Clinical Impact of Double Protease Inhibitor Boosting with Lopinavir/Ritonavir and Amprenavir as Part of Salvage Antiretroviral Therapy

Mona Loutfy, MD, MPH,[1] Janet Raboud, PhD,[2,3] Courtney Thompson,[3] Alice Tseng, PharmD,[3] Zainab Abdurrahman,[4] Colin Kovacs, MD,[2,5] Anita Rachlis, MD, MEd,[2,6] Elizabeth Phillips, MD,[2,6] Gary Rubin, MD,[5] Kevin Gough, MD,[2,7] and Sharon Walmsley, MD, MSc[2,3]

[1]Immune Deficiency Treatment Centre, Montreal General Hospital, McGill University, Montreal, Quebec; [2]Department of Medicine, University of Toronto, Toronto, Ontario; [3]Division of Infectious Diseases, University Health Network, Toronto, Ontario; [4]Department of Statistics and Actuarial Science, University of Waterloo, Waterloo, Ontario; [5]Canadian Immunodeficiency Research Collaborative, Toronto, Ontario; [6]Division of Infectious Diseases, Sunnybrook and Women's College Health Sciences Centre, Toronto, Ontario; [7]St. Michael's Hospital, Toronto, Ontario, Canada

Purpose: Double protease inhibitor (PI) boosting is being explored as a new strategy in salvage antiretroviral (ARV) therapy. However, if a negative drug interaction leads to decreased drug levels of either or both PIs, double PI boosting could lead to decreased virologic response. A negative drug interaction has been described between amprenavir (APV) and lopinavir/ritonavir (LPV/r). This observational cohort study assessed the virologic impact of the addition of APV to a salvage ARV regimen, which also contains LPV/r, compared to a regimen containing LPV/r alone. **Method:** Patients initiated on a salvage ARV regimen that included LPV/r obtained from the expanded access program in Toronto, Canada, were evaluated. APV (600–1,200 mg bid) was added at the discretion of the treating physician. **Results:** Using multivariate Cox proportional hazards models, we found that the addition of APV to a LPV/r-containing salvage regimen was not significantly associated with time to virologic suppression (< 50 copies/mL; adjusted hazard ratio [HR] = 0.75, $p = .12$) or with time to virologic rebound (adjusted HR = 1.46, $p = .34$). Those patients who received higher doses of APV had an increased chance of virologic suppression ($p = .03$). In a subset of 27 patients, the median LPV C_{trough} was significantly lower in patients receiving APV ($p = .04$), and the median APV C_{trough} was reduced compared to reported controls. **Conclusion:** Our data do not support an additional benefit in virologic reduction of double boosting with APV and LPV/r relative to LPV/r alone in salvage ARV therapy. Our study's limitations include its retrospective nature and the imbalance between the two groups potentially confounding the results. Although these factors were adjusted for in the multivariate analysis, a prospective randomized controlled trial is warranted to confirm our findings. **Key words:** *amprenavir, lopinavir/ritonavir, salvage therapy*

Antiretroviral (ARV) treatment failure is a common and significant problem in human immunodeficiency virus (HIV) disease, and as many as 50% of previously ARV-naïve patients have detectable plasma HIV RNA after 1 year despite combination therapy.[1] The choice of drugs in a salvage regimen to manage treatment failure is guided by prior ARV drug history and resistance testing and often exploits pharmacokinetic (PK) interactions by boosting protease inhibitor (PI) trough levels with low-dose ritonavir.[2] A new strategy under evaluation in heavily ARV-experienced patients is the use of double PI boosting, in which ritonavir is used to simultaneously boost the levels of two PIs. Although randomized studies are lacking, small pilot studies have shown that double boosting with saquinavir (SQV) and lopinavir/ritonavir (LPV/r)

For correspondence or reprints contact: Sharon Walmsley, 200 Elizabeth Street, EN-219, Toronto, Ontario Canada, M5G 2C4. Email: sharon.walmsley@uhn.on.ca

HIV Clin Trials 2003;4(5):301–310

www.thomasland.com

resulted in a good chance of virologic response, which suggests an additive response.[3–7] Another double PI boosting combination that has been used in salvage ARV therapy is amprenavir (APV) and LPV/r. Although there have been many in vitro studies on the PK interaction of APV and LPV/r, there have been no data on the virologic response or clinical outcome of double boosting with these two PIs.[8–11] The PK studies on the drug interaction between LPV/r and APV are conflicting, which results in a poor understanding of how best to use these two PIs in combination in clinical practice.[8–11] The in vitro data suggest that both LPV and APV can induce cytochrome p450 3A4 enzyme activity, providing a mechanism by which each PI lowers the level of the other when used in combination; this potential two-way drug interaction could result in a poorer virologic response.[8–11]

The PK studies have shown that APV minimum concentration (C_{min}) is either lowered by 33%–81% or is unchanged when combined with LPV/r.[8–18] Some studies have shown that LPV levels were decreased by simultaneous use with APV, whereas other studies found that LPV levels were not lowered by APV.[8,13–18] Given these data, it remains unclear whether the doses of the two PIs should be increased when used in combination.

