

Pharmacokinetics and short-term efficacy of a double-boosted protease inhibitor regimen in treatment-naïve HIV-1-infected adults

Jasper van der Lugt^{1,2*}, Reshma Saskia Autar^{1,2}, Sasiwimol Ubolyam¹, Evian Fernandez Garcia², Jongkol Sankote¹, Anchalee Avihingson¹, Theshinee Chuenyam¹, David A. Cooper^{1,3}, Joep Lange^{1,2,4}, Praphan Phanuphak^{1,5}, Ferdinand Wit^{2,4}, Kiat Ruxrungtham^{1,5} and David Burger⁶ on behalf of the HIV-NAT 019 Study Team

¹HIV Netherlands Australia Thailand (HIV-NAT) Research Collaboration, Thai Red Cross AIDS Research Center Bangkok, 104 Ratchadamri Road, Pathumwan, Bangkok 10330, Thailand; ²International Antiretroviral Therapy Evaluation Center, Pietersbergweg 9, 1105 BM Amsterdam, The Netherlands; ³National Center in HIV Epidemiology and Clinical Research, University of New South Wales, Level 2, 376 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia; ⁴Department of Internal Medicine, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands; ⁵Department of Medicine, Faculty of Medicine, Chulalongkorn University, 104 Ratchadamri Road, Pathumwan, Bangkok 10330, Thailand; ⁶Radboud University Nijmegen Medical Center, 864 Department of Clinical Pharmacy and Nijmegen University Centre for Infectious Diseases (NUCI), Geert Grooteplein 10, 6525 GA Nijmegen, The Netherlands

Received 6 November 2007; returned 5 December 2007; revised 27 December 2007; accepted 20 January 2008

Objectives: To study the pharmacokinetics and short-term efficacy of low and standard dose lopinavir/ritonavir and saquinavir combinations in Thai, human immunodeficiency virus (HIV)-infected, treatment-naïve patients.

Methods: In this open-label, 24-week, prospective study, 48 treatment-naïve patients were randomized to lopinavir/ritonavir 400/100 mg+saquinavir 1000 mg twice daily (arm A), lopinavir/ritonavir 400/100 mg+saquinavir 600 mg twice daily (arm B), lopinavir/ritonavir 266/66 mg+saquinavir 1000 mg twice daily (arm C), or lopinavir/ritonavir 266/66 mg+saquinavir 600 mg twice daily (arm D). A 12 h pharmacokinetic profile in all patients was performed. Plasma concentrations of saquinavir and lopinavir were determined using an HPLC technique. HIV-1 RNA was measured over 24 weeks.

Results: Forty-three subjects were included in the pharmacokinetic analysis. The total exposure differed significantly for the different arms. Median values for lopinavir area under the curve at 0–12 h were 128.2, 119.2, 66.1 and 68.5 mg·h/L for arms A–D, respectively. For saquinavir, the median values were 36.9, 19.2, 25.3 and 12.4 mg·h/L for arms A–D, respectively. The proportion of patients having a viral load below 50 copies/mL at week 24 was 39% for arm A, 63% for arm B, 55.0% for arm C, and 69% for arm D.

Conclusions: The pharmacokinetic parameters for the different treatment arms were adequate. However, the proportion of subjects with an undetectable viral load at week 24 was lower than anticipated.

Keywords: viral dynamics, lopinavir, efficacy, dose reduction, saquinavir

*Correspondence address. HIV-NAT, Thai Red Cross AIDS Research Centre, 104 Rajdamri Road, Pathumwan, Bangkok 10330, Thailand. Tel: +66-2-652-3040, ext. 128; Fax: +66-2-254-7574/+66-2-252-5779; E-mail: jasper.v@hivnat.org

Introduction

The use of potent combination antiretroviral (ARV) therapy, also referred to as highly active retroviral therapy (HAART), has been associated with dramatic reductions in mortality and morbidity in the population infected with the human immunodeficiency virus (HIV).¹ Despite the virological and immunological benefits observed with HAART, failure rates of 15% to 50% are reported within the first year of HAART in ARV-naïve patients.^{2–4} This rate of failure only increases with successive treatment regimens.^{5–7} With scaling up of the ARV, these figures may not apply to the current situation in developed countries anymore. However, in Thailand, where the most used first-line regimen is still the fixed-dose combination of nevirapine, stavudine and lamivudine, the failing rate can still be high due to possible toxicity and poor monitoring.^{8,9} In order to improve this situation, more tailor-made strategies have to be explored for different populations, to avoid rapid development of resistance and reduce costs.

With the introduction of HAART as the standard of care in Thailand, it became evident that the pharmacokinetics of distinctive protease inhibitors (PIs) in this population differ significantly from Caucasians. Dose-finding studies for saquinavir and indinavir showed the benefits of dose reduction in the Thai population.^{10–12} With lower dose regimens, similar plasma levels of the PIs as in the Caucasian population were achieved, with similar potency and potentially less toxicity and lower costs. As a result, ritonavir-boosted indinavir and saquinavir are widely used in a lower dose (400/100 mg twice daily and 1600/100 mg once daily, respectively) in Thailand. More data are required for further dose adjustment of other frequently used combination regimens. A potentially interesting regimen that can be explored is the double-boosted PI combination of saquinavir together with lopinavir/ritonavir (Kaletra[®]) in a dose-reduced setting. It is essential to investigate the pharmacokinetic interactions of any potential PI combination because all the currently available PIs are metabolized by cytochrome P450 isozymes and can act both as inducers and inhibitors of this enzyme system, giving rise to sometimes unpredictable interactions when their use is combined.

The combination of lopinavir/ritonavir+saquinavir has been investigated in salvage therapy settings. These studies demonstrated no significant interaction between lopinavir and saquinavir, while the boosting effect of ritonavir (RTV) is maintained for both drugs.^{13–15} In the study of Staszewski *et al.*,¹⁶ the lopinavir/ritonavir+saquinavir combination, without nucleoside reverse transcriptase inhibitor (NRTI) backbone, showed good immunological and virological activity in a salvage setting over 48 weeks. Moreover, *in vitro* data showed a synergistic effect on the inhibition of HIV-1 viral replication when saquinavir was combined with lopinavir.¹⁷ The need for salvage therapy options in Thailand is urgent. The limited access to resistance and viral load testing enhances resistance development, due to prolonged use of failing non-NRTI (NNRTI)-based HAART regimens.^{8,18,19} Especially, at the start of our study, the access to drugs was heavily constrained in the Thai setting.

