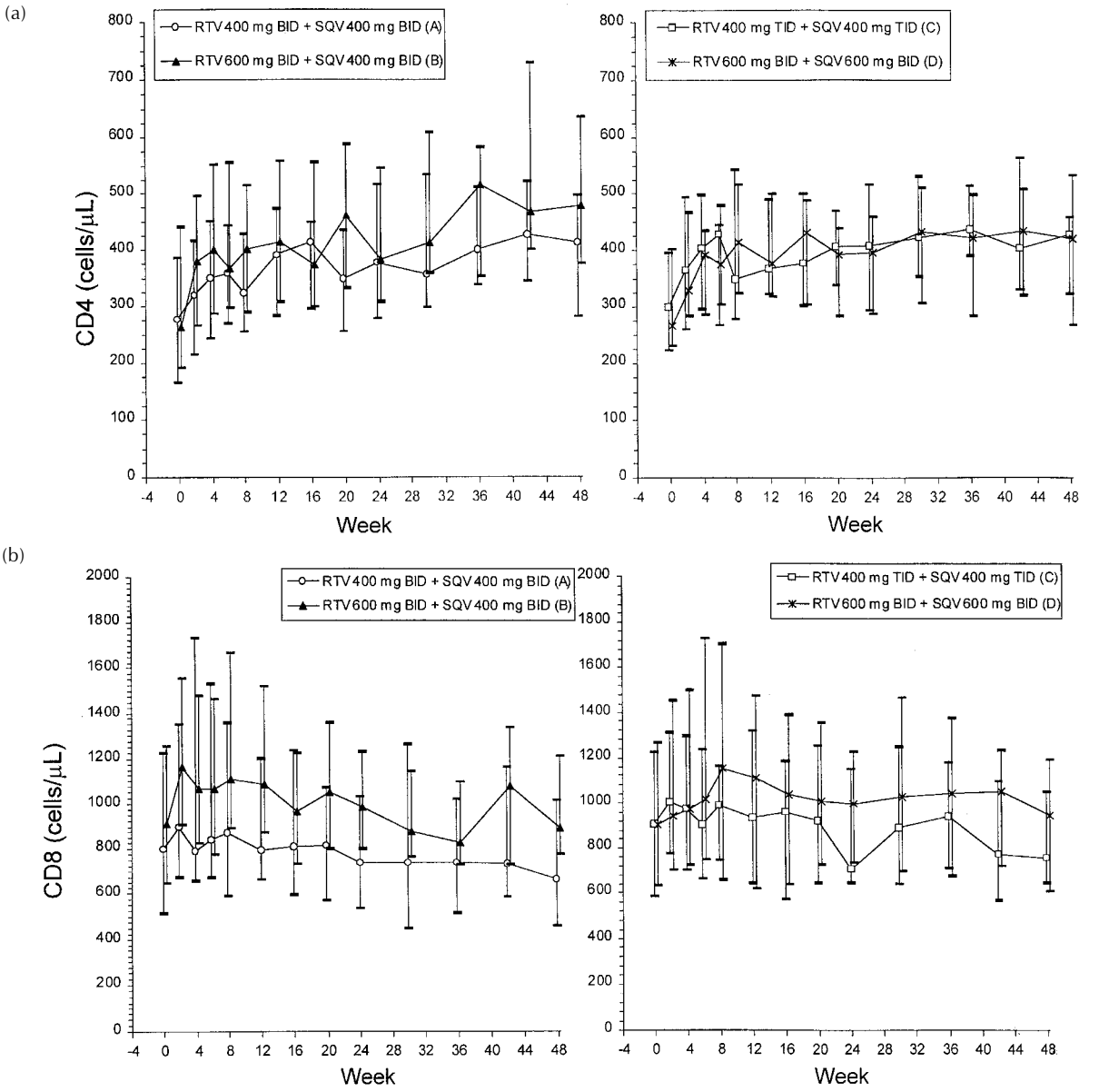

Fig. 4. Changes in T-lymphocytes median values ± interquartile range by week of the study. (a) CD4+ T lymphocytes. (b) CD8+ T lymphocytes.

One patient took a single dose of ergotamine tartrate for migraine headache and developed peripheral vasoconstriction and cyanosis. Ritonavir and saquinavir were interrupted during the acute illness but were subsequently restarted and the patient remained on therapy through 48 weeks without further incident. Subsequently, it was recognized that HIV protease inhibitors interfere with the metabolism of ergotamines, and their use has been contraindicated with this class of drug.