We report a retrospective observational cohort analysis of the virologic response and clinical outcome of the combination of LPV/r and APV compared to the use of LPV/r as the sole PI as part of a salvage regimen in a group of ARV-experienced HIV-infected patients with virologic failure. When controlling for important confounders, we did not find that double PI boosting with APV and LPV/r increased the rate of virologic suppression or decreased the rate of virologic rebound compared to the use of LPV/r alone in salvage therapy. Our data do not support an additional clinical benefit of double boosting with APV and LPV/r relative to LPV/r alone in salvage ARV therapy.

METHOD

Patient Population and Data Collection

The patient population included HIV-infected patients enrolled in the LPV/r expanded access program (EAP) from two primary and three tertiary care HIV centers in Toronto, Ontario, Canada. Patients were considered eligible for this cohort study if they had previously received ARV therapy, were experiencing treatment failure defined as a plasma viral load (VL) ≥ 50 copies/mL (Chiron 3.0; Chiron Corp., Emeryville, California, USA) on at least two consecutive occasions at least 1 month apart, were switching to a salvage regimen that included LPV/r, and had follow-up laboratory evaluation for at least 1 month after the initiation of salvage therapy. The other ARV agents and doses in the patients' salvage regimen were chosen at the discretion of the treating physician and could be from all available classes. The dose of LPV/r used was 400/100 mg bid but was increased to 533/133 mg bid when a nonnucleoside reverse transciptase inhibitor (NNRTI) was used in the regimen. The dose of APV ranged from 600–1,200 mg bid and was chosen at the discretion of the physician. Eligible patients were divided into two groups based on whether APV was or was not included in the salvage regimen for analysis.

Charts of eligible patients entering the LPV/r EAP from February 1, 2000, to March 31, 2001, were reviewed with follow-up to January 15, 2002. Demographic, laboratory, virologic, and immunologic factors were recorded. Demographic variables included date of birth, gender, risk factor for acquisition of HIV, duration of HIV infection, and ARV treatment history. Laboratory variables included baseline plasma VL (Chiron 3.0), baseline CD4 cell count, and the results of viral genotypic resistance testing (Antivirogram; Virco, Mechelen, Belgium) if performed. The ARV agents and doses in the patients' salvage regimen were recorded. All follow-up CD4 cell counts and plasma VLs (Chiron 3.0) were recorded, and their frequency was at the discretion of the treating physician; typically they were performed 1 month after initiation of the salvage regimen and then every 3 months. The disposition of the patients at the end of the follow-up was determined including survival, development of an AIDS-defining illness, discontinuation or switching of ARV drugs, reasons for discontinuation, and drug toxicity. All the data were double entered into an Access database (Microsoft Corp.).

Statistical Analysis

All statistical analyses were performed using SAS Version 8.2 statistical software (SAS Institute, Cary, North Carolina, USA). Characteristics were compared between the two groups of patients,

those receiving and those not receiving APV. Categorical variables were compared using Fisher exact test, and the medians of continuous variables were compared using Wilcoxon rank sum test. A p value of $< .05$ was considered to be statistically significant.

The goal of the primary analysis was to determine the impact of the presence of APV in the LPV/r-containing salvage regimen on time to virologic suppression (plasma VL < 50 copies/mL), which was defined as the number of months from starting the salvage regimen to the first month when the plasma VL was < 50 copies/mL. Participants who never achieved virologic suppression were censored at the month of last follow-up. Cox proportional hazards models were used to evaluate the effect of each potential confounder on time to virologic suppression. The final multivariate model was selected based on stepwise elimination and clinical significance. The assumptions of the Cox proportional hazards model were tested for each variable. Kaplan-Meier curves, stratified by NNRTI experience, were plotted for the two groups. Stratification by NNRTI experience was chosen, because it had the most significant effect on VL suppression. Because the current use of an NNRTI in the salvage regimen was collinear with NNRTI experience and did not alter the analysis, it was not included as an additional stratification variable. The median times to virologic suppression for the two groups were compared using the log rank test.

The second analysis used Cox proportional hazards models to identify predictors of time to virologic rebound among patients who achieved virologic suppression. Time to virologic rebound was defined as the number of months from the first plasma VL < 50 copies/mL to the next plasma VL ≥ 50 copies/mL. Virologic suppression was considered to be maintained if the plasma VL rose above 50 copies/mL at a single visit and returned to < 50 copies/mL by the next sampling. If the plasma VL rose above 50 copies/mL at two consecutive samplings, then virologic rebound was said to have occurred. If patients experienced more than one period of virologic suppression during the study, then the duration of the longer period was used in the analysis. Patients who did not experience virologic rebound during the study period were censored at the month of last follow-up.

