Predictive factors for response to a boosted dual HIV-protease inhibitor therapy with saquinavir and lopinavir in extensively pre-treated patients

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Objective: To evaluate predictive factors for therapy outcome of a boosted double-protease inhibitor (PI) regimen in 58 extensively pre-treated patients with HIV. Methods: Patients received lopinavir/ritonavir 400/100 mg and saquinavir 1,000 mg twice daily without reverse transcriptase inhibitors (RTI). The primary outcome parameter was HIV RNA <400 copies/ml at week 48, secondary parameters were HIV-1 RNA and CD4$^+$ T-cell count changes from baseline to week 48. Pharmacokinetics, genotypic resistance and clinical and individual parameters were correlated with the clinical outcome in regression analyses. Covariates for the analyses were minimum plasma concentration (C_{min}), maximum plasma concentration, area under the concentration versus time curve, half-life and clearance of lopinavir and saquinavir, the genotypic inhibitory quotients (GIQ) of archived (GIQ_{arch}) and baseline PI resistance mutations, previously taken antiretrovirals, archived and baseline viral resistance mutations, baseline HIV-1 RNA and CD4$^+$ T-cell count.

Results: The analyses detected correlations between the primary outcome parameter and several factors: baseline CD4$^+$ T-cell count (P=0.001); absence of mutations at V82T/A/F/I/S plus I54M/V/L (P=0.002) or K20M/R (P=0.010); and lopinavir $C_{min}GIQ_{arch}$ (P=0.046). This regression model had a predictability of 97.0% for response to therapy. Covariates for the decrease of HIV-1 RNA from baseline to week 48 were baseline HIV-1 RNA (P<0.001), lopinavir $C_{min}GIQ_{arch}$ (P=0.013), presence/absence of mutations at V82T/A/F/I/S or I84A/V plus L10I/R/V/F, I54M/V/L or L63P (P=0.018), and previously taken antiretrovirals (P=0.034).

Conclusions: Baseline HIV-1 RNA <5.0 $\log_{10}$ and CD4$^+$ T-cell count >200 cells/µl, lopinavir $C_{min}GIQ_{arch}$ >2,000 ng/ml and the absence of viral resistance mutations at V82T/A/F/I/S and I54M/V/L are highly predictive for therapeutic success of a regimen of saquinavir/lopinavir/ ritonavir without RTI in a heterogenic cohort of patients with an extensive pre-treatment history and highly variable pharmacokinetics.

Introduction

Combinations of protease inhibitors (PIs) with reverse transcriptase inhibitors are currently the most widely administered and recommended antiretroviral second-line therapy regimen [1,2]. However, as adverse effects related to mitochondrial toxicity [3–5] or viral resistance [6] impede the administration of nucleoside analogues in some patients, an alternative therapy option could be a boosted double PI regimen of lopinavir/ritonavir and saquinavir without the addition of reverse transcriptase inhibitors. This combination appears to be promising because of its synergistic antiretroviral activity *in vitro* [7], and has been proposed as one possible therapy option for extensively pre-treated patients who require a nucleoside-sparing regimen [8–12]. However, the use of a lopinavir/ritonavir plus saquinavir regimen is controversial and has been discussed; it is not explicitly recommended in international treatment guidelines [13–19] and only a few studies have been conducted evaluating this regimen. None of these studies were prospective controlled clinical trials, and the use of lopinavir/ritonavir plus saquinavir has been restricted to special clinical settings, including salvage therapy. Furthermore, recent study results indicated that an addition of lamivudine, despite documented nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations, decreases viral fitness and improves therapy outcome [20,21]; however, this addition was not considered in

boosted double PI studies. Thus, experience is limited regarding who qualifies for this regimen with a high chance for therapeutic success, and how a double PI regimen without the addition of NRTI performs in clinical practice.

The present analyses therefore focused on predictors of therapeutic outcome of lopinavir/ritonavir plus saquinavir regimen. We included patient demographics and treatment history, baseline viral load and $CD4^+$ T-cell count, and previously archived PI resistance mutations in the analysis. In addition, we monitored PI plasma concentrations because of reported associations of the development of viral resistance mutations with sustained low plasma concentrations [22–24]. When a virus becomes partially resistant, the genotypic inhibitory quotient (GIQ), which is the ratio of trough antiretroviral plasma concentrations to the number of HIV resistance mutations, has been shown to predict the therapy outcome in multiple pretreated patients [25–28]. Moreover, the GIQ has been shown to be a reasonable target for drug-monitoring-based therapy adjustment, which, when exceeded by the antiretroviral plasma concentrations, is able to overcome HIV resistance.

We analysed these candidate predictors of antiretroviral therapy success in 58 extensively pretreated patients who received lopinavir/ritonavir and saquinavir twice daily without reverse transcriptase inhibitors. Although the clinical and immunological results in a large cohort of patients were recently published [11], the present analysis provides additional data and integrates all available information on PI plasma concentrations, viral resistance and clinical data in order to establish parameters that can be used to identify patients in whom the lopinavir/ritonavir plus saquinavir antiretroviral regimen will be successful.

