Successful Pharmacogenetics-based Optimization of Unboosted Atazanavir Plasma Exposure in HIV-positive Patients: a Randomized, Controlled, Pilot Study (The REYAGEN Study).

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Synopsis

Background: Atazanavir without ritonavir, despite efficacy and tolerability, shows low plasma concentrations that warrant optimization.

Methods: In a randomized, controlled, pilot trial, stable HIV-positive patients on atazanavir/ritonavir (with tenofovir/emtricitabine) were switched to atazanavir. In the standard dose arm atazanavir was administered as 400 mg once-daily, while according to patients' genetics (*PXR, ABCB1* and *SLCO1B1)* in the pharmacogenetic arm: patients with unfavourable genotypes received atazanavir 200 mg twice-daily.

Results: Eighty patients were enrolled with balanced baseline characteristics. Average atazanavir exposure was 253 ng/mL (150-542) in the pharmacogenetic arm versus 111 ng/mL (64-190) in the standard arm ($p<0.001$); 28 patients in the pharmacogenetic arm (75.7%) had atazanavir exposure >150 ng/mL versus 14 patients (38.9%) in the standard arm ($p=0.001$). Immunovirological and laboratory parameters had a favourable outcome throughout the study with non-significant differences between study arms.

Conclusions: Atazanavir plasma exposure is higher when the schedule is chosen according to the patient's genetic profile.

INTRODUCTION

In the lifelong perspective of anti-HIV treatment, individual tailoring of the antiretroviral regimen is going to be increasingly required. Although never formally approved in Europe, the use of atazanavir without concurrent intake of ritonavir has been shown to be effective and well tolerated in two induction-maintenance clinical trials of relevant size and several retrospective studies. [1-4] However in a significant proportion of patients the pharmacokinetic (PK) exposure of atazanavir might be potentially insufficient to guarantee long-term HIV inhibition.[5,6] atazanavir lower exposure when combined with tenofovir disoproxil fumarate has been shown in healthy volunteers but subsequently found to be less relevant in HIV-positive patients.[7-9] atazanavir pharmacokinetics is significantly influenced by genetic polymorphisms in the region coding for the pregnane X receptor (*PXR*, controlling the expression of several genes involved in drug metabolism and transport); additionally polymorphisms in *ABCB1* (coding for P-glycoprotein) and *SLCO1B1* (coding for OATP1B1) were shown to have a comparable effect on atazanavir exposure.[10-12] Furthermore we observed that the pharmacokinetic exposure of atazanavir was significantly improved when administered 200 mg twice-daily instead of 400 mg once-daily.[13]

We report here the results of a randomized comparative study on the clinical use of unboosted atazanavir with or without pharmacogenetic guide in patients also taking co-formulated tenofovir/emtricitabine.

METHODS

HIV-positive adult patients on treatment with atazanavir/ritonavir (300/100 mg) plus tenofovir/emtricitabine with HIV RNA <50 copies/mL for at least six months were eligible for enrolment at two sites in Italy. Switch to atazanavir was proposed for toxicity/tolerability or for simplification, according to physicians' evaluation in clinical practice. Exclusion criteria were:

previous virological failure, genotypic resistance-associated mutations, ongoing opportunistic infections/neoplasias, liver cirrhosis, chronic renal failure, self-reported adherence <90% (Visual Scale) and consumption of potentially interacting drugs.

The study was approved by the institutional review board at both participating centres, and each participant provided signed informed consent before enrolment; the procedures were in accordance with the ethical standards of the Helsinki Declaration of 1975 (as revised in 1983).

The study was a randomized, controlled, open-label, pilot trial. Patients were randomized 1:1 (block randomization) to either standard-dose arm ["SD"; atazanavir 400 mg *once daily*] or pharmacogenetic-based arm ["PG"; atazanavir 400 mg *once daily* in patients with favourable genetic profile or atazanavir 200 mg *twice daily* in patients with unfavourable genetic profile].At enrolment genomic DNA was extracted using QIAamp whole blood mini kit (Qiagen, Valencia, CA, USA) and genotyping was conducted by real time-based allelic discrimination with the use of standard methods (BIORAD, Milano, Italy). The following single nucleotide polymorphisms were analysed: C63396T in *PXR* (rs2472677), C3435T in *ABCB1* (rs1045642) and C521T in *SLCO1B1* (rs4149056). *PXR* 63396 TT, *ABCB1* 3435 CT/TT and *SLCO1B1* 521 TT were codified as 1 (associated with lower plasma concentrations). On the basis of the PG results patients were given a score (min zero - max three) and a different dosing schedule according to favourable (≤1) or unfavourable genetic profiles (≥2).