No cases of lumps, humps, or Cushing's syndrome were reported; however, one patient was reported to have developed abdominal lipoma at week 48 of the study.

Laboratory evaluations

Fourteen of the 141 (10%) patients developed grade 3 or 4 (> 5 × ULN) elevations in liver function tests. The majority of these patients (8 of 14) were enrolled to arm D (arms A, B, and C had two cases each). While two cases of elevated liver function tests occurred in arm A, one patient had serologically confirmed acute hepatitis A virus and another patient had the ritonavir dose escalated from 400 mg twice daily to 600 mg twice daily in violation of the protocol prior to the development of liver function abnormalities. Baseline elevations in liver function test(s) above the ULN were seen in 7 of the 14 patients, while 5 of the 14 were HBV surface antigen-positive at baseline and 4 of 14

Table 2. Patients with moderate or severe drug-related events.

Toxicity-related event	Group I		Group II	
	A	B	C	D
At least moderate severity and of possible, probable, or unknown relation to drug(s)*				
Non-site-specific symptoms, n (%)				
Asthenia	2 (5.7)	3 (8.3)	8 (24.2)	10 (27.0)
Gastrointestinal symptoms, n (%)				
Diarrhea	4 (11.4)	11 (30.6)	5 (15.1)	12 (32.4)
Nausea	4 (11.4)	7 (19.4)	4 (12.1)	11 (29.7)
Vomiting	2 (5.7)	2 (5.6)	4 (12.1)	2 (5.4)
Neurologic symptoms, n (%)				
Circumoral paresthesia	1 (2.9)	3 (8.3)	1 (3.0)	4 (10.8)
Depression	1 (2.9)	0 (0.0)	5 (15.1)	4 (10.8)
Dizziness	2 (5.7)	4 (11.1)	3 (9.0)	3 (8.1)
Peripheral parasthesia	1 (2.9)	4 (11.1)	1 (3.0)	2 (5.4)
Grade 3 or 4 toxicity-related laboratory event				
Hepatic transaminase elevation	2[†]	2	2	8
Triglyceride elevation (> 1500 mg/dl)	3	5	3	5
Discontinuation because of adverse events	1	6	9	6

Treatment groups divided into four arms: A (n = 35), ritonavir 400 mg twice daily, saquinavir 400 mg twice daily; B (n = 36), ritonavir 600 mg twice daily, saquinavir 400 mg twice daily; C (n = 33), ritonavir 400 mg three times daily, saquinavir 400 mg three times daily; D (n = 37), ritonavir 600 mg twice daily, saquinavir 600 mg twice daily. *At least 5% among all the patients in the study. [†]Includes one patient that had ritonavir dose escalated to 600 mg twice daily and one patient with serologically confirmed acute hepatitis A.

were HCV antibody-positive at baseline. Two patients with baseline HCV antibody-positive serologies had normal liver function tests at baseline. In 4 of 86 (5%) of the patients who enrolled with normal baseline liver function tests and negative serologies for HBV surface antigen and HCV antibody, grade 3 or 4 elevations in liver function tests developed. An increased risk of developing grade 3 or 4 elevations in hepatic tranaminases was associated with baseline abnormalities in liver function tests, and positive results for baseline HBV surface antigen or HCV antibody [relative risk (RR) 5.0, 95% confidence interval (CI) 1.5–16.9). A liver biopsy was performed in one patient with a history of HBV surface antigen-positivity and abnormal baseline liver function tests. The histopathologic changes were consistent with severe acute hepatitis with widespread immunostaining for HBV surface antigen. After discontinuing study drugs, liver function tests returned to normal.

Patients with asymptomatic triglyceride elevations were allowed to continue treatment at the discretion of the clinical investigator. No patient discontinued the study for elevated triglycerides. Further, no case of hypertriglyceridemia-associated pancreatitis was observed during the 48-week treatment period. Sixteen of 141 (11%) patients developed hypertriglyderidemia [> 1500 mg/dL (> 15 g/l)]. Six of these 16 patients were treated with antihyperlipidemic agents (of the fibrate class), which resulted in a lowering of triglyceride levels in all cases. Increases in mean cholesterol levels above baseline were observed in all treatment arms. Mean increases in cholesterol were between 67 and 83 mg/dL (670–830 mg/l); however, there was no significant difference between arms within either group.

