New Microbiologica, 41, 2, 106-111, 2018, ISN 1121-7138

FULL PAPER

Potential associations between atazanavir exposure and clinical outcome: a pharmacokinetic sub-study from the MODAt randomized trial

Elisa Colella[1‡#], Dario Cattaneo[2#], Laura Galli[3#], Sara Baldelli[2], Emilio Clementi[4,5], Massimo Galli[1], Adriano Lazzarin[3], Antonella Castagna[3], Stefano Rusconi[1], Vincenzo Spagnuolo[3]

[1]Infectious Diseases Unit, Department of Biomedical and Clinical Sciences 'Luigi Sacco', Università degli Studi di Milano, Italy; [2]Clinical Pharmacology Unit, Luigi Sacco University Hospital, Milan, Italy; [3]Department of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy; [4]Unit of Clinical Pharmacology, Department of Biomedical and Clinical Sciences 'Luigi Sacco' University Hospital, Consiglio Nazionale delle Ricerche Institute of Neuroscience, Università degli Studi di Milano, Italy; [5]E. Medea Scientific Institute, Bosisio Parini, Italy

 # These 3 authors contributed equally to this work
‡ Present address: Infectious Diseases Unit, San Gerardo Hospital, Monza (MB), Italy

SUMMARY

The 96-week results of the Monotherapy Once a Day with Atazanavir/r (MODAt) study [NCT01511809] showed an inferior virological efficacy of atazanavir (ATV)/ritonavir monotherapy *versus* triple therapy, which was promptly retrieved by the reintroduction of nucleoside/nucleotide inhibitors of reverse transcriptase [N(n)RTIs].

We aimed to identify potential relationships between ATV exposure and clinical outcome in HIV-1 subjects treated with ATV/ritonavir monotherapy [ATV/r 300/100 mg] *versus* ATV/ritonavir triple therapy [ATV/r 300/100 mg+2NRTIs].

A chromatographic method coupled with tandem mass spectrometry was applied to analyze ATV plasma concentrations in a pharmacokinetic sub-study from the MODAt trial. Mixed linear models were used to examine the ATV plasma concentration trend during follow-up and to assess the association between ATV plasma concentrations trajectories with the study arm or the occurrence of treatment failure or drug-related adverse events or the grading of baseline total bilirubin (<3 vs ≥3). The analyses were performed using SAS Software, release 9.4 (SAS Institute, Cary, NC, USA).

Overall, ATV plasma Ctrough concentration did not vary during follow-up (slope: +0.75 ng/mL/week, 95%CI: -0.97 to 2.47, p=0.387); trajectories did not differ between study arms (p=0.527). The unadjusted model-based means (95%CI) of ATV Ctrough during follow-up were 835 (95%CI: 657-1012) ng/ml in the ATV/r monotherapy arm as compared to 911 (95%CI: 740-1082) ng/mL in the ATV/r triple therapy arm (p=0.621).

Mean ATV Ctrough was similar in subjects with or without adverse events (AEs). Subjects treated with ATV/r monotherapy showed significantly higher ATV concentrations as compared to subjects without adverse events or treated with ATV/r triple therapy. ATV concentrations were associated with the grading of baseline total bilirubin and the occurrence of drug-related AEs but not with HCV infection.

Our findings showed a lack of association between ATV concentrations and treatment failure both in ATV/r monotherapy and triple therapy. Conversely, these data emphasized that ATV concentrations are associated with the development of side-effects in both subjects treated with ATV/r monotherapy and subjects treated with ATV/r triple therapy.

Received September 12, 2017 *Accepted* December 22, 2017

INTRODUCTION

Due to the risk of long-term toxicity, patient's perception, treatment fatigue, and costs, an increasing frequency of switches from standard to non-standard regimens has been observed in clinical practice (The Antiretroviral Therapy Cohort Collaboration (ART-CC), 2013; Castagna *et al.,* 2014) and prompted the search for an alternative and affordable antiretroviral regimen in fully suppressed patients. (Baril *et al.,* 2014; Colasanti *et al.,* 2014; Di Giambenedetto *et al.,* 2013; Perez-Molina *et al.,* 2015).