Materials and methods

Patients

In the context of the LOPSAQ study [11], all patients received lopinavir/ritonavir 400/100 mg (Kaletra®, Abbott Laboratories, Queensborough, UK) plus saquinavir mesylate 1,000 mg (either as Invirase®, *n*=14 or Fortovase®, *n*=44; Hoffmann-La Roche, Basel, Switzerland) twice daily, without the addition of reverse transcriptase inhibitors. Criteria for eligibility into the LOPSAQ study [11] were (i) a lack of therapy options with reverse transcriptase inhibitors due to previous experience of severe toxicity or due to viral resistance, and (ii) genotypic resistance testing [6] showing sufficient sensitivity of HIV-1 to PIs. Patients at any $CD4^+$ T-cell count or viral load were enrolled, but patients taking co-medication known to relevantly modify CYP3A activity [2] and patients with hepatic impairment according to Child–Pugh classification B or C were not enrolled.

Patients from the LOPSAQ cohort [11] were enrolled in the present analysis if (i) they consented to participation in a 12 h pharmacokinetic assessment, (ii) viral resistance analyses were available from the patient's enrolment into the LOPSAQ study and (iii) resistance data from failing therapy regimens was available in the patient's medical history. This resulted in 58 of the 128 LOPSAQ participants qualifying for the present analysis. The legal and ethical standards for observational studies have been observed and adhered to, in accordance with the Declaration of Helsinki.

Pharmacokinetic assessments

Twelve-hour plasma concentration versus time profiles of the three PIs were assessed after ≥2 weeks (median 10 weeks) on therapy. Prior to this assessment, the patients had to document the intake of any medication including herbal medicines and nutritive supplements for 3 days.

On the day of the pharmacokinetic assessment, fasting trough levels were obtained immediately before dosing. Subsequently, blood samples were collected at 1, 2, 4, 6, 9 and 12 h after the drug intake. A standardized breakfast of approximately 2,500 kJ (23% from fat) was offered immediately after drug intake. Blood was centrifuged for 10 min at 2,000 min^{-1} within 20 min after sampling. Plasma was separated and stored at -80°C pending analysis. Lopinavir, saquinavir and ritonavir plasma concentrations were determined by high-performance liquid chromatography tandem mass spectrometry methods (equipment from Merck-Hitachi, Mannheim, Germany, and Applied Biosystems, Streetsville, Canada, at HIV-Lab, TherapiaGmbH, Berlin, Germany), as described elsewhere [29]. The lower limit of quantification was 20 ng/ml; linearity for the calibration curves of all compounds was proven up to 20,000 ng/ml.

The PI plasma concentration versus time profiles were analysed by non-compartmental methods. The minimum and maximum concentrations during the observation period of 12 h (C_{min} and C_{max}, respectively) were read from the data. The area under the plasma concentration versus time curve at steady state (AUC_{ss} 0–12) was calculated using the logarithmic trapezoidal rule.

The genotypic inhibitory quotient

Genotypic resistance data were obtained at therapy baseline and from all previously recorded archived PI resistance mutations (detected with ViroSeq ™ HIV-1 Genotyping System V2, purchased from Abbott, Wiesbaden, Germany as described elsewhere [30]). All HIV-1 resistance mutations against PIs were analyzed.

Specifically, this included L10I/L/R/V, G48V, I54M/V/L, A71T/V, G73S, V77I, V82T/A/F/I/S, I84A/V and L90M, reported to confer resistance to saquinavir [31–33], and L10I/L/R/V, K20M/R, L24I/V, V32I, L33F, M46I/F/V/L, I47V/A, I50V, F53L, I54M/V/L, L63P, A71V/T, G73S, V82T/A/F/I/S, I84A/V and L90M, reported to confer resistance to lopinavir [34–36], with some mutations shared between both [6].

The GIQ was calculated as the ratio of C_{min} to the number of relevant PI mutations, separately for saquinavir and lopinavir. This approach treated one and zero mutations similarly, which is in accordance with the demonstration that viral susceptibility to lopinavir did not differ between one or no mutations [25,37]. For each patient the number and relevance of resistance mutations were defined according to the International AIDS Society USA (IAS–USA) Drug Resistance Mutations Group algorithm [6]. Several variants of the GIQ were calculated and analysed for prediction of therapy success. Thus, additional values of GIQ were calculated from C_{max} or AUC, and with either the complete number of archived PI resistance mutations or the number of viral mutations present at baseline of therapy. This distinction was made because the baseline resistance tests in the LOPSAQ study were performed in the absence of treatment and the disappearance of PI mutations subsequent to a structured treatment interruption was an inclusion criteria for PI pre-treated patients. Therefore, the analysis of the archived mutations takes into consideration that the harboured resistance mutations may quickly be reselected under the investigated therapy regimen.

