



HAL
open science

The relation between treatment outcome and efavirenz, atazanavir or lopinavir exposure in the NORTHIV trial of treatment-naïve HIV-1 infected patients

Filip Josephson, Maria C. H. Andersson, Leo Flamholz, Magnus Gisslén, Lars Hagberg, Vidar Ormaasen, Anders Sönnnerborg, Jan Vesterbacka, Ylva Böttiger

► To cite this version:

Filip Josephson, Maria C. H. Andersson, Leo Flamholz, Magnus Gisslén, Lars Hagberg, et al.. The relation between treatment outcome and efavirenz, atazanavir or lopinavir exposure in the NORTHIV trial of treatment-naïve HIV-1 infected patients. *European Journal of Clinical Pharmacology*, 2009, 66 (4), pp.349-357. 10.1007/s00228-009-0763-z . hal-00543001

HAL Id: hal-00543001

<https://hal.science/hal-00543001>

Submitted on 5 Dec 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The relation between treatment outcome and efavirenz, atazanavir or lopinavir exposure in the NORTHIV trial of treatment-naïve HIV-1 infected patients

Filip Josephson · Maria C. H. Andersson · Leo Flamholz · Magnus Gisslén · Lars Hagberg · Vidar Ormaasen · Anders Sönnnerborg · Jan Vesterbacka · Ylva Böttiger

Received: 21 September 2009 / Accepted: 11 November 2009 / Published online: 5 December 2009
© Springer-Verlag 2009

Abstract

Purpose The relation between treatment outcome and trough plasma concentrations of efavirenz (EFV), atazanavir (ATV) and lopinavir (LPV) was studied in a pharmacokinetic/pharmacodynamic substudy of the NORTHIV trial—a randomised phase IV efficacy trial comparing antiretroviral-naïve human immunodeficiency virus-1-infected patients treated with (1) EFV+2 nucleoside reverse transcriptase inhibitors (2NRTI) once daily, (2) ritonavir-boosted ATV+

2NRTI once daily or (3) ritonavir-boosted LPV+2NRTI twice daily. The findings were related to the generally cited minimum effective concentration levels for the respective drugs (EFV 1,000 ng/ml, ATV 150 ng/ml, LPV 1,000 ng/ml). The relation between atazanavir-induced hyperbilirubinemia and virological efficacy was also studied.

Methods Drug concentrations were sampled at weeks 4 and 48 and optionally at week 12 and analysed by high-performance liquid chromatography with UV detector. When necessary, trough values were imputed by assuming the reported average half-lives for the respective drugs. Outcomes up to week 48 are reported.

Results No relation between plasma concentrations of EFV, ATV or LPV and virological failure, treatment withdrawal due to adverse effects or antiviral potency (viral load decline from baseline to week 4) was demonstrated. Very few samples were below the suggested minimum efficacy cut-offs, and their predictive value for treatment failure could not be validated. There was a trend toward an increased risk of virological failure in patients on ATV who had an average increase of serum bilirubin from baseline of <25 µmol/l.

Conclusions The great majority of treatment-naïve and adherent patients on standard doses of EFV, ritonavir-boosted ATV and ritonavir-boosted LPV have drug concentrations above that considered to deliver the maximum effect for the respective drug. The results do not support the use of routine therapeutic drug monitoring (TDM) for efficacy optimisation in treatment-naïve patients on these drugs, although TDM may still be of value in some cases of altered pharmacokinetics, adverse events or drug interactions. Serum bilirubin may be a useful marker of adherence to ATV therapy.

F. Josephson · M. C. H. Andersson · Y. Böttiger
Department of Clinical Pharmacology,
Division of Laboratory Medicine,
Karolinska University Hospital and Karolinska Institutet,
Stockholm, Sweden

L. Flamholz
Department of Infectious Diseases, Malmö University Hospital,
Malmö, Sweden

M. Gisslén · L. Hagberg
Department of Infectious Diseases,
The Sahlgrenska Academy at the University of Gothenburg,
Gothenburg, Sweden

V. Ormaasen
Department of Infectious Diseases, Ullevål University Hospital,
Oslo, Norway

A. Sönnnerborg · J. Vesterbacka
Department of Infectious Diseases, Institution of Medicine,
Karolinska University Hospital and Karolinska Institutet,
Stockholm, Sweden

F. Josephson (✉)
Department of Clinical Pharmacology,
Karolinska University Hospital at Huddinge,
141 86 Stockholm, Sweden
e-mail: filip.josephson@karolinska.se

Keywords Atazanavir · Concentration · Effect · Efavirenz · HIV-1 · Lopinavir

Introduction

Therapeutic drug monitoring (TDM) of antiretroviral agents may be performed under a wide range of different circumstances. These include altered or unpredictable pharmacokinetics (PK; e.g. drug–drug interactions, pregnancy, organ failure etc), the presence of concentration-dependent adverse effects (where a lowered dose may be a treatment option in the case of a high drug exposure) as well as routine drug monitoring to optimise drug exposure in treatment naïve or -experienced patients. The strength of the rationale for TDM in each of these settings varies, but in all cases assumptions about the relation between drug exposure and drug effects are necessary. These are conveniently codified in the form of a “therapeutic plasma concentration interval” with a lower limit for efficacy and, in some cases, an upper limit for toxicity. The identification of such targets, however, is problematic due to factors such as co-treatment with several active drugs, variable drug susceptibility (resistance), variable sensitivity to the toxic effects of a drug and, in some cases, very considerable intra-individual day-to-day variability of drug exposure [1]. Minimum effective concentration levels of 1,000 ng/ml for efavirenz (EFV), 150 ng/ml for atazanavir (ATV) and 1,000 ng/ml for lopinavir (LPV) are currently widely cited as targets when these antiretroviral drugs are used for treating antiretroviral-naïve subjects with full drug susceptibility [2, 3]. However, evidence for these respective cut-offs is variable (see below), and the need for their validation in a larger number of datasets is generally recognised [2].