Several clinical trials analysed the 96 week efficacy and safety of lopinavir/ritonavir (Arribas *et al.,* 2009; Nunes *et al.,* 2009; Meynard *et al.,* 2010; Ghosn *et al.,* 2010; Gutmann *et al.,* 2010; Cahn *et al.,* 2011; Bernardino *et al.,* 2013; Gianotti, *et al.,* 2014) or darunavir/ritonavir (Clumeck *et al.,* 2011; Valantin *et al.,* 2012; Antinori *et al.,* 2015) monotherapy in virologically suppressed HIV patients and their results showed a lower efficacy of ritonavir-boosted PI (PI/r) monotherapy than triple therapy in maintaining virological suppression. Similarly, the recently published

Key words:
HIV-1, Atazanavir, Monotherapy, Pharmacokinetics, Bilirubin, HCV.

Corresponding author:
Stefano Rusconi, MD
E-mail: stefano.rusconi@unimi.it

96-week results of the Monotherapy Once a Day with Atazanavir/r (MODAt) study showed an inferior virological efficacy of atazanavir/ritonavir monotherapy [ATV/r 300/100 mg + 2NRTIs] in comparison with triple therapy [ATV/r 300/100 mg + 2NRTIs], which was promptly retrieved by the reintroduction of NRTIs (Galli *et al.,* 2016).
Considerable inter-individual variability has been observed in plasma concentrations of ATV after standard dosing, mainly related to drug-to-drug interactions and genetic variants affecting the enzymes involved in the hepatic metabolism of ATV (Van Schaik *et al.,* 2002; Evans and McLeod, 2003; Wempe and Anderson, 2011; Le Tiec *et al.,* 2005; Solas *et al.,* 2008). This may be clinically relevant considering that significant correlations have been reported between plasma ATV concentrations and clinical outcome. Indeed, the highest probability of achieving undetectable viral load in treatment-experienced as well as naïve HIV patients has been associated with ATV plasma Ctrough >150 ng/mL (Gonzalez de Requena *et al.,* 2005; Bonora *et al.,* 2011; Gervasoni *et al.,* 2015). Conversely, thresholds ATV Ctrough of around 700-1000 ng/mL have been proposed as risk factors for ATV-related complications, such as hyperbilirubinemia (Bonora *et al.,* 2011), dyslipidemia or nephrolitiasis (Gervasoni *et al.,* 2015; Giacomelli *et al.,* 2016).
The aim of this MODAt sub-study was to investigate whether significant relationships exist between ATV plasma concentrations and clinical outcome either in terms of efficacy and safety among patients enrolled in the MODAt study.

MATERIALS AND METHODS

Study design and population

This was a sub-study of the MODAt trial: a multicentre, randomized, open-label, non-inferiority trial in adult HIV-1-infected subjects (Galli et al., 2016). Patients underwent clinical assessment and routine laboratory tests at screening, baseline and at weeks 4, 8, 12, 16, every 8 weeks until week 48 and then every 12 weeks until week 96 or discontinuation (Galli *et al.,* 2016). The sub-study protocol was approved by the ethics committee of each participating site and all the enrolled patients provided additional written informed consent to use their stored plasma samples, all collected before the administration of the next dose of the randomized treatment. Patients' adherence to therapy was verified through direct questioning during every outpatient visit. Data on self-reporting adherence were cross-checked with the refill data from the Pharmacy Department in order to validate self-reported data. Only patients with high adherence to antiretroviral medications (above 95% of the doses) were considered in the present study.
Plasma samples for the assessment of plasma ATV concentrations were collected at different time-points during follow-up, with an average of 4 measurements/patient; at all time-points, plasma samples were collected after an overnight fast (at least 8 hours since midnight: range 8-12 hours). Treatment failure (TF) was defined as the occurrence of a virological failure (VF, defined as 2 consecutive values of HIV-RNA >50 copies/mL) or discontinuation for any reason. Treatment tolerability was defined as the occurrence of ATV drug-related adverse events of any grade recorded during the MODAt trial and judged by the investigators as potentially related to ATV.