Primary end point was the prevalence of atazanavir average trough concentrations (geometric mean of the first three determinations at weeks 4, 8 and 12) above 150 ng/mL (suggested target plasma level) in the two arms. Secondary end points were the comparison of the proportion of patients with HIV RNA <50 copies/mL and of the changes in indirect bilirubin, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides at 48 weeks.

atazanavir trough plasma concentrations [12/24 hours after drug intake according to drug schedule (± two hours)] were measured by a previously validated HPLC-PDA (Photo Diode Array) method and performed in Torino.[14]

A sample size of 80 patients (40 per group) was calculated to provide a statistical power of at least 80%, in order to identify a difference in mean atazanavir Ctrough below the MEC of 150 ng/mL between the two study arms. It was assumed a 20% of atazanavir Ctrough under MEC in the PG arm, and a 50% in the control arm from previous studies results.[10-12] Standard non-parametric tests were usd for all analysis and performed using SPSS 20.0 software for Mac (SPSS, IBM Inc.).

RESULTS

Eighty patients were enrolled (2009-2011): demographic and immunovirological characteristics were well balanced between study arms (Table 1). Patients' disposition is shown in Figure S1: no subject dropped out of the study due to toxicity, virological failure or major clinical events. The prevalence of single nucleotide polymorphisms is reported in Table 1; all variants were in Hardy-Weinberg equilibrium. 27 patients in the PG arm received atazanavir 200 mg twice daily.

Atazanavir plasma trough concentrations are shown in Figure S2 and Table S1. Atazanavir Ctrough was slightly higher at baseline in the PG arm [1034 ng/mL (592-1935) versus. 587 ng/mL (77-1290), Mann-Whitney p=0.06] as compared to SD arm; it was significantly higher at every time point after randomization ($p<0.001$ for all comparisons, Mann-Whitney) in the PG arm.

Geometric mean of week 4 to 12 atazanavir Ctroughs was 253 ng/mL (150-542) in the PG arm versus 111 ng/mL (64-190) in the SD arm, favouring the former ($p<0.001$, Mann-Whitney). As for the primary endpoint 28 patients in the PG arm (75.7%) had an average atazanavir Ctrough above 150 ng/mL versus 14 patients (38.9%) in the SD arm (p=0.001, RR 4.89, 95%CI 1.79-13.38) (Fig. 1).

No difference in plasma HIV-RNA <50 copies/mL was observed in 37 patients (100%) in the PG arm versus 33 patients (97%) in the SD arm at week 48. Three patients (8.1%) and 4 patients (11.7%) in the PG and SD arm presented a viral blip during the study (p=0.703, Fisher's exact test). Patients in both arms had similar CD4+ T lymphocytes recovery at week 48: 39 cells/mm^3 in the PG versus 53 cells/mm^3 in the SD arm (p=0.744, Mann-Whitney).

At 48 weeks significant decreases (all p<0.05, Wilcoxon's) in safety markers were noted as compared to baseline: no significant differences between study arms were found (Mann-Whitney), (Table S2).

DISCUSSION

In this pilot, randomized and controlled study we found that the pharmacokinetic exposure of atazanavir, when co-administered with tenofovir/emtricitabine was significantly higher and closer to the desired target concentration when the frequency of administration was chosen according to the patient's genetic profile. The proportion of patients with atazanavir Ctrough above the cut-off concentration rose from 40% (previous studies and the standard arm) to 75.7% (study arm) when the frequency of atazanavir administration (400 mg once daily or 200 mg twice daily) was decided on the basis of the individual genotypic profile.[10-12] Although not all patients had a Ctrough level above the pre-specified cut-off value of 150 ng/mL, the pharmacokinetic exposure in the study arm was found significantly more appropriate than in patients in the control arm. In the PG arm baseline atazanavir levels were higher than those recorded in the SD arm: it is possibly due to unbalanced factors between study arms (such as *CYP3A5* genotype and adherence levels) and unexpected atazanavir exposures according to genotype (Supp.Tab.1) may support this hypothesis.[15] It must however be considered that the 150 ng/mL threshold resulted from the analysis of a moderately experienced population of HIV-infected patients that was no longer formally re-assessed in

203 treatment-naïve patients: it appears possible that it could be lower in patients not harbouring virus
204 with resistance associated mutations and after achieving viral suppression.[6,16,17] The documented
205 higher intracellular accumulation of atazanavir as compared to other PIs might also support this
206 hypothesis. [18,19] No significant difference in the prevalence of viral control or in the changes in
207 safety markers between study arms was seen: it is possible the longer follow-up may be required to
208 observe the effect of improved pharmacokinetic exposure or that lower atazanavir concentrations
209 may be adequate.