NORTHIV is a phase IV, randomised, open label, multicentre efficacy trial carried out in Sweden and Norway, in which human immunodeficiency virus-1 (HIV-1)-infected patients naïve to antiretroviral drugs were randomised to combination antiretroviral therapy based on EFV, ritonavir-boosted ATV (ATV/r) or ritonavir-boosted LPV (LPV/r). The primary outcomes of the NORTHIV trial will be reported elsewhere. Here, we describe the relationship between plasma EFV, ATV and LPV concentrations and treatment outcome in a PK/pharmacodynamic (PD) substudy to NORTHIV. We also report the relation between ATV-induced increase in serum bilirubin (s-bilirubin) from baseline and virological efficacy, based on previously reported findings of a relation between s-bilirubin levels during ATV therapy and virological outcome [4, 5].

Materials and methods

The NORTHIV trial

The NORTHIV trial recruited a total of 242 patients in Sweden and Norway. Of these, 239 received at least one

dose of the study drugs. The patients were recruited to the study between 2004 and 2007, and they randomised to one of three different treatment regimens: (1) EFV 600 mg daily (q.d.)+2 nucleoside reverse transcriptase inhibitors (2NRTI) q.d., (2) ATV 300 mg q.d.+ritonavir 100 mg q.d.+2NRTI q.d., or (3) LPV 400 mg b.i.d.+ritonavir 100 mg b.i.d.+2NRTI twice daily (b.i.d). Most patients on LPV used the Kaletra soft gel capsules (SGC). However, during the NORTHIV trial, the tablet formulation of Kaletra was introduced, and some patients started with tablets or switched from SGC to tablets during the course of the study.

The study design was such that subjects were to be followed for a protocol-stated 144 weeks. In NORTHIV, virological failure is defined as: (1) HIV-1 RNA >50 copies/ml in two consecutive measurements from week 24 onwards, or (2) any rise in HIV-1 RNA >50 copies/ml in two consecutive measurements after a viral load <50 copies/ml has been reached, or (3) HIV-1 RNA never becomes <50 copies/ml after week 24, or (4) <1 log copies/ml drop in HIV-1 RNA from baseline to week 4 (provided HIV-1 RNA >50 copies/ml), or (5) <2 log copies/ml drop in HIV-1 RNA from baseline to week 12 (provided HIV-1 RNA >50 copies/ml). Patients for whom plasma concentrations were available for analysis and interpretation (see below) were included in this PK/PD substudy. We report outcomes in this subset of patients up to week 48.

The PK/PD substudy

Venous blood was sampled according to protocol for the determination of plasma EFV, ATV and LPV concentrations at study weeks 4 and 48 and, optionally, at week 12. ATV and LPV concentrations were preferably to be sampled at the end of the dosing interval, just prior to the next dose (trough concentrations), and EFV concentrations were preferably to be sampled at least 12 h after drug intake. This, however, was not always the case. For drug concentrations not sampled at the end of the dosing interval, trough concentrations were imputed by assuming a uniform log-linear elimination derived from the average half-lives for the respective drugs: for EFV 50 h, for ATV/r 8.6 h and for LPV/r 5.5 h [6–8]. Samples obtained prior to the T_{max} (EFV 5 h, ATV/r 3 h, LPV/r 4 h) [6–8] were excluded from analysis.

Endpoints

Virological failure up to 48 weeks was the primary endpoint. We primarily related our drug concentration data to the consensus minimum target trough concentrations (C_{trough}) for EFV (1,000 ng/ml), ATV (150 ng/ml) and LPV (1,000 ng/ml) in treatment-naïve HIV-1 infected patients with wild-type virus (as defined at the 7th

International Workshop on Clinical Pharmacology of HIV Therapy [2]). These targets are also suggested in the Department of Health and Human Services (USA) *Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents* and are widely cited in the literature [3]. However, the power of our study to validate these cut-offs was very low, primarily due to a low number of samples below these cut-offs and a low frequency of virological failure. Furthermore, drug concentration measurements were not blinded to the treating physician, and in some cases the results prompted dosage changes. Therefore, we also report the predictive value of the cut-offs for regimen potency, defined as the decline in plasma HIV-RNA from baseline to week 4 (the time prior to the first plasma concentration measurement). We also performed explorative analyses of (1) the relations between drug concentrations and virological efficacy or side effects, (2) the relation between self-reported adherence, drug concentration and virological outcome and (3) the relation between the increase in s-bilirubin and virological outcome in ATV-treated patients. For this latter analysis, we used an on-treatment population in which nine of 72 patients failed virologically; that is, four ATV-treated patients in NORTHIV were included who were not part of the PK/PD substudy population as defined above.

Analytical methods

Venous blood was sampled in a sodium heparin tube; the sample was centrifuged and plasma separated, frozen prior to transport and stored at -20° prior to analysis.

We used two high-performance liquid chromatography (HPLC) methods routinely applied for TDM: one for determining the plasma concentrations of EFV and LPV and the other for ATV. The samples were precipitated by acetonitrile. As a first step, 100 μ l plasma and 200 μ l acetonitrile were mixed in a capped vial for 10 s and centrifuged at 3000 rpm for at least 3 min. A 6- μ l aliquot of the supernatant was injected into the HPLC system equipped with a UV detector (model 1100; Agilent Technologies, Santa Clara, CA). The analytical column used was an Ace 3 C18 3 μ m 50 \times 3.0 mm (Advanced Chromatography Technologies, Aberdeen, Scotland) and was kept at 50 $^{\circ}$ C. The samples were run under isocratic conditions with diode array detection (EFV and LPV at 210 nm and ATV at 280 nm for 11 and 9 min, respectively). The mobile phase consisted of 60% of a mixture of acetonitrile/methanol(50:50, v/v) 40% 10 mM acetic acid and 4 mM potassium hydroxide, pH 4.4, with flow rate of 0.8 ml/min. The standard curves were linear over a working range of 158–31,568 ng/ml for EFV, 314–62,881 for LPV and 141–28,194 ng/ml for ATV. The intra-assay and inter-assay precision were studied respectively at 1,000 and