The third analysis examined the effect of the dose of APV (600 to 1,200 mg po bid) on the probability of ever achieving virologic suppression (HIV RNA < 50 copies/mL) during the study period using the chi-square test for trend. Stepwise logistic regression was used to adjust for important confounders including present NNRTI use and baseline VL.

Pharmacokinetic Analysis

Patients from two of the tertiary care centers were eligible for therapeutic drug monitoring (TDM), and 27 accepted based on patient willingness and convenience. Pre-dose plasma drug levels of the PIs were obtained for these 27 patients who had been on their salvage therapy for at least 2 weeks. Details of the salvage ARV regimen including LPV/r and APV doses and use of NNRTIs were recorded. The pre-drug levels were collected just prior to observed doses of PIs. Samples were spun frozen at –80°C until analysis. Concentrations of ritonavir, APV, and LPV were simultaneously measured in plasma by a validated high performance liquid chromatography with ultraviolet detection (HPLC-UV).[19] The median LPV trough concentrations were compared between the patients on a salvage regimen containing LPV/r alone (10 patients) or LPV/r and APV in combination (17 patients) using the Wilcoxon rank sum test. The median ritonavir trough concentrations were also assessed. The median APV trough concentrations in the LPV/r and APV combination group were compared to reported historical controls. The relationship between virologic response and APV and LPV trough concentrations was assessed using Wilcoxon rank sum test. The median LPV and median APV trough concentrations were compared for patients who did and did not ever achieve viral suppression < 50 copies/mL. Multivariate logistic regression was carried out adjusting for baseline plasma VL and NNRTI use, because NNRTI use affects the dose and drug levels of both PIs.

RESULTS

Patient Characteristics

During the study period, 328 patients were enrolled in the Toronto LPV/r EAP. Seventy-four patients were excluded from the analysis for the following reasons: 3 patients were previously ARV

Table 1. Baseline characteristics of patient groups

Characteristic	LPV/r-only group	LPV/r + APV group	*p*
n	154	100	
Age[a]	40.7 (36.3–46.4)	42.8 (38.2–48.7)	.03
Male	143 (93%)	95 (95%)	.27
Caucasian	127 (83%)	87 (87%)	.33
MSM	107 (70%)	70 (70%)	.93
IVDU	6 (4%)	5 (5%)	.76
AIDS diagnosis	64 (42%)	49 (49%)	.23
Years on ARVs	5.7 (4.4–9.1)	7.5 (5.6–10.3)	<.001
Previous ARVs	7 (5– 9)	9 (7–10)	<.0001
Previous PIs[a]	2 (2–3)	3 (3–4)	<.0001
Previous NNRTI use[a]	74 (48%)	79 (79%)	<.0001
PI mutations[a]	5 (2–6)	6 (4–7)	<.01
RT mutations[a]	4 (2–7)	7 (5–9)	<.0001
Current NNRTI use	106 (69%)	51 (51%)	.004
Baseline log VL[a]	4.7 (3.8–5.2)	4.7 (4.2–5.3)	.19
Baseline CD4[a]	171 (50–280)	137 (48–242)	.19

Note: LPV/r = lopinavir/ritonavir; APV = amprenavir; MSM = men who have sex with men; IVDU = intravenous drug users; ARVs = antiretrovirals; PI = protease inhibitor; RT = reverse transciptase; NNRTI = nonnucleoside reverse transcriptase inhibitor; VL = viral load; CD4 = CD4 cell count.

[a]Median (interquartile range).

naïve, 36 patients had baseline plasma HIV RNA levels < 50 copies/mL, and 35 patients had no follow-up data available. The study sample consisted of 254 patients, including 100 in the double PI (APV and LPV/r) group and 154 in the LPV/r-only group. The baseline characteristics of the two groups are presented in **Table 1**. Genotypic resistance testing was done in 162 patients. The two groups were similar with respect to age, gender, race, HIV risk factor, baseline plasma VL, and baseline CD4 count. Patients were followed for a median of 9 months (interquartile range [IQR] 5–12) and had a median of four follow-up plasma VL measurements (IQR 3–6). The patients in the APV group were more ARV-experienced as evident by a longer duration on ARV therapy, a greater number of prior ARV drugs and PIs, an increased number of baseline reverse transciptase (RT) and PI mutations, and a higher proportion with NNRTI experience. A higher proportion of patients in the LPV/r-alone group had an NNRTI included in their salvage regimen. Both groups received a median of two nucleoside reverse transciptase inhibitors (NRTIs) in the salvage regimen, which were mostly recycled with a median of one new NRTI (p = .99). None of the patients received tenofovir.

Virologic Suppression

One hundred and forty-four of 254 (56.7%) patients achieved HIV RNA suppression (HIV RNA < 50 copies/mL): 45/100 (45%) of the double PI group and 99/154 (64%) of the LPV/r-only group. The crude Kaplan-Meier curves comparing the two groups stratified by NNRTI experience are shown in **Figure 1**. The unadjusted median time to virologic suppression for the combination group was 12 months (95% CI 7,17 months); for the LPV/r-only group, it was 5 months (95% CI 4, 5 months) (log rank p = .003). Other variables including current use of an NNRTI, number of previous ARVs or PIs, and number of RT and PI mutations were not included as additional stratification variables, because they were collinear with NNRTI experience but they were examined in Cox proportional hazards models.