HIV-related events and deaths

A single Center for Disease Control (CDC) AIDS-defining events occurred during the observation period of this study (Kaposi's sarcoma); no deaths were reported. Oral hairy leukoplakia was the most commonly reported HIV-related event and occurred in 12 patients (9%); other events included oropharyngeal candidiasis in seven patients (5%), herpes zoster in three patients (2%), and vulvovaginal candidiasis in one patient.

Pharmacokinetic substudy

A total of 32 patients (7, 11, 4, and 11 from arms A, B, C, and D, respectively) participated the pharmacokinetic study. One patient in arm D did not receive the assigned ritonavir dose during the day that the pharmacokinetic study was performed; therefore, this patient's data were excluded from statistical analysis. Figure 5 shows the ritonavir and saquinavir steady-state plasma concentrations, which exceed the inhibitory concentrations (EC_{50}) for all four dose regimens adjusted for the presence of human serum [23]. The mean values of the pharmacokinetic parameters for the four arms are shown in Table 3. No significant correlation was found between ritonavir and saquinavir pharmacokinetics and body weight, baseline CD4+ and CD8+ T-lymphocyte cell counts, or plasma HIV RNA as a surrogate of disease state.

The AUC, C_{max}, and C_{min} values of ritonavir for the two regimens in group I were approximately proportional with ritonavir dose. Among the regimens with the same daily ritonavir dose of 1200 mg (i.e., arms B, C, D), the AUC_{24} and C_{max} of ritonavir were not significantly different; however, the C_{min} value for arm C (400 mg ritonavir three times a day) was significantly

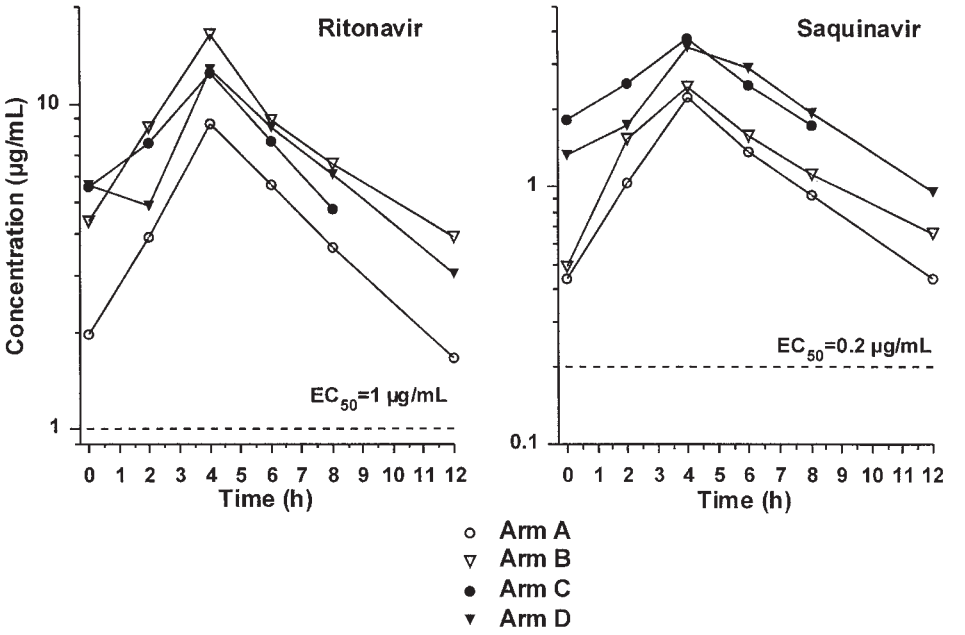

Fig. 5. Ritonavir and saquinavir plasma concentrations at week 4.

Table 3. Pharmacokinetic parameters (mean ± SD) for ritonavir and saquinavir after oral dosing for approximately 4 weeks.

Dosage regimen*	n	AUC_τ (µg × h/ml)	$C_{max\,\tau}$ (µg × h/ml)	$C_{min\,\tau}$ (µg × h/ml)	$t_{max\,\tau}$ (h)	DFL
Ritonavir						
Arm A	7	55 ± 11	9.17 ± 2.53	1.62 ± 0.57	4.9 ± 1.1	1.7 ± 0.5
Arm B	11	103 ± 33	15.13 ± 4.42	3.97 ± 2.68	4.0 ± 0.9	1.4 ± 0.4
Arm C	4	68 ± 23	11.87 ± 3.66	4.05 ± 2.05	3.0 ± 2.0	0.9 ± 0.2
Arm D	10	85 ± 16	12.40 ± 2.62	2.51 ± 0.98	4.6 ± 1.3	1.4 ± 0.4
Saquinavir						
Arm A	7	16 ± 8	2.50 ± 1.03	0.48 ± 0.36	4.9 ± 1.6	1.6 ± 0.4
Arm B	11	20 ± 11	2.83 ± 1.33	0.72 ± 0.67	3.8 ± 0.6	1.3 ± 0.4
Arm C	4	21 ± 3	3.54 ± 0.69	1.49 ± 0.26	4.0 ± 0.0	0.8 ± 0.2
Arm D	10	27 ± 9	3.85 ± 1.31	0.99 ± 0.60	4.2 ± 0.6	1.3 ± 0.3