4,000 ng/ml for EFV, 1,000 and 8,000 ng/ml for LPV and 200 and 1,000 ng/ml for ATV. The intra-assay coefficient of variations (CVs) were 1.3 and 1.8% for EFV ($n = 5$), 2.4 and 1.7% for LPV ($n = 5$) and 2.8 and 1.4% for ATV ($n = 5$), respectively. The inter-assay CVs were 1.9 and 2.3% for EFV ($n = 25$), 2.7 and 1.8% for LPV ($n = 25$) and 4.5 and 2.7% for ATV ($n = 25$), respectively. The limit of detection signal-to-noise ratio of 3) was 47 ng/ml for EFV, 75 ng/ml for LPV and 42 ng/ml for ATV. The limit of quantification (signal-to-noise ratio of 10) was 158 ng/ml for EFV, 252 ng/ml for LPV and 141 ng/ml for ATV.

The laboratory is accredited by the Swedish accreditation body SWEDAC and participates in the KKGTT quality assurance program for the analysis of antiretroviral drugs.

Plasma HIV-RNA was quantified at baseline and at study weeks 4, 12, 24 and 48 using the Roche Amplicor Ultrasensitive Assay ver. 1.5 (Roche Molecular Systems Branchburg NJ).

Serum bilirubin concentration was determined at baseline and at study weeks 4, 12, 24 and 48 by methods routinely used at the clinical chemistry departments of the respective centres where the patients were treated.

Adherence assessment

Adherence was self-reported by patients at weeks 4, 12, 24 and 48, using a modified version of the validated ACTG adherence questionnaire [9, 10].

Statistical analysis

All statistical calculations were performed using StatView software (SAS Institute, Cary, NC). For between-group comparisons of continuous variables, we used the independent two-sample t test or the Mann-Whitney U test, as considered appropriate. For categorical variables, we used the Fisher exact test. Correlations were analysed by Spearman's rank correlation coefficient.

Ethics

Subjects gave their informed written consent, and the study was approved by the local Research Ethics Committees, the Swedish Medical Products Agency and the Norwegian Medicines Agency.

Results

The demographics and baseline characteristics of the patients in the PK/PD substudy are shown in Table 1. Plasma trough concentrations of EFV, ATV and LPV at week 4, week 12 and week 48 are shown in Table 2.

Table 1 Baseline characteristics

Baseline parameters	EFV	ATV/r	LPV/r
<i>n</i> (Male/female)	71 (45/26)	68 (50/18)	65 (48/17)
Age, median, years (range)	40 (22–74)	42 (27–63)	39 (21–69)
Ethnicity (<i>n</i>)	25 Caucasian	39 Caucasian	39 Caucasian
	28 African	21 African,	18 African
	12 Asian	5 Asian,	7 Asian
	5 Other	1 Other	1 Other
	1 Unknown	2 Unknown	
Mode of transmission (<i>n</i>)	11 Homosexual	29 Homosexual	21 Homosexual
	48 Heterosexual	37 Heterosexual	32 Heterosexual
	5 i.v. drug use	1 i.v drug use	6 i.v. drug use
	1 Transfusion	1 Unknown	6 Unknown
	6 Unknown		
log ₁₀ viral load, median (IQR)	5.30 (4.72–5.69)	5.17 (4.80–5.47)	5.22 (4.88–5.59)
CD4-cell count, median (IQR)	150 (80–201)	170 (117.5–220.25)	150 (100–219)
Co-treatment (<i>n</i>) ^a	TDF+3TC/FTC 51	TDF+3TC/FTC: 43	ZDV+3TC: 56
	ABC+3TC: 10	ABC+3TC: 23	TDF+3TC/FTC: 3
	Other NRTIs: 10	Other NRTIs: 2	ABC+3TC: 5
			Other NRTIs: 1

EFV, Efavirenz; ATV, atazanavir; LPV, lopinavir; i.v., intravenous; IQR, interquartile range

^a NRTI, Nucleoside reverse transcriptase inhibitor; ABC, abacavir; FTC, emtricitabine; TDF, tenofovir; ZDV = zidovudine; 3TC, lamivudine

Efavirenz

Of the 71 patients on EFV, four experienced virological failure according to criteria. One patient failed at week 4. For this patient, EFV was not detectable in plasma, and poor adherence was suspected (though the patients reported not missing any doses in the adherence questionnaire). Another patient never reached <50 copies/ml and was a protocol-defined virological failure at week 12; the EFV trough concentration of this patient at week 4 was 725 ng/ml. A third patient experienced a virological rebound at week 48; the week 4 concentration was 1,529 ng/ml. A fourth patient failed virologically at week 24 and had a week-12 trough concentration of 12,223 ng/ml

Five patients discontinued EFV due to adverse events, of which four were due to central nervous system (CNS) effects—one at week 8, one at week 9 and two at week 24, respectively. The EFV concentrations of the patients

at week 4 were ordinary and ranged from 1,122 to 1,950 ng/ml.

The median decrease in viral load at week 4 was 2.55 log₁₀ [inter-quartile range (IQR) 2.17–2.95]. The proportion of patients with <50 HIV-RNA copies/ml plasma at week 4 was 9/64.