Pharmacokinetic evaluations

ATV plasma concentrations were assessed by a chromatographic method coupled with tandem mass spectrometry (lower limit of quantification 20 ng/mL). Method performance was verified during each analytical run by means of internal and external quality controls (the KKGT Association for Quality Assessment in TDM and Clinical Toxicology program, The Netherlands).
Some patients did not take ATV in the morning. Therefore, the plasma samples were randomly drawn during the dosing interval and ATV trough plasma concentrations were not always available. To overcome this limitation, trough plasma concentrations were predicted using the interval between last dose intake and blood sampling and the mean ATV elimination half-life using the following formula:

$$[ATV]24h = [ATV]t \times 0.5^{EXP(24-interval)/half\text{-}life}$$

where [ATV]t is the measured ATV concentration and "interval" is the time between the last dose intake and blood sampling. All the ATV plasma samples were collected in the elimination phase. ATV plasma trough concentrations were evaluated according to the therapeutic window (set at 150-800 ng/mL) (Bonora *et al.,* 2011; Gervasoni *et al.,* 2015).

Statistical analyses

Demographic, immune-virological, haematological and other biochemical parameters were described by median (IQR) or frequency (%). Comparisons of demographic, haematological and biochemical parameters, as well as immune-virologic status between groups were carried out using the Wilcoxon rank-sum test or chi-square/Fisher exact test.
We used linear mixed modelling with random intercept and slope to evaluate ATV plasma Ctrough concentration trend over time (trajectories), accounting for the within-patient correlation of measurements obtained at different weeks on the same patient (by use of an unstructured covariance matrix). Linear mixed models were also applied to assess the effect of study arm (monotherapy vs triple therapy), grading of baseline total bilirubin (<3 vs ≥3), treatment failure occurrence (yes vs no) or drug-related adverse events occurrence (yes vs no) on ATV plasma Ctrough concentration trajectories. In case of non-significant changes over time, unadjusted model-based (linear mixed model) means of ATV plasma Ctrough concentrations were reported for each of the previous groups with the corresponding 95% confidence interval (95%CI). All two-tailed P values were considered significant at a threshold of $P < 0.05$. The analyses were performed using SAS Software, release 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Patients' characteristics

One hundred and one out of the 103 patients enrolled in the MODAt trial (51 in the ATV/r monotherapy arm [ATV/r 300/100 mg] and 52 in the ATV/r triple therapy arm [ATV/r 300/100 mg+2NRTIs]) completed the study 96 weeks. Seventy-four out of the 103 patients (72%) patients gave their consent to participate in this pharmacokinetic sub-study (35 patients in the ATV/r monotherapy arm and 39 in the ATV/r triple therapy arm). Samples for the determination of ATV Ctrough were available in 60/74 (81%) patients and

were considered for these analyses: 28/35 (80%) patients randomized to the ATV/r monotherapy arm and 32/39 (82%) to the ATV/r triple therapy arm.
Among the 28 patients randomized to ATV/r monotherapy, 9 subjects had virological failure and were re-intensified with their previous NRTIs. Four patients in the monotherapy arm (2 with previous re-intensification) and 4 patients in the triple therapy arm discontinued during follow-up. The baseline characteristics of the 60 patients enrolled in this sub-study are shown in *Table 1*. Twenty-eight patients did not have a grade ≥3 elevation at baseline [12 (43%) subjects in the ATV/r monotherapy arm and 16 (50%) subjects in the ATV/r triple therapy arm]. Among these subjects, 8/12 (75%) subjects of the ATV/r monotherapy arm vs 10/16 (63%) subjects of the ATV/r triple therapy arm had a grade ≥3 elevation in total bilirubin during follow-up (p=0.999).

Trend over time of measured ATV concentrations

A total of 215 samples were analyzed and provided valid values of ATV plasma concentrations: the overall median (IQR) number of measurements per patient was 4 (3-4) [4 (3-4) and 3 (2-4) measurements per patients in the ATV/r monotherapy arm and in the ATV/r the triple therapy arm, respectively (p=0.021)].
Overall, ATV plasma Ctrough concentration did not vary during follow-up (slope: +0.75 ng/mL/week, 95%CI: -0.97 to 2.47, p=0.387); trajectories did not differ between study arms [ATV/r monotherapy arm: +0.96, 95%CI: -1.00 to 2.92, p=0.330; ATV/r triple therapy arm: +0.51, 95%CI: -1.53 to 2.55, p=0.622; comparison between study arms: p=0.527]. The unadjusted means (95%CI) of ATV Ctrough during follow-up were 835 (95%CI: 657-1012) ng/ml in the ATV/r monotherapy arm as compared to 911 (95%CI: 740-1082) ng/mL in the ATV/r triple therapy arm (p=0.621). All ATV plasma determinations from both arms were >150 ng/mL, except for two values which resulted below the detection limit [patient T01-033 (arm A) at week 132 followed by values >3000 ng/mL at week 144 and week 180; patient T01-045 (arm B) at week 144 with no further values thereafter].