*Treatment dosages: arm A, ritonavir 400 mg twice daily, saquinavir 400 mg twice daily; arm B, ritonavir 600 mg twice daily, saquinavir 400 mg twice daily; arm C, ritonavir 400 mg three times daily, saquinavir 400 mg three times daily; arm D, ritonavir 600 mg twice daily, saquinavir 600 mg twice daily. AUC_τ, area under the plasma concentration–time profile for a dose interval τ; C_{max}, observed peak plasma concentration; C_{min} observed minimum plasma concentration; t_{max} time to reach C_{max}; DFL, degree of fluctuation,calculated as $(C_{max} - C_{min})/(AUC_\tau/\tau)$.

higher than that of arm D. The AUC_{24}, C_{max}, and C_{min} of saquinavir were not significantly different between the two arms in group I or between the two arms of group II. For the two arms (B and D) that received ritonavir 600 mg twice daily, the AUC, C_{max}, and C_{min} values of saquinavir were approximately proportional with saquinavir dose.

Cerebrospinal fluid substudy

A cross-sectional analysis of CSF HIV RNA was performed in a subset of 15 patients from three clinical centers who signed consent to participate in the substudy. All had been treated with ritonavir and saquinavir alone for at least 48 weeks and had a plasma HIV RNA that was below the limit of detection at two consecutive visits. CSF drug levels were assesssed in 12 patients. As predicted by the highly protein-bound nature of these protease inhibitors [23], none had detectable saquinavir in the CSF [lower quantitation limit of 10-33 ng/ml (10–33 µg/l)] and only five patients had detectable [12–21 ng/ml (12–21 µg/l)] ritonavir concentrations [lower quantitation limit of 10–33 ng/ml (10–33 µg/l)]. Fourteen of the fifteen (93%) patients we evaluated had CSF HIV RNA levels below the limit of quantitation (< 400 copies/ml) at week 48.

Discussion

The combination of ritonavir and saquinavir with selective use of RTI was shown to be a highly potent and durable antiretroviral regimen for up to 48 weeks in more than 80% of the protease inhibitor-naive HIV-

positive patients using an on-treatment analysis. In addition, on-treatment and intent-to-treat analyses gave comparable results. The study population included individuals with CD4+ T-lymphocyte counts of $100–500 \times 10^6$ cells/l, most of whom had been previously treated with an RTI for a median duration of 28 months prior to entry into the study. CD4+ T-lymphocyte counts increased over 100×10^6 cells/l from baseline and to a similar degree across all treatment arms. There was no apparent difference in antiretroviral activity between the four treatment regimens. Most patients who had plasma HIV RNA below the limit of quantitation also had plasma HIV RNA below the detection limit. Because all the treatment arms appeared to perform equally well, we could not determine on the basis of potency or durability one regimen that was significantly better than the others. Nevertheless, the activity of ritonavir–saquinavir compares favorably with other two- and three-drug regimens for the treatment of HIV infection [2,24].

During the course of the study, it became apparent that the ritonavir–saquinavir regimens were highly potent and durable. There was concern, however, that, because of the highly protein-bound nature of ritonavir and saquinavir, drug concentrations would be sufficient to result in HIV suppression in plasma and tissue compartments, but low CSF drug concentrations would result in significant viral replication occurring within the central nervous system. This hypothesis does not take into account the fact that the free concentration of ritonavir and saquinavir available to HIV-infected CD4+ T lymphocytes is likely to be similar to the concentration of drug available to cross the blood–brain barrier. Ritonavir and saquinavir are both highly lipophilic drugs that would be expected to cross the blood–brain barrier and the bilayer lipid membrane of cells. Our cross-sectional study found that nearly all of the patients had HIV RNA below the limit of quantitation in the CSF as well as in plasma. It is important to note that no pre-treatment lumbar punctures were performed as part of the original study design; therefore, our results must be interpreted with caution. Nevertheless, results show that the patients taking ritonavir–saquinavir alone did not have significant persistent CSF viremia in the setting of plasma HIV RNA suppression.

