

1 **Influence of alpha 1 glycoprotein acid concentrations and variants on atazanavir**
2 **pharmacokinetics in HIV-infected patients included in ANRS107 trial**

3
4 A. Barrail-Tran^{1*}, F. Mentré², C. Cosson³, C. Piketty⁴, C. Chazallon⁵, L. Gérard⁵, P.M. Girard⁶,
5 A.M. Taburet¹

6
7 ¹ Clinical Pharmacy, Hôpital Bicêtre, AP-HP, France, ² INSERM, U738, Paris, France ;
8 Université Paris 7, UFR de Médecine, Paris, France; AP-HP, Hôpital Bichat, UF de
9 Biostatistiques, Paris, France, ³ Biochemistry, Hôpital Bicêtre, AP-HP, France, ⁴ Department of
10 immunology, Hôpital Georges Pompidou, AP-HP, France, ⁵ Inserm SC10, Villejuif, France, ⁶
11 Department of infectious diseases, Hôpital Saint Antoine, AP-HP, Paris, France

12
13 Correspondance : Dr Aurélie Barrail-Tran

14 Hôpital Bicêtre, Clinical Pharmacy

15 78 rue du Général Leclerc

16 94275 Le Kremlin Bicêtre cedex, France

17 Tel : +33 1 45 21 29 64

18 Fax : +33 1 45 21 28 60

19 E-mail: aurelie.barrail-tran@bct.aphp.fr

20 Running title: alpha 1 glycoprotein acid polymorphism and atazanavir pharmacokinetics

21 27 pages, 2 tables, 2 figures

22 This work was presented in part at the 14th Conference on Retroviruses and opportunistic
23 infections, Los Angeles, February 2007.

24 **Abstract**

25 Atazanavir is an HIV-1 protease inhibitor (PI) with high protein binding in human plasma. The
26 objectives were first to determine the *in vitro* binding characteristics of atazanavir, second to
27 evaluate whether plasma protein binding to albumin and to orosomucoid (alpha 1 glycoprotein
28 acid) influence the pharmacokinetics of atazanavir in HIV-infected patients. For the *in vitro*
29 study, atazanavir protein binding characteristics were determined in alpha 1 glycoprotein acid and
30 albumin purified solutions. Atazanavir was found to bind alpha 1 glycoprotein acid on a high
31 affinity saturable site (association constant $4.61 \cdot 10^5$ L/mol) and albumin on a low-affinity non-
32 saturable site. For the *in vivo* study, blood samples from 51 patients included in the ANRS107–
33 Puzzle 2 trial were drawn prior to drug intake at week 6. For 10 patients included in the
34 pharmacokinetic substudy, five additional blood samples were collected during one dosing
35 interval at week 6. Atazanavir concentrations were assayed by LC-MS/MS. Albumin
36 concentrations, alpha 1 glycoprotein acid concentrations and phenotypes were also measured in
37 these patients. Concentrations of atazanavir were modelled using a population approach. A one-
38 compartment model with first-order absorption and elimination best described atazanavir
39 pharmacokinetics. Atazanavir pharmacokinetic parameters and their interindividual variabilities
40 (%) were as follows: absorption rate constant (k_a) 0.73 h^{-1} (139.3%), apparent clearance (Cl/F)
41 13.3 L/h (26.7%) and apparent volume of distribution (V/F) 79.7 L (27.0%). Atazanavir Cl/F
42 decreased significantly when alanine aminotransferase and/or alpha 1 glycoprotein acid levels
43 increased ($p < 0.01$). ORM1*S alpha 1 glycoprotein acid phenotype also significantly increased
44 atazanavir V/F ($p < 0.05$). These *in vivo* results indicate that atazanavir pharmacokinetics is
45 moderately influenced by its protein binding, especially to alpha 1 glycoprotein acid without
46 expected clinical consequences.

47

48 **Introduction**

49 Atazanavir is an azapeptide protease inhibitor, with a distinct resistance and tolerance profile
50 approved for use in combination treatment of HIV-1 in the US and Europe (11, 12, 17). In the
51 US, one of the recommended antiretroviral regimens is the combination of atazanavir / ritonavir
52 with tenofovir / emtricitabine. Recommended doses of atazanavir are 400 mg once daily taken
53 with food without ritonavir in therapy-naive patients or 300 mg in combination with low-dose
54 ritonavir (100 mg) once daily in antiretroviral-experienced patients, or when combined with
55 tenofovir (7). The major advantages of atazanavir are its simplicity of administration and a
56 favourable adverse effects profile, especially on lipid parameters.

