

Effect of a Modified Saquinavir/Ritonavir Dosing Regimen with Lower Dose Lead-In Phase on QTc Interval, Pharmacokinetics, Antiviral Activity and Safety in Treatment-Naïve HIV-1-Infected Patients

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Abstract

Background Saquinavir/ritonavir (1000/100 mg twice daily [BID]) is associated with dose- and exposure-dependent prolongation of the QT interval. The QT risk is considered higher during the first week of therapy, when saquinavir peak exposure has been observed. A modified regimen with a lower dose lead-in phase may reduce potential saquinavir/ritonavir-induced QT prolongations.

Objective To explore the effect of the modified saquinavir/ritonavir regimen on QT interval, pharmacokinetics, antiviral activity, and safety in treatment-naïve HIV-1-infected patients.

Methods Twenty-three HIV-1-infected treatment-naïve patients received saquinavir/ritonavir 500/100 mg BID on days 1–7 and 1000/100 mg BID on days 8–14 in combination with two nucleoside reverse transcriptase inhibitors. The primary endpoint was mean maximum change from dense predose baseline in QT values corrected using Fridericia's formula ($\Delta\text{QTcF}_{\text{dense}}$) across study days. Secondary endpoints included maximum change from time-matched baseline in QTcF, antiviral activity, pharmacokinetics, and safety over the 14 days.

Results The mean maximum $\Delta\text{QTcF}_{\text{dense}}$ was 3, 1, 7, 12, and 7 ms on days 3, 4, 7, 10, and 14, respectively. Across all study days, 2/21 patients had a maximum $\Delta\text{QTcF}_{\text{dense}} \geq 30$ ms (on day 10); the highest mean $\Delta\text{QTcF}_{\text{dense}}$ was <10 ms. During week 1, saquinavir exposure was highest on day 3 and lowest on day 7. All patients showed continuous declines in HIV-RNA; none experienced virologic breakthrough/rebound. The modified regimen was generally well tolerated.

Conclusion Treatment initiation with the modified saquinavir/ritonavir regimen in treatment-naïve HIV-1-infected patients reduced saquinavir exposure during week 1, potentially mitigating/reducing QT liability while suppressing HIV-RNA during the course of treatment.

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Key Points

QT prolongation by protease inhibitors, including saquinavir, has been demonstrated to be a result of dose-dependent blockage of the human ether-a-go-go-related gene (hERG) channels.

A modified saquinavir/ritonavir dosage regimen is recommended in the EU for treatment-naïve patients initiating therapy (500/100 mg twice daily for the first 7 days, then 1000/100 mg twice daily thereafter). This modified regimen is predicted to reduce the risk of saquinavir-associated QT prolongation in the first week of treatment.

The results of this trial demonstrate that treatment initiation with the modified saquinavir/ritonavir is associated with reduced saquinavir exposure during the first week, potentially mitigating or reducing QTc liability while suppressing HIV RNA levels.

1 Introduction

In 1995, saquinavir became the first HIV-1 protease inhibitor approved for the treatment of HIV-1 infection, and it was subsequently approved in combination with low-dose ritonavir in the member states of the European Union and in a number of other countries. The oral bioavailability of saquinavir is limited by extensive first-pass metabolism, mediated primarily by cytochrome P450 3A4 enzyme (CYP3A4) [1]. Coadministration of low-dose ritonavir, another protease inhibitor that is also a potent inhibitor of CYP3A4 and P-glycoprotein [2], increases saquinavir bioavailability and exposure, thereby improving therapeutic efficacy. The approved recommended therapeutic dose in the US and Europe for adults is saquinavir 1000 mg twice daily (BID) in combination with ritonavir 100 mg BID. The efficacy and safety of saquinavir/ritonavir 1000/100 mg BID have been demonstrated in several large phase III clinical trials [3, 4] and, since its approval in 1995, approximately 1,200,000 patients have received saquinavir (Roche, personal communication).

There is uncertainty in the literature as to whether there is an effect of HIV protease inhibitors on cardiac conductivity as measured by prolongation of QT and PR interval durations. Despite this apparent uncertainty, the results of a previous TQT study in healthy subjects demonstrated significant dose-dependent QT and PR interval prolongation with therapeutic and suprathreshold doses of saquinavir/ritonavir 1000/100 mg BID and 1500/100 mg BID,

respectively [5]. In response to the findings of the TQT study and risk of significant QT prolongation in the first week of treatment with saquinavir/ritonavir 1000/100 mg BID, the recommended dosing regimen for treatment-naïve patients initiating ritonavir-boosted saquinavir therapy was amended in 2011 by the European Medicines Agency. The revised European label recommends that treatment-naïve patients initiate therapy with saquinavir/ritonavir at a dosage of 500/100 mg BID for the first 7 days followed by the standard dosage of saquinavir/ritonavir 1000/100 mg BID thereafter [6]. No changes to dosing recommendations were requested by the US FDA. The current study was carried out at the request of the European Medicines Agency to explore the effect of the modified saquinavir/ritonavir regimen (500/100 mg BID on days 1–7 followed by 1000/100 mg BID on days 8–14) on the QTc interval, pharmacokinetics, antiviral activity, and safety in treatment-naïve HIV-1-infected patients.

2 Methods

This single-center, open-label, multiple-dose, single-arm study was conducted between January and June 2012 (ClinicalTrials.gov number NCT01638650). The primary objective of the study was to measure the maximum change from dense predose baseline in QTcF ($\Delta\text{QTcF}_{\text{dense}}$) (see Sect. 2.4). Secondary objectives included the collection of information on the pharmacokinetics, antiviral activity, and safety of the modified saquinavir/ritonavir regimen and to evaluate the effect of this regimen on other electrocardiogram (ECG) parameters (heart rate, RR interval, QT interval, PR interval, QRS interval). Exploratory analyses were also planned to explore the relationship, if any, between changes in QTc interval and saquinavir plasma concentrations.