A novel study design was employed whereby patients were treated with the dual protease inhibitor regimen for 12 weeks, after which time the treatment regimen could be intensified with the addition of up to two RTI. While 25% of the patients continuing therapy required the addition of RTI to the dual protease regimen, 75% of the patients did not. The design of the study was intended to allow flexibility for the investigators (or primary-care physicians) in choosing the RTI that they felt was likely to be effective given the unique pre-treatment history of each patient. Notably, frequent monitoring (on a monthly basis) allowed rapid response to virologic failure. These visits provided an opportunity for on-going patient education, management of early adverse events, and sharing of surrogate marker and safety laboratory data with patients, all of which may have improved patient compliance and long-term outcome.

The adverse event profile in this study was similar to that observed in other studies with protease inhibitors in combination with RTI drugs [1,3,12]. Dose reductions appeared to alleviate or reduce the severity of these reactions. During the course of the study, it was recognized that patients with underlying liver abnormalities had a significant risk of developing grade 3 or 4 elevations in hepatic transaminases, particularly those patients receiving higher doses of ritonavir–saquinavir. Subsequently, patients with baseline positive serology for HBV surface antigen or HCV antibody had their dose reduced to ritonavir–saquinavir 400–400 mg twice daily and continued on treatment without interruption. The underlying mechanism for the liver function test elevations among patients with pre-existing liver disease is unclear. The possibilities include exposure to high saquinavir levels not previously achieved in other studies, additive toxicity between ritonavir and saquinavir, or immune reconstitution following initiation of protease inhibitors, as previously reported with other co-infections among patients with HIV infection [25-27]. The excellent tolerability and safety profile combined with the potent and durable antiretroviral activity of the ritonavir–saquinavir 400-400 mg twice daily regimen suggests that this dose is optimal.

A key feature of the ritonavir–saquinavir combination is ritonavir's potent inhibition of the major cytochrome P450 drug-metabolizing enzyme CYP3A. The results of this study confirm previous observations that dose-normalized steady-state saquinavir plasma concentrations were increased more than 20-fold by ritonavir compared with the mean AUC observed in patients receiving saquinavir alone 600 mg every 8 h [28]. As a weak CYP3A inhibitor, saquinavir showed no significant effect on ritonavir pharmacokinetics in this study. A new formulation of saquinavir (Fortovase) increases bioavailability compared with the hard gel formulation (Invirase). When combined with ritonavir, the same dose of either preparation resulted in mean plasma exposures that were not significantly different [29].

In summary, ritonavir plus saquinavir along with the selective addition of RTIs reduces plasma HIV RNA levels to below the limit of quantitation and increases CD4+ T-lymphocyte counts in most protease inhibitor-naive HIV-positive patients treated for up to 48 weeks. While all dose regimens appeared safe and effective, the ritonavir–saquinavir 400–400 mg twice

daily dose was the best tolerated and resulted in surrogate marker improvements that were similar to the higher dose regimens evaluated in this study.