57 Major pharmacokinetic characteristics of atazanavir are a variable absorption through the gut,
58 86% plasma protein binding on albumin and alpha 1 glycoprotein acid (orosomuroid) and
59 elimination through biotransformation which involved CYP3A. Although consequences of
60 CYP3A metabolism on first pass effect and drug-drug interactions have been largely studied,
61 there is a lack of information on consequences of protein binding on atazanavir pharmacokinetics.
62 It is now recognized that protein binding is an important modulator of protease inhibitor
63 disposition and unbound concentration inhibitor is considered as the active moiety which is
64 available to cross cell membranes. Therefore variations in the concentrations and structures of
65 orosomuroid and albumin under physiological or pathological infections such as HIV infection
66 are likely to influence protease inhibitors pharmacokinetics (3). Albumin concentrations could be
67 significantly reduced in patients with liver disease such as co-infections with hepatitis B virus or
68 C virus. Orosomuroid, also called alpha 1 glycoprotein acid, is a glycoprotein which is controlled
69 by a cluster of three adjacent genes : AGP-A which codes for the major protein ORM1; AGP-B
70 and AGP-B' which code for the protein ORM2 (14). The proteins ORM1 and ORM2 are

71 different by a sequence of 22 amino-acids. The protein ORM1 is polymorphic with three variants:
72 ORM1*F1, ORM1*S and ORM1*F2, whereas the protein ORM2 is generally monomorphic with
73 one variant: ORM2*A (8). This polymorphism could be responsible for interindividual variation
74 in the plasma binding of protease inhibitors, which might influence their pharmacokinetic
75 parameters.

76 The purpose of this work was to determine the *in vitro* binding characteristics of atazanavir. Then
77 we evaluate whether albumin concentration and/or alpha 1 glycoprotein acid concentration and
78 alpha 1 glycoprotein acid variants are pertinent covariates in the population pharmacokinetics
79 analyses of atazanavir used in combination with ritonavir tenofovir in HIV-infected patients
80 included in a clinical trial (ANRS 107 – Puzzle 2) (22).

81 **Methods**

82 *Atazanavir in vitro binding experiments*

83 Alpha 1 glycoprotein acid and serum albumin solutions were prepared in pH 7.4 phosphate-
84 buffered saline. Concentrations of alpha 1 glycoprotein acid and serum albumin used were those
85 found in normal patients and were 0.7 g/L and 40 g/L respectively. These solutions were spiked
86 with known amounts of atazanavir to yield the following final concentrations: 0, 500, 1000, 1500,
87 2000, 5000, 10000, 15000 and 30000 ng/mL. Bound and unbound atazanavir were separated by
88 ultrafiltration of 500 µL samples using Centrifree® devices (Amicon, YM-300 filter system,
89 Millipore Corp., Bedford, Massachussets, USA) at 3000 g during two hours at 30°C. Atazanavir
90 was then measured in the ultrafiltrate according to a method developed for amprenavir (1). The
91 Amicon Centrifree YM-300 filter system (Millipore Corp. Bedford, MA) with a membrane
92 molecular weight cut off of 30,000 Daltons was used to ultrafiltrate plasma samples. The driving

93 force for ultrafiltration was provided by centrifugation (Jouan, GR 4.11) at 2000 g, at 30°C.
94 Duration of centrifugation was 120 minutes to obtain an ultrafiltrate volume of at least 200 mL
95 from a 500-mL. Atazanavir concentrations in ultrafiltrate were measured by HPLC with
96 separation on a C18 column after liquid-liquid extraction and UV detection at 220 nm. The
97 mobile phase consisted of pH 5.6 phosphate buffer/acetonitrile/methanol (480/110/110, vol/vol)
98 and the flow rate was 1.1 mL/min. Retention times for atazanavir and 6,7-dimethyl-2,3-di-(2-
99 pyridyl)-quinoxaline (internal standard from Sigma Aldrich Chemicals) were 15 and 30 minutes
100 respectively in our chromatographic system.

101 Unbound fraction was the ratio of unbound atazanavir and total atazanavir concentrations. The
102 graph of the drug bound concentration as a function of the drug unbound concentration depicts
103 the protein binding system (24).