The univariate analyses identified several im-

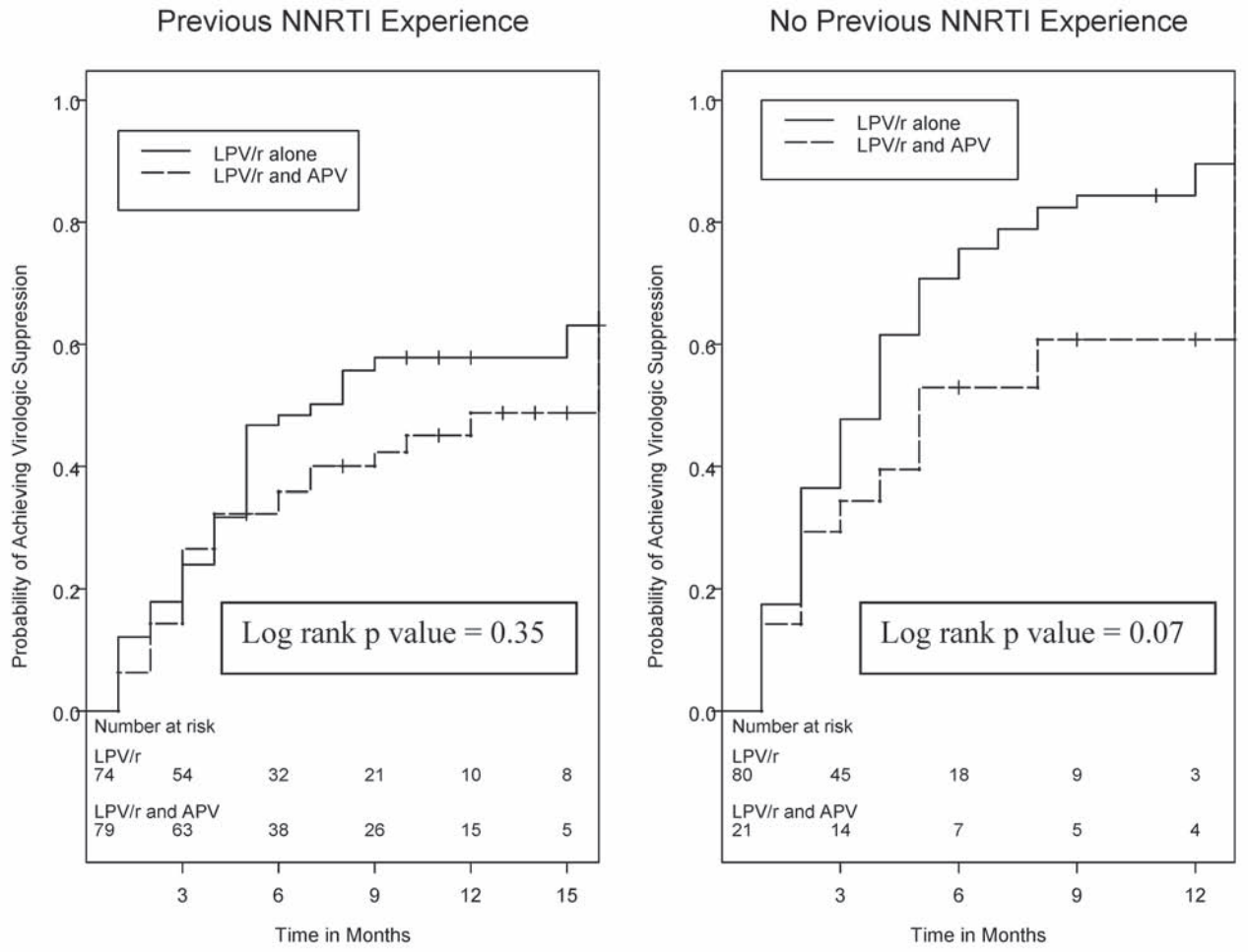

Figure 1. Kaplan-Meier curves of probability of patients with virologic failure achieving a plasma HIV plasma viral load (VL) of < 50 copies/mL according to the presence of amprenavir (APV) in patients taking lopinavir/ritonavir (LPV/r)-containing salvage regimens stratified by nonnucleoside reverse transcriptase inhibitor (NNRTI) experience.

portant predictors of virologic suppression, including the number of previous ARV drugs, number of previous HAART regimens, NNRTI experience, number of baseline PI mutations, baseline plasma VL, and baseline CD4 count (**Table 2**). The crude hazard ratio for the presence of APV in the salvage regimen was 0.61 (95% CI 0.43, 0.87; $p = .01$). After adjusting for baseline plasma VL and NNRTI experience in a multivariate Cox regression model, we found that the presence of APV in the salvage regimen was not significantly associated with time to virologic suppression (HR = 0.75; 95% CI 0.52, 1.08; $p = .12$). The adjusted hazard ratios for baseline plasma VL and previous NNRTI use were 0.68 per $\log_{10}$ copies/mL (95% CI 0.58, 0.80; $p < .0001$) and 0.60 (95% CI 0.42, 0.85; $p = .004$), respectively. Other clinically important covariates such as number of previous ARV drugs, previous number of HAART regimens, number of baseline RT and PI mutations, baseline CD4 count, and AIDS diagnosis were not statistically significant in multivariate models and did not alter the hazard ratio of APV and were therefore not included in the final multivariate model.