Statistical analyses

The primary efficacy target parameter was the sustained virological response to therapy at week 48, expressed as HIV-1 RNA <400 copies/ml, in an intention-to-treat analysis. Response or non-response was dichotomized into '0' or '1', respectively, and submitted to binary logistic regression (SPSS 12.0 for Windows, SPSS Inc. Chicago, IL, USA). Demographic parameters (age and sex) and pharmacokinetic and virological parameters were included as regressors after univariable analysis at a significance level of P=0.05. Specifically, pharmacokinetic parameters tested were the lopinavir and saquinavir C_{min}, C_{max} and AUC. Virological parameters tested were the presence or absence of the HIV mutations as described above, and combinations of mutations known to be associated with an above-average loss of viral susceptibility to lopinavir (that is, combinations of either V82T/A/F/I/S or I84A/V plus L10I/R/V/F, I54M/V/L and L63P, or combinations of I54V plus V82A/F and of M46I/V/F/L, I54V plus V82A/F, respectively [36–40]), C_{min} GIQ, C_{max} GIQ and AUC GIQ. In addition, baseline CD4$^+$ T-cell count, HIV-1 RNA at baseline, the number of previously taken antiretrovirals and PIs, the experience of a structured treatment interruption and the accumulated duration of antiretroviral therapy prior to onset of the actual therapy were included. Most interval-scaled variables were included after log-transformation because of the log-normal rather than normal distribution. Logistic regression was performed with stepwise deletion of variables, using minus two times the log likelihood as significance criteria, with α-levels for inclusion and deletion of 0.01 and 0.05, respectively. Differences between responders and non-responders to therapy in the presentation of viral resistance mutations were tested performing Fisher's exact and Pearson χ^2 tests for the single mutation on a significance level of P=0.05. Subsequently, significant variables are selected by the stepwise deletion of variables during the logistic regression analysis, which removes covariates from the model if they are non-significant and not a confounder.

Secondary efficacy target parameters were (i) the reduction in viral load at week 48 as compared with baseline and (ii) increase in CD4$^+$ T-cell count from baseline to week 48. These parameters were analysed by means of multiple linear regression using the same regressors as in the logistic regression. Backward stepwise deletion was employed, with an α-level of 0.01 to remain in the model and of 0.05 to be deleted.

Results

Patients characteristics

The mean (range) baseline data of patients were: men/women (n=48/10); age 42 years (27–67); CD4$^+$ T-cell count 212 cells/μl (1–998); HIV-1 RNA 4.86 $\log_{10}$ copies/ml (1.28–6.00); accumulated time on previous treatments 6.2 years (4.6–8.3); number of previously taken antiretroviral treatments 8.5 (3–14) and previously taken PIs 2.2 (0–6); and structured treatment interruption of >4 weeks prior to baseline (n=38). All patients had at least one resistance assay performed at the end of the last failing regimen. Nevertheless, previously accumulated, and at that time not shown, resistance mutations were also included in the number of archived PI mutations: the mean (interquartile range (IQR) number of resistance tests performed were 3.1 (1–7) in the group of non-responders and 2.1 (1–6) in the group of responders (P=0.023). Seven/two non-responders/ responders had previously taken lopinavir (Fisher's exact P=0.031) and 12/11 non-responders/responders had previously taken saquinavir (Fisher's exact P=0.290). However, the accumulation of genotypic resistance variants M46I/F/V/L, V82T/A/F/I/S plus I54L/T/F was neither correlated to lopinavir experience (Fisher's exact P=0.231) nor saquinavir experience (Fisher's exact P=0.673).

Virological and immunological response to therapy

Thirty-three patients responded to therapy, showing a viral load <400 copies/ml at week 48 (responders, group 1) and 25 patients did not match the primary efficacy criterion (non-responders, group 2). The mean (95% confidence interval [CI]) decrease of HIV RNA from baseline to week 48 in responders to therapy was -2.82 $\log_{10}$ copies/ml (2.38–3.25) versus -1.21 copies/ml (0.58–1.83) in non-responders (P<0.001). Twenty-four patients responding to therapy showed a viral load <50 copies/ml at week 48 and nine patients had a viral load between 50–399 copies/ml with a mean HIV-RNA polymerase chain reaction of 202 copies/ml.

Mean (95%CI) CD4$^+$ T-cell increase from baseline to week 48 differed significantly between the groups (P=0.003): responders showed an increase of 197 cells/µl (132–263), while non-responders gained 75 cells/µl (42–108). The percentage change of the CD4$^+$ T-cell increase from baseline to week 48 showed differences that were not statistically significant: the mean (95% CI) CD4$^+$ T-cell count of responders increased 72.4% (49.3–95.6), whereas non-responders showed an increase of 56.7% (40.0–67.0).

Primary efficacy parameter: prediction of response and non-response to therapy

Predictors identified for therapy response at week 48 (HIV-1 mRNA <400 copies/ml) (Table 1) were: (i) baseline CD4$^+$ T-cell count (χ^2 after logistic regression; P=0.001), (ii) absence of genotypic resistance mutations V82T/A/F/I/S plus I54L/T/F (P=0.002), (iii) absence of the genotypic resistance mutation K20M/R (P=0.010) and (iv) lopinavir C_{min} GIQ of archived mutations (P=0.046). The prediction of therapeutic response based on these parameters was 97.0%. The CD4$^+$ T-cell count at baseline was 274 versus 140 cells/µl in responders and non-responders, respectively (t-test P=0.004; Table 2). The mean (95% CI) lopinavir GIQ, C_{min} of responders and non-responders to therapy was 2,611 ng/ml (1,857–3,365) versus 1,320 ng/ml (712–1928) (t-test P=0.006; Table 3; Figure 1). A combination of mutations at the positions I54V/V82AF was found in seven patients with therapy failure and one patient responding to therapy (Fisher's exact P=0.016; Table 4), the K20M/R was found in five patients with therapy failure but in none of the patients who responded to therapy (Fisher's exact P=0.048; Table 4).