2.1 Patients

HIV-1-infected treatment-naïve patients who were at least 18 years of age were included in the study if they were eligible and willing to initiate a saquinavir (Invirase®; F. Hoffmann-La Roche, Basel, Switzerland) plus ritonavir (Norvir®; Abbott, Chicago, IL, USA) regimen in combination with two nucleoside reverse transcriptase inhibitors (NRTIs), in accordance with current clinical HIV treatment guidelines at the time of the study, for the first 14 days, and had a body mass index (BMI) of 18–32 kg/m², HIV-1 RNA levels below 500,000 copies/mL, CD4+ T lymphocyte count above 200 cells/mm³, and a resting heart rate of 50–100 beats per minute, and had not smoked tobacco products for at least 3 months prior to day 1 of the study. Patients were excluded if they were co-infected with

hepatitis B or C virus, if they had received (4 weeks prior to baseline [day -1]) medications known to alter cardiac conduction, interfere with the human ether-a-go-go-related gene (hERG) channel or to be associated with QT prolongation, if they were receiving medications that had the potential to interact with saquinavir or ritonavir, if they had a cardiac pacemaker, or if they had screening or baseline ECG abnormalities or were at increased risk of ECG abnormalities because of electrolyte disturbances, if they had a history or evidence of symptomatic arrhythmias, long QT syndrome, atrial fibrillation or flutter, complete right or left bundle branch block, or Wolff–Parkinson–White syndrome.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and good clinical practice guidelines. The study was approved by the Independent Ethics Committee, London–Riverside Research Ethics Committee, Bristol, United Kingdom and the European Committee for Medicinal Products for Human Use (CHMP). All patients provided written informed consent.

2.2 Study Design

Patients reported to the study unit on the evening before baseline and before study days 3, 7, 10, and 14 and remained at the study unit until the completion of last ECG assessment specified for each study day (34–38 h to cover both baseline and day 1, 46–50 h to cover both days 3 and 4, and 22–26 h for days 7, 10, and 14). All patients received the modified saquinavir/ritonavir regimen (500/100 mg BID on days 1–7 followed by 1000/100 mg BID on days 8–14). Study medications were to be taken with 240 mL of water, 15–30 min after the consumption of a meal. On days 3, 4, 7, 10, and 14, a standardized breakfast was provided 45 min prior to the morning dosing. On days 3, 4, 7, 10, and 14, a standard lunch was provided 15 min after the 4-h ECG extraction time point, and a standard dinner was provided 15 min after the 12-h extraction time point. Meals were to be completed within 20–30 min. During the stay at the study unit, the scheduled saquinavir/ritonavir doses were administered by the study staff; dosing on other study days was administered by the patients. Patients received study medications in combination with two NRTIs (tenofovir/emtricitabine). Fluid intake, meals, environment, and physical activity level remained as controlled and as consistent as possible during the specified stay at the study unit.

Subjects maintained a study diary that tracked their meals and study drug administration. Additionally, subjects had stays in the phase I unit on days 1–4, 7, 10, and 14, where they were monitored by the study staff. Subject compliance to study medications was assessed by

maintaining adequate study drug dispensing records. The investigator was responsible for ensuring that dosing was administered in compliance with the protocol and for following up with study volunteers.

2.3 Assessments

2.3.1 Electrocardiograms (ECGs)

The primary and secondary endpoints were determined by analysis of digital ECG data by cardiologists. All scheduled ECGs were performed after the patients had rested for at least 10 min in a supine position. Continuous Holter 12-lead ECG recordings (Mortara H-12+™ digital Holter recorder, Mortara Instruments, Inc. Milwaukee, WI, USA) were obtained at baseline from -25 h predose up to -11.75 h predose, on day 1 from -1.5 h predose up to 1 h postdose, and on days 3, 4, 7, 10, and 14 from -1 h predose up to 12.25 h postdose.

These data were forwarded to a central laboratory for evaluation (Cardiocre, Rockville, MD, USA). After delivery of the Holter data, replicate 12-lead ECG recordings that were 10 s in duration and corresponded to specific time points after dosing (2, 4, 6, 8, and 12 h), were extracted to determine the primary and secondary end-points in the study.

The digital (Holter monitor data) ECGs were reviewed for each patient by the same team of two cardiologists to control variability in interpretation. All measurements were blinded with respect to time, treatment, and subject identifier. The following intervals were measured and recorded: heart rate, RR, PR, QRS, and QT intervals. The ECG machine provided corrected QT intervals (QTc), and, for each subject and extraction point, the QTc values were corrected using Bazett's formula (QTcB) and Fridericia's formula (QTcF). T- and U-wave morphology was also assessed using predefined criteria. T waves were labelled abnormal if they were inverted, flat, biphasic, peaked, had non-specific changes, or were considered to be altered due to ventricular conduction or due to left ventricular hypertrophy. If present, U waves were labelled as normal or abnormal, and abnormal U waves were described as being large, inverted or fusion of T–U waves/early after depolarization. ECG intervals were analysed semi-automatically: fiducial points were placed by an algorithm and then confirmed or adjusted by the cardiologist as recommended by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use [7].

The Holter monitor data were not available to study personnel to determine eligibility or monitor safety. Thus, triplicate paper ECGs were recorded within 10 min of each other (3–5 min apart) once at screening, on day -2, and at

follow-up (3–7 days after last dose) to determine eligibility and monitor safety. The paper ECGs were not forwarded to Cardiacore, were not seen by the cardiologists, and were not used to determine the primary or secondary end-points in the study.

2.3.2 Pharmacokinetics

Blood samples (3 mL) for measurements of plasma saquinavir and ritonavir concentrations were collected from all patients predose and at 2, 4, 6, 8, and 12 h post-dose on days 3, 4, 7, 10, and 14, and analyzed using validated liquid chromatography with tandem mass spectrometry detection methods (range 50.0–10,000 ng/mL) as described previously [5]. For saquinavir, assay precision based on the quality control samples ranged from ± 4.6 to 7.4 % while accuracy ranged from -0.7 to 2.1 %. For ritonavir, assay precision based on quality control samples ranged from ± 3.3 to 3.4 % and accuracy ranged from -1.3 to 4.0 %.

2.3.3 Pharmacodynamics (antiviral activity)

Blood samples (5 mL) were collected for HIV RNA quantification at predose on days 1, 3, 4, 7, 10, and 14. The total volume of blood required for the HIV RNA testing was approximately 35 mL per patient. HIV RNA quantification was performed using a validated, commercial polymerase chain reaction-based assay (COBAS[®] AmpliPrep/COBAS[®] TaqMan[®] HIV-1 Test).

2.3.4 Safety

All patients who received at least one dose of the study medication were included in the safety population and were assessed by monitoring ECGs, vital signs, laboratory tests, and adverse events (AEs) throughout the study. AEs were graded on a 3-point scale (mild, moderate, and severe). Patients with ECGs showing a significant increase in QT/QTc (>500 ms or >60 ms from baseline) that persisted for an hour or more were withdrawn from the study and ECG recordings were repeated until two successive ECGs showed QTc values below the threshold value of the initial increase.