Virologic Rebound

The 144 patients who achieved virologic suppression during the study period were included in the secondary analysis. Thirty-four (24%) patients experienced virologic rebound during the study period: 17/45 (38%) of the double PI group and 17/

Table 2. Univariate Cox proportional models with time to plasma VL < 50 copies/mL as the outcome

Covariate	Hazard ratio	95% CI	*p*
APV in salvage regimen	0.61	0.43, 0.87	.01
Age	1.01	0.99, 1.03	.44
Female	0.97	0.66, 1.40	.04
Caucasian	0.89	0.57, 1.41	.63
MSM	0.91	0.64, 1.30	.61
IVDU	0.87	0.35, 2.12	.75
AIDS diagnosis	0.73	0.52, 1.02	.06
Years on ARVs	0.95	0.90, 1.01	.07
No. of previous ARV drugs	0.87	0.81, 0.93	<.0001
No. of previous PIs	0.82	0.72, 0.94	.004
Previous NNRTI use	0.48	0.34, 0.66	<.0001
No. of PI mutations	0.89	0.81, 0.98	.01
Baseline $\log_{10}$ VL	0.64	0.54, 0.75	<.0001
Baseline CD4 (/100 cells/μL)	1.16	1.06, 1.27	.001
Current NNRTI in regimen	1.55	1.12, 2.15	.01

Note: APV = amprenavir; MSM = men who have sex with men; IVDU = intravenous drug users; ARV = antiretroviral; PI = protease inhibitor; NNRTI = nonnucleoside reverse transcriptase inhibitor; VL = viral load; CD4 = CD4 cell count.

99 (17%) of the LPV/r alone group. The univariate analyses identified important predictors of virologic rebound including baseline CD4 count, NNRTI experience, and years on ARV therapy. Current NNRTI use was not identified as a significant predictor. The crude hazard ratio for the presence of APV in the salvage regimen was 2.35 (95% CI 1.20, 4.61; $p = .01$), suggesting that the addition of APV to a salvage regimen containing LPV/r tended to shorten the time to virologic rebound. However, after adjusting for baseline CD4 count, previous NNRTI use, and number of years of taking ARV drugs in the multivariate analysis, the association of APV to virologic rebound decreased in magnitude and statistical significance with an adjusted hazard ratio for the presence of APV in the LPV/r-containing salvage regimen of 1.46 (95% CI 0.67, 3.20; $p = .34$). The adjusted hazard ratios for baseline CD4 cell count, previous NNRTI use, and years of ARV therapy were 0.68 per 100 cells/μL (95% CI 0.51, 0.92; $p < .01$), 1.76 (95% CI 0.78, 3.98; $p = .18$), and 1.10 per year on ARV therapy (95% CI, 0.97, 1.25; $p = .13$), respectively. Other covariates were not statistically significant and were not included in the multivariate model.

Effect of Amprenavir Dose on Virologic Suppression

This analysis was limited to 79 of the 100 patients taking the double PI salvage regimen who had the dose of APV recorded. Of the 79, 53.2% (42/79) had an NNRTI included in the salvage regimen, and the dose of LPV/r was increased accordingly to 533/133 mg bid. There was an increase in the proportion of patients achieving a VL < 50 copies/mL at every dose increase of APV as seen in **Table 3** ($p = .03$, chi-square for trend). Using logistic regression, the crude odds ratio for each additional 150-mg pill given twice a day was 2.25 (95% CI 1.20, 4.18; $p = .01$). After adjusting for baseline plasma VL and present NNRTI use, the odds ratio per 150 mg of APV was 2.00 (95% CI 1.03, 3.90; $p = .04$). Therefore, the odds of achieving virologic suppression among patients receiving 750-, 900-, and 1,200-mg doses of APV relative to patients receiving 600 mg of APV would be 2, 4, and 8, respectively.