104

105 *Population pharmacokinetic analysis of atazanavir in patients*

106 **Study design and population**

107 ANRS 107–Puzzle 2 was a randomised, open-label, multiple-dose trial evaluating the efficacy
108 and safety of a combination of atazanavir/ritonavir and tenofovir in HIV-infected patients with
109 multiple antiretroviral treatment failures. The study design is detailed elsewhere (22). From week
110 3 to week 26, all patients received atazanavir/ritonavir (300 mg / 100 mg QD) plus tenofovir
111 disoproxil fumarate (300 mg QD equivalent to 245 mg of tenofovir disoproxil or 136 mg of
112 tenofovir) and nucleoside reverse transcriptase inhibitors (NRTIs) selected according to the
113 baseline reverse transcriptase genotype of the HIV isolate infecting each patient. Drugs were
114 administered in the morning with a light continental breakfast.

115 Before inclusion, all patients gave their written informed consent. The protocol was approved by
116 the Institutional Review Board of Saint Antoine Hospital, Paris VI University. HIV-infected
117 patients were eligible for inclusion if they met the following criteria: documented treatment
118 failure with at least two PIs and one non-nucleoside reverse transcriptase inhibitor, HIV RNA
119 >10 000 copies/mL, no change in antiretroviral treatment within the last month before inclusion
120 in this study, normal liver function.

121 **Plasma collection**

122 Blood samples for biochemical and immuno-virological measurements were drawn at screening
123 and at regular time intervals after inclusion. Blood samples for atazanavir plasma concentrations
124 assay were collected prior to drug intake in the morning at week 6, 1 month after starting the full
125 antiretroviral treatment, atazanavir + ritonavir + tenofovir + optimized background treatment,
126 according to the design of the ANRS107 study (22). For 10 patients included in the
127 pharmacokinetic substudy, additional blood samples were collected after dosing at times 1, 2, 3,
128 5, 8 and 24 hours (23). Plasma samples were kept at -20°C until analysis. The actual times of
129 drug administration and sampling were recorded.

130 **Drug assays**

131 Atazanavir concentrations were measured in plasma patients samples by validated LC/MS/MS
132 assays (Bristol-Myers Squibb, Saint Nazaire, France). The lower limits of quantification (LOQ)
133 were 1 ng/mL. Day-to-day variabilities for the quality control samples were 5.7%.

134 **Biochemical and virological measurements**

135 Absolute numbers of CD4 lymphocytes, plasma HIV RNA levels, blood chemistry parameters
136 (albumin, total bilirubin, total cholesterol, triglycerides, alanine and aspartate aminotransferases)
137 were determined according to standard assays (22).

138 **Determination of alpha 1 glycoprotein acid concentrations and phenotypes**

139 Alpha 1 glycoprotein acid plasma concentrations were measured by nephelometry (BN Prospec,
140 Dade Behring). Alpha 1 glycoprotein acid phenotypes (ORM1*S-ORM2*A, ORM1*F1S-
141 ORM2*A and ORM1*F1-ORM2*A) were determined on plasma samples by isoelectric focusing
142 according to the method developed by Eap and Baumann, with some modifications (10).

143 **Population pharmacokinetic modelling**

144 Data at week 6 were analysed using a population approach with the first-order method
145 (WinNonMix version 2.0.1, Pharsight Corporation, Mountain View, CA, USA) and
146 parameterised with the apparent volume of distribution (V/F), the first order absorption rate
147 constant (k_a) and the apparent clearance (Cl/F). The statistical model for the observed plasma
148 concentrations of the drug C_{ij} in patient i at time t_{ij} was given by: $C_{ij} = f(t_{ij}, \theta_i) + \varepsilon_{ij}$ where θ_i is the
149 pharmacokinetic parameter vector of patient i , ε_{ij} the residual error and f the pharmacokinetic
150 model.

151 An exponential random-effect model was chosen to describe inter-individual variability: $\theta_i = \theta$
152 $\exp(\eta_i)$ where θ is the population mean vector of the pharmacokinetic parameters and η_i
153 represents the random effect vector. Random effects were assumed to follow a normal
154 distribution with zero mean and variance matrix Ω which was supposed to be diagonal. Residual
155 variability was modelled using a proportional error model.