Studying lopinavir/ritonavir and the saquinavir regimen in treatment-naïve patients will generate useful and clean data about the pharmacokinetics and short-term tolerability of this combination in different doses, without the contamination of other ARVs. Data can be used to conduct trials in a salvage

setting as well as for larger trials in treatment-naïve patients. If the preliminary efficacy data look promising, using lopinavir/ritonavir together with saquinavir in treatment-naïve patients might be an option for future first-line treatment, preserving other drug classes for future second-line treatment. In the current ARV guidelines, the double-boosted PI combination is not incorporated as an option at any time during the course of the disease.

Therefore, we conducted a pilot study to explore the pharmacokinetics and efficacy of a double-boosted PI treatment for different dosing regimens in Thai HIV-1-infected adults.

Methods

Study design

This study was approved by the Ethics Committee of the Faculty of Medicine Chulalongkorn Hospital (approval number 235/2004), and written informed consent was obtained for all patients.

Eligible patients were ARV-naïve, HIV-1-infected Thai patients, who were recruited from the Thai Red Cross Society's Anonymous Clinic and the HIV Outpatient Immune Clinic of King Chulalongkorn Memorial Hospital. The study took place from October 2004 to March 2006.

This was an open label, randomized, pilot study in which co-administration of saquinavir hard gel capsules (Invirase[®], Roche, Basel Switzerland) and lopinavir/ritonavir (Kaletra[®], Abbott Laboratories, Abbott Park, IL, USA), fixed-dose combination (soft gel capsules) was evaluated in 48 patients. The study participants were randomized to four arms (A–D).

Arms A and B received lopinavir/ritonavir 400/100 mg twice daily, whereas arms C and D received lopinavir/ritonavir 266/66 mg twice daily. Saquinavir was administered in a 1000 mg twice-daily dose for arms A and C and 600 mg twice-daily dose for arms B and C. No NRTI backbone was part of the ARV treatment. At the discretion of the study physician, therapy could be intensified with stavudine plus lamivudine, which currently is no longer the standard of practice. Guidance for intensification was less than a one-fold decrease of the viral load in combination with good adherence at week 12. No concomitant medication, known to interfere with saquinavir and lopinavir/ritonavir pharmacokinetics, was used. Baseline data, including demographic data, and prior AIDS-defining diseases, according to the 1993 Centre for Disease Control and Prevention classification, were obtained. The safety and tolerability of the study medication were assessed throughout the study on the basis of clinical and laboratory adverse events. World Health Organization toxicity grading scales were used to characterize abnormal laboratory results (liver and kidney function tests, fasting blood lipids and haematology) and physical examination. Study visits were scheduled at baseline, weeks 2, 4, 9, 12 and 24. CD4 count was obtained at every visit; routine laboratory tests were obtained every visit, except for weeks 4 and 9. Plasma HIV-1 RNA concentrations (viral load) were obtained at days –14, –7, 0, 1, 3, 7, 10, 14, 17 and at weeks 3, 4, 6, 9, 12, 18 and 24 in order to gain enough data to calculate a first- and second-phase decay of the viral load. Viral loads were assessed using the Roche Amplicor HIV-1 monitor assay (version 1.5, Roche).

Pharmacokinetic analysis

Blood samples for the PK analysis were obtained 2 weeks after initiation to ensure steady state. After one night of fasting, patients

ingested lopinavir/ritonavir and saquinavir, together with their provided standardized breakfast. Blood samples were drawn just before ingestion of the drugs and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10 and 12 h after drug intake. Plasma was isolated by centrifugation (1500 rpm) on the same day and stored at -80°C until analysis.

Plasma concentrations of lopinavir, saquinavir and ritonavir were measured in all available samples using a validated HPLC method.²⁰ The HIV-NAT pharmacokinetic laboratory participates in an international quality control and quality assessment programme and therefore has been cross-validated with other pharmacokinetic laboratories.²¹ A UV detection was used with a lower limit of quantification of 0.04 mg/L. Calculations of pharmacokinetic parameters such as area under the plasma concentration–time curve (AUC) from 0 to 12 h, maximum concentration (C_{max}), minimum concentration (C_{trough}), time of maximum concentration (T_{max}) and half-life ($t_{1/2}$) of the PIs were made by non-compartmental methods using WinNonlin software (version 5.0.1, Pharsight Corporation, Mountain View, CA, USA).

Statistical analysis

Descriptive statistics were generated for all pharmacokinetic measures. Statistical analysis was performed using SPSS version 12.01 (SPSS, Chicago, IL, USA). For the comparison between four different treatment arms, the Kruskal–Wallis test was used, and for a two-arm comparison, the Mann–Whitney *U*-test was used. Proportion of patients with undetectable viral load at week 24 was assessed according to intention-to-treat (ITT) as well as on-treatment analysis (OT) principles; patients with missing values were imputed as virological failures. Patients who required intensification of their regimen were considered as failures.

The dynamics of viral decline were determined using a non-linear least square fitting model for the first phase and a linear spline model for the second phase. The limit of the first and second phase was determined by the model with the best goodness of fit. No imputation techniques were used in this analysis; therefore, only observed data were included in the model.

No sample size calculation was performed as it was a pilot study. Ten patients per arm are generally sufficient for pharmacokinetic pilot studies. Taking drop outs into account, 12 patients per arm were included. The study was not powered to demonstrate differences in tolerability, toxicity and efficacy. Therefore, these measurements are only secondary endpoints.