Table 1 - *The Pharmacokinetic MODAt sub-study: baseline characteristics.*

	ATV/r monotherapy (arm A) (N=28)	*ATV/r triple therapy (arm B) (N=32)*	*P-value*
Age, *years*	41 (36-47)	42 (34-48)	0.994[a]
Males	25 (89%)	30 (94%)	0.657[b]
Race			
Caucasian	27 (96%)	29 (91%)	0.565[b]
Black	0	1 (3%)	
Hispanic	1 (4%)	2 (8%)	
BMI, *kg/m²*	23.9 (21.5-25.2)	24.3 (21.7-25.3)	0.630[a]
Years of HIV infection	6 (3-8)	4 (2-6)	0.060[a]
HIV risk factor			
IDU	0	2 (6%)	0.255[b]
MSM	17 (61%)	15 (47%)	
Heterosexual	4 (14%)	7 (22%)	
Other/unknown	7 (25%)	8 (25%)	
CDC C Stage	0	0	-
CD4+ nadir, *cells/μL*	274 (224-365)	307 (260-348)	0.499[a]
HCV co-infection	3 (11%)	3 (9%)	0.998[b]
ART duration, *months*	25 (17-56)	20 (18-45)	0.667[a]
Months on ATV/r + 2 NRTIs	21 (15-32)	20 (17-29)	0.813[a]
TDF/FTC backbone	26 (93%)	27 (84%)	0.459[b]
HIV-1 RNA <50 cp/mL, *months*	22 (14-55)	18 (13-25)	0.205[a]
HIV-1 RNA at ART start, *log10 copies/mL*	4.91 (4.41-5.35)	4.82 (3.85-5.27)	0.673[a]
CD4+, *cells/μL*	626 (466-825)	570 (417-684)	0.166[a]
Fasting glucose (mg/dL)	82 (77-91)	82 (79-91)	0.888[a]
Total cholesterol (mg/dL)	185 (160-212)	181 (158-209)	0.807[a]
LDL-cholesterol (mg/dL)	127 (99-136)	119 (103-135)	0.998[a]
HDL-cholesterol (mg/dL)	42 (36-48)	45 (38-50)	0.365[a]
Triglycerides (mg/dL)	112 (100-183)	110 (82-129)	0.187[a]
ALT (UI/L)	29 (21-39)	24 (18-35)	0.230[a]
AST (UI/L)	29 (21-39)	20 (14-26)	0.859[a]
Creatinine (mg/dL)	0.87 (0.80-0.99)	0.90 (0.78-1.00)	0.700[a]
ALP (U/mL)	94 (72-114)	99 (85-116)	0.332[a]
Total bilirubin (mg/dL)	1.63 (1.30-3.12)	2.20 (1.57-3.53)	0.445[a]
grade ≥3	16 (57%)	16 (50%)	0.613[b]

Results are expressed as median (IQR) or frequency (%).
Abbreviations: ATV/r, atazanavir/ritonavir; NRTIs, nucleoside reverse transcriptase; BMI, body mass index; IDU, intravenous drug user; MSM, men who have sex with men; ART, antiretroviral treatment; TDF, tenofovir; FTC, emtricitabine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.
[a]by Wilcoxon rank-sum test.
[b]by Fisher exact test, as appropriate.
Mean values (95% confidence intervals) were calculated by use of the mixed linear model.

Association of ATV concentrations with treatment failure

No significant difference in ATV Ctrough trajectories were found according to treatment failure (TF) [without TF: +0.67, 95%CI: -1.09 to 2.43, p=0.450; with TF: +1.23, 95%CI: -1.52 to 3.99, p=0.377; comparison between groups: p=0.616]. The unadjusted means of ATV Ctrough during follow-up was 885 (95%CI: 644-1126) ng/mL in subjects with TF and 871 (95%CI: 728-1014) ng/mL in subjects without TF (p=0.917). We examined in detail subjects randomized to ATV/r monotherapy who had virological failure (n=9/11): in 4/9 (44%) subjects, the lowest value of ATV Ctrough was observed at the date of re-intensification with previous NRTIs, although these values were all in the therapeutic range.