156 Goodness of fit plots (observed versus predicted population and individual concentrations,
157 weighted residuals versus predicted concentrations and versus time) were examined for each
158 model. Tested covariates were age, weight, body mass index, creatinine clearance at week 6,
159 alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) at baseline, plasma
160 alpha 1 glycoprotein acid and albumin concentrations at week 6, alpha 1 glycoprotein acid
161 phenotypes, coinfections (hepatitis B infection and/or hepatitis C infection), ritonavir trough and
162 average concentrations and combined NRTIs,. Continuous variables were centred on their
163 median. The sex of the patients was not tested as a covariate because there were only two females
164 in the population. The effects of covariates were tested on each individual parameter (correlation
165 test for continuous variables and ANOVA for categorical variables) using Statgraphics version
166 5.1 (Manugistics, Inc. Rockville, Maryland, USA). The covariates that were found to have a
167 significant effect ($p < 0.05$) were then evaluated in the population analysis. The effect of a
168 covariate was assessed by the likelihood ratio test. In the forward inclusion process, a covariate
169 was retained in the model if there was a decrease greater than 3.84 in the objective function ($p <$
170 0.05 , 1 degree of freedom) and if there was a decrease in the interindividual variability of the
171 associated pharmacokinetic parameter. From the best model including covariates, a backward
172 elimination procedure was then used to test whether all covariates selected should remain in the
173 final model. When deletion of a covariate ($p < 0.05$) significantly increased the log-likelihood ($>$
174 3.84), that covariate was kept in the model.

175 **Results**

176 *Atazanavir in vitro binding*

177 In a solution of 0.7 g/L of alpha 1 glycoprotein acid, atazanavir was found to bind to one high
178 affinity saturable site at about 31.1%. The association constant was $4.61 \cdot 10^5$ L/mol and the
179 number of sites 0.61. In a solution of 40 g/L of serum albumin, mean unbound fraction of
180 atazanavir was found to be 21.3% and was constant up to 15000 ng/mL of total atazanavir. This
181 suggested that atazanavir binds to one low-affinity non-saturable site. The number of sites and the
182 affinity constant were not differentiated and the product was estimated as 6352 L/mol. When
183 atazanavir total concentrations were above 15000 ng/mL, atazanavir unbound fraction increase to
184 30.9%, protein binding sites becoming saturated (figure 1).

185 *Population pharmacokinetic analysis of atazanavir in patients*

186 **Patients**

187 Fifty-one patients completed the study at week 6 and ten patients were included in the
188 pharmacokinetic substudy. The characteristics of these 51 patients are summarised in table 1. The
189 NRTIs combined with ritonavir-boosted atazanavir plus tenofovir were lamivudine (n=40),
190 abacavir (n=30), didanosine (n=18), zidovudine (n=15), zalcitabine (n=5) and stavudine (n=2).
191 Twenty-three patients were hepatitis virus B and/or C co-infected.

192

193 **Alpha 1 glycoprotein acid concentrations and phenotypes**

194 Alpha 1 glycoprotein acid concentrations were 1.0 g/L (0.6-1.5) at week 6 and were elevated in
195 15 patients (28.8%) accounting for their age and sex.

196 Five patients had the phenotype ORM1*S-ORM2*A, 19 ORM1*F1-ORM2*A and 28 were
197 heterozygous and presented ORM1*F1S-ORM2*A. The allele ORM1*F2 was not observed in
198 this rather small sample. The allele ORM2*A was monomorphic and observed in all sera of
199 patients. The ORM1 allele frequencies were as follows: 0.635 for ORM1*F1 and 0.365 for
200 OMR1*S. Good agreement was found between observed and expected values, assuming a Hardy-
201 Weinberg equilibrium ($p > 0.5$). Total plasma concentrations of alpha 1 glycoprotein acid were
202 not found to be significantly dependant on alpha 1 glycoprotein acid phenotypes.

203 **Atazanavir model**

204 *Basic model*

205 A one-compartment model with first-order absorption and elimination was used to describe
206 atazanavir pharmacokinetics. The population parameter estimates, their relative standard error of
207 estimation (RSE %) and their inter-individual variability for this basic model are shown in table
208 2.