Excluding the one patient without detectable plasma EFV who was judged to be non-adherent by the treating physician, there was no significant difference in viral load decline between those above and below 1,000 ng/ml (2.61 log₁₀ vs. 2.34 log₁₀, *p* = 0.25, Student's *t* test). In an exploratory analysis, there was a trend toward a lower viral response in individuals with concentrations <800 ng/ml, with a median decline of 2.07 log₁₀ vs. 2.56 for those above (*p* = 0.0963, Mann–Whitney *U* test). However, only four samples were below this limit. There was no increase in the effect on plasma viremia with increasing drug concentrations above this limit (Fig. 1).

Table 2 Drug concentrations at weeks 4, 12 and 48 in the respective treatment groups, and proportion of samples suggesting efficacy cut-offs

	EFV	ATV/r	LPV/r
Week 4, median concentration ^a	1,852 (1,202–2,517)	691 (461–1,125)	6,029 (4,239–8,387)
Proportion below suggested efficacy cut/off	11/64 < 1,000 ng/ml	3/58 < 150 ng/ml	1/49 < 1,000 ng/ml
Week 12, median concentration ^a	1,983 (1,465–2,715)	673 (432–975)	5,604 (3,705–11,307)
Proportion below suggested efficacy cut/off	1/19 < 1,000 ng/ml	0/19 < 150 ng/ml	0/16 < 1,000 ng/ml
Week 48, median concentration ^a	2,011 (1,539–2,556)	621 (469–903)	5,314 (3,828–6,425)
Proportion below suggested efficacy cut/off	3/47 < 1,000 ng/ml	1/52 < 150 ng/ml	0/48 < 1,000 ng/ml

ATV/r, Ritonavir-boosted ATV; LPV/r, ritonavir-boosted LPV

^a Median concentration in nanograms per millilitre, with the IQR given in parenthesis

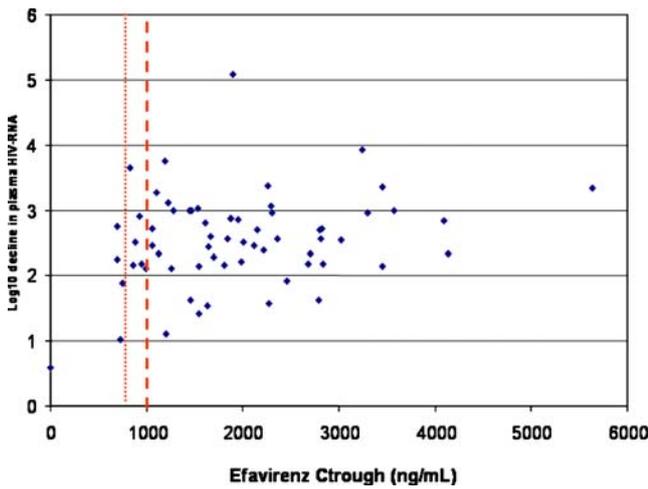


Fig. 1 The relation of efavirenz C_{trough} to the \log_{10} decline in human immunodeficiency virus (HIV)-RNA at week 4. The *thick dotted line* denotes the suggested efficacy cut-off of 1,000 ng/ml, whereas the *thin line* denotes 800 ng/ml, below which there is a trend towards lower antiviral potency (excluding the patient without detectable EFV). C_{trough} Plasma concentration at the end of the dosing interval, just prior to the next dose

Three of the eleven patients with low plasma EFV concentrations at week 4 received dose increases of EFV; all of these patients reached virological suppression. Another patient with an EFV $C_{trough} > 1,000$ ng/ml temporarily had a dose increase when the viral load increased from week 3 to week 4. This patient was also subsequently virologically suppressed.

Atazanavir

Of the 68 patients on ATV/r, six experienced protocol-defined virological failure. One of these failed at week 12; the other five failed at week 24. Their median ATV C_{trough} at week 4 was 604 ng/ml (range 453–1,072 ng/ml). For three of these patients, week-12 concentrations were available and ranged from 501 to 948 ng/ml.

None of the ATV-treated patients in the PK/PD substudy discontinued due to adverse events.

The median decrease in plasma viral load at week 4 was 2.11 \log_{10} (IQR 1.80–2.45). The proportion of patients with >50 HIV-RNA copies/ml plasma at week 4 was 3/58. The virological response at week 4 of the three patients with plasma ATV below the suggested cut-off of 150 ng/ml was similar to that of those above. There was no apparent increase in the effect on plasma viremia with increasing drug concentrations (Fig. 2).

Serum bilirubin was measured at baseline and at treatment weeks 4, 12, 24 and 48. There was a statistically significant correlation between the ATV C_{trough} at week 4 and the increase in s-bilirubin at week 4 (Spearman's $\rho = 0.445$, $p = 0.0008$) as well as between the 4-week

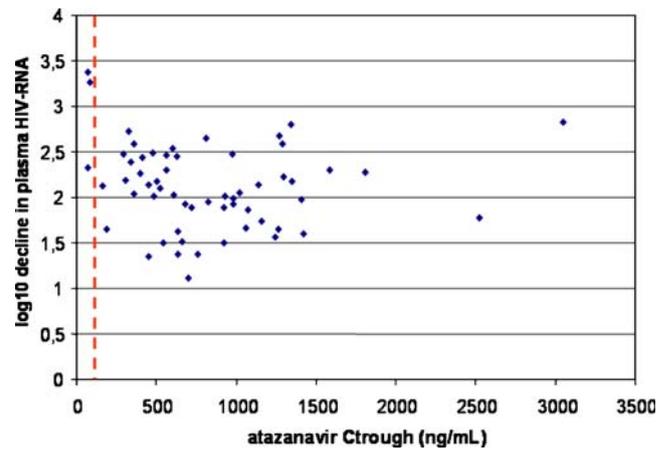


Fig. 2 The relation of atazanavir C_{trough} to the \log_{10} decline in HIV-RNA at week 4. The *dotted line* denotes the suggested efficacy cut-off of 150 ng/ml

ATV concentration and the mean increase in bilirubin at weeks 4 through 48 or to study discontinuation (Spearman's $\rho = 0.410$, $p = 0.0020$). Although the median increase in bilirubin was higher in patients successfully treated for 48 weeks than in those who failed virologically (29.75 vs. 22.7 $\mu\text{mol/l}$), this difference was not statistically significant ($p = 0.1454$, Mann–Whitney U test). Exploring the relation between the mean increase in serum bilirubin and the risk of virological failure in patients treated with ATV/r, there was a trend towards a higher rate of virological failure in patients with a mean bilirubin increase of <25 $\mu\text{mol/l}$ (1.46 mg/dl) ($p = 0.0746$, Fisher's exact test) (Fig. 3). This cut-off predicted virological failure with a sensitivity of 78% and a specificity of 57%.