Association of ATV concentration with treatment tolerability

There were 20/60 (33%) subjects with at least one drug-related adverse event (AE) grade 1-3 related to ATV, for a total of 20 drug-related adverse events: 8 and 12 AEs in the ATV/r monotherapy and triple therapy arm, respectively (*Table 2*). Overall, no difference in trajectories of ATV Ctrough between subjects with or without AEs was found [subjects with AEs: +1.31, 95%CI: -0.95 to 3.58, p=0.252; subjects without AEs: +0.45, 95%CI: -1.43 to 2.34, p=0.632; comparison between groups: p=0.522]. The unadjusted means of ATV Ctrough according to the occurrence of drug-related AEs are shown in *Table 3:* ATV Ctrough concentrations were 945 (95%CI: 730-1161) ng/mL versus 838 (95%CI: 692-983) ng/mL in subjects with or without AEs, respectively (p=0.403).

Table 2 - *Patients' characteristics with drug-related Adverse Events.*

Patient ID	*Study arm*	*Description of adverse event*	*Grade*	*Mean ATV C_{trough} during follow-up*
T01-006	A	Hypertriglyceridemia	2	875.72
T01-007	A	Diarrhea	1	662.23
T01-018	A	Asthenia	1	1149.32
T01-031	A	Nephrolithiasis	2	1386.68
T01-033	A	Hypertriglyceridemia	2	1666.06
T01-048	A	Nephrolithiasis	3	931.19
T01-052	A	Hypercholesterolemia	2	1066.41
T01-060	A	Nephrolithiasis	2	1847.90
T01-001	B	Creatinine increase	1	484.54
T01-010	B	Hypertriglyceridemia	1	311.53
T01-014	B	Nephrolithiasis	2	754.13
T01-024	B	Hypercholesterolemia	1	1130.22
T01-029	B	Nephrolithiasis	2	523.70
T01-045	B	Hemoglobinuria	1	425.47
T01-056	B	Hypercholesterolemia	1	775.85
T01-058	B	Hypertriglyceridemia	2	1437.60
T01-072	B	Diarrhea	1	413.39
T01-076	B	GFR decrease	3	689.40
T01-077	B	Nephrolithiasis	2	1414.42
T01-082	B	Nephrolithiasis	2	1196.88

Arm A: ATV/r monotherapy; arm B: ATV/r triple therapy.
Abbreviations: ATV, atazanavir.

Table 3 - *Predicted atazanavir C_{trough} according to study arm and occurrence of drug-related adverse events.*

Characteristic	*Group*	*N*	*Mean atazanavir C_{trough} (95% CI), ng/mL[§]*	*P-value[§]*
Study Arm	ATV/r monotherapy	28	835 (657-1012)	0.621
	ATV/r triple therapy	32	911 (740-1082)	
Treatment failure occurrence	No	45	871 (728-1014)	0.917
	Yes	15	885 (644-1126)	
Occurrence of drug-related adverse events	No	40	838 (692-983)	0.403
	Yes	20	945 (730-1161)	
Baseline total bilirubin grading	<3	28	704 (537-871)	0.012
	≥3	32	1007 (852-1162)	
HCVAb	Negative	54	914 (775-1052)	0.124
	Positive	6	602 (215-989)	

[§]Unadjusted model-based mean values (95% confidence intervals) were calculated and compared by use of the mixed linear model.
Abbreviations: ATV/r, atazanavir/ritonavir

Table 4 - *Predicted atazanavir C_{trough} according to the occurrence of drug-related adverse events and grading of baseline total bilirubin.*

Occurrence of drug-related adverse events during follow-up	*Grading of baseline total bilirubin*	*N*	*Mean atazanavir C_{trough} (95% CI), ng/mL*[§]	*P-value*[§]
No	Grade <3	20	716 (557-874)	0.098
	Grade ≥3	20	902 (742-1061)	
Yes	Grade <3	8	572 (276-868)	0.002
	Grade ≥3	12	1183 (970-1395)	

[§]Unadjusted model-based mean values (95% confidence intervals) were calculated and compared by use of the mixed linear model.