209 *Covariates model building*

210 From this basic model, we tested the effects of the covariates on the individual estimates of the
211 random effects. Using the estimated individual parameters, significant effects of hepatitis B
212 and/or C infection on k_a ($p = 0.030$), of plasma alpha 1 glycoprotein acid concentration,
213 didanosine, ALAT level, ASAT level, ritonavir trough and average concentrations on Cl/F ($p =$
214 $0.001 ; 0.028 ; 0.037 ; 0.017 ; 0.009 ; 0.006$ respectively) and of plasma alpha 1 glycoprotein acid
215 concentration, ORM1*S alpha 1 glycoprotein acid phenotype on V/F ($p = 0.009; 0.008$
216 respectively) were found.

217 According to the likelihood ratio test, the final population model had plasma alpha 1 glycoprotein
218 acid concentration (AAG), ALAT level effects on Cl/F ($p < 0.01$; $p < 0.01$ respectively) and
219 ORM1*S alpha 1 glycoprotein acid phenotype (ORM1*S) effects on V/F ($p < 0.05$). The
220 equations are:

$$221 \quad Cl/F_i = (Cl/F - \beta^{ALAT}_{CL/F} (ALAT_i/29) - \beta^{AAG}_{CL/F} (AAG/1.0)) \times \exp(\eta_{Cl/F_i})$$

222 where 29 (IU/L) and 1.0 (g/L) are the median ALAT and AAG levels.

$$223 \quad V/F_i = (V/F \times \beta^{ORM1*S}_{V/F}) \times \exp(\eta_{V/F_i})$$

224 The population parameters of this final model and their relative standard errors of estimation are
225 given in table 2. The goodness of fit plots (not shown) were all very satisfactory for the basic and
226 final models.

227 The apparent volume of distribution was estimated to be 79.7 L and increased about 2-fold in
228 patients with the ORM1*S phenotype. Absorption rate constant was estimated to be 0.73 /h. The
229 apparent clearance was estimated to be 8.1 L/h when ALAT was 29 IU/L and alpha 1
230 glycoprotein acid level was 1.0 g/L. Median (range) half-life was 6.9 h (4.0-18.2). We found an
231 increase of 34% in mean atazanavir area under the curve (AUC, 47410 ng.h/mL versus 35432
232 ng.h/mL, $p=0.0004$) and of 12% in mean half-life (7.8 h versus 6.9 h, $p=0.03$) in patients who had
233 an elevated ALAT level (≥ 40 IU/L), compared with patients with a normal ALAT level. A 36%
234 increase in mean atazanavir AUC (49644 ng.h/mL versus 36529 ng.h/mL) and a 28% increase in
235 mean half-life (9 h versus 7 h) were observed in patients with elevated alpha 1 glycoprotein acid
236 (according to their age and sex), compared with those with a normal alpha 1 glycoprotein acid
237 level.

238 The inter-individual variabilities of absorption rate constant, apparent volume of distribution and
239 apparent clearance were 139.3%, 27.0% and 26.7%, respectively. They were slightly decreased
240 from the basic model by the incorporation of the covariates. Residual variability was 19.9%.
241 Figure 2 shows the model-predicted concentrations and the observed concentrations versus time
242 when ALAT and plasma alpha 1 glycoprotein acid concentrations were 29 IU/L and 1.0 g/L,
243 respectively, in patients with or without the alpha 1 glycoprotein acid ORM1*S phenotype.

244 **Discussion**

245 Atazanavir is a potent and safe HIV PI with a pharmacokinetic profile that allows once daily oral
246 administration and which can be optimised by adding low-dose ritonavir. All patients included in
247 this study received ritonavir boosted atazanavir and a relationship was found between atazanavir
248 clearance and ritonavir concentrations. However variation in ritonavir concentrations was not
249 found to impact atazanavir pharmacokinetics to a significant extent and therefore was not retained
250 in the final model. It is known that protein binding affects PI disposition (3) and could reduce the
251 antiviral effect of these drugs (13). It was reported that atazanavir binds to both alpha 1
252 glycoprotein acid and albumin to a similar extent, but binding parameters were unknown (12).
253 Our *in vitro* data demonstrate that atazanavir has a higher affinity to alpha 1 glycoprotein acid
254 than to albumin. However, alpha 1 glycoprotein acid is present at about 1/40 the concentration of
255 albumin in plasma. Consequently, the binding of atazanavir to alpha 1 glycoprotein acid was
256 saturable above 6000 ng/mL whereas the saturation of albumin appears above 15000 ng/mL of
257 atazanavir. A similar result was already found for saquinavir (15). As for many basic drugs, alpha
258 1 glycoprotein acid has a high affinity but albumin has a higher capacity for binding (16). In
259 patients treated by atazanavir/ritonavir at the recommended daily dose of 300/100 mg, trough
260 plasma atazanavir concentrations are around 1000 ng/mL. Consequently, alpha 1 glycoprotein