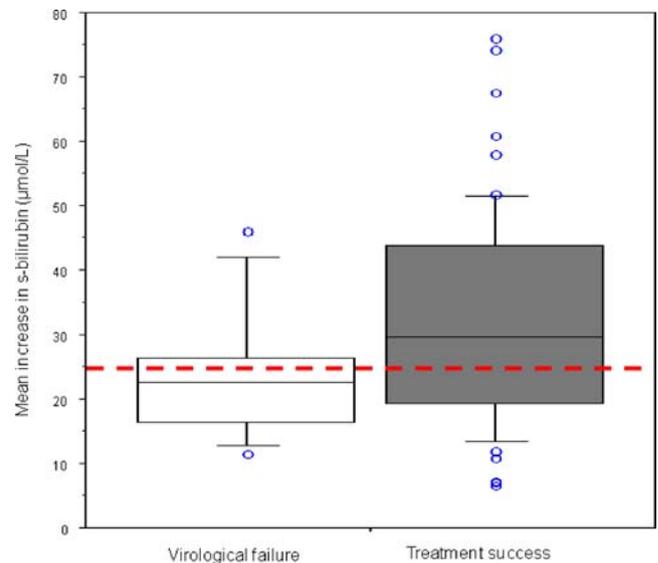


Fig. 3 The mean on-treatment increase in serum bilirubin (*s-bilirubin*) from baseline in patients treated with ATV, by virological success or failure. *Dashed line* s-bilirubin level of 25 $\mu\text{mol/l}$

Two patients on ATV had dose increases to 400/100 mg q.d; in one of these cases the increase was due to a low plasma concentration. Two patients at various times reported taking 150/100 mg q.d, due to misunderstanding of dosing instructions.

Lopinavir

Of the 65 patients in the LPV/r group, three experienced virological failure. All of these patients failed at week 24. The LPV concentrations at week 4 or 12 of these patients were 5,063, 7,929 and 5,005 ng/ml, respectively.

Five patients discontinued LPV due to adverse events. Four of these adverse events consisted of gastrointestinal symptoms, at study weeks 7, 8, 16 and 22, respectively. The median plasma LPV concentration of these patients at week 4 or 12 was 7,252 ng/ml, and the range was 570–11,628 ng/ml.

The median decrease in plasma viral load at week 4 was 2.33 log₁₀ (IQR 2–2.61). The proportion of patients with <50 HIV-RNA copies/ml plasma at week 4 was 6/49. The one patient with a LPV concentration <1,000 ng/ml experienced roughly the same virological effect as the sample mean. There was no apparent increase in the effect on plasma viremia with increasing drug concentrations (Fig. 4).

Eleven patients started treatment with Kaletra tablets rather than the SGCs. Another eight patients changed from capsules to tablets during the course of the study reported here. Three patients had dose increases to 533/133 mg b.i.d. One of these experienced virological failure. Three patients had dose decreases to 266/66 mg b.i.d.

Self-reported adherence

Self-reported adherence data at one or more visits were available for 75/77 patients on EFV, 79/81 patients on ATV/r

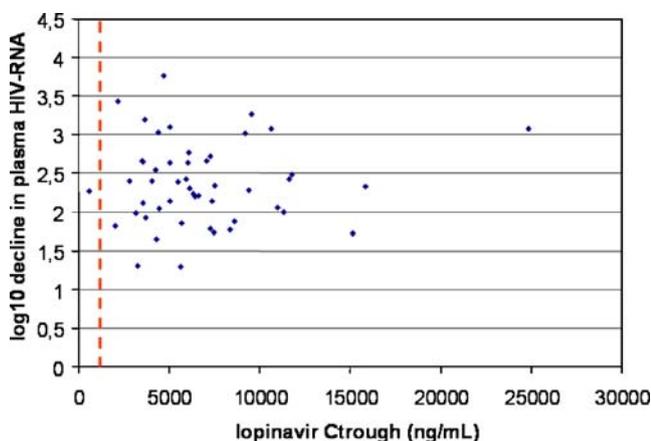


Fig. 4 The relation of lopinavir C_{trough} to the log₁₀ decline in HIV-RNA at week 4. Dotted line Suggested efficacy cut-off of 1000 ng/ml

and 78/81 patients on LPV/r from among the total population of the NORTHIV trial. Overall, reported adherence was very good, with only 7/239 patients at any time reporting seven or more missed doses the preceding month. The proportion of patients with any treatment failure or with virological treatment failure reporting at any one visit that at least one dose had been missed during the past month was 22 and 25%, respectively, compared with 26% of patients with successful treatment outcome. The number of missed doses reported was therefore not predictive of virological failure, treatment failure or trough plasma concentration at any time point, nor was it predictive of the viral load decline from baseline to week 4, in any of the treatment groups (data not shown).

Sensitivity analysis

A separate analysis was made on the subset of patients on EFV with sampling times of 16–28 h post-dose, ATV/r with sampling times of 20–28 h post-dose and LPV/r with sampling times of 10–14 h post-dose. No trends different from those reported on the whole dataset with imputed trough values were noted (data not shown).