The predictability for non-response to therapy in the final logistic regression model on the basis of a viral load of <400 copies/ml at week 48 was 68.0%. However, when re-including the number of archived saquinavir mutations, which had been excluded in the next-to-last iteration step of the logistic regression, the predictability for non-response increased to 80.0%. Twelve of 25 non-responders had three archived saquinavir mutations compared with only 7/33 responders (Fisher's exact test P=0.048). Because of the increase in productivity, and because it is feasible from a clinical point of view to take the saquinavir mutations into account, this variable was included in the final set of predictors of non-response.

Table 1. Factors significantly correlated with therapy outcome after 48 weeks of therapy with boosted lopinavir and saquinavir without reverse transcriptase inhibitor co-medication

Factor	P-value
Response or non-response to therapy at week 48* (HIV-1 RNA <400 copies/ml or ≥400 copies/ml)	
Baseline CD4$^+$ T-cell count	0.001
V82T/A/F/I/S plus I54M/V/L	0.002
K20M/R	0.010
GIQ, lopinavir C_{min}	0.046
HIV-1 RNA decrease from baseline to week 48†	
Baseline HIV-1 RNA	<0.001
GIQ, lopinavir C_{min}	0.013
V82T/A/F/I/S or I84A/V plus L10I/R/V/F, I54M/V/L, L63P	0.018
Previous exposure to antiretrovirals	0.034

*Binary logistic regression analysis. †Multiple linear regression analysis. C_{max}, maximum plasma concentration within the dosing interval; C_{min}, minimum plasma concentration within the dosing interval; GIQ, genotypic inhibitory quotient (calculated as ratio of measured plasma concentration to the number of previously archived relevant genotypic protease inhibitor resistance mutations, IAS drug resistance mutations 2005).

If a viral load of <50 copies/ml at week 48 (n=24) was taken as efficacy parameter, the logistic regression analysis identified (i) baseline CD4$^+$ T-cell count (χ^2 after logistic regression; P=0.007) and (ii) lopinavir C_{min} GIQ of archived mutations (P=0.046) as predictors for success of therapy. The mean (95% CI) baseline CD4$^+$ T-cell count of non-responders and responders to therapy was 300 cells/µl (214–385) versus 150 (98–201) cells/µl (t-test P=0.002). The mean (95% CI) lopinavir GIQ, C_{min} of responders and non-responders to therapy was 2,805 (1,823–3,787) ng/ml versus 1,525 (1,006–2,043) ng/ml (t-test P=0.013) (pharmacokinetics of lopinavir and saquinavir are shown in detail in Figure 1). Differences in the absence or presence of the genotypic resistance mutations M46I/F/V/L and K20M/R remained as significant covariates in the logistic regression analysis (P=0.046 and P=0.048, respectively). A mutation at the positions M46I/F/V/L was found in 15 patients with therapy failure and four patients responding to therapy, the K20M/R was found in five patients with therapy failure but in none of the patients who responded to therapy.

Secondary efficacy parameter: decrease of HIV-1 RNA and CD4$^+$ T-cell increase

Predictors (Table 1) identified for therapy response at week 48 (HIV-1 mRNA <400 copies/ml) were (Table 2;

Table 2. Baseline data of 58 extensively pre-treated HIV-1-infected adults, receiving a double protease inhibitor therapy regimen with lopinavir/ritonavir 400/100 mg and saquinavir 1,000 mg twice daily without nucleoside reverse transcriptase inhibitors

Parameter	Responders group 1 *n*=33 mean (95% CI)	Non-responders group 2 *n*=25 mean (95% CI)	Difference: group 1 versus group 2 *P*-value[†]
Gender: male/female, *n* (%)	29/4 (87.9/12.1)	19/6 (76.0/24.0)	0.201[‡]
STI yes/no, *n* (%)	20/13 (60.6/39.4)	18/7 (72.0/28.0)	0.267[‡]
Age, years*	40.4 (38.3–42.6)	43.8 (40.1–47.5)	0.262
ART cumulative, years*	5.7 (4.6–6.8)	6.7 (5.7–8.3)	0.370
CD4$^+$ T-cell count, cells/µl (95% CI)	271 (202–341)	133 (74–192)	0.004
HIV-RNA PCR, $\log_{10}$ (95% CI)	4.58 (4.10–5.04)	5.23 (4.85–5.60)	0.028
Previous GRTest, *n**	2.1 (1.7–2.6)	3.1 (2.4–3.8)	0.023
Saquinavir MUTarch, *n**	1.6 (1.0–2.1)	2.6 (1.9–3.9)	0.012
Lopinavir MUTarch, *n**	1.8 (1.1–2.5)	4.2 (2.9–5.5)	0.001
Previous ARVs, *n**	7.7 (6.7–8.7)	9.4 (8.2–10.6)	0.032
Previous PI, *n**	1.8 (1.2–2.4)	2.6 (1.9–3.4)	0.078
Previous saquinavir, *n**	11	12	0.290[‡]
Previous lopinavir, *n**	2	7	0.031[‡]
PI-naive, *n**	10	3	0.122[‡]

*Mean value (95% confidence interval). [†]ANOVA analysis. [‡]Fisher´s exact test. ART cumulative, cumulative time on antiretroviral therapy; MUTarch, archived resistance mutations against saquinavir/lopinavir; PI-naive, HIV protease inhibitor naive; previous ARV, previously taken antiretrovirals; previous GRTest, previously performed genotypic resistance tests; previous PI, previously taken HIV protease inhibitors; previous lopinavir, previous exposure to lopinavir; previous saquinavir, previous exposure to saquinavir; STI, structured treatment interruption.