261 acid is the major protein involved in atazanavir binding. These findings are essential because
262 alpha 1 glycoprotein acid concentration can vary considerably as a result of disturbances of
263 homeostasis. It increases during acute or chronic inflammation and infectious disease. HIV-
264 infected patients are therefore likely to exhibit increased concentrations of alpha 1 glycoprotein
265 acid (3, 16, 21). Moreover the alpha 1 glycoprotein acid polymorphism could be responsible for
266 interindividual variation in the plasma binding of protease inhibitors, which might influence their
267 disposition.

268 The impact of proteins that bind atazanavir on atazanavir pharmacokinetics was evaluated in 51
269 HIV infected patients having failed several lines of previous antiretroviral treatment and included
270 in the ANRS 107-Puzzle 2 trial. Unfortunately, atazanavir unbound fraction could not be
271 measured in these patients as remaining samples volumes was too low

272 This is the first population model analysis to explore the possible influence of alpha 1
273 glycoprotein acid concentrations, alpha 1 glycoprotein acid phenotypes, albumin concentrations,
274 ALAT and ASAT levels and/or hepatitis B and C coinfections on atazanavir pharmacokinetic
275 parameters.

276 In this very advanced population, alpha 1 glycoprotein acid concentrations remained elevated in
277 29% of the patients even though they had received a new treatment for 6 weeks. This could be
278 explained by the weak virological response to the new treatment and the lack of effect of the
279 antiretroviral treatment on the inflammation due to HIV infection (22). The relative frequencies
280 of alpha 1 glycoprotein acid phenotypes found here are close to those previously described in
281 healthy subjects or in HIV-infected patients (5, 8). We did not find ORM1*F2 on this population
282 but this variant is present only at a low allelic frequency (9, 19). As already described in healthy
283 subjects, alpha 1 glycoprotein acid plasma concentrations were not found to differ between the 3
284 phenotypes (18).

285 To determine atazanavir pharmacokinetic parameters and the influence of protein binding, we
286 used a population approach because only ten patients had a complete pharmacokinetic profile.
287 The pharmacokinetics of atazanavir was described by a one-compartment model with first-order
288 absorption and first-order elimination with random effects on k_a , V/F and Cl/F . This structural
289 model was similar to that of Dailly et al. and Colombo et al., although Colombo et al. described a
290 first-order absorption with a lag time (4, 6). Our estimation of atazanavir pharmacokinetic
291 parameters, apparent volume of distribution, absorption rate constant, apparent clearance and
292 half-life are in the same range as those of previous studies (4, 6, 17, 23). The atazanavir apparent
293 clearance of 9.8 L/h demonstrates that atazanavir has low extraction ratio with clearance
294 depending on the unbound fraction and intrinsic clearance. The atazanavir apparent volume of
295 distribution of 78 L (1.2 L/kg) indicates that plasma protein binding is not a restricting factor to
296 body distribution. Assuming that pharmacologic effect is related to exposure to unbound drug
297 concentrations (AUC_u), which after oral administration, depends on the fraction absorbed
298 through the gut wall, the dose and the intrinsic clearance as demonstrated by Benet and Hoener
299 (2), any change in total drug concentrations should not have clinical consequences. Large inter-
300 individual variability was found for k_a (139.3%) as variable absorption of PIs has already been
301 described.

302 We found that the apparent clearance of atazanavir decreases with increasing plasma alpha 1
303 glycoprotein acid concentration. A similar effect was previously reported for lopinavir and
304 indinavir clearance (5). This result suggests that elevated alpha 1 glycoprotein acid
305 concentrations could decrease the protein unbound fraction of atazanavir, leading to increased
306 total atazanavir concentrations, but without change in unbound atazanavir concentrations linked
307 to drug efficacy (2).