Discussion

In our study, plasma concentrations of EFV, ATV or LPV were neither predictive of virological efficacy nor of discontinuations due to adverse events. Since the majority of patients had drug concentrations above currently accepted target concentrations, our study was not powered to validate these. Thus, presumably most of the patients in our study had drug exposures far above E_{max} , and failures were not due to insufficiency of the prescribed doses.

In our material, 17% of EFV-treated patients at week 4 had trough concentrations below the suggested cut-off of 1,000 ng/ml. We were unable to detect any difference in virological effect in this group. There was a trend towards lower antiviral potency in the few patients with concentrations <800 ng/ml. We present this exploratory finding since, given the other datasets available, this could represent a true decreasing antiviral effect in this range, although its clinical relevance is not shown here.

In one study which used a dose ranging procedure for EFV in antiretroviral-naive patients, the authors did not distinguish any clear difference in the antiretroviral activity of the three doses tested (200 mg q.d, 400 mg q.d. and 600 mg q.d.) [11]. In another study, where EFV was added to the treatment regimen of patients failing on dual NRTI therapy, however, the efficacy of 600 mg q.d was superior to that of 400 mg [12]. A combined PK/PD analysis of

these and other premarketing EFV trials showed that treatment failure was threefold more common in patients with $C_{\text{trough}} < 1,100$ ng/ml [13]. These data are not easy to interpret because they include data from the two other studies mentioned [11, 12], but the summary implication of the results is that the minimal effective concentration of EFV is likely to vary depending on the activity of the other drugs used in the combination. In the PK/PD substudy of 2NN, comprising only previously treatment-naïve patients, a $C_{\text{trough}} < 1,100$ ng/ml was associated with an increased risk of virological failure, although the positive predictive value for virological failure was very low [14]. In this study, 1,100 ng/ml was the imputed median C_{trough} of the population; thus, the EFV exposure in that study seems to be lower than in other study populations (see [7, 15, 16]).

The literature is conflicting in terms of the ability of EFV plasma concentrations to predict CNS adverse effects. Some investigators have found positive relations [15, 17, 18], whereas others have not [19, 20]. This difference may be method-dependent. There are, however, interesting data on the relation between CYP2B6 pharmacogenetics, drug exposure and CNS side effects. In one study, a positive relation between EFV exposure and CNS effects was shown at week 1, but it was no longer apparent at week 4 post-treatment initiation, implying a classical pharmacological tolerance effect [21]. We could not find any suggestion of a relation between drug concentrations and discontinuation due to CNS effects in our dataset

Only 3/58 of our ATV samples were below the suggested cut-off of 150 ng/ml at week 4. This is in line with previous reports on the use of ritonavir to boost ATV [22–25]. The support cited for the suggested cut-off level of 150 ng/ml in treatment-naïve patients was generated in an observational cohort of whom only 33% were protease inhibitor (PI)-naïve [2, 26], and other researchers have not been able to validate it [24]. The premarketing dose-ranging monotherapy study of ATV was performed in antiretroviral-naïve subjects with unboosted doses of 200–500 mg q.d., and the results of this study do suggest an increasing potency with increasing exposure over that same dose range [27]. The main source of data on the exposure-response relation of ATV in treatment-naïve subjects, however, is probably the BMS-089 study, where unboosted ATV 400 mg q.d. was compared with ATV/r 300/100 mg q.d [28]. This study shows a trend toward greater virologic efficacy for the ritonavir-boosted regimen and, importantly, a greater frequency of resistance mutations in patients failing virologically on unboosted ATV. A PK/PD analysis from this study has been presented [25] in which the ATV C_{trough} was associated with the probability of virological success, with 74 and 87% of individuals in the lowest and highest quartiles of C_{trough} , respectively, reaching <50 copies/ml. Of note, the upper limit of the lowest quartile

was 118 ng/ml, and all patients below this limit received unboosted ATV. In summary, these results support the higher efficacy of the concentration range seen when ATV is given with a ritonavir-booster, but they do not support the specific cut-off of 150 ng/ml nor the utility of TDM when treatment-naïve patients are treated with ritonavir-boosted ATV. Our results are in agreement with the latter conclusion; the lack of power to define a cut-off largely being a function of the fact that ritonavir-boosting yields ATV drug concentrations far in excess of the minimum effective concentration in the great majority of treatment-naïve patients.

It is well known that ATV causes a concentration-dependent increase in s-bilirubin due to the inhibition of the enzyme UGT1A1, which is responsible for the conjugation of bilirubin, an effect that is influenced by genetic polymorphisms of UGT1A1 [29–31]. Still, an inverse relation between ATV-associated hyperbilirubinemia and the risk of virological failure has been reported, prompting the suggestion that this widely available and inexpensive test may be potentially useful as a biomarker of ATV exposure and predictor of the risk of treatment failure. In a retrospective cohort with a median treatment experience of six agents, an increase of s-bilirubin of >0.4 mg/dl (6.84 $\mu\text{mol/l}$) from baseline was predictive of virological response [5]. In a study of ATV maintenance monotherapy, median s-bilirubin, but not ATV concentration, was significantly higher in responders than in failures [4]. This has led to the suggestion that s-bilirubin may be a more accurate marker of ATV exposure over time than the ATV plasma concentration. Although the results on this aspect of our study in treatment-naïve patients is inconclusive, it is interesting that the mean increase in s-bilirubin from baseline was lower in patients with virological failure on ATV treatment, and that there was a trend towards a statistically significantly increased risk of failure in patients who did not have a mean s-bilirubin increase of >25 $\mu\text{mol/l}$ (1.46 mg/dl). This cut-off is different from that proposed by Petersen et al. [5], but again there is a suggestion that s-bilirubin may reflect ATV drug exposure in a clinically valuable way. Evaluation of the trend in s-bilirubin may be a valuable part of the assessment of adherence in patients treated with ATV.