Twenty out of the 40 patients (50%) without drug-related adverse events during follow-up and 12/20 (60%) subjects with AEs had a grade ≥3 total bilirubin at baseline. Subjects with a grade ≥3 in total bilirubin at baseline showed a statistically significant increase in atazanavir Ctrough concentrations during follow-up as compared to those with a grade <3 in total bilirubin at baseline [subjects with a baseline total bilirubin of grade <3: -0.77, 95%CI: -2.87 to 1.32, p=0.463; subjects with a baseline total bilirubin of grade ≥3: +2.10, 95%CI: +0.06 to 4.15, p=0.044; comparison between groups: p=0.012]. The unadjusted means atazanavir Ctrough values according to the grading of total bilirubin at baseline are shown in *Table 3:* differences in the mean values of atazanavir Ctrough between grading classes of total bilirubin at baseline (p=0.012) were detected.

Among subjects who had AEs (*Table 4*), those with a grade <3 as compared to those with a grade ≥3 in total bilirubin at baseline had lower atazanavir Ctrough (p=0.002). Among subjects who had no AEs, grading of total bilirubin at baseline was not associated with different atazanavir Ctrough (p=0.098).

No differences were observed in the ATV Ctrough trajectories during follow-up between subjects with (n=6) or without (n=54) hepatitis C [subjects with of hepatitis C: -2.56, 95%CI: -6.94 to 1.81, p=0.247; subjects without of hepatitis C: +1.04, 95%CI: -0.99 to 3.07, p=0.310; comparison between groups: p=0.157]. Mean atazanavir Ctrough values according to the presence of hepatitis C are shown in *Table 3*. The same findings were obtained also when the comparison was performed taking into account the study arm (data not shown).

DISCUSSION

HAART has radically improved HIV patients' life expectancy, so it will be necessary to move forward to new tolerable HAART regimens, with the aim to be life-long and economically sustainable, both for younger and older HIV-infected patients. A PI-based monotherapy seemed to be a good compromise for maintaining a genetic barrier, to preserve the pool of antiretroviral drugs and minimize metabolic iatrogenic disorders in view of a life-long therapy.

In this pharmacokinetic sub-study, we investigated whether the results of MODAt trial, which showed a lower efficacy of the ATV-based monotherapy versus the ATV-based triple therapy, could have been affected, at least in part, by differences in ATV exposure. ATV Ctrough concentrations in both arms did not significantly differ from values reported in naïve patients (150-800 ng/mL) (Gonzalez de Requena *et al.,* 2005). Overall, we found no differences in ATV trough concentrations between the two treatment arms of the MODAt trial and, most importantly, no association between ATV plasma concentrations and study outcome in terms of therapeutic success. This finding allows us to conclude that the observed differences in efficacy between ATV-based monotherapy and triple therapy were not related to differences in ATV exposure and bioavailability.

This MODAT sub-study partially confirmed previous findings (Gervasoni *et al.,* 2015; Giacomelli *et al.,* 2016) by documenting that higher ATV plasma concentrations have been shown to play a role in adverse events occurrence, with a significant relationship between baseline bilirubin grade (when ≥3), ATV concentrations and adverse events occurrence. This sub-study has important limitations. First of all, the limited sample size of this study and the lack of some plasma samples at the predefined time-points may have masked differences between groups (according to study arm, treatment failure, occurrence of AEs, grading of baseline total bilirubin) that might indeed exist. In addition to this caveat, there is a difference in numbers between HCV-positive and HCV-negative patients who gave their additional consent to participate in this pharmacokinetic sub-study, thus the role of HCV infection could not be properly weighted. Another possible limitation is baseline bilirubin role as a confounder in interpreting data of adverse events. As is well known, ATV has the characteristic to produce hyperbilirubinemia: for this reason, the high prevalence of baseline hyperbilirubinemia makes it difficult to interpret adverse events data. Moreover, our patients were exposed to ATV for a median time of roughly 2 years and this could have selected a group of subjects who tolerated ATV quite well. Nonetheless, as in every study, AEs could arise throughout the observation time in an open-label trial.