Table 3. Genotypic inhibitory quotients of protease inhibitors for responders and non-responders to therapy after 48 weeks of therapy with boosted lopinavir and saquinavir without reverse transcriptase inhibitor comedication

Parameter	Responders group1; *n*=33 mean (95% CI)	Non-responders group 2; *n*=25 mean (95% CI)	Difference group 1 versus group 2 *P*-value*
Saquinavir			
GIQ, C_{min}, ng/ml	508 (330–688)	301 (171–431)	0.026
GIQ, C_{max}, ng/ml	1,815 (1,254–2,376)	1,002 (546–1,457)	0.005
GIQ, AUC, ng*h/ml	15,741 (11,200-20,283)	8,813 (5,604–12,021)	0.005
Lopinavir			
GIQ, C_{min}, ng/ml	2,611 (1,857–3,365)	1,320 (712–1,928)	0.006
GIQ, $C_{max,}$ ng/ml	4,408 (3,216–5,560)	2,692 (1,732–3,653)	0.026
GIQ, AUC, ng*h/ml	41,689 (29,909–53,469)	26,560 (16,846-36,275)	0.005

*ANOVA analysis of log-values. AUC, area under the time versus concentration curve; GIQ, genotypic inhibitory quotient (calculated as ratio of measured plasma concentrations to the number of previously archived relevant genotypic protease inhibitor resistance mutations, IAS drug resistance mutations 2005); C_{max}, maximum plasma drug concentration within the 12 h dosing interval; C_{min}, minimum plasma drug concentration within the 12 h dosing interval.

Figure 2) (i) a significant correlation of the baseline viral load (analysis of variance after multiple regression; $P<0.001$), (ii) the log-transformed lopinavir C_{min} archived GIQ (GIQ_{arch}) ($P=0.013$), (iii) absence of genotypic resistance mutations V82T/A/F/I/S or I84A/V plus L10F, I54L/T/F, L63P ($P=0.018$) and (iv) the number of previously taken antiretrovirals in a patients' medical history ($P=0.034$). The mean (95% CI) viral load was 4.58 $\log_{10}$ copies/ml (4.10–5.04) in responders versus 5.23 $\log_{10}$ copies/ml (4.85–5.60) in non-responders to therapy (*t*-test $P=0.028$). The described combination of mutations was detected in eight patients not responding and in none of the patients responding to therapy (Fisher's exact $P=0.002$; Table 4), the mean (95% CI) number of previously taken antiretrovirals was 7.7 (6.7–8.7) in the group of responders versus 9.4 (8.2–10.6) in the non-responder group (*t*-test $P=0.032$; Table 3).