2.4 Statistical Methods

No formal sample size calculation was performed. The sample size was based on practical considerations and discussions with regulatory agencies. All patients who received at least one dose of study medication were included in the ECG analysis. For all ECG variables, the average of the triplicate measurements obtained at each time point was

used as a single observation. The primary endpoint was the maximum change from dense predose baseline in QTcF ($\Delta\text{QTcF}_{\text{dense}}$) at time points 2, 4, 6, 8, and 12 h postdose on days 3, 4, 7, 10, and 14. The dense predose baseline was defined as the average of the three triplicate measurements (nine measurements overall collected at 1, 0.75, and 0.5 h predose on day 1). This is identical to the dense predose baseline measurement as defined in the previous QT study of saquinavir in healthy volunteers [5]. Maximum $\Delta\text{QTcF}_{\text{dense}}$ was described using summary statistics. Secondary ECG variables included absolute and change from baseline in QT, QTcB, RR, heart rate, PR, QRS, T-wave, and U-wave at the same time points defined above. In addition, maximum change from time-matched baseline in QTcF ($\Delta\text{QTcF}_{\text{tmcb}}$) was also used as a secondary variable to measure the maximal increase in baseline-corrected QTcF.

All patients treated and adherent to the protocol were included in the pharmacokinetics and pharmacodynamics analysis. The trough drug concentration, defined as the concentration at the end of the dosing interval (C_{12}), maximum concentration (C_{max}), time to reach C_{max} (t_{max}), and area under the plasma concentration–time curve from 0 to 12 h ($\text{AUC}_{0-12\text{h}}$) for saquinavir and ritonavir, were estimated using noncompartmental methods using WinNonlin software (version 5.2, Pharsight Corporation, Mountain View, CA, USA) for days 3, 4, 7, 10, and 14. Pharmacokinetics measurements were presented by descriptive summary statistics. Predose actual sampling times were set to zero. Actual sampling times were used for the non-compartmental analyses and for the individual plasma concentration versus time profiles. Scheduled sampling times were used for the creation of summary tables and mean plasma concentration versus time profiles. Individual and mean serum HIV RNA levels at each sampling time point were described by summary statistics. Changes from baseline in HIV RNA in plasma on days 3, 4, 7, 10, and 14 were calculated.

3 Results

3.1 Patient Disposition and Demographics

Twenty-three patients enrolled in the study, all of whom were male and most of whom were White (83 %). The mean age was 33 ± 9 years, mean BMI was 24 ± 3 kg/m², and mean baseline HIV RNA was 4.69 ± 0.47 log₁₀ copies/mL. Twenty-one patients completed the study as planned; one patient discontinued due to a non-drug-related serious adverse event (gonococcal proctitis), and one patient withdrew consent.

3.2 ECG Evaluation

Available ECG data from all 23 patients enrolled in the study were included in the analyses. For the two patients who withdrew from the study, no ECG data were available for one patient after day 3 predose and for one patient after day 3 at 8 h predose. The modified saquinavir/ritonavir regimen was associated with a mean maximum $\Delta QTcF_{dense}$ of 3, 1, 7, 12, and 7 ms on days 3, 4, 7, 10, and 14, respectively (Table 1). The mean maximum $\Delta QTcF_{tmcb}$ was slightly higher than the mean maximum $\Delta QTcF_{dense}$ but followed a similar pattern. Only 9.5 % (2/21) and 19 % (4/21) of patients demonstrated a maximum $\Delta QTcF_{dense} \geq 30$ ms and $\Delta QTcF_{tmcb} \geq 30$ ms, respectively, across all study days, but all were <60 ms (Table 1). Only one patient had an absolute QTcF of >450 ms but <480 ms. This individual had an absolute QTcF of 454 ms on day 10 at predose which subsequently declined to 428 ms 2 h postdose. No patient had an absolute QTcF of >500 ms.

The maximum mean changes from baseline for $QTcF_{dense}$ and $QTcF_{tmcb}$ were highest on day 10, but remained below 10 and 12 ms, respectively, at all time points (Table 1; Fig. 1).

Maximum mean $\Delta QTcF_{dense}$ and $\Delta QTcF_{tmcb}$ were 9 and 12 ms, respectively, at 6 h postdose on day 10.

Maximum changes from dense predose and time-matched baselines were observed at 6 h postdose for heart rate, RR interval, and QT interval, and at 4 h postdose for PR interval (data not shown). However, these ECG parameters, including QRS interval, were not affected by treatment with saquinavir/ritonavir at either 500/100 mg BID or 1000/100 mg BID and changes were small and not clinically significant.

The mean maximal changes in secondary ECG parameters (heart rate, RR, PR, QRS and QT intervals) are presented in Table 2.

3.3 Pharmacokinetic Analysis and Effect on QTcF

The mean (\pm standard deviation) plasma concentration-time profiles of saquinavir and ritonavir throughout the study are shown in Fig. 2 and key pharmacokinetic parameters are summarized in Table 3. Following administration of saquinavir/ritonavir 500/100 mg BID during the first 7 days, the mean saquinavir plasma concentrations peaked on day 3 and declined on days 4 and 7, with the lowest concentrations observed on day 7. Doubling the dose of saquinavir to the standard dose of 1000/100 mg BID during the second week resulted in higher mean saquinavir plasma concentrations on day 10, as expected, with only slightly lower mean saquinavir plasma concentrations observed on day 14 (Fig. 2). Consistent with the