In conclusion, the present study documented that differences in the efficacy of patients enrolled in the MODAt trial were not related to different ATV concentrations. Rather, our findings emphasized an association of ATV plasma concentrations with the development of side-effects in both subjects treated with ATV/r monotherapy and subjects treated with ATV/r triple therapy. Side-effects were more frequently observed in subjects treated with ATV/r monotherapy and with higher ATV plasma concentrations.

Acknowledgements

This pharmacokinetic sub-study was made possible through an unrestricted research grant from Bristol-Myers Squibb.

Conflict of interest statement

S.R. received grants, compensation for CME activities or speaker's bureau from ViiV, MSD, BMS, Gilead, Janssen and AbbVIE. D.C. received educational grants from ViiV, MSD.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

References

Antinori A., Clarke A., Svedhem-Johansson V., Arribas J.R., Arenas-Pinto A., et al. (2015). Week 48 efficacy and central nervous system analysis of darunavir/ritonavir monotherapy versus darunavir/ritonavir with two nucleoside analogues. *AIDS*. **10**, 1811-1820.

Arribas J.R., Delgado R., Arranz A., Muñoz R., Portilla J., et al. (2009). Lopinavir/ritonavir monotherapy versus lopinavir-ritonavir and 2 nucleosides for maintenance therapy of HIV: 96-week analysis. *J Acquir Immune Defic Syndr*. **51**, 147-152.

Baril J., Conway B., Giguère P., Ferko N., Hollmann S., Angel J.B. (2014). A meta-analysis of the efficacy and safety of unboosted atazanavir compared with ritonavir-boosted protease inhibitor maintenance therapy in HIV-infected adults with established virological suppression after induction. *HIV Med*. **15**, 301-310.

Bernardino J.I., Pulido F., Martinez E., Arrizabalaga J., Domingo P., et al. (2013). Switching to lopinavir/ritonavir with or without abacavir/lamivudine in lipoatrophic patients treated with zidovudine/abacavir/lamivudine. *J Antimicrob Chemother*. **68**, 1373-1381.

Bonora S., Gonzalez de Requena D., D'Avolio A., Calcagno A., Tettoni M., et al. (2011). Pharmacokinetics of switching unboosted atazanavir coadministered with tenofovir disoproxil fumarate from 400 mg once daily to 200 mg twice daily in HIV-positive patients. *Antivir Ther*. **16**, 499-504.

Cahn P., Montaner J., Junod P., Patterson P.., Krolewiecki A, et al. (2011). Pilot, randomized study assessing safety, tolerability and efficacy of simplified LPV/r maintenance therapy in HIV patients on the 1st PI-based regimen. *PLoS One*. **6**, e23726.

Castagna A., Spagnuolo V., Galli L., Vinci C., Nozza S., et al. (2014). Simplification to atazanavir/ritonavir monotherapy for HIV-1 treated individuals on virological suppression: 48-week efficacy and safety results. *AIDS*. **28**, 2269-2279.

Clumeck N., Rieger A., Banhegyi D., Schmidt W., Hill A., et al. (2011). 96 week results from the MONET trial: a randomized comparison of darunavir/ritonavir with versus without nucleoside analogues, for patients with HIV RNA <50 copies/mL at baseline. *J Antimicrob Chemother*. **66**, 1878-1885.

Colasanti J., Marconi V.C., Taiwo B. (2014). Antiretroviral reduction: is it time to rethink the unthinkable? *AIDS*. **28**, 943-947.

Di Giambenedetto S., Fabbiani M., Colafigli M., Ciccarelli N., Farina S., et al. (2013). Safety and feasibility of treatment simplification to atazanavir/ritonavir R lamivudine in HIV infected patients on stable treatment with twonucleos(t)ide reverse transcriptase inhibitors + atazanavir/ritonavir with virological suppression (Atazanavir and Lamivudine for treatment Simplification, AtLaS pilot study). *J Antimicrob Chemother*. **68**, 1364-1372.

Evans W.E., McLeod H.L. (2003). Pharmacogenomics-drug disposition, drug targets, and side effects. *N Engl J Med*. **348**, 538-549.

Galli L., Spagnuolo V., Bigoloni A., D'Arminio Monforte A., Montella F., et al. (2016). Atazanavir/ritonavir monotherapy: 96 week efficacy, safety and bone mineral density from the MODAt randomized trial. *J Antimicrob Chemother*. **71**, 1637-1642.