Results

Baseline characteristics

A total of 48 patients were randomized to the four treatment arms of the study (13 to arm A, 11 to arm B, 11 to arm C and 13 to arm D). There were a total of 28 women and 20 men with a median age of 35.5 years. Median CD4 count at baseline was 114 [interquartile range (IQR), 55–196] cells/mm³. Baseline characteristics were not different between the arms (Table 1). During the 24 weeks of follow-up, five patients discontinued the study medication. One was lost to follow-up (week 1, arm B). Two patients discontinued their medication because of personal reasons (week 13, arm A; week 21 arm D), one hepatitis B virus co-infected subject had to stop due to grade 4 transaminase elevation (week 5, arm B), and one subject was switched to a simplified standard-of-care regimen (fixed-dose stavudine,

lamivudine, nevirapine: GPO-vir) by the physician due to non-adherence (week 6, arm C). After interim analysis at week 12, the treatment of two patients, one from arm C and one from arm D, was intensified; stavudine and lamivudine were added as viral load decline was insufficient, whereas self-reported adherence was adequate.

Pharmacokinetics

A total of 43 curves were used for the analysis, two patients were excluded from the analysis due to non-adherence (one arm B and one arm C), and one patient was lost to follow-up before week 2 (arm B). Two other cases (both arm A) showed very inconsistent pharmacokinetic curves, highly suspected for non-adherence, during the first 2 weeks of the study and therefore excluded from the analysis. The baseline characteristics of the five patients excluded from the PK analysis did not differ significantly. Lopinavir subtherapeutic trough concentrations (defined as <1.0 mg/L) were observed in four patients: one in each arm. Saquinavir trough concentrations were above the minimum effective concentration (0.1 mg/L) for all patients, except one (arm B). The median plasma concentration–time profiles of lopinavir are shown in Figure 1, and for saquinavir, the median plasma concentration–time levels are plotted in Figure 2. Median plus IQR values for the relevant pharmacokinetic parameters are listed in Tables 2 and 3.

The median AUC of lopinavir/ritonavir 400/100 mg combined with saquinavir 1000 mg twice daily (arm A) was 49% higher ($P < 0.001$) than lopinavir/ritonavir 266/66 mg with saquinavir 1000 mg twice daily (arm C). When accompanied with the lower dose of saquinavir (arms B and D), the lopinavir AUC for arm B is 42% higher when compared with arm D ($P < 0.001$). Looking into the different doses of saquinavir, about the same percentages were found; however, no statistical significance was reached. When comparing saquinavir 1000 mg/lopinavir 400/100 mg twice daily (arm A) with saquinavir 600 mg/lopinavir/ritonavir 400/100 mg twice daily (arm B), a 42% higher AUC was found for arm A ($P = 0.15$). A 54% higher increase in AUC was found when comparing saquinavir 1000 mg together with lopinavir/ritonavir 266/66 mg with the saquinavir 600 mg/lopinavir/ritonavir 266/66 mg combination ($P = 0.10$).

Comparing the equally dosed saquinavir arms with each other (arms A–C and B–D), a 31% higher AUC was found when saquinavir 1000 mg was combined with lopinavir/ritonavir 400/100 mg (arm A) in comparison with the saquinavir 1000 mg/lopinavir/ritonavir 266/66 mg arm ($P = 0.39$). A similar trend was found when comparing the two saquinavir 600 mg arms: a 36% higher exposure for arm B, which was accompanied with lopinavir/ritonavir 400/100 mg ($P = 0.01$).

In contrast, no large quantitative differences in lopinavir AUC were found when the equally dosed lopinavir arms were evaluated.

Irrespective of the used dose of saquinavir, the use of lopinavir/ritonavir 400/100 mg resulted in higher C_{max} and C_{trough} when compared with the use of lopinavir/ritonavir 266/66 mg; *P* values were less than 0.001. For saquinavir, the differences for C_{trough} and C_{max} were less straightforward: the most outspoken difference was found between arm A (high-dose saquinavir, high-dose lopinavir/r) and arm D (low-dose saquinavir, low-dose

Table 1. Subject demographic data and baseline characteristics, medians (IQRs), or *n* where appropriate

Parameters	LPV/RTV/SQV 400/100/ 1000 twice daily (arm A)	LPV/RTV/SQV 400/100/ 600 twice daily (arm B)	LPV/RTV/SQV 266/66/ 1000 twice daily (arm C)	LPV/RTV/SQV 266/66/600 twice daily (arm D)	<i>P</i> value
<i>n</i>	13	11	11	13	
Male/female	6/7	4/7	5/6	5/8	0.820
Age (years)	35 (1.97)	37 (3.53)	38 (1.89)	35 (2.98)	0.641
Weight (kg)	60.0 (49.8–70.0)	55 (41–60)	60 (47–70)	55 (48–62)	0.654
Height (cm)	160 (156–165)	159 (153–165)	165 (159–170)	158 (152–66)	0.654
CD4 (cells/ mm ³)	175 (93–238)	114 (102–265)	107 (35–192)	74 (24.5–178)	0.196
Log ₁₀ HIV-RNA	4.86 (4.68–5.00)	4.87 (4.52–4.98)	4.90 (4.01–5.16)	4.80 (4.41–5.20)	0.964

LPV, lopinavir; RTV, ritonavir; SQV, saquinavir.
n, number of patients.

lopinavir/r), with $P < 0.001$ for both values. The other pairwise comparisons were more variable (Tables 2 and 3).

Virological and immunological activity

Immunological response. The overall median rise in the CD4 count was +125 cells/mm³ (IQR 58–165) after 24 weeks ($P < 0.001$). The absolute increase per arm was +140 (IQR 65–224) for arm A, +130 (IQR 92–206) for arm B, +69 (IQR 25–136) for arm C, and +108 (IQR 67–130) for arm D.

Virological response. Viral load changes in individual patients and the mean changes in the four arms are shown in Figure 3. Overall, plasma HIV-1 RNA titres fell from baseline to week 24 by an average of $-2.62 \log_{10}$ (IQR -3.04 to -2.13). By the ITT analysis, the proportion of patients reaching a plasma HIV-1 RNA concentration below 50 copies/mL was 39%, 63%, 55% and 62% for arms A–D, respectively. The overall

proportion was 56.2%, whereas the OT analysis showed a proportion of 62.5%.

Estimated virological decay. We found no significant difference for the estimated half-life for viral decay of the first and second phase between study arms in both the ITT and OT analyses. Estimates of the half-life values in all arms of the study for two distinct periods (first phase, second phase, OT) are shown in Table 4. Only the OT was depicted as this gives a better picture of the intrinsic ARV capacity.