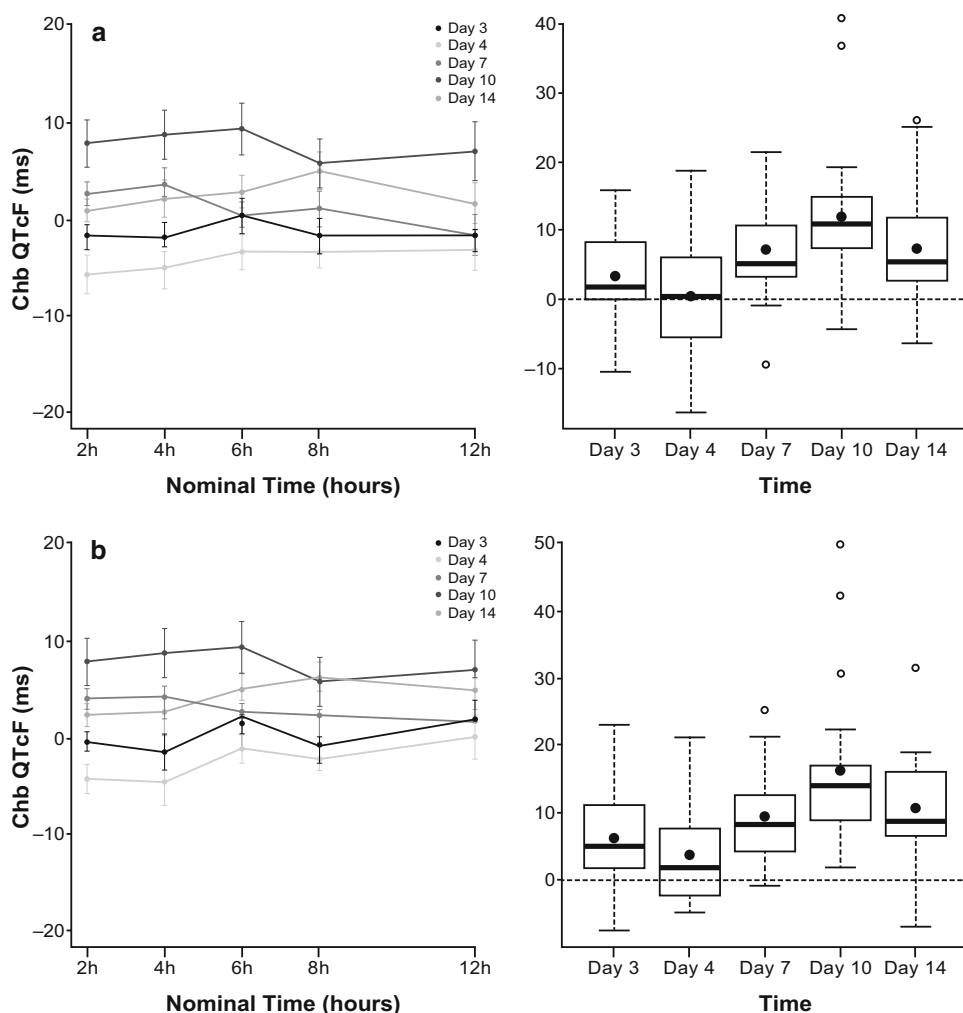
Table 1 Summary of ECG parameters

	Day 3 (n = 22) ^a		Day 4 (n = 21)		Day 7 (n = 21)		Day 10 (n = 21)		Day 14 (n = 21)	
	$\Delta QTcF_{dense}$	$\Delta QTcF_{tmcb}$	$\Delta QTcF_{dense}$	$\Delta QTcF_{tmcb}$	$\Delta QTcF_{dense}$	$\Delta QTcF_{tmcb}$	$\Delta QTcF_{dense}$	$\Delta QTcF_{tmcb}$	$\Delta QTcF_{dense}$	$\Delta QTcF_{tmcb}$
Mean maximum change (SD), ms	3 ± 7	6 ± 8	1 ± 9	4 ± 8	7 ± 7	9 ± 7	12 ± 12	16 ± 12	7 ± 8	11 ± 8
Range, ms	-10 to 16	-7 to 23	-16 to 19	-5 to 21	-9 to 22	-1 to 25	-4 to 43	2 to 52	-6 to 26	-7 to 31
Maximum change from baseline, n (%)										
<5 ms	14 (63.6)	11 (50.0)	15 (71.4)	14 (66.7)	8 (38.1)	6 (28.6)	4 (19.0)	2 (9.5)	9 (42.9)	2 (9.5)
5–10 ms	3 (13.6)	4 (18.2)	2 (9.5)	3 (14.3)	7 (33.3)	6 (28.6)	6 (28.6)	5 (23.8)	5 (23.8)	9 (42.9)
10–15 ms	3 (13.6)	4 (18.2)	2 (9.5)	2 (9.5)	2 (9.5)	5 (23.8)	5 (23.8)	6 (28.6)	4 (19.0)	4 (19.0)
15–30 ms	2 (9.1)	3 (13.6)	2 (9.5)	2 (9.5)	4 (19.0)	4 (19.0)	4 (19.0)	5 (23.8)	3 (14.3)	5 (23.8)
≥30 ms	0	0	0	0	0	0	2 (9.5)	3 (14.3)	0	1 (4.8)
Maximum mean change from baseline on a given day, ms	<1	2	-3	<1	4	4	9	12	5	6

ECG electrocardiogram, $\Delta QTcF_{dense}$ change from dense predose baseline in QT interval corrected using Fridericia's formula, $\Delta QTcF_{tmcb}$ change from time-matched baseline in QT interval corrected using Fridericia's formula, SD standard deviation

^a No ECG data were available for one patient after predose and for one patient after the 8-h predose

Fig. 1 Change from **a** dense predose and **b** time-matched baselines in QT values corrected using Fridericia's formula (QTcF). *Line plots* represent mean change (SEM) over time. *Box plots* represent distribution of maximum change from baseline in QTcF by study day. *Solid horizontal bars* indicate median values; *solid dots* indicate mean values; *boxes* indicate interquartile ranges; *vertical dotted lines (whiskers)* indicate maximum and minimum values; *open circles* indicate outliers. *Chb* change from baseline



mean plasma concentrations, the highest saquinavir mean C_{\max} and AUC_{0-12h} following administration of saquinavir/ritonavir 500/100 mg BID was observed on day 3 (4030 ng/mL and 27,100 ng·h/mL, respectively) and declined on days 4 and 7, with the lowest exposure on day 7. Similarly, doubling the dose of saquinavir to 1000/100 mg BID during the second week resulted in higher mean C_{\max} and AUC_{0-12h} on days 10 and 14 with highest C_{\max} and AUC_{0-12h} on day 10 (5300 ng/mL and 34,200 ng·h/mL, respectively). Median time to C_{\max} (i.e., T_{\max}) for saquinavir was reached approximately 2–4 h after dosing (Table 3).

Mean ritonavir plasma concentrations and mean ritonavir exposures (C_{\max} and AUC_{0-12h}) fluctuated across study days but were generally similar when considering the interindividual variability (Table 3). Highest mean ritonavir plasma concentrations were observed on day 3 while the lowest mean ritonavir plasma concentrations were observed on day 7, coinciding with the lowest observed saquinavir exposure (Table 3; Fig. 2). Median T_{\max} was

reached approximately 3–4 h after dosing (Table 3). The pharmacokinetics of ritonavir generally appeared to be independent of saquinavir exposure.

The relationship between saquinavir concentration and QTcF was evaluated using C_{\max} because a delay in maximum $\Delta QTcF_{\text{dense}}$ compared with maximum saquinavir plasma concentrations is observed manifesting as a counter-clockwise hysteresis (data not shown). No strong correlation was observed between the maximum $\Delta QTcF_{\text{dense}}$ and C_{\max} of saquinavir (Fig. 3).