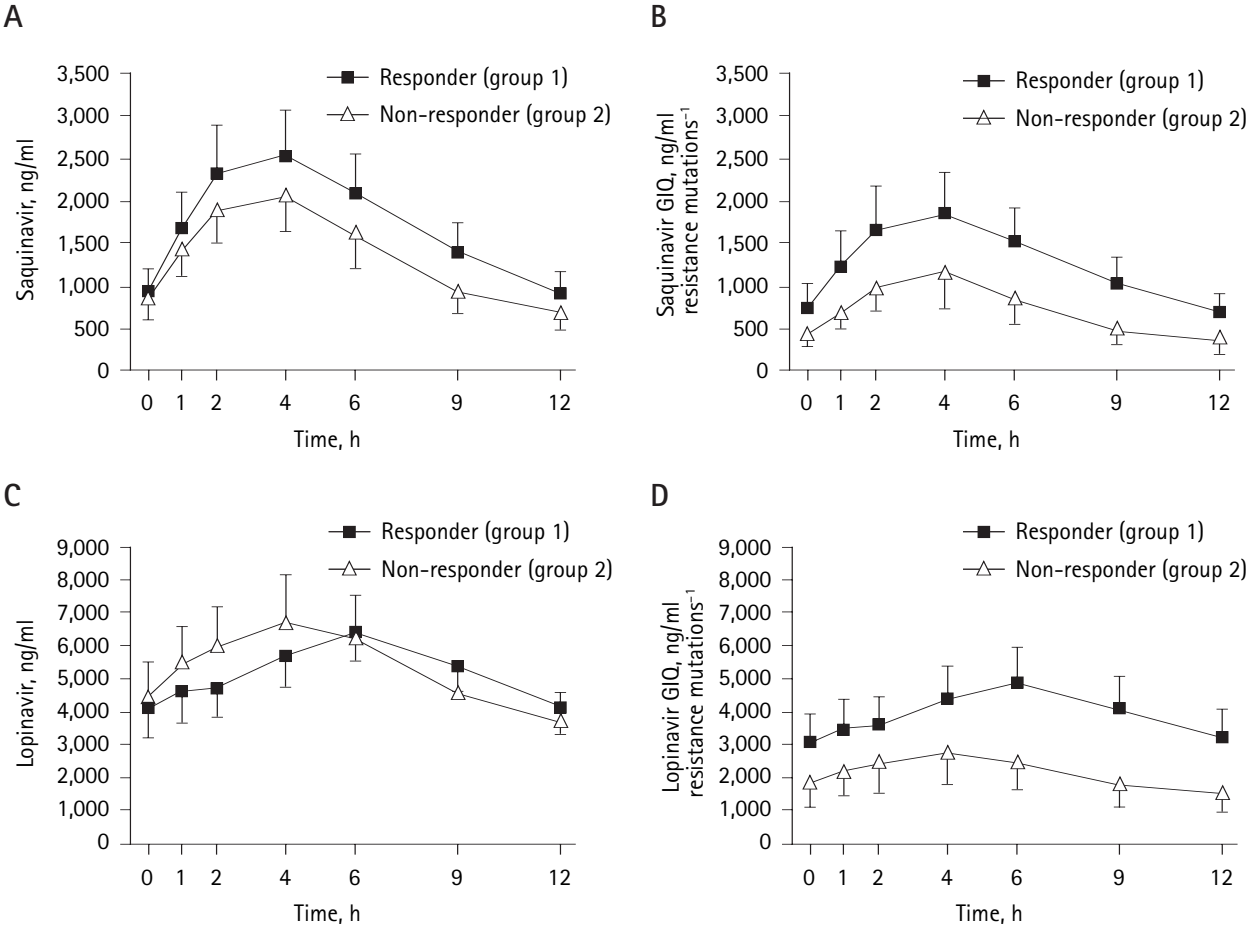
The increase of CD4$^+$ T-cells from baseline to week 48 was not correlated to any of the tested variables.

Discussion

Integration of virological, clinical and pharmacokinetic data into a comprehensive analysis successfully

identified variables which, in combination, were highly predictive for therapy success of a boosted double PI combination of saquinavir and lopinavir in extensively pre-treated patients in whom administration of nucleoside analogues was not prescribed. We showed that HIV-1 mutations known to confer viral resistance especially to lopinavir [37,41,42] play a prominent role in the success of the investigated therapeutic regimen. The reported [37] 14-fold increase in the lopinavir IC_{50} caused by the HIV-1 mutation V82A/F/T and the 16-fold increase in the lopinavir IC_{50} caused by the I54L/T/V variant as compared to wild type represent a clinically important modulator of therapeutic success. Although differences exist in the exact valuation of phenotypic viral resistance in relation to these genotypes, a combination of both variants is considered to decrease viral susceptibility by 10- to >40-fold [40]. However, it remains unclear whether the additional K20M/R mutation, which was detected in the viral genome of five non-responders but none of the responders and was found to be a significant covariate for non-response in the regression models, confers to viral resistance against the tested regimen. Although results from *in vitro* testing reported a significantly reduced susceptibility of HIV-1 against lopinavir in the presence of K20M/R [37], current algorithms (HIV db [40], REGA v7.1.1 [43], ANRS [44], IAS [6]) qualify the potency of the single K20M/R mutation to reduce viral susceptibility against different PIs [40,44], among them lopinavir and saquinavir [40,45]. Detecting the K20M/R mutation as a significant covariate in viral resistance analysis could be due to the fact that K20M/R is often combined to major mutations such as the cited I54L/T/V and V82A/F/T. The appearance of the K20M/R mutation was also not correlated to a non-B HIV-1 subtype [46], which was detected in two responders (subtypes G and CRF02-AG, respectively) and two non-responders (subtypes C and D, respectively); none of these exhibited a K20M/R mutation.

Figure 1. Time versus concentration curves of lopinavir and saquinavir, GIQ excluded **(A, C)** and included **(B, D)**



Lopinavir and saquinavir time versus concentration curves **(A,C)**. Lopinavir and saquinavir mean genotypic inhibitory quotient for responder and non-responder to therapy, calculated as ratio of the mean plasma concentrations to the mean numbers of archived genotypic resistance mutations of the HIV against lopinavir and saquinavir **(B,D)**.

The predominant role of resistance mutations for the therapeutic outcome was indicated by a 90.9% predictability of therapy success, which remained after the exclusion of the GIQ from the regression analysis. Moreover, the improvement of predictability by the lopinavir C_{min} GIQ up to 97% was not obtained until the resistance mutations against lopinavir and saquinavir were analysed separately. This emphasizes the importance of assessing viral resistance mutations separately for each PI when calculating the GIQ. The V82T/A/F/I/S, I54M/V/L and K20M/R mutations confer HIV-1 resistance mainly to lopinavir [37], and therefore GIQs calculated without distinction between the antiretrovirals to which a particular mutation is active evidently lose predictability for the therapeutic outcome. Exclusively the GIQ calculated as ratio of the lopinavir C_{min} to GIQ_{arch} was significantly associated with therapy outcome after 48 weeks.