Pharmacokinetic Analysis

Among the 27 patients with PK data, 55.6% (15/27) were taking an NNRTI, including 41% (7/17) in

Table 3. Proportion of patients on APV and LPV/r in salvage regimens achieving virologic suppression by dose of APV

APV dose bid	% with virologic suppression
600 mg	25.0% (2/8)
750 mg	34.8% (16/46)
900 mg	45.0% (9/20)
1,200 mg	100.0% (5/5)

Note: APV = amprenavir; LPV/r = lopinavir/ritonavir. $p = .03$, chi-square for trend.

the APV group and 80% (8/10) in the LPV/r-alone group ($p = .10$, Fisher exact test). The dose of LPV/r was 533/133 mg and 400/100 mg bid with and without an NNRTI in the salvage regimen, respectively. None of the 27 patients received additional ritonavir. The APV dose in the 17 patients ranged from 750 to 1,200 mg bid, with 70.6% (12/17) taking 750 mg bid. The median trough concentrations of LPV, ritonavir, and APV of the two groups are presented in **Table 4**. The APV trough level of 0.75 µg/L is lower than the value reported for historical controls who took APV 600 mg bid with ritonavir 100 mg bid (1.90 µg/L; range 0.52–5.69).[20] The median LPV C_{trough} in the 7 patients who achieved VL < 50 copies/mL was 5.87 µg/L (IQR 4.91–6.55), and in the 20 who did not it was 3.03 µg/L (IQR 2.27–4.49) ($p = .02$). The median APV C_{trough} in the 2 patients who achieved VL < 50 copies/mL was 1.15 µg/L (IQR 1.03–1.26), and in the 15 who did not it was 0.69 µg/L (IQR 0.40–1.09) ($p = .28$). Using logistic regression, the crude odds ratio per µg/L LPV C_{trough} was 2.76 (95%CI 1.12, 6.79; $p = .03$). After adjusting for baseline plasma VL and present NNRTI use, the odds ratio per µg/L LPV C_{trough} was 3.82 (95% CI 1.01, 14.41; $p = .05$). The odds ratio for baseline plasma VL was 0.03 (95% CI <0.001, 1.35; $p = .07$), and for present NNRTI use it was 0.89 (95% CI 0.034, 20.18; $p = .94$). Using logistic regression, the crude odds ratio per µg/L APV C_{trough} was 8.12 (95% CI 0.18, 377.83; $p = .28$). It was not possible to determine the adjusted odds ratio for APV C_{trough} due to the small sample size of 17 and due to the fact that only 2 patients achieved virologic suppression.

Clinical Outcomes

Six percent (6/100) of patients taking the double boosted PIs (APV and LPV/r) in their salvage regimen compared to 1.3% (2/154) of the patients taking salvage regimens containing only LPV/r died by the end of the study period ($p = .06$, Fisher exact test). Twenty-four percent of patients (24/100) in the double boosted group and 11.7% (18/154) of the patients taking LPV/r alone discontinued their salvage regimen ($p = .01$, Fisher exact test), including 8% (8/100) of patients in the double boosted PI group and 5.8% (9/154) of the patients taking LPV/r alone who discontinued their salvage regimen due to side effects ($p = .60$) and 2/100 patients in the double boosted PI group and none of the patients taking LPV/r alone who discontinued due to virologic failure. The reasons for the remainder of the discontinuations were not recorded.

Table 4. The trough concentrations of lopinavir, ritonavir, and amprenavir in the two study groups

Protease inhibitor	LPV/r-only group	LPV/r + APV group	*p*
n	10	17	
Lopinavir (µg/L)	4.73 (1.66–7.65)	2.78 (1.21–6.18)	.04
Ritonavir (µg/L)	0.19 (0.01–0.40)	0.15 (0.01–0.87)	.82
Amprenavir (µg/L)	N/A	0.75 (0.22–1.63)	N/A

Note: Values given are median (range). LPV/r = lopinavir/ritonavir; APV = amprenavir, N/A = not applicable.

DISCUSSION

Double boosting of two PIs with ritonavir is a new strategy being evaluated for ARV-experienced HIV-infected patients who require salvage therapy. In order for double boosting to be maximally effective, there should be significant pharmaco-enhancement of both PIs by ritonavir, a lack of negative PK effects between the two PIs, an additive or synergistic antiviral effect, and the PIs should inhibit different resistant viral subpopulations. However, the increased pill burden and adverse effects potentially associated with double PI boosting may be deleterious to patient adherence. The double PI combination of APV and LPV/r may not meet many of these requirements. The available PK data on the drug interaction between LPV/r and APV are conflicting.[8–18] Different studies showed that APV levels were either decreased, unchanged, or increased and LPV levels were either decreased or unchanged.[8–18] However, these studies are limited to PK analysis, involve small numbers of patients or healthy volunteers, are retrospective in design, and typically compared their results to historical controls. In contrast, a recent in vitro study suggested that the combination of APV and LPV/r resulted in additive inhibition of wild-type virus.[21] Given these findings, physicians remain uncertain about the effectiveness of and the appropriate doses of LPV/r and APV when used together.