3.4 Pharmacodynamics

Individual patients' HIV RNA profiles are illustrated in Fig. 4. Continuous declines in HIV RNA were observed in all treatment-naïve patients receiving the modified saquinavir/ritonavir regimen over the 14-day treatment period. The mean HIV RNA level declined from $4.69 \pm 0.47 \log_{10}$ copies/mL at baseline to $2.83 \pm 0.48 \log_{10}$ copies/mL on day 14 and the mean change from baseline was

Table 2 Summary of changes from baseline in secondary ECG parameters

Parameter ^a	Day 3	Day 4	Day 7	Day 10	Day 14
Heart rate, bpm	12 ± 7 (-4 to 28) [6]	9 ± 7 (-4 to 22) [6]	11 ± 6 (-1 to 23) [6]	12 ± 6 (-3 to 25) [6]	8 ± 7 (-9 to 22) [8]
RR interval, ms	-137 ± 100 (-345 to 26) [0]	-127 ± 90 (-308 to 39) [6]	-146 ± 80 (-320 to -17) [6]	-161 ± 81 (-319 to -40) [6]	-117 ± 92 (-234 to 91) [6]
PR interval, ms	5 ± 9 (-14 to 27) [4]	2 ± 9 (-13 to 19) [6]	5 ± 6 (-3 to 25) [4]	8 ± 8 (-9 to 24) [4]	9 ± 7 (-10 to 18) [4]
QRS interval, ms	1 ± 3 (-7 to 9) [0]	1 ± 3 (-5 to 9) [0]	2 ± 4 (-5 to 9) [0]	3 ± 3 (-4 to 9) [0]	2 ± 4 (-5 to 11) [0]
QT interval, ms	-18 ± 16 (-42 to 11) [2]	-21 ± 16 (-55 to 8) [2]	-20 ± 14 (-51 to 3) [6]	-14 ± 17 (-51 to 20) [6]	-14 ± 17 (-42 to 17) [6]

^a Mean maximum change from dense pre-dose baseline ± standard deviation (range) [time post-dose in hours]

-1.93 ± 0.28 log₁₀ copies/mL on day 14 (Table 4). No resistance testing was conducted, since no patient experienced either a viral breakthrough (detectable HIV RNA after undetectable HIV RNA) or viral rebound (increase in HIV RNA following a decline in HIV RNA).

3.5 Safety

All patients enrolled in the study were included in the safety analysis. The modified saquinavir/ritonavir regimen was generally well tolerated. There were no deaths during the study. One patient discontinued study drug due to gonococcal proctitis which was classified as a serious AE but was not considered to be drug-related. A total of 55 treatment-emergent AEs were reported during the study, of which most were mild (37; 67 %) or moderate (16; 29 %) in severity. Only two events were considered to be severe (one case of lethargy and one case of gonococcal proctitis; each in one patient). The most commonly reported AEs (reported by ≥10 % of patients) during the study were diarrhea (48 %), nausea (35 %), headache (35 %), vomiting (22 %), and lethargy (13 %).

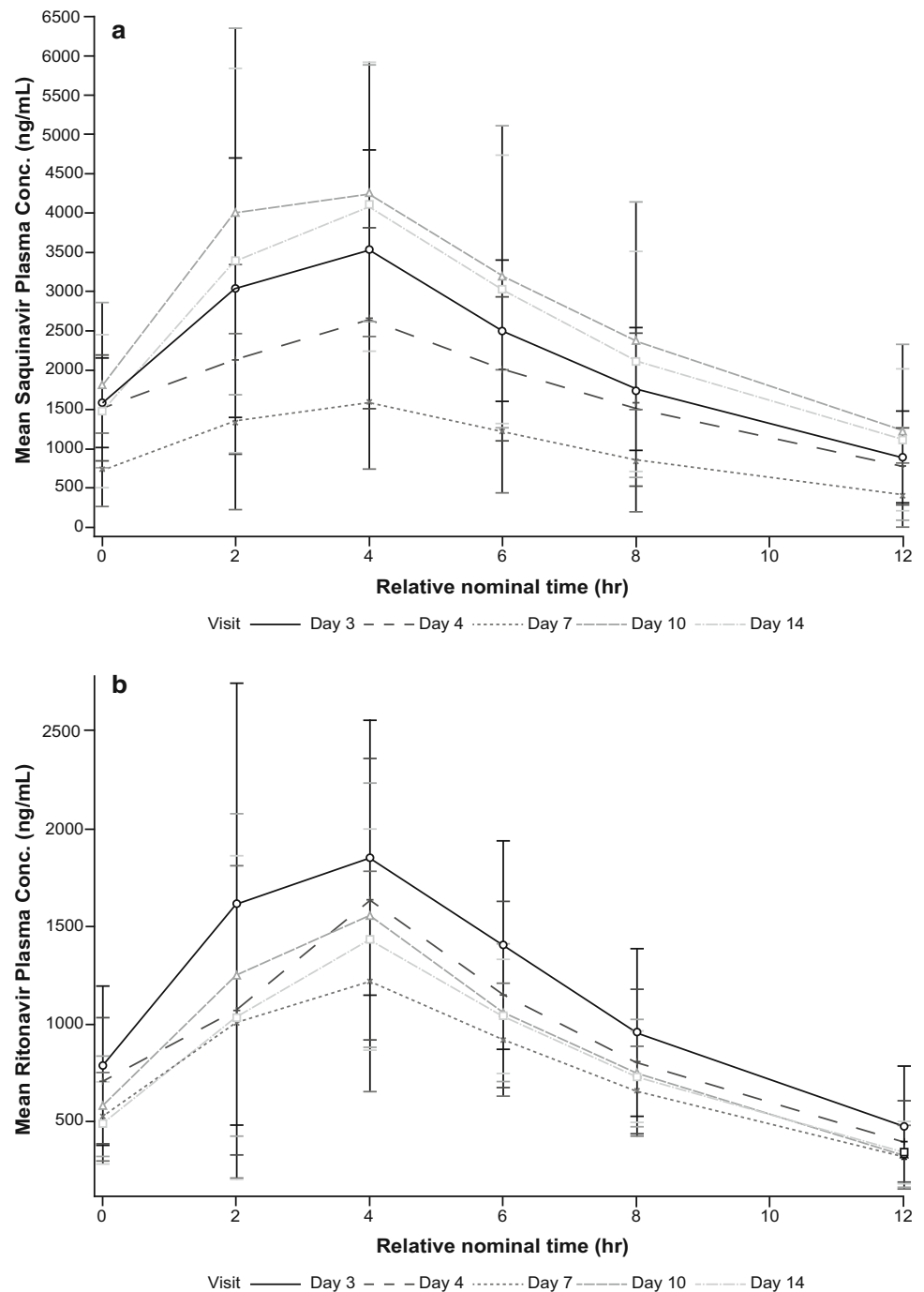
There were no clinically meaningful changes in mean values of laboratory chemistry parameters or mean vital sign values during the study.