We assessed several possible variants used in the literature to calculate the GIQ [25,47]. Among GIQ trough concentration (C_{trough}), GIQ C_{min} and GIQ AUC, only the lopinavir C_{min} GIQ was a significant regressor in the present analysis. The GIQ calculated with C_{trough} determined as C_{12h} (the plasma drug concentration 12 h after the previous dosing interval) of the previous dosing interval [26,48] was not correlated to therapy outcome. If included into the binary logistic regression analysis instead of the GIQ of lopinavir C_{min}, leaving out the C_{min} GIQ, the GIQ was no longer identified as predicting the therapeutic outcome. Lopinavir C_{trough} GIQ calculated with the number of archived resistance mutations was deleted from the logistic regression analysis after the third iteration step (P=0.413) and only the viral resistance mutations remained in the final logistic regression model (Table 1). Thus, our data indicate a favouring of the GIQ C_{min} to other possible GIQs.

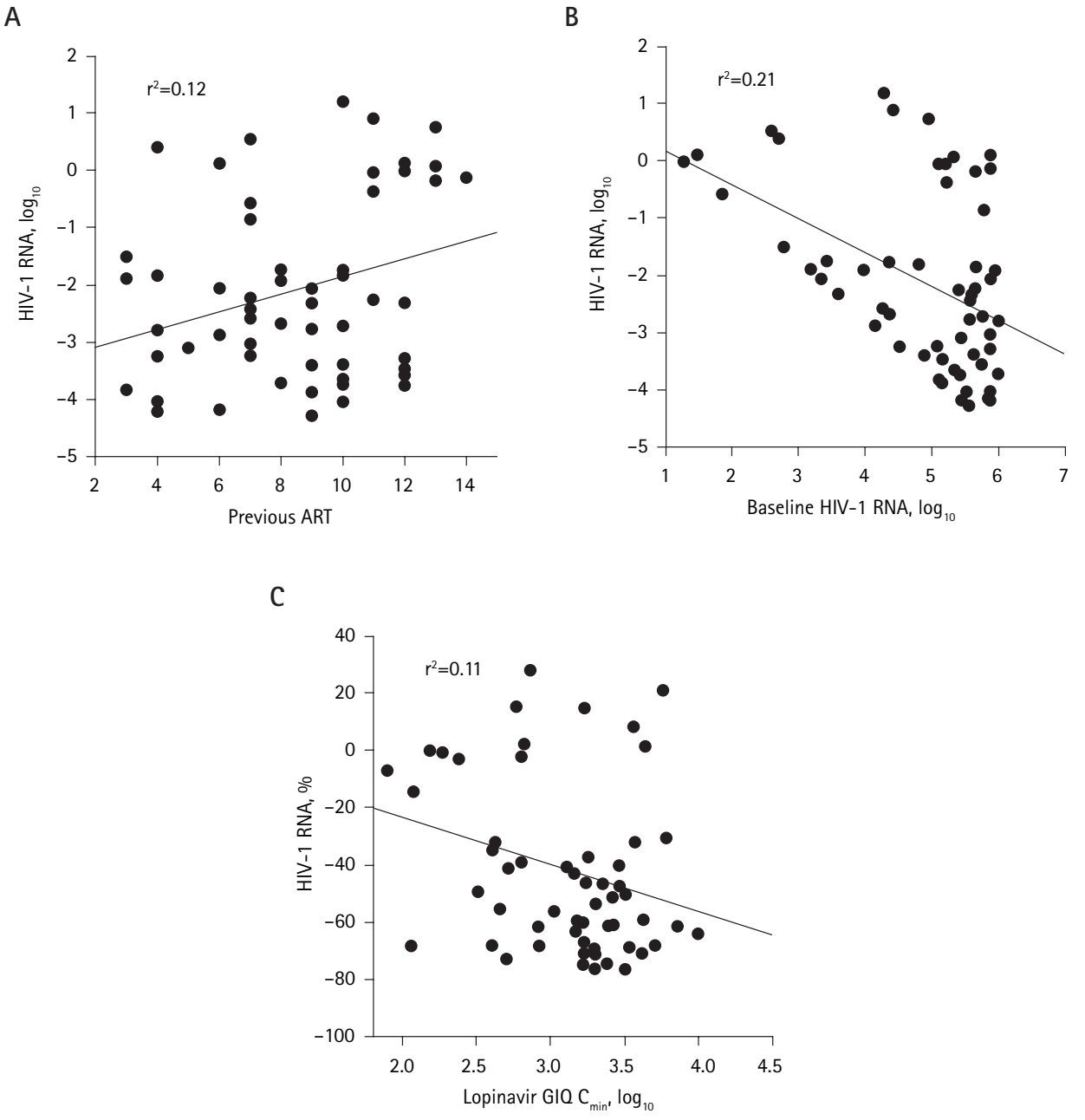
From a practical perspective, clinicians would like to see the results calculated with C_{trough} and not C_{min}, as C_{min} requires a full pharmacokinetic assessment to be performed. However, as the evaluated regimen is for patients with restricted therapy options and must be seen in relation to its high predictivity for therapy outcome, a higher diagnostic effort may be justifiable.