Our study provides the first clinical data on the use of this PI combination. The addition of APV to a LPV/r-containing salvage regimen in ARV-experienced patients experiencing virologic failure was of no additional benefit in terms of time to virologic suppression and time to preventing virologic rebound. On univariate analysis, the LPV/r-only group had a superior virologic response to the combination group; however, this result is confounded by the significantly more ARV experience in the combination PI group. After adjusting for these confounders using multivariate analysis, we found the difference was nonsignificant. With the 95% CIs of 0.52 and 1.08, there is a 93.8% likelihood that the conclusion that the addition of APV to a LPV/r-containing salvage regimen is of no benefit to virologic suppression is true.

The risk of virologic rebound among patients who achieved virologic suppression was not significantly different among patients who received APV as part of their LPV/r-containing salvage regimen than among those who did not receive APV, although the confidence interval for the hazard ratio was wide due to the relatively small number of patients experiencing virologic rebound. Baseline CD4 count was the only statistically significant predictor of risk of virologic rebound, which is consistent with previous data.[22]

The evidence that this PI combination did not result in improved virologic response was supported by the PK analysis, which revealed lower median LPV trough concentration in patients receiving APV in addition to LPV/r in their salvage regimen. The median APV trough concentration was also reduced in this population as compared to historical controls. The LPV trough concentration was significantly associated with virologic response. The APV trough concentration was not significantly associated with virologic response; but because of the small sample size, this association cannot be excluded. The association between APV drug levels and virologic response is supported by the observation of increasing APV dose and increasing virologic response. The lack of improved virologic response in salvage therapy that used LPV/r and APV in combination may be due to reduced levels of both drugs. Therefore, if these two PIs are used together, clinicians may want to consider higher doses of APV (900 to 1,200 mg twice a day) and LPV/r (533/133 mg twice a day). However, this suggestion needs to be confirmed in prospective studies.

The limitations of this study include its retrospective design, lack of randomization, limited sample size, and lack of adherence data, which is an important confounder. The increased pill burden in the double PI boosted group could lead to lower adherence, resulting in lower drug concentrations and poorer virologic outcome. However, our finding of an association between increased virologic response and increased APV dose would not support lower adherence as the reason for the reduced virologic response in the double boosted PI group. A controlled trial randomizing patients to a salvage regimen containing a single boosted PI or double boosted PI with clinical relevant outcomes such as drug discontinuation in addition to viral response as the endpoint would clarify these findings.

In conclusion, this study could not demonstrate that the addition of APV to salvage regimens containing LPV/r benefits the time to or duration of

virologic suppression in highly ARV-experienced patients. These results are consistent with the reported negative PK interaction between LPV/r and APV. Given our observations of higher response rates with higher APV and of lower drug concentrations of both PIs, further studies are required to determine whether there is an additive benefit in antiviral activity when both these PIs are combined in higher doses.

ACKNOWLEDGMENTS

The authors thank Marija Trpeski for assistance with data collection and data entry.

This project was funded in part by an unrestricted research grant from Abbott Laboratories, Canada, and salary support from the Canadian Institutes of Health Research (ML), Ontario HIV Treatment Network (SW, EP), The Skate Dream Fund, Toronto General and Western Hospital Foundation (JR), and Merrill Lynch studentship (CT).

Drs. Walmsley, Loutfy, Rachlis, Tseng, and Phillips have served as consultants, received honoraria, speaker's fees, and educational grants and have participated in clinical trials with both Abbott Laboratories and Glaxo Smith Kline.