4 Discussion

This study was conducted to determine the effect of the modified saquinavir/ritonavir regimen (500/100 mg BID for the first week followed by 1000/100 mg BID for the second week) on the QTc interval, pharmacokinetics, antiviral activity, and safety in treatment-naïve HIV-1-infected patients. The rationale for the study, study design, and dosing regimen was to assess the impact of a change in the saquinavir/ritonavir dosing in treatment-naïve patients as recommended by the European Medicines Agency [6].

In a previous TQT study in healthy volunteers, the maximum least-squares mean estimated study-specific QTc (QTcS) change from predose baseline (ddQTcS_{dense}) was 19 ms at 12 h postdose with a saquinavir/ritonavir 1000/100 mg BID regimen, and 30 ms at 20 h postdose with a 1500/100 mg BID regimen on day 3 of treatment with saquinavir/ritonavir BID compared with 12 ms 4 h after a single 400 mg dose of the positive control moxifloxacin [5]. Day 3 was selected for evaluation in the healthy volunteer TQT study based on another completed healthy volunteer multiple ascending dose (MAD) study, which showed that saquinavir exposure would be highest on day 3 of treatment with saquinavir/ritonavir and would decline with further administration [8]. A post hoc pharmacokinetic/pharmacodynamic analysis of available day 3

Fig. 2 Mean plasma concentration–time profiles following administration of the modified saquinavir/ritonavir regimen for **a** saquinavir and **b** ritonavir



pharmacokinetic and QTc data from the TQT study demonstrated a linear exposure–response relationship between $ddQTcS_{dense}$ and C_{max} (Roche, personal communication). Based on this relationship, a modified saquinavir/ritonavir dosing regimen of 500/100 mg BID for the first 7 days followed by the full therapeutic dose regimen (1000/100 mg BID) thereafter in treatment-naïve patients was expected to reduce QTc liability while maintaining efficacy with regard to concentration/exposure and consequently virologic suppression.

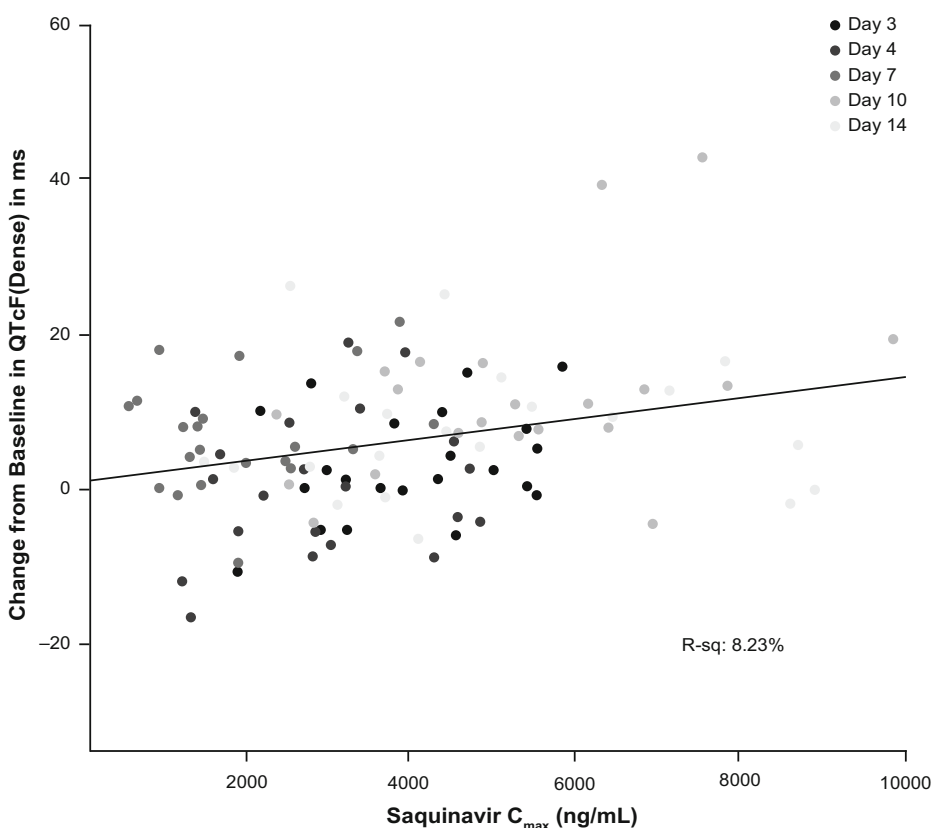
QTc results for the primary endpoint from this study (maximum $\Delta QTcF_{dense}$) illustrated that the modified saquinavir/ritonavir regimen resulted in a mean maximum $\Delta QTcF_{dense}$ of 3 ms on day 3 with mean values peaking at 12 ms on day 10. Some day-to-day fluctuations were observed in mean maximum $\Delta QTcF_{dense}$ that may be due to the observed high interpatient variability. It is also important to note that in vitro experiments have demonstrated that saquinavir results not only in dose-dependent blockage of hERG channels heterologously expressed in HEK293

Table 3 Summary of pharmacokinetic parameters following administration of the modified saquinavir/ritonavir regimen in HIV-1-infected patients

	Drug	Day 3	Day 4	Day 7	Day 10	Day 14
AUC _{0-12h} ng·h/mL, mean ± SD	Saquinavir	27,100 ± 9650	20,300 ± 8100	12,600 ± 6850	34,200 ± 16,500	31,100 ± 15,400
	Ritonavir	14,400 ± 5940	11,600 ± 4930	9670 ± 3520	11,500 ± 3510	10,400 ± 3580
C _{max} ng/mL, mean ± SD	Saquinavir	4030 ± 1170	2960 ± 1190	1960 ± 1040	5300 ± 1910	4860 ± 2270
	Ritonavir	2130 ± 843	1720 ± 690	1410 ± 600	1800 ± 675	1620 ± 615
T _{max} h, median (range)	Saquinavir	1.98 (1.98–6.02)	3.98 (0–7.98)	3.98 (0–7.98)	3.98 (1.98–7.98)	3.98 (1.98–5.98)
	Ritonavir	2.98 (0–5.98)	3.98 (0–5.98)	3.98 (0–7.98)	3.98 (1.98–7.98)	3.98 (1.98–5.98)
C ₁₂ ng/mL, mean ± SD	Saquinavir	899 ± 583	782 ± 488	416 ± 409	1220 ± 1120	1120 ± 910
	Ritonavir	476 ± 309	401 ± 208	322 ± 162	336 ± 167	346 ± 159