Safety and tolerability

No statistically significant differences between treatment arms in frequency of specific adverse events of any severity or relationship to study drug were noted. Mild gastrointestinal adverse events were reported frequently (>80% of the patients), but did not differ among the study arms.

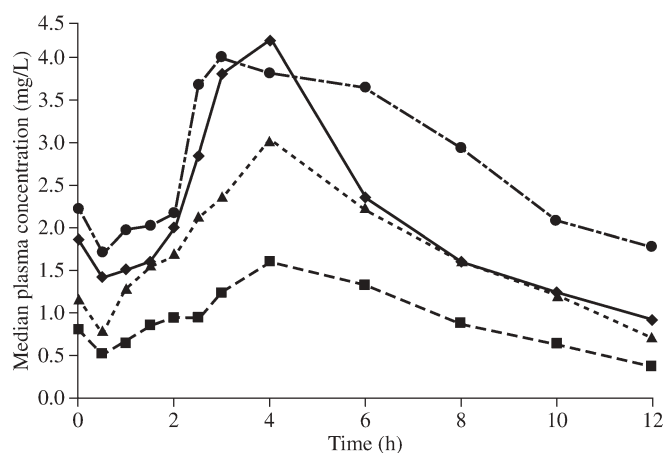


Figure 1. Median concentration–time curve of saquinavir. Filled circles, lopinavir/ritonavir (400/100 twice daily)+saquinavir (1000 twice daily), arm A; filled triangles, lopinavir/ritonavir (400/100 twice daily)+saquinavir (600 twice daily), arm B; filled diamonds, lopinavir/ritonavir (266/66 twice daily)+saquinavir (1000 twice daily), arm C; filled squares, lopinavir/ritonavir (266/66 twice daily)+saquinavir (600 twice daily), arm D.

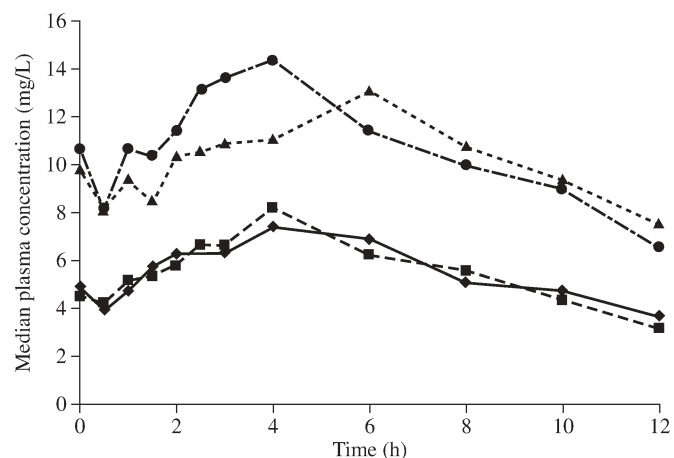


Figure 2. Median concentration–time curve of lopinavir. Filled circles, lopinavir/ritonavir (400/100 twice daily)+saquinavir (1000 twice daily), arm A; filled triangles, lopinavir/ritonavir (400/100 twice daily)+saquinavir (600 twice daily), arm B; filled diamonds, lopinavir/ritonavir (266/66 twice daily)+saquinavir (1000 twice daily), arm C; filled squares, lopinavir/ritonavir (266/66 twice daily)+saquinavir (600 twice daily), arm D.

Double-boosted protease inhibitor regimen in a naive population

Table 2. Steady-state pharmacokinetic parameters of saquinavir for all study arms

Parameters	LPV/RTV/SQV 400/100/ 1000 twice daily (arm A)	LPV/RTV/SQV 400/100/ 600 twice daily (arm B)	LPV/RTV/SQV 266/66/ 1000 twice daily (arm C)	LPV/RTV/SQV 266/66/ 600 twice daily (arm D)	<i>P</i> value Kruskal–Wallis
<i>n</i>	11	9	10	13	
<i>C</i> _{trough} (mg/L)	1.70 (0.52–2.06)	0.61 (0.56–1.27)	0.92 (0.37–1.51)	0.36 (0.24–074)	0.018 ^a
<i>C</i> _{max} (mg/L)	5.97 (3.25–8.48)	3.02 (2.44–4.32)	4.39 (1.90–5.70)	1.68 (1.18–3.17)	0.0013 ^b
AUC (mg·h/L)	36.89 (19.54–68.51)	19.20 (18.40–39.78)	25.33 (15.24–38.31)	12.35 (7.67–22.52)	0.0018 ^c
<i>t</i> _{1/2} (h)	3.95 (3.17–5.30)	4.02 (3.70–5.82)	3.89 (2.88–5.90)	3.53 (2.83–4.36)	0.656
<i>T</i> _{max} (h)	3.0 (3–4)	4.0 (3–4)	4.0 (3–4)	4.0 (4–6)	0.169

LPV, lopinavir; RTV, ritonavir; SQV, saquinavir.

Values are expressed as medians with IQRs.

^a*P* < 0.001 (Mann–Whitney *U*-test) for comparison A to D; *P* < 0.05 (Mann–Whitney *U*-test) for comparison B to D.

^b*P* < 0.001 (Mann–Whitney *U*-test) for comparison A to D; *P* < 0.05 (Mann–Whitney *U*-test) for comparisons A to B and B to D; *P* < 0.05 (Mann–Whitney *U*-test) for comparison C to D.

^c*P* < 0.001 (Mann–Whitney *U*-test) for comparison A to D; *P* < 0.05 (Mann–Whitney *U*-test) for comparison B to D.

There were a total of 12 adverse events in nine patients who were classified as moderate or severe and were reported as at least possibly related to the study drugs (two in arm A, six in B, one in C and three in D). Four gastrointestinal events (diarrhoea/vomiting), four liver enzyme elevations, one hypertriglyceridaemia, one rash, one hypercholesterolaemia and one hypokalaemia were reported.