308 Interestingly, we found that atazanavir apparent volume of distribution decreases with increased
309 with alpha 1 glycoprotein acid concentrations and increases with the ORM1*S phenotype. This
310 suggests that atazanavir may preferentially bind to the ORM1*F1 variant, compared with the
311 ORM1*S variant. Unfortunately, this hypothesis could not be tested *in vitro* as human plasma
312 samples tested for alpha 1 glycoprotein acid variants were not available. In contrast, it was
313 suggested that lopinavir and indinavir may preferentially bind to the ORM1*S variant (5). There
314 are conflicting data on preferential binding of neutral and basic drugs such as quinidine to
315 ORM1*S and ORM1*F1 (18, 20). *In vitro* drug-binding studies are warranted to determine the
316 possible different capacities of the ORM1 variants.

317 Overall, increases in alpha 1 glycoprotein acid lead to a modest 28% increase in atazanavir half
318 life which should not have any clinical consequence, but could contribute to the interindividual
319 variability of atazanavir pharmacokinetics although to a lesser extent than absorption or CYP3A
320 activity.

321 We found that the apparent clearance of atazanavir decreases with increasing ALAT. This was
322 expected as atazanavir, like other PIs, is extensively metabolised by hepatic CYP3A isoenzymes,
323 which are decreased in patients with liver failure and directly affect the intrinsic clearance.
324 However, such a relationship is remarkable as liver dysfunction was mild to moderate in our
325 patients. A 42% increase in AUC and longer terminal half-life (12 versus 6 hours) have been
326 reported in volunteers with hepatic impairment (Child-Pugh grade B or C), compared with
327 healthy volunteers (12). In the present study, we found a similar increase in atazanavir AUC and
328 half-life in patients who had an elevated ALAT level. Our data, although limited, could suggest
329 that high atazanavir concentrations are expected with severe liver dysfunction. As previously
330 described, age, body weight and body mass index did not influence atazanavir pharmacokinetics
331 (4). Most of our patients were males, so no influence of sex on atazanavir pharmacokinetics could

332 be detected. In keeping with our *in vitro* data, atazanavir pharmacokinetics was not influenced by
333 albumin concentrations; however in these patients, there was a small variability in albumin
334 concentrations.

335

336 In conclusion, this study demonstrates that atazanavir pharmacokinetics is modestly influenced
337 by its protein binding, especially to alpha 1 glycoprotein acid. The effect of alpha 1 glycoprotein
338 acid concentrations or polymorphisms or liver enzyme elevations on the unbound moiety able to
339 cross biologic membranes and exert a pharmacologic effect is unknown as is therefore, the
340 potential clinical significance, and that this is a limitation of this work.

341

342 Acknowledgements

343 This study was supported by ANRS (National Agency for AIDS Research), Paris, France.

344 We thank Gilead sciences and Bristol-Myers Squibb for helpful discussions and support for
345 carrying out this trial.