With LPV, sufficient drug concentrations cannot be reached in the absence of CYP3A inhibition, such as with ritonavir-boosting. Therefore, this drug has only been given to patients with ritonavir boosting. LPV/r was dose-ranged at 200/100, 400/100 and 400/200 mg b.i.d., without any clear differences in virological effect [6]. LPV/r has also been studied and is registered in the USA at the dose 800/200 q.d in treatment-naïve patients. Although this dose has been demonstrated to be “non-inferior” to 400/100 b.i.d [32, 33], it is still to some extent a matter of contention

whether it is equally efficacious [34]. A published PK/PD analysis on the relation between LPV concentration and effect in patients receiving these doses, as well as 800/200 q.d., states that no relation between drug concentration and antiretroviral effect was observed within the range of LPV exposures investigated [35]. The direct clinical evidence cited for the suggested efficacy cut-off of 1,000 ng/ml in treatment-naïve patients appears to be restricted to a study of 20 children with prior NRTI experience, who were treated with a dual-boosted PI regimen, including saquinavir and LPV [36]. Our study was unable to validate this cut-off; indeed, since only one of 49 samples was below this level, we were unable to test it at all. In a recently published dataset from the MaxCmin trials, the experience was similar: only five of 70 LPV trough concentrations were below 1,000 ng/ml [37]. Yet, even though it is rare that adherent patients with LPV/r 400/100 mg b.i.d. have troughs below this level, the issue of the—hitherto undefined—lower efficacy target of LPV is of substantial importance in a range of clinical situations, such as in relation to the higher oral clearance seen in pregnancy, and the consequent question of the appropriate dose of LPV under these circumstances [38]. In our view, the question of the minimal effective concentration for LPV in treatment-naïve patients remains unanswered. However, we believe that the definition and validation of a minimum effective concentration for LPV through observational studies in patients treated with recommended doses may not be practically feasible.

Our patients had high self-reported adherence. While in some cases this was not in agreement with the virological and pharmacological findings, on a whole the low rate of virological failure and the low proportion of drug concentrations below the suggested lower efficacy targets support the view that this cohort indeed had good adherence. As mentioned, our study had very low statistical power to validate or define a lower efficacy cut-off. Importantly, however, this is related to the relation between recommended drug doses, drug exposure and virological effects, which is such that indeed very few adherent patients have insufficient drug exposure, regardless of the exact correct minimum effective concentration. In this respect, our data are in agreement with published results.

Apart from power issues, we recognise a number of other weaknesses in our study. Drug concentrations for most patients were only sampled on two occasions, at week 4 (or 12) and at week 48, and only the earlier samples could be related to treatment efficacy, since all patients sampled at week 48 were virologically suppressed. Furthermore, in some cases, doses were changed after week 4 with knowledge of the plasma drug concentration, which may have affected the outcome of the study.

Conclusion

Drug concentrations at week 4 did not relate to treatment outcome in our cohort, and a clear relation between treatment-limiting toxicities and drug concentration was absent in our material. These results are congruent with other datasets cited, implying that the utility of routine TDM in treatment-naïve HIV-1 infected patients is questionable. Whereas no clear concentration–effect relations emerged, the monitoring of s-bilirubin as an adherence marker may be of value in patients treated with ATV.

Acknowledgements This study was supported by grants from the Sahlgrenska Academy at the University of Gothenburg (ALFGBG-11067), the Research Foundation of Swedish Physicians Against AIDS, The Health & Medical Care Committee of the Region Västra Götaland (VGFOUREG 25921), the Swedish Research Council (2007–7092), and the Stockholm County Council ALF-grant (581513).

References

1. Nettles RE et al (2006) Marked intraindividual variability in antiretroviral concentrations may limit the utility of therapeutic drug monitoring. *Clin Infect Dis* 42(8):1189–1196
2. la Porte CJL et al (2006) Updated guidelines to perform therapeutic drug monitoring for antiretroviral agents. *Rev Antiviral Therapy* 3:4–14
3. Hammer SM et al (2008) Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society–USA panel. *JAMA* 300(5):555–570
4. Karlstrom O, Josephson F, Sonnerborg A (2007) Early virologic rebound in a pilot trial of ritonavir-boosted atazanavir as maintenance monotherapy. *J Acquir Immune Defic Syndr* 44(4):417–422
5. Petersen K et al (2005) Use of bilirubin as a marker of adherence to atazanavir-based antiretroviral therapy. *AIDS* 19(15):1700–1702
6. Abbott Laboratories (2008) European SPC Kaletra. Available at: <http://www.emea.europa.eu/humandocs/PDFs/EPAR/kaletra/H-368-PI-en.pdf>
7. Bristol-Myers Squibb Pharma (2009) European SPC Sustiva. Available at: <http://www.emea.europa.eu/humandocs/PDFs/EPAR/Sustiva/H-249-PI-en.pdf>
8. Bristol-Myers Squibb Pharma (2009) European SPC Reyataz. Available at: <http://www.emea.europa.eu/humandocs/PDFs/EPAR/reyataz/H-494-PI-en.pdf>
9. Chesney MA et al (2000) Self-reported adherence to antiretroviral medications among participants in HIV clinical trials: the AACTG adherence instruments. Patient Care Committee & Adherence Working Group of the Outcomes Committee of the Adult AIDS Clinical Trials Group (AACTG). *AIDS Care* 12(3):255–266
10. Reynolds NR et al (2007) Optimizing measurement of self-reported adherence with the ACTG Adherence Questionnaire: a cross-protocol analysis. *J Acquir Immune Defic Syndr* 46(4):402–409
11. Haas D et al (1998) A phase II, double-blind, placebo-controlled, dose ranging study to assess the antiretroviral activity and safety of efavirenz in combination with open label zidovudine with lamivudine at 36 weeks (DMP 266–055). In: 12th Int Conf AIDS. Abstract no. 22334. Geneva, Switzerland