Of these 12 events, 2 were severe: the hypokalaemia and a grade 4 elevated liver enzymes. The hypokalaemia was induced by diarrhoea, which already existed before the start of the study, but worsened after taking the study drugs. The patient was treated with potassium chloride and loperamide orally and was able to continue the study. The patient with grade 4 aspartate aminotransferase, alanine amino transferase and bilirubin elevation in the presence of hepatitis B permanently discontinued the study drugs. Another, not study drugs related, serious adverse event was reported. This patient developed insulin-induced hypoglycaemia grade 4 and later in the study non-fatal cardiac failure.

Discussion

In this pilot study of double-boosted lopinavir/saquinavir/ritonavir in ARV-naive patients, adequate PK parameters were observed for all four different dose regimens.

The observation that lower dosages of PIs result in adequate plasma levels in Thai individuals is consistent with earlier dose-finding studies carried out in the region.^{11,12} For lopinavir, however, it was the first time that its use was investigated in a reduced dose in Thai patients.

As reported earlier, saquinavir does not seem to have a significant effect on the lopinavir levels.^{15,22} The two high-dose lopinavir groups (arms A and B) showed similar PK results as did the two reduced-dose arms (arms C and D), irrespective of the dose of saquinavir.

In contrast, the dose of lopinavir/ritonavir did have an influence on the AUC of saquinavir. Although no statistical significance was reached between the saquinavir 1000 mg twice-daily arms (A and C), the median of arm A is 11 mg·h/L higher than that for arm C.

Table 3. Steady-state pharmacokinetic parameters of lopinavir for all study arms

Parameters	LPV/RTV/SQV 400/100/ 1000 twice daily (arm A)	LPV/RTV/SQV 400/100/ 600 twice daily (arm B)	LPV/RTV/SQV 266/66/ 1000 twice daily (arm C)	LPV/RTV/SQV 266/66/ 600 twice daily (arm D)	<i>P</i> value Kruskal–Wallis
<i>n</i>	11	9	10	13	
<i>C</i> _{trough} (mg/L)	6.57 (5.44–8.18)	5.39 (4.58–10.91)	3.43 (1.34–4.31)	2.77 (1.55–3.76)	<0.001 ^a
<i>C</i> _{max} (mg/L)	14.98 (11.97–16.36)	13.06 (10.28–19.08)	8.74 (5.85–10.11)	8.86 (6.54–11.24)	<0.001 ^a
AUC (mg·h/L)	128.20 (119.53–135.11)	119.20 (92.87–179.46)	66.10 (44.09–88.43)	68.47 (52.03–86.42)	<0.001 ^a
<i>t</i> _{1/2} (h)	7.01 (6.26–14.14)	9.23 (7.10–13.23)	7.66 (3.86–13.09)	5.46 (3.26–7.83)	0.151
<i>T</i> _{max} (h)	4.0 (1.5–4.0)	4 (1.8–5.0)	2.0 (1.5–3.3)	4 (1.5–6.0)	0.532

LPV, lopinavir; RTV, ritonavir; SQV, saquinavir.

Values are expressed as medians with IQRs.

^a*P* < 0.001 (Mann–Whitney *U*-test) for comparisons A to C, A to D, B to C and B to D.