346 We thank the following investigators who participated in the ANRS trial 107-Puzzle 2 trial:

347 Hôpital Saint Louis, Paris (E Oksenhendler, O Carel, JM Molina, M Tourneur, P Palmer, I

348 Madelaine-Chambrin, N Mezreb); Hôpital de Bicêtre, Kremlin Bicêtre (JF Delfraissy, C Goujard,

349 M Mole, Y Quertainmont, N Idri, C Le Tiec); Hôpital Européen Georges Pompidou, Paris (M

350 Kazatchkine, A Aouba, N Bengrait, A Si-Mohamed, B Sabatier); Hôpital Saint-Antoine, Paris (D

351 Bollens, F Besse, B Gaujour, L Morand-Joubert, A Charrois, A Daguanel-Nguyen); Hôpital

352 Sainte-Marguerite, Marseille (JA Gastaut, I Poizot-Martin, V Marin-Frixon, C Tamalet, P

353 Colson, C Penot-Ragon); Hôpital de l'Archet, Nice (JP Cassuto, M Poirée, C Ceppi, S

354 Benhamou, MC Rigault); Hôpital Purpan, Toulouse (P Massip, L Cuzin, M Obadia, J Izopet, A

355 Jaafar, I Peyranne).

356

357

References

- 358 1. **Barrail, A., C. Le Tiec, S. Paci-Bonaventure, V. Furlan, I. Vincent, and A. M.**
359 **Taburet.** 2006. Determination of amprenavir total and unbound concentrations in plasma
360 by high-performance liquid chromatography and ultrafiltration. *Ther Drug Monit* **28**:89-
361 94.
- 362 2. **Benet, L. Z., and B. A. Hoener.** 2002. Changes in plasma protein binding have little
363 clinical relevance. *Clin Pharmacol Ther* **71**:115-21.
- 364 3. **Boffito, M., D. J. Back, T. F. Blaschke, M. Rowland, R. J. Bertz, J. G. Gerber, and V.**
365 **Miller.** 2003. Protein binding in antiretroviral therapies. *AIDS Res Hum Retroviruses*
366 **19**:825-35.
- 367 4. **Colombo, S., T. Buclin, M. Cavassini, L. A. Decosterd, A. Telenti, J. Biollaz, and C.**
368 **Csajka.** 2006. Population pharmacokinetics of atazanavir in patients with human
369 immunodeficiency virus infection. *Antimicrob Agents Chemother* **50**:3801-8.
- 370 5. **Colombo, S., T. Buclin, L. A. Decosterd, A. Telenti, H. Furrer, B. L. Lee, J. Biollaz,**
371 **and C. B. Eap.** 2006. Orosomucoid (alpha1-acid glycoprotein) plasma concentration and
372 genetic variants: effects on human immunodeficiency virus protease inhibitor clearance
373 and cellular accumulation. *Clin Pharmacol Ther* **80**:307-18.
- 374 6. **Dailly, E., O. Tribut, P. Tattevin, C. Arvieux, P. Perre, F. Raffi, and P. Jolliet.** 2006.
375 Influence of tenofovir, nevirapine and efavirenz on ritonavir-boosted atazanavir
376 pharmacokinetics in HIV-infected patients. *Eur J Clin Pharmacol* **62**:523-6.
- 377 7. **Department of Health and Human Services (DHHS), and**
378 <http://aidsinfo.nih.gov/guidelines/>, posting date. Guidelines for the use of antiretroviral
379 agents in HIV-1 infected adults and adolescents. [Online.]

- 380 8. **Duche, J. C., F. Herve, and J. P. Tillement.** 1998. Study of the expression of the genetic
381 variants of human alpha1-acid glycoprotein in healthy subjects using isoelectric focusing
382 and immunoblotting. *J Chromatogr B Biomed Sci Appl* **715**:103-9.
- 383 9. **Dulmer, M., G. Reker, T. T. Nguyen, L. Henke, and J. Henke.** 1998. Human
384 orosomuroid (ORM1) subtyping: further population genetic data and reports on the
385 feasibility to type aged blood samples and stains. *J Forensic Sci* **43**:413-6.
- 386 10. **Eap, C. B., and P. Baumann.** 1988. Isoelectric focusing of alpha-1 acid glycoprotein
387 (orosomuroid) in immobilized pH-gradients with 8M urea: detection of its desialylated
388 variants using an alkaline phosphatase-linked secondary antibody system. *Electrophoresis*
389 **9**:650-4.
- 390 11. **European Medicines Agency, and**
391 <http://www.emea.eu.int/humandocs/Humans/EPAR/reyataz/reyataz.htm>, posting
392 date. [Online.]
- 393 12. **FDA, and** <http://www.fda.gov/cder/foi/label/2006/021567s0071bl.pdf>, posting date.
394 CDER for Reyataz. [Online.]
- 395 13. **Ford, J., S. H. Khoo, and D. J. Back.** 2004. The intracellular pharmacology of
396 antiretroviral protease inhibitors. *J Antimicrob Chemother* **54**:982-90.
- 397 14. **Fournier, T., N. N. Medjoubi, and D. Porquet.** 2000. Alpha-1-acid glycoprotein.
398 *Biochim Biophys Acta* **1482**:157-71.
- 399 15. **Holladay, J. W., M. J. Dewey, B. B. Michniak, H. Wiltshire, D. L. Halberg, P. Weigl,**
400 **Z. Liang, K. Halifax, W. E. Lindup, and D. J. Back.** 2001. Elevated alpha-1-acid
401 glycoprotein reduces the volume of distribution and systemic clearance of saquinavir.
402 *Drug Metab Dispos* **29**:299-303.

- 403 16. **Kremer, J. M., J. Wilting, and L. H. Janssen.** 1988. Drug binding to human alpha-1-
404 acid glycoprotein in health and disease. *Pharmacol