REFERENCES

1. Bartlett J, Demasi R, Quinn J, Moxham C, Rousseau F. Meta-analysis of efficacy of triple combination therapy in antiretroviral-naive HIV-1 infected adults. In: Proceedings of the Seventh Conference on Retroviruses and Opportunistic Infections; January 2000; San Francisco, CA. Abstract I519.
2. Loutfy MR, Walmsley SL. Salvage therapy in HIV infection. *Exprt Opin Pharmacother.* 2002;3:81–90.
3. Smith GHR, Klein M, Leblanc R, MacCleod J, Routy JP, Lalonde R. Salvage therapy for HIV-1 infection with lopinavir/ritonavir (LOP/RIT), saquinavir-SGC (SQR), and nucleoside analogues in patients having failed all three antiretroviral classes. In: Proceedings of the 10th Canadian Conference on HIV/AIDS Research; May 2001; Toronto, Canada. Abstract I212.
4. Ruiz L, Ribera E, Bonjochl A, et al. Virological and immunological benefit of a salvage therapy that includes Kaletra plus Fortovase preceded or not by antiretroviral therapy interruption (TI) in advanced HIV-infected patients (6-month follow-up). In: Proceedings of the Ninth Conference on Retroviruses and Opportunistic Infections; February 2002; Seattle, WA. Abstract 421-W.
5. Helliger J, Morris AB, Piscitelli S, et al. Pilot study of saquinavir-SGC (Fortovase, SQV) 1000 mg twice daily and lopinavir/ritonavir (Kaletra, LPV/r) in protease inhibitor-experienced HIV+ individuals: dose escalation and combined normalized inhibitory quotient (cNIQ). In: Proceedings of the Ninth Conference on Retroviruses and Opportunistic Infections; February 2002; Seattle, WA. Abstract 421-W.
6. Staszewski S, Dauer B, Stephan C, Gute P, Klauke S, Sturmer M. Switch to a simple boosted double protease inhibitor regimen of lopinavir/r and saquinavir without reverse transcriptase inhibitors after multiple therapy failures. In: Proceedings of the XIV International AIDS Conference; July 2002; Barcelona, Spain. Abstract TuPeB4474.
7. Zala C, Patterson P, Coll P, et al. Virological response and safety at 48 weeks of double boosted protease inhibitors with Lopinavir/R plus either Saquinavir or Amprenavir in heavily pretreated HIV infected patients. In: Proceedings of the XIV International AIDS Conference; July 2002; Barcelona, Spain. Abstract TuPeB4492.
8. Khanlou H, Graham E, Brill M, Farthing C. Drug interaction between amprenavir and lopinavir/ritonavir in salvage therapy [letter]. *AIDS.* 2002;16:797–798.
9. Stein AJ, Brothers CH, Scott TR. Effect of reduced-dose amprenavir in combination with lopinavir on plasma levels of amprenavir in patients infected with HIV. *Clin Ther.* 2001;23:513–515.
10. Duval X, Race E, Lamotte C, et al. Inhibitory quotient and efficacy of amprenavir-lopinavir containing HAART in heavily pretreated HIV infected patients. In: Proceedings of the Second International Workshop on Clinical Pharmacology of HIV Therapy; April 2001; Noordwijk, The Netherlands. Abstract 5.11.
11. Peytavin G, Lamotte C, Duval X, et al. Amprenavir (APV) plasma concentrations are dramatically decreased by association with ABT378/r in HIV-infected patients. In: Proceedings of the Second International Workshop on Clinical Pharmacology of HIV Therapy; April 2001; Noordwijk, The Netherlands. Abstract 1.14.
12. Solas C, Quinson AM, Couprie C, et al. Pharmacokinetic interaction between lopinavir/r and amprenavir in salvage therapy. In: Proceedings of the Ninth Conference on Retroviruses and Opportunistic Infections; February 2002; Seattle, WA. Abstract 440-W.
13. Baldini F, Rizzo MG, Hoetelmans R, et al. A prospective study of deep salvage therapy with lopinavir/r, amprenavir, and NRTIs: final 24-week data, pharmacokinetics, and association of drug levels/drug susceptibility with virologic response. In: Proceedings of the Ninth Conference on Retroviruses and Opportunistic Infections; February 2002; Seattle, WA. Abstract 423-W.
14. Mauss S, Schmutz G, Kuschak D. Unfavourable interaction of amprenavir and lopinavir in combination with ritonavir? *AIDS.* 2002;16:296–297.
15. Romer K, Fatkenheuer G, Kamps R, Salzberger B, Burger D. Pharmacokinetics of amprenavir and lopinavir in combination with ritonavir and nevirapine in highly pretreated HIV infected patients. In: Proceedings of the Eighth European Conference on Clinical Aspects and Treatment of HIV Infection; October 2001; Athens, Greece. Abstract P166.
16. Fatkenheuer G, Romer K, Kamps R, Salzberger B, Burger D. Pharmacokinetics of amprenavir and lopinavir in combination with nevirapine in highly pretreated HIV-infected patients. *AIDS.* 2001;15:2334–2335.
17. Hsu A, Bertz R, Ashbrenner E, et al. Interaction of ABT-378/

ritonavir with protease inhibitors in healthy volunteers. In: Proceedings of the First International Workshop on Clinical Pharmacology of HIV Therapy; March 2000; Noordwijk, The Netherlands. Abstract 2.4.

18. Mauss S, Schmutz G, Kuschak D. Unfavourable interaction of lopinavir and amprenavir in combination with ritonavir. In: Proceedings of the Eighth European Conference on Clinical Aspects and Treatment of HIV Infection; October 2001; Athens, Greece. Abstract P170.
19. van Heeswijk RP, Hoetelmans RM, Harms R, et al. Simultaneous quantitative determination of the HIV protease inhibitors amprenavir, indinavir, nelfinavir, ritonavir and saquinavir in human plasma by ion-pair high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl.* 1998;719:159–168.
20. Lamotte C, Duval X, Peytavin G, Farinotti R. Amprenavir (APV) plasma concentrations are dramatically increased by association to ritonavir (RTV) baby-doses in HIV infected patients: possible combination with efavirenz (EFV). In: Proceedings of the XIII International AIDS Conference; July 2000; Durban, South Africa. Abstract WePeB123.
21. Molla A, Mo H, Vasavanonda S, et al. In vitro antiviral interaction of lopinavir with other protease inhibitors. *Antimicrob Agents Chemother.* 2002;46:2249–2253.
22. Miller V, Staszewski S, Sabin C, et al. CD4 lymphocyte count as a predictor of the duration of highly active antiretroviral therapy-induced suppression of human immunodeficiency virus load. *J Infect Dis.* 1999;180:530–533.