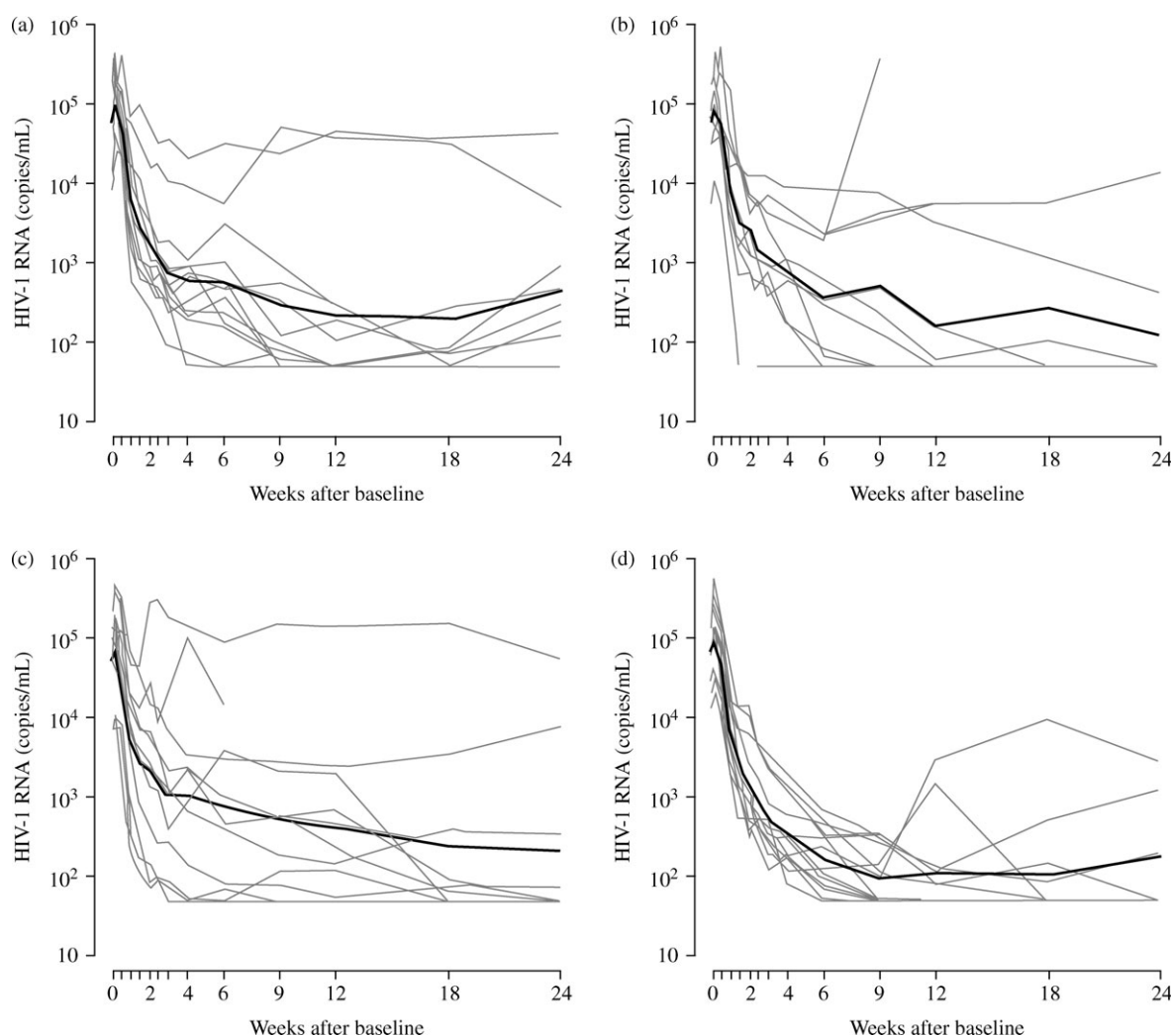


Figure 3. Viral decline for the different treatment arms. In grey, the individual curves for each patient; in black, the mean decline for each arm. (a) Lopinavir/ritonavir 400/100 mg+saquinavir 1000 mg twice daily. (b) Lopinavir/ritonavir 400/100 mg+saquinavir 600 mg twice daily. (c) Lopinavir/ritonavir 266/66 mg+saquinavir 1000 mg twice daily. (d) Lopinavir/ritonavir 266/66 mg+saquinavir 600 mg twice daily.

When comparing the two 600 mg arms (B and D), a significantly higher AUC is found when combined with lopinavir 400/100 mg. This suggests that the higher dose of lopinavir/ritonavir has a substantial effect on the concentrations of saquinavir. The most reasonable explanation for this phenomenon is the difference in ritonavir dosing, more than lopinavir itself, as the inhibition of the P450 cytochrome by ritonavir is much stronger.^{23,24} The idea that

lopinavir does not affect the saquinavir parameters was also suggested in the study of Stephan *et al.*,¹³ in which saquinavir co-administered with standard dose lopinavir/ritonavir did not reveal different PK values for saquinavir when compared with a control group with saquinavir/ritonavir plus a regular backbone.

As far as we know, there is only very limited information on the boosting effect on saquinavir of ritonavir dosed below

Table 4. Mean viral decay in days (SD), on-treatment analysis for the different treatment arms

Parameters	LPV/RTV/SQV 400/100/1000 twice daily (arm A)	LPV/RTV/SQV 400/100/600 twice daily (arm B)	LPV/RTV/SQV 266/66/1000 twice daily (arm C)	LPV/RTV/SQV 266/66/600 twice daily (arm D)	P value ANOVA
<i>n</i>	12	10	9	11	
First phase (days)	5.55 (0.06)	5.53 (0.08)	5.45 (0.15)	5.57 (0.11)	0.880
Second phase (days)	47.98 (1.62)	51.33 (1.66)	48.02 (1.86)	52.43 (1.76)	0.167

LPV, lopinavir; RTV, ritonavir; SQV, saquinavir.

100 mg. However, the literature on the saquinavir-boosting effect of different doses of ritonavir is conflicting. Some studies, in which different booster doses of ritonavir in combination with saquinavir were studied, showed a significant increase in the PK parameters for saquinavir when the ritonavir dose was increased.^{25,26} However, other studies do not support these conclusions; in a pilot study of Kilby *et al.*,²⁷ in healthy volunteers, different dose levels of ritonavir did not show an increase in any of the PK parameters. This could be possibly explained by a complete inhibition of cytochrome P450 at a dose of 100 mg ritonavir, resulting in no additional boosting effect when a higher dose of ritonavir is added. Our study did show that higher doses of ritonavir (and lopinavir) significantly increased the saquinavir exposure.

When comparing the pharmacokinetic parameters of lopinavir that we observed with the findings of Cameron *et al.*,²⁸ in which the same combination of PIs was used, the PK values of this Caucasian population appear to be lower than that in the Thai population. The parameters of the study of Cameron *et al.* for lopinavir 400/100 mg were 99.7 mg·h/L, 5.6 mg/L and 10.8 mg/L for AUC, C_{trough} and C_{max} , respectively, compared with 128.2 mg·h/L, 6.57 mg/L and 14.98 mg/L for these parameters in our study (arm A).

In the Cameron study, the same combination and the same dose, as our arm C, were used (lopinavir/ritonavir 400/100+saquinavir 600 twice daily). The C_{max} (mg/L), C_{trough} (mg/L) and AUC (mg·h/L) for saquinavir in this dose regimen were 2.44, 0.51 and 15.59, respectively, compared with 2.98, 0.59 and 19.20 in our study. Body weight and environmental factors may explain these differences, and also a racial or genetic effect cannot be ruled out.

Another remarkable finding was the lower than expected virological efficacy. Although the increase in CD4 count can be considered to be adequate, the virological response is disappointing when considering the proportion of patients with plasma HIV-1 RNA levels below 50 copies/mL. Other studies have shown good ARV responses in a salvage setting as well as in a naive population with similar regimens. Two studies report efficacy and safety of the saquinavir/lopinavir/ritonavir combination in a PI-naive population.^{28,29} Both are 48-week pilot studies. In the study of Hellinger *et al.*, the proportion of patients with undetectable HIV-1 RNA levels was 90%. The study of Cameron *et al.* showed a somewhat more similar proportion as we did, 63%, showing no inferiority when compared with a standard HAART regimen.