In contrast to the good prediction of therapy response by the presently assessed parameters, the prediction of non-response remained unsatisfactory. Twenty percent of the patients with therapy failure were not predicted to be non-responders. We therefore searched the records of these patients for probable additional reasons [49] for therapy failure, which resulted in non-compliance documented in two patients and perhaps declined drug absorption due to prolonged episodes of diarrhoea in another two patients, whereas for one patient no likely reason for

Table 4. Numbers of viral resistance mutations apparent in genotypic resistance tests at the end of previously failing therapies, archived over the cumulated time on antiretroviral treatment

Resistance mutations of the HIV genome	Responders group 1; *n*=33	Non-responders group 2; *n*=25	Fisher´s exact *P*-value
L10I/R/V/F	13	6	**0.011**
K20M/R	0	5	**0.048**
L24I/V	1	0	1.00
V32I	2	0	0.181
L33F	3	1	0.305
M46I/V/F/L	14	5	0.431
I47V/A	0	1	0.431
G48V	2	1	0.572
I50V	0	1	0.431
I54M/V/L	8	2	**0.014**
L63P	21	17	0.786
A71TV	11	7	0.087
G73S	6	2	0.065
V77I	18	10	0.301
V82T/A/F/I/S	9	4	0.054
I84A/V	4	5	0.459
L90M	10	7	0.151
V82T/A/F/I/S or I84A/V plus L10I/R/V/F, I54M/V/L, L63P	0	7	**0.002**
V32I plus I47V/A	0	1	0.439
V82T/A/F/I/S + I54M/V/L	1	7	**0.016**

Figure 2. Parameters correlated with viral load decrease over 48 weeks



Variables of the multiple linear regression analysis correlated significantly with viral load decrease from baseline to week 48: the number of previously taken antiretrovirals **(A)** (P=0.034), baseline HIV-1 RNA **(B)** (P<0.001), and the genotypic inhibitory quotient of lopinavir minimum plasma concentration **(C)** (P=0.013). ART, antiretroviral therapy; GIQ, genotypic inhibitory quotient.

therapy failure was detected. The fact that adherence has not been assessed consequently in this study may explain the remaining proportion of patients in whom therapy failure could not be predicted with this model.

The presently observed significant correlation between the lopinavir C_{min} GIQ and the HIV-1 RNA decrease from baseline, and moreover the significant association of therapy outcome with the lopinavir C_{min} GIQ, apparently suggest monitoring of PI plasma concentrations at the beginning of therapy. In cases where a lopinavir C_{min} GIQ of <1,800 ng/ml are found (Table 2), dose escalation might be employed to overcome phenotypic viral resistance. However, this reasoning bears certain clinical difficulties. On the one

hand, the high intra-individual variability of PI plasma concentrations [50] decreases the reliability at which the intended C_{min} change can be maintained. On the other hand, the association of high lopinavir C_{max} with side effects [51,52], for example, diarrhoea, indicates a narrow therapeutic window for a dose escalation. Therefore, the addition of a second class of antiretrovirals such as enfuvirtide [53,54] may be preferred in order to improve the patient's response to therapy.

Taken together, this shows that the presently investigated boosted dual PI regimen may be advised for extensively pre-treated patients who no longer have therapy options with reverse transcriptase inhibitors when certain prerequisites are met (Table 2); specifically, if these patients had seven previous antiretrovirals, three saquinavir mutations and no V82T/A/F/I/S, I54M/V/L or K20M/R mutations in their treatment history, and if they have a $CD4^+$ T-cell count of >200 cells/µl and a viral load of <5.0 $\log_{10}$ HIV-1 RNA at baseline of therapy. After 4 weeks on treatment a 12 h pharmacokinetic assessment should provide additional data for the calculation of the lopinavir C_{min} GIQ, which should exceed 1,800 ng/ml. The early individual prediction of response or non-response to this therapy offers the chance to select the applicable subjects prior to baseline and to modify the therapy regimen after the assessment of the lopinavir C_{min} GIQ, thus avoiding sustained viral replication under therapy with the selection of new viral resistance mutations [55] and the loss of the remaining treatment options.

In conclusion, viral resistance mutations are most predictive for success or failure of HIV therapy in extensively pre-treated patients taking a standard dose combination of saquinavir, lopinavir and ritonavir, but without the addition of NRTIs. Also, baseline $CD4^+$ T-cell count and HIV-1 RNA are predictors of response to therapy. The pharmacokinetics of lopinavir only gains significance as GIQ, calculated as the ratio of C_{min} to the archived lopinavir mutations. This provides a rational basis to select this nucleoside-sparing, single-class therapeutic regimen among the few therapeutic options for extensively pre-treated patients in whom nucleoside analogues cannot be administered because of side effects or viral resistance.

Although in the year 2007 new therapeutic options are available, such as the novel PIs tipranavir and darunavir, the investigational non-nucleoside reverse transcriptase inhibitor etravirine or the upcoming integrase inhibitors and CCR5 receptor blockers, the recycling of combinations of established antiretrovirals may be considered in certain clinical settings. Well-known side-effect profiles, pharmacokinetics and interaction potential, lack of therapeutic options with other drug classes, patient's decision or the concept of keeping new options for later stages of an infection which has to be treated life long, argue for the use of a boosted double PI regimen prior to switching to new or investigational drugs.

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