AUC_{0-12h} area under the plasma concentration-time curve from 0–12 h, C₁₂ trough drug concentration, C_{max} maximum observed plasma concentration, SD standard deviation, T_{max} time to reach C_{max}

Fig. 3 Saquinavir concentration (C_{max})–time profile for change from dense pre-dose baseline in QT values corrected using Fridericia’s formula (QTcF)



cells, but also has inhibitory effects on hERG trafficking [9, 10]. Thus, a potentially complex contribution/interplay between direct hERG blocking and hERG trafficking by saquinavir may potentially contribute to the day-to-day variability seen in mean maximum Δ QTcF_{dense}. Importantly, only 2/21 (9.5 %) patients had a maximum Δ QTcF_{dense} \geq 30 ms, which occurred on day 10. The maximum mean Δ QTcF_{dense} was less than 10 ms across all sampling days. Secondary analyses using QTcF_{tmcb}

illustrated trends similar to the primary analysis using QTcF_{dense}, though values were slightly larger, potentially due to the baseline correction used and any consideration of diurnal variations. The results from the present study confirm and extend the results from the TQT study in healthy volunteers, although the primary endpoints differ in the two studies [5]. In the TQT study, in healthy volunteers the mean maximum Δ QTcF_{dense} (a secondary endpoint in the TQT study) with the full therapeutic dose (1000/

Fig. 4 **a** Absolute and **b** change from baseline \log_{10} HIV RNA of individual patients across the study

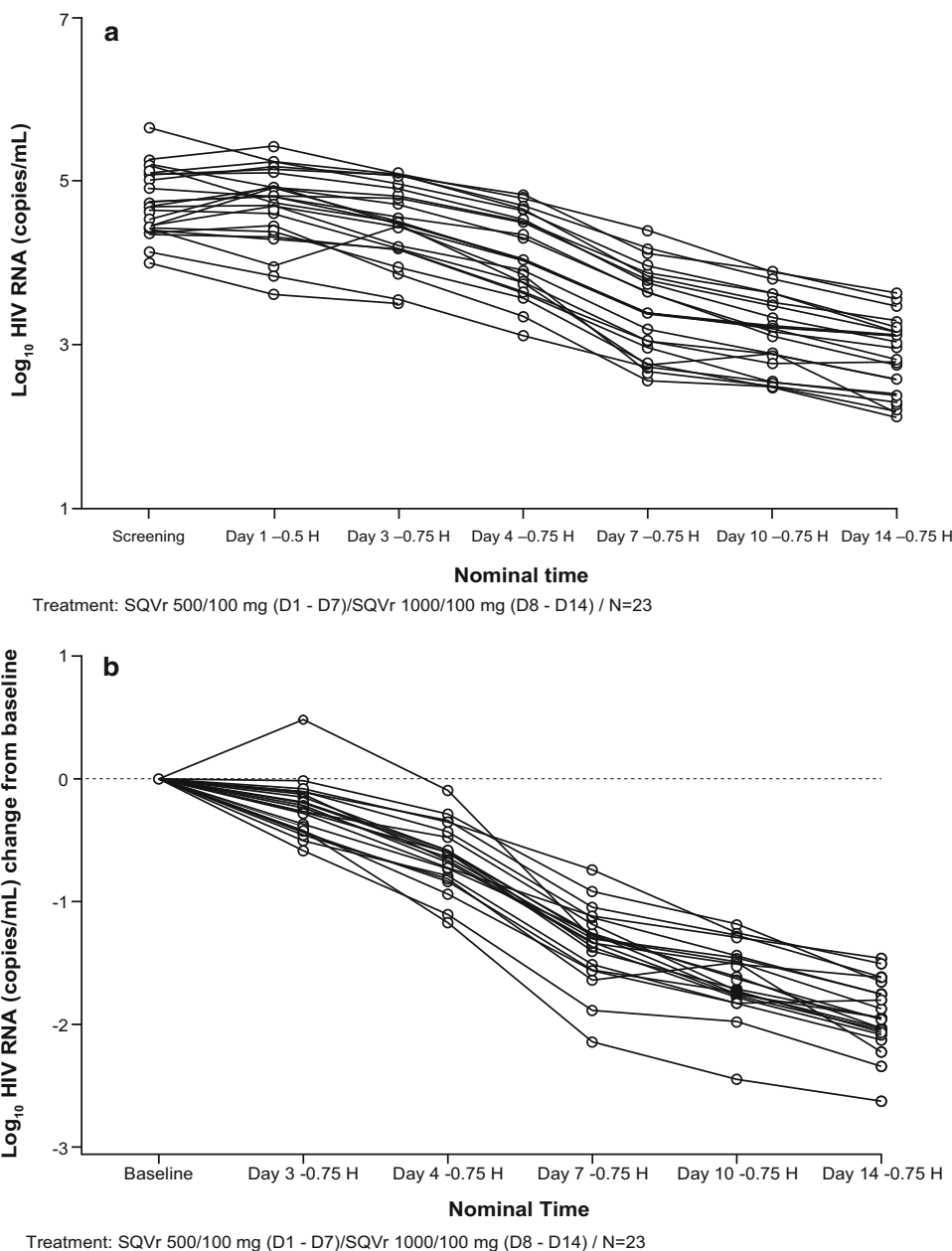


Table 4 Effect of the modified saquinavir/ritonavir regimen on \log_{10} HIV RNA in HIV-1-infected patients

	Baseline	Day 3	Day 4	Day 7	Day 10	Day 14
Mean \log_{10} HIV RNA \pm SD	4.69 \pm 0.47	4.46 \pm 0.49	4.12 \pm 0.51	3.42 \pm 0.56	3.13 \pm 0.49	2.83 \pm 0.48
Change from baseline, mean \pm SD	-	-0.24 \pm 0.22	-0.64 \pm 0.26	-1.35 \pm 0.31	-1.63 \pm 0.29	-1.93 \pm 0.28

SD standard deviation

100 mg BID) was 32 ms and occurred on day 3 (Roche, personal communication). Fifty-one percent of the population receiving the full therapeutic dose had a Δ QTcF_{dense} of >30 ms (Roche, personal communication). The results of both studies taken together suggest that the

profile of QTc prolongation is beneficially impacted with the modified saquinavir/ritonavir dosing regimen that reduces saquinavir exposure in the first week; however, it is important to note how differences in study designs, study endpoints, study populations, and subject numbers limit

comparisons between studies. Also, notably, no placebo- or positive-control treatment was included in the current study.