Regarding the fact that the treatment arm with the highest PK values (arm A) had the worse efficacy, a positive correlation between plasma levels and viral efficacy is very unlikely. The most likely explanation is non-adherence due to toxicity and/or pill burden (16 pills a day in the high/high group). Although the self-reported adherence was good, no drug accountability was done, and no other extensive adherence measurements were obtained. With 19% of the patients suffering from one or more moderate or severe adverse events and 80% with a mild event (in arm A even 100%), the tolerability can be qualified as poor and a reason for the high proportion of failures. Especially, when compared with the current first-line options, the amount of adverse events was large.^{30,31} However, as stated earlier, the most commonly used ARV combination in Thailand is the fixed-dose combination of nevirapine, stavudine and lamivudine (GPO-vir), a regimen that has shown significant toxicity as

well.³² Nevertheless, the efficacy of GPO-vir appears more adequate than that in our study.³³

As other studies showed good predictive correlation between fast, early viral decay, and long-term efficacy, early viral decay was calculated in order to look into the potency of the different regimen.^{34,35} No difference in potency was found. The viral decay, during the first and the second phase, appears much slower than previously reported in the literature.^{36,37} All previously reported decay rates were from patients using standard dual-class HAART regimens, which might be a possible explanation for the difference. PIs prevent the production of new virions. Therefore, infection by virions produced before the start of treatment still occurs. In case the HIV protease is not suppressed fully, production of infectious cells will continue, resulting in a slower decline of plasma virus. The mathematical model used is very sensitive to outliers, especially with the small sample size used. Owing to these outliers, the ITT analysis resulted even in a negative number for arm B, and when recalculated with an OT analysis, the figures became more realistic.

In summary, this study reinforced that more research towards a population-driven treatment is indicated. With adequate PK parameters for the low-dosed PIs, this pilot study justifies further well-powered prospective randomized trials to confirm that low-dose PIs achieve non-inferior efficacy and safety outcomes in Thai patients. Especially, the expensive PI-based regimens need further attention, preferably in a HAART setting, as, in terms of efficacy and tolerability, this double-boosted PI regimen appears inferior to a standard HAART regimen in ARV-naive patients.

Acknowledgements

We would like to thank the HIV-NAT 019 study team for their huge contribution to this study and for following-up the patients after the completion of the study. We also gratefully acknowledge the participants of the study.

Funding

This study is funded by Roche Pharmaceuticals.

Transparency declarations

K. R. has received travel grants, grants, consultancy fees, and honoraria from various pharmaceutical companies including Hoffmann-LaRoche, Merck Sharp & Dohme, Bristol-Myers-Squibb and Abbott. D. A. C. has received research grants/funding, honoraria or lecture sponsorships from, or is a consultant or advisor to, Abbott, Boehringer-Ingelheim, Bristol-Myers-Squibb, Chiron, Gilead, GlaxoSmithKline, Merck Sharpe & Dohme, Pfizer and Hoffmann-LaRoche. P. P. has received honoraria from Bristol-Meyers-Squibb as a scientific consultant and research grants from Bristol-Myers-Squibb, Hoffman-LaRoche GlaxoSmithKline and Merck Sharp & Dohme. J. L. has received consultancy fees and honoraria from GlaxoSmithKline, Boehringer-Ingelheim, Bristol-Myers-Squibb, Hoffmann-LaRoche, Merck Sharp & Dohme, Schering-Plough, Bayer, Shire Pharmaceuticals,

Agouron/Pfizer and Virco/Tibotec. Other authors: none to declare.