210 Independently of study arm atazanavir-based regimens were well tolerated and associated with
211 improved safety profiles. Even if the drug is nowadays less used given the availability of safe and
212 very compact antiretroviral regimens it may be very useful in the long-term treatment of HIV-
213 positive patients. The absence of ritonavir (associated with side effect even at low doses) and the
214 uncommon incidence of hyperbilirubinmia (being the main determinant of atazanavir/ritonavir
215 inferior performance in naïve patients) support the attractiveness of atazanavir-containing
216 regimens.[20] Even if the need for genetic testing prior to start atazanavir might no be commonly
217 accepted it can be a tool for avoiding unnecessary treatment interruptions and side effects.[21]
218 Although some patients (those with unfavourable genetic profile) would necessitate to take the drug
219 twice daily instead of once daily, the advantage in terms of side effects reduction might compensate
220 the higher frequency of administration.

221 We have to recognize some limitations of this study: the limited sample size, the restricted number
222 of included genetic polymorphisms as well as a casual impaired factors distribution between the
223 study arms, the potential need for therapeutic drug monitoring even in the PG-based arm.

224 Once in a lifetime performed genetic testing offers the possibility to know in advance the likelihood
225 of an individual patient to achieve a more appropriate atazanavir pharmacokinetic exposure and to

choose the frequency of administration accordingly; if confirmed, this observation supports the use of pharmacogenetics for treatment tailoring in atazanavir-receiving HIV-positive patients.

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SB, SR, MS, AD and GDP designed the study and contributed to data collection. AC performed data interpretation and statistical analysis and generated the random allocation sequence. SR, MB, OV, ML, AT, LM contributed to data collection. AC and MB drafted the first version of the manuscript and finalized the manuscript. JC, MS and AD performed the pharmacokinetic and pharmacogenetic analysis and revised the technical details of the paper. SB, SR, GDP and MG contributed to study design, supervision and critical revision of the manuscript for intellectual content. All authors read and approved the final manuscript.

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Tables

Characteristic	Standard dose arm (n= 40)	Pharmacogenetic arm (n= 40)	p values
Age (years): median (IQR)	43 (37-47)	44 (38-50)	0.424
Male gender: n (%)	28 (70%)	30 (75%)	0.783
Ethnicity: n (%)			
White	37 (92.5%)	34 (85%)	0.487
Black	1 (2.5%)	3 (7.5%)	
Other	2 (5%)	3 (7.5%)	
BMI (Kg/m^2): median (IQR)	22.9 (20.2-25.3)	23.9 (21-26.2)	0.421
Duration of HIV infection (years): median (IQR)	5.9 (3.7-12.4)	7.3 (3.7-12.3)	0.665
CD4+ T lymphocytes ($cells/mm^3$): median (IQR)	541 (428-628)	467 (320-600)	0.063
CD4+/CD8+ T lymphocytes ratio: median (IQR)	0.65 (0.53-1.1)	0.60 (0.5-1.29)	0.864
Hepatitis B surface antigen positive: n (%)	6 (15%)	1 (2.5%)	0.049
Hepatitis C antibody positive: n (%)	8 (20%)	8 (20%)	0.823
Single nucleotide polymorphisms: n (%)			
***PXR 63396* TT**	12 (30%)	10 (25%)	0.848
***ABCB1 3435* CT/TT**	28 (70%)	29 (72.5%)	0.364
***SLCO1B1 521* TT**	30 (75%)	33 (82.5%)	0.848
Favorable pharmacogenotypic score (<=1): n (%)	14 (35%)	13 (32.5%)	0.797

Table 1. Demographics, immunovirological and pharmacogenetic characteristics of randomized patients. Values were compared between the two arms using Chi-square (Fisher's exact test where appropriate) for categorical values and Mann-Whitney test for continuous variable; two-sided p values are shown in the last column. "IQR": interquartile range.

Figures:

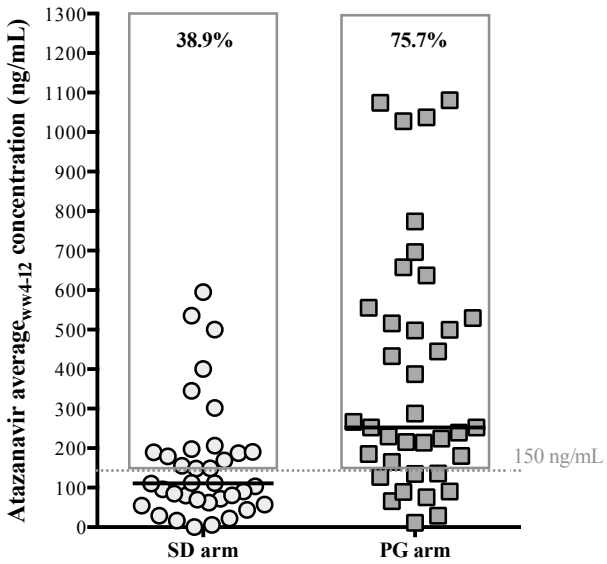

Figure 1. Atazanavir average concentration (weeks 4 to 12) according to study arm. Symbols indicate geometric mean of trough concentration obtained at weeks 4, 8 and 12; the horizontal lines represent median values. The gray boxes represent the percentage of patients with average exposure above 150 ng/mL.