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References

1. Hammer S, Squires K, Hughes M, *et al.*: **A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less**. *N Engl J Med* 1997, **337**:725–733.
2. Gulick RM, Mellors JW, Havlir D, *et al.*: **Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy**. *N Engl J Med* 1997, **337**:734–739.
3. Cameron D, Heath-Chiozzi M, Danner S, *et al.*: **Randomised placebo-controlled trial of ritonavir in advanced HIV-1 disease**. *Lancet* 1998, **351**:543–549.
4. Deeks S, Smith M, Holodniy M, Kahn J: **HIV-1 protease inhibitors**. *JAMA* 1997, **277**:145–153.
5. Kempf D, Rode R, Xu Y, *et al.*: **The duration of viral suppression during protease inhibitor therapy for HIV-1 infection is predicted by plasma HIV-1 RNA at the nadir**. *AIDS* 1998, **12**:F9–F14.
6. Kohl NE, Emini EA, Schleif WA, *et al.*: **Active human immunodeficiency virus protease is required for viral infectivity**. *Proc Natl Acad Sci USA* 1988, **85**:4686–4690.
7. Peng C, Ho BK, Chang TW, Chang NT: **Role of human immunodeficiency virus type 1-specific protease in core protein maturation and viral infectivity**. *J Virol* 1989, **63**:2550–2556.
8. Seelmeier S, Schmidt H, Turk V, von der Helm K: **Human immunodeficiency virus has an aspartic-type protease that can be inhibited by pepstatin A**. *Proc Natl Acad Sci USA* 1988, **85**:6612–6616.
9. Roberts NA, Martin JA, Kinchington D, *et al.*: **Rational design of peptide-based HIV proteinase inhibitors**. *Science* 1990, 248:358–361.
10. Kempf D, Marsh KC, Denissen J, *et al.*: **ABT-538 is a potent inhibitor of human immunodeficiency virus protease and has high oral bioavailability in humans.** *Proc Natl Acad Sci USA* 1995, **92**:2484–2488.
11. Collier AC, Coombs RW, Schoenfeld DA, *et al.*: **Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group**. *N Engl J Med* 1996, **334**:1011–1017.
12. Lalezari J, Haubrich R, Burger H, *et al.*: **Improved survival and decreased progression of HIV in patients treated with saquinavir plus zalcitabine**. *XI International Conference on AIDS*. Vancouver, British Columbia 1996, LB.B.6033.
13. Shapiro J, Winters M, Stewart F, *et al.*: **The effect of high-dose saquinavir on viral load and CD4+ T-cell counts in HIV-infected patients**. *Ann Int Med* 1996, **124**:1039–1050.
14. Fortovase™ package insert. Roche Laboratories, Inc., 1998.
15. Kempf D, Marsh K, Kumar G, *et al.*: **Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by coadministration with ritonavir**. *Antimicrob Agents Chemother* 1997, **41**:654–660.
16. Hsu A, Granneman G, Cao G, *et al.*: **Pharmacokinetic interactions between two HIV-protease inhibitors, ritonavir and saquinavir.** *Clin Pharmacol Ther* 1998, **63(4)**:453–464.
17. Merry C, Barry MG, Mulcahy F, *et al.*: **Saquinavir pharmacokinetics alone and in combination with ritonavir in HIV-infected patients**. *AIDS* 1997, **11**:F29–F33.
18. Molla A, Korneyeva M, Gao Q, *et al.*: **Ordered accumulation of mutations in HIV protease confers resistance to ritonavir**. *Nat Med* 1996, **2**:760–766.
19. Jacobsen H, Haenggi M, Ott M, Duncan I, Vella S, Mous J: **In vivo resistance to HIV protease inhibitor saquinavir – an update**. *AIDS Res Hum Retroviruses* 1995, **11**:S169.
20. Division of AIDS: *Division of AIDS table for grading severity of adult adverse experiences 1996*. National Institute of Allergy and Infectious Diseases, Rockville, MD.
21. Mulder J, McKinney N, Christopherson C, Sninsky J, Greenfield L, Kwok S: **Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection**. *J Clin Microbiol*. 1994, **32**:292–300.
22. Marsh KC, Eiden E, McDonald E: **Determination of ritonavir, a new HIV protease inhibitor, in biological samples using reversed-phase high-performance liquid chromatography**. *J Chromatogr* 1997, **704**:307–313.
23. Molla A, Granneman G, Sun E, Kempf D: **Recent developments in HIV protease inhibitor therapy**. *Antiviral Res* 1998, **39**(1):1–23.
24. Hammer SM, Katzenstein DA, Hughes MD, *et al.*: **A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. AIDS Clinical Trials Group Study 175 Study Team**. *N Engl J Med* 1996, **335**:1081–1090.
25. Race E, Adelson-Mitty J, Kriegel G, *et al.*: **Focal mycobacterial lymphadenitis following initiation of protease-inhibitor therapy in patients with advanced HIV-1 disease.** *Lancet* 1998, **351**:252–255.
26. Carr A, Cooper D: **Restoration of immunity to chronic hepatitis B infection in HIV-infected patient on protease inhibitor**. *Lancet* 1997, **349**:996–997.
27. Rutschmann O, Negro F, Hirschel B, Hadengue A, Anwar D, Perrin L: **Impact of treatment with human immunodeficiency virus (HIV) protease inhibitors on hepatitis C in patients coinfected with HIV.** *J Infect Dis*. 1998, **177**:783–785.
28. Invirase™ package insert. Roche Laboratories, Inc., 1998.
29. Buss N, on behalf of the Fortovase Study Group: **Saquinavir soft gel capsule (Fortovase): pharmacokinetics and drug interactions**. *5th Conference on Retroviruses and Opportunistic Infections,* Chicago, IL, 1998: Abstract 354, p.145.