12. Mayers D et al (1998) A double-blind, placebo-controlled study to assess the safety, tolerability and antiretroviral activity of efavirenz in combination with open-label zidovudine and lamivudine in HIV-1 infected patients. The Efavirenz Clinical Team. In: 12th Int Conf AIDS. Abstract no. 22340. Geneva, Switzerland
13. Joshi AS et al (1999) Population pharmacokinetics of efavirenz in phase II studies and relationship with efficacy. In: 39th Intersci Conf Antimicrobial Agents Chemother. Abstract no. 1201. San Francisco
14. Leth FV et al (2006) Pharmacokinetic parameters of nevirapine and efavirenz in relation to antiretroviral efficacy. *AIDS Res Hum Retroviruses* 22(3):232–239
15. Marzolini C et al (2001) Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. *AIDS* 15(1):71–75
16. Stahle L et al (2004) Efavirenz plasma concentrations in HIV-infected patients: inter- and intraindividual variability and clinical effects. *Ther Drug Monit* 26(3):267–270
17. Gallego L et al (2004) Analyzing sleep abnormalities in HIV-infected patients treated with Efavirenz. *Clin Infect Dis* 38(3):430–432
18. Gutierrez F et al (2005) Prediction of neuropsychiatric adverse events associated with long-term efavirenz therapy, using plasma drug level monitoring. *Clin Infect Dis* 41(11):1648–1653
19. Kappelhoff BS et al (2005) Are adverse events of nevirapine and efavirenz related to plasma concentrations? *Antivir Ther* 10(4):489–498
20. Takahashi M et al (2007) No observable correlation between central nervous system side effects and EFV plasma concentrations in Japanese HIV type 1-infected patients treated with EFV containing HAART. *AIDS Res Hum Retroviruses* 23(8):983–987
21. Haas DW et al (2004) Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS* 18(18):2391–2400
22. Molto J et al (2007) Monitoring atazanavir concentrations with boosted or unboosted regimens in HIV-infected patients in routine clinical practice. *Ther Drug Monit* 29(5):648–651
23. Smith DE, Jeganathan S, Ray J (2006) Atazanavir plasma concentrations vary significantly between patients and correlate with increased serum bilirubin concentrations. *HIV Clin Trials* 7(1):34–38
24. Cleijnsen RM et al (2007) Therapeutic drug monitoring of the HIV protease inhibitor atazanavir in clinical practice. *J Antimicrob Chemother* 60(4):897–900
25. Bertz R et al (2007) Assessment of pharmacokinetic/pharmacodynamic relationships through 48 weeks from a study in HIV+, ART-naive subjects receiving antiretroviral regimens containing atazanavir 400 mg or atazanavir/ritonavir 300/100 mg once daily. In: 14th Conf Retroviruses Opportunistic Infect. Abstract no. 565. Los Angeles
26. Gonzalez de Requena D et al (2005) Atazanavir C_{trough} is associated with efficacy and safety at 24 weeks: definition of therapeutic range. In: 6th Int Workshop Clin Pharmacol HIV Therapy 2005. Abstract no. 60. Quebec City
27. Sanne I et al (2003) Results of a phase 2 clinical trial at 48 weeks (AI424-007): a dose-ranging, safety, and efficacy comparative trial of atazanavir at three doses in combination with didanosine and stavudine in antiretroviral-naive subjects. *J Acquir Immune Defic Syndr* 32(1):18–29
28. Malan DR et al (2008) Efficacy and safety of atazanavir, with or without ritonavir, as part of once-daily highly active antiretroviral therapy regimens in antiretroviral-naive patients. *J Acquir Immune Defic Syndr* 47(2):161–167
29. Rotger M et al (2005) Gilbert syndrome and the development of antiretroviral therapy-associated hyperbilirubinemia. *J Infect Dis* 192(8):1381–1386
30. Lankisch TO et al (2006) Gilbert's disease and atazanavir: from phenotype to UDP-glucuronosyltransferase haplotype. *Hepatology* 44(5):1324–1332
31. Rodriguez-Novoa S et al (2007) Genetic factors influencing atazanavir plasma concentrations and the risk of severe hyperbilirubinemia. *AIDS* 21(1):41–46
32. Johnson MA et al (2006) A once-daily lopinavir/ritonavir-based regimen provides noninferior antiviral activity compared with a twice-daily regimen. *J Acquir Immune Defic Syndr* 43(2):153–160
33. Gathe J et al (2009) A once-daily lopinavir/ritonavir-based regimen is noninferior to twice-daily dosing and results in similar safety and tolerability in antiretroviral-naive subjects through 48 weeks. *J Acquir Immune Defic Syndr* 50:474–481
34. Clumeck N et al (2007) ARTEMIS—efficacy and safety of lopinavir (BID vs QD) and darunavir (QD) in antiretroviral-naive patients. In: 11th Eur AIDS Conf. Abstract no. LPBS7/5. Madrid, Spain
35. Chiu YL et al (2007) Trough lopinavir concentrations do not predict response to lopinavir/ritonavir-based three-drug regimens in antiretroviral-naive patients. In: 8th Int Workshop Clinical Pharmacol HIV Therapy. Abstract no. 38. Budapest
36. Ananworanich J et al (2005) Pharmacokinetics and 24-week efficacy/safety of dual boosted saquinavir/lopinavir/ritonavir in nucleoside-pretreated children. *Pediatr Infect Dis J* 24(10):874–879
37. Justesen US et al (2007) Pharmacokinetics of two randomized trials evaluating the safety and efficacy of indinavir, saquinavir and lopinavir in combination with low-dose ritonavir: the MaxCmin1 and 2 trials. *Basic Clin Pharmacol Toxicol* 101(5):339–344
38. Stek AM et al (2006) Reduced lopinavir exposure during pregnancy. *AIDS* 20(15):1931–1939