Limitations of the present study include the small number of patients, the lack of a control group, the absence of female participants and the low number of non-White participants. The results of both the present study and the earlier healthy volunteer study by Zhang and colleagues [5] support the evidence that saquinavir is associated with QT interval prolongation despite the considerable uncertainty and contradictory reports in the literature on the effect of the HIV protease inhibitor class of antiretrovirals on cardiac repolarization. It is not entirely clear why there are contradictory reports in the literature, but it is possible that the effect of protease inhibitors on cardiac repolarization is seen predominantly during the first few days or weeks of treatment (as seen in the TQT studies) as opposed to cross-sectional studies of patients on chronic therapy. However, QT interval prolongation and torsades de pointes are rare events among the large number of patients receiving HIV protease inhibitor therapy, including those treated with saquinavir. Furthermore, it has been reported that duration of HIV infection is independently associated with QT interval prolongation [11] and that risk factors for QT interval prolongation for HIV patients are similar to those seen in non-HIV-infected patients, including factors such as age, female sex, ethnicity, cardiac conditions, diabetes mellitus, and use of drugs known to cause QT interval prolongation [12]. However, the clinical consequences of such prolongation remain unclear.

Pharmacokinetic data from this study showed that following administration of the modified saquinavir/ritonavir dose regimen of saquinavir/ritonavir 500/100 mg BID in the first week, mean saquinavir exposure peaked on day 3 and declined to the lowest exposure on day 7 as expected with ritonavir induction effects. Moreover, saquinavir exposure in the first week of treatment with the modified saquinavir/ritonavir dosing regimen in the present study was substantially lower than that observed on day 3 of treatment with the full therapeutic dose (1000/100 mg BID) in the healthy volunteer TQT study [5]. Following administration of the full saquinavir/ritonavir dose in the second week, saquinavir exposure was higher on days 10 and 14 compared with the first week, as expected, and was slightly lower on day 14 compared with day 10, suggesting a continued decline in saquinavir exposure toward steady state. This is consistent with results from the healthy volunteer MAD study, which showed that mean saquinavir predose concentrations were slightly lower on day 14 compared with day 10 [8]. Day 14 pharmacokinetic parameters approached the range of historical means for saquinavir steady-state values in HIV-1-infected patients [13–20].

The observed mean saquinavir exposure (AUC and C_{12}) following the modified saquinavir/ritonavir regimen exceeded across all study days the clinical EC_{50} value (AUC_{0–24} 3.23 $\mu\text{g}\cdot\text{h}/\text{mL}$ and C_{min} 0.05 $\mu\text{g}/\text{mL}$) determined from the previous pharmacokinetic/pharmacodynamic analysis of saquinavir monotherapy data in 84 patients by several-fold [21]. Further, mean saquinavir C_{12} values in this study exceeded those recommended in the US Department of Health and Human Services (DHHS) guidelines on antiretroviral treatment for adults and adolescents (100–250 ng/mL) with drug-susceptible virus [22].

HIV RNA was collected across study days to evaluate the effect of the modified saquinavir/ritonavir dosing regimen when combined with two NRTIs on antiviral activity in the treatment-naïve patients. Continuous declines in HIV RNA were observed in all treatment-naïve patients over the 2-week treatment duration. The mean decline in viral load on day 14 following administration of the modified saquinavir/ritonavir regimen observed in the current study ($-1.93 \pm 0.28 \log_{10}$ copies/mL) was comparable to the day 14 viral load decline reported in the GEMINI phase III study evaluating the full saquinavir/ritonavir dosing regimen (1000/100 mg BID) in a similar patient population (-1.88 ; 95 % CI -2.0 to -1.8) [23, 24]. However, it is important to note that, although short-term continuous declines in HIV RNA were observed and were comparable to those obtained with standard doses in the GEMINI study, the long-term efficacy of the modified regimen was not evaluated. Adherence to antiretroviral therapy has been correlated strongly with HIV viral suppression and reduced rates of resistance [22, 25]. For most patients who are adherent to therapy and who do not harbor resistance mutations to the prescribed drugs, complete viral suppression is generally achieved in 12–24 weeks [22]. The patients in the current study were treated at a single study center, and study medication was administered by the study staff while patients were in the study unit. Thus, while a continuous decline in HIV RNA or viral load was observed in all patients, it is likely that the modified saquinavir/ritonavir regimen in combination with NRTIs and adherence also contributed to HIV viral suppression during the time of the study.

Safety results from the study indicated that oral saquinavir/ritonavir 500/100 mg BID for the first week followed by saquinavir/ritonavir 1000/100 mg BID in the second week, in combination with two NRTIs, was generally well tolerated. Although nearly all of the patients experienced AEs, most were mild or moderate in severity. Results of laboratory examinations, vital signs assessments, and ECGs did not reveal any clinically significant changes. Mean changes over time in heart rate and RR, PR, QRS, and QT intervals were small and not clinically significant.

5 Conclusion

In summary, results from this study demonstrated that the modified saquinavir/ritonavir regimen reduced mean maximum $\Delta\text{QTcF}_{\text{dense}}$ in the first week of treatment when compared with historical data obtained with the standard dose regimen in a different population of healthy volunteers enrolled in a TQT study; only 2/21 (9.5 %) patients across all study days had maximum $\Delta\text{QTcF}_{\text{dense}} \geq 30$ ms and the maximum mean $\Delta\text{QTcF}_{\text{dense}}$ remained below 10 ms across all study days. Saquinavir pharmacokinetic parameters were lower than those observed on day 3 in the TQT study in healthy volunteers, while day 14 saquinavir exposures approached historical mean (and target exposure) values for steady-state saquinavir exposures in HIV-1-infected patients receiving full doses of saquinavir/ritonavir. Continuous declines in HIV RNA were observed in all treatment-naïve patients receiving the modified saquinavir/ritonavir dosing regimen over the 2-week treatment period, suggesting HIV viral suppression during the time of the study. No patient experienced virologic breakthrough or rebound, so no resistance testing was conducted. Safety results showed that saquinavir/ritonavir regimens were well tolerated. Thus, treatment initiation with the modified saquinavir/ritonavir regimen in treatment-naïve, HIV-1-infected patients starting de novo ritonavir-boosted saquinavir treatment resulted in reduced saquinavir exposure (C_{max} and AUC) during the first week, potentially mitigating or reducing QTc liability while suppressing HIV RNA during the period of observation.

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