Gervasoni C., Meraviglia P., Minisci D., Ferraris L., Riva A., et al. (2015). Metabolic and kidney disorders correlate with high atazanavir concentrations in HIV-infected patients: is it time to revise atazanavir dosages? *PLoS One*. **10**, e0123670.

Ghosn J., Flandre P., Cohen-Codar I., Girard P.M., Chaix M.L., et al. (2010). Long-term (96-week) follow-up of antiretroviral-naive HIV-infected patients treated with first-line lopinavir/ritonavir monotherapy in the MONARK trial. *HIV Med*. **11**, 137-142.

Giacomelli A., Oreni L., Franzetti M., Di Cristo V., Colella E., et al. (2016). Factors involved in continuance of atazanavir-based regimens: Results from a cohort of HIV1-positive patients. *Antiviral Res*. **129**, 52-57.

Gianotti N., Poli A., Galli M., Pan A., Rizzardini G., et al. (2014). Monotherapy with lopinavir/ritonavir versus standard of care in HIV-infected patients virologically suppressed while on treatment with protease inhibitor-based regimens: results from the MoLo study. *New Microbiol*. **37**, 439-448.

Gonzalez de Requena D., Bonora S., Canta F., Marrone R., D'Avolio A., Sciandra M., et al. (2005). Atazanavir Ctrough is associated with efficacy and safety: definition of therapeutic range. 12th Conference on Retroviruses and Opportunistic Infections, Boston, February 22–25, 2005. Abstract 646.

Gutmann C., Cusini A., Günthard H.F., Fux C., Hirschel B., et al. (2010). Randomized controlled study demonstrating failure of LPV/r monotherapy in HIV: the role of compartment and CD4-nadir. *AIDS*. **24**, 2347-2354.

Le Tiec C., Barrail A., Goujard C., Taburet A.M. (2005). Clinical pharmacokinetics and summary of efficacy and tolerability of atazanavir. *Clin Pharmacokinet*. **44**, 1035-1050.

Meynard J.L., Bouteloup V., Landman R., Bonnard P., Baillat V., et al. (2010). Lopinavir/ritonavir monotherapy versus current treatment continuation for maintenance therapy of HIV-1 infection: the KALESOLO trial. *J Antimicrob Chemother*. **65**, 2436-2444.

Nunes E.P., Santini de Oliveira M., Merçon M., Zajdenverg R., Faulhaber J.C., et al. (2009) Monotherapy with lopinavir/ritonavir as maintenance after HIV-1 viral suppression: results of a 96-week randomized, controlled, open-label, pilot trial (KalMo study). *HIV Clin Trials*. *10*, 368-374.

Perez-Molina J.A., Rubio R., Rivero A., Pasquau J., Suárez-Lozano, et al. (2015). Dual treatment with atazanavir-ritonavir plus lamivudine versus triple treatment with atazanavir-ritonavir plus two nucleos(t)ides in virologically stable patients with HIV-1 (SALT): 48 week results from a randomised, open-label, non-inferiority trial. *Lancet Infect Dis*. **15**, 775-784.

Solas C., Gagnieu M.C., Ravaux I., Drogoul M.P., Lafeuillade A., et al. (2008). Population pharmacokinetics of atazanavir in human immunodeficiency virus-infected patients. *Ther Drug Monit*. **30**, 670-673.

The Antiretroviral Therapy Cohort Collaboration (ART-CC). (2013). Durability of first ART regimen and risk factors for modification, interruption or death in HIV-positive patients starting ART in Europe and North America 2002-2009. *AIDS*. **27**, 803-813.

Valantin M.A., Lambert-Niclot S., Flandre P., Morand-Joubert L., Cabiè A., et al. (2012). Long-term efficacy of darunavir/ritonavir monotherapy in patients with HIV-1 viral suppression: week 96 results from the MONOI-ANRS 136 study. *J Antimicrob Chemother*. **67**, 691-695.

Van Schaik R.H., van der Heiden I.P., van den Anker J.N., Lindemans J. (2002). CYP3A5 variant allele frequencies in Dutch Caucasians. *Clin Chem*. **48**, 1668-1671.

Wempe M.F., Anderson P.L. (2011). Atazanavir metabolism according to CYP3A5 status: an in vitro-in vivo assessment. *Drug Metab Dispos*. **39**, 522-527.