References

- Palella FJ, Jr, Delaney KM, Moorman AC *et al.* Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998; **338**: 853–60.
- Bartlett JA, Fath MJ, Demasi R *et al.* An updated systematic overview of triple combination therapy in antiretroviral-naïve HIV-infected adults. *AIDS* 2006; **20**: 2051–64.
- Paredes R, Mocroft A, Kirk O *et al.* Predictors of virological success and ensuing failure in HIV-positive patients starting highly active antiretroviral therapy in Europe: results from the EuroSIDA study. *Arch Intern Med* 2000; **160**: 1123–32.
- Bartlett JA, Buda JJ, von Scheele B *et al.* Minimizing resistance consequences after virologic failure on initial combination therapy: a systematic overview. *J Acquir Immune Defic Syndr* 2006; **41**: 323–31.
- Howard AA, Arnsten JH, Lo Y *et al.* A prospective study of adherence and viral load in a large multi-center cohort of HIV-infected women. *AIDS* 2002; **16**: 2175–82.
- Palella FJ Jr, Chmiel JS, Moorman AC *et al.* Durability and predictors of success of highly active antiretroviral therapy for ambulatory HIV-infected patients. *AIDS* 2002; **16**: 1617–26.
- Kaufmann GR, Khanna N, Weber R *et al.* Long-term virological response to multiple sequential regimens of highly active antiretroviral therapy for HIV infection. *Antivir Ther* 2004; **9**: 263–74.
- Sungkanuparph S, Manosuthi W, Kiertiburanakul S *et al.* Options for a second-line antiretroviral regimen for HIV type 1-infected patients whose initial regimen of a fixed-dose combination of stavudine, lamivudine, and nevirapine fails. *Clin Infect Dis* 2007; **44**: 447–52.
- Getahun A, Tansuphasawadikul S, Desakorn V *et al.* Efficacy and safety of generic fixed-dose combination of stavudine, lamivudine and nevirapine (GPO-vir) in advanced HIV infection. *J Med Assoc Thai* 2006; **89**: 1472–8.
- Autar RS, Boffito M, Hassink E *et al.* Interindividual variability of once-daily ritonavir boosted saquinavir pharmacokinetics in Thai and UK patients. *J Antimicrob Chemother* 2005; **56**: 908–13.
- Autar RS, Ananworanich J, Apateerapong W *et al.* Pharmacokinetic study of saquinavir hard gel caps/ritonavir in HIV-1-infected patients: 1600/100 mg once-daily compared with 2000/100 mg once-daily and 1000/100 mg twice-daily. *J Antimicrob Chemother* 2004; **54**: 785–90.
- Boyd M, Moosikapun P, Burger D *et al.* Pharmacokinetics of reduced-dose indinavir/ritonavir 400/100 mg twice daily in HIV-1-infected Thai patients. *Antivir Ther* 2005; **10**: 301–7.
- Stephan C, Hentig N, Kourbeti I *et al.* Saquinavir drug exposure is not impaired by the boosted double protease inhibitor combination of lopinavir/ritonavir. *AIDS* 2004; **18**: 503–8.
- Hammer SM, Vaida F, Bennett KK *et al.* Dual vs single protease inhibitor therapy following antiretroviral treatment failure: a randomized trial. *JAMA* 2002; **288**: 169–80.
- Ribera E, Lopez RM, Diaz M *et al.* Steady-state pharmacokinetics of a double-boosting regimen of saquinavir soft gel plus lopinavir plus minidose ritonavir in human immunodeficiency virus-infected adults. *Antimicrob Agents Chemother* 2004; **48**: 4256–62.
- Staszewski S, Babacan E, Stephan C *et al.* The LOPSAQ study: 48 week analysis of a boosted double protease inhibitor regimen containing lopinavir/ritonavir plus saquinavir without additional antiretroviral therapy. *J Antimicrob Chemother* 2006; **58**: 1024–30.
- Molla A, Mo H, Vasavanonda S *et al.* *In vitro* antiviral interaction of lopinavir with other protease inhibitors. *Antimicrob Agents Chemother* 2002; **46**: 2249–53.
- Chetchotisakd P, Anunnatsiri S, Kiertiburanakul S *et al.* High rate multiple drug resistances in HIV-infected patients failing nonnucleoside reverse transcriptase inhibitor regimens in Thailand, where subtype A/E is predominant. *J Int Assoc Physicians AIDS Care (Chic Ill)* 2006; **5**: 152–6.
- Sukasem C, Churdboonchart V, Chasombat S *et al.* Surveillance of genotypic resistance mutations in chronic HIV-1 treated individuals after completion of the National Access to Antiretroviral Program in Thailand. *Infection* 2007; **35**: 81–8.
- Droste JA, Verweij-Van Wissen CP, Burger DM. Simultaneous determination of the HIV drugs indinavir, amprenavir, saquinavir, ritonavir, lopinavir, nelfinavir, the nelfinavir hydroxymetabolite M8, and nevirapine in human plasma by reversed-phase high-performance liquid chromatography. *Ther Drug Monit* 2003; **25**: 393–9.
- Droste JA, Aarnoutse RE, Koopmans PP *et al.* Evaluation of antiretroviral drug measurements by an interlaboratory quality control program. *J Acquir Immune Defic Syndr* 2003; **32**: 287–91.
- la Porte CJ, Wasmuth JC, Schneider K *et al.* Lopinavir/ritonavir plus saquinavir in salvage therapy; pharmacokinetics, tolerability and efficacy. *AIDS* 2003; **17**: 1700–2.
- Zeldin RK, Petruschke RA. Pharmacological and therapeutic properties of ritonavir-boosted protease inhibitor therapy in HIV-infected patients. *J Antimicrob Chemother* 2004; **53**: 4–9.
- Moyle GJ, Back D. Principles and practice of HIV-protease inhibitor pharmacoenhancement. *HIV Med* 2001; **2**: 105–13.
- Hsu A, Granneman GR, Cao G *et al.* Pharmacokinetic interactions between two human immunodeficiency virus protease inhibitors, ritonavir and saquinavir. *Clin Pharmacol Ther* 1998; **63**: 453–64.
- Boffito M, Dickinson L, Hill A *et al.* Steady-state pharmacokinetics of saquinavir hard-gel/ritonavir/fosamprenavir in HIV-1-infected patients. *J Acquir Immune Defic Syndr* 2004; **37**: 1376–84.
- Kilby JM, Hill A, Buss N. The effect of ritonavir on saquinavir plasma concentration is independent of ritonavir dosage: combined analysis of pharmacokinetic data from 97 subjects. *HIV Med* 2002; **3**: 97–104.
- Cameron DW, Becker S, King MS *et al.* Exploratory study comparing the metabolic toxicities of a lopinavir/ritonavir plus saquinavir dual protease inhibitor regimen versus a lopinavir/ritonavir plus zidovudine/lamivudine nucleoside regimen. *J Antimicrob Chemother* 2007; **59**: 957–63.
- Hellinger J, Cohen C, Morris A *et al.* Pilot study of saquinavir and lopinavir/ritonavir twice daily in protease inhibitor-naïve HIV-positive patients. *HIV Clin Trials* 2005; **6**: 107–17.
- Eron J Jr, Yeni P, Gathe J Jr *et al.* The KLEAN study of fosamprenavir–ritonavir versus lopinavir–ritonavir, each in combination with abacavir–lamivudine, for initial treatment of HIV infection over 48 weeks: a randomised non-inferiority trial. *Lancet* 2006; **368**: 476–82.
- Pozniak AL, Gallant JE, DeJesus E *et al.* Tenofovir disoproxil fumarate, emtricitabine, and efavirenz versus fixed-dose zidovudine/lamivudine and efavirenz in antiretroviral-naïve patients: virologic, immunologic, and morphologic changes—a 96-week analysis. *J Acquir Immune Defic Syndr* 2006; **43**: 535–40.
- Zhou J, Paton NI, Ditangco R *et al.* Experience with the use of a first-line regimen of stavudine, lamivudine and nevirapine in patients in the TREAT Asia HIV Observational Database. *HIV Med* 2007; **8**: 8–16.
- Kiertiburanakul S, Khongnorasat S, Rattanasiri S *et al.* Efficacy of a generic fixed-dose combination of stavudine, lamivudine and nevirapine (GPO-VIR) in Thai HIV-infected patients. *J Med Assoc Thai* 2007; **90**: 237–43.
- Notermans DW, Jurriaans S, de Wolf F *et al.* Decrease of HIV-1 RNA levels in lymphoid tissue and peripheral blood during treatment

Double-boosted protease inhibitor regimen in a naive population

with ritonavir, lamivudine and zidovudine. Ritonavir/3TC/ZDV Study Group. *AIDS* 1998; **12**: 167–73.

- 35.** Reijers MH, Weverling GJ, Jurriaans S *et al.* Maintenance therapy after quadruple induction therapy in HIV-1 infected individuals: Amsterdam Duration of Antiretroviral Medication (ADAM) study. *Lancet* 1998; **352**: 185–90.
- 36.** Boyd MA, Dixit NM, Siangphoe U *et al.* Viral decay dynamics in HIV-infected patients receiving ritonavir-boosted saquinavir and efavirenz with or without enfuvirtide: a randomized, controlled trial (HIV-NAT 012). *J Infect Dis* 2006; **194**: 1319–22.
- 37.** Louie M, Hogan C, Di Mascio M *et al.* Determining the relative efficacy of highly active antiretroviral therapy. *J Infect Dis* 2003; **187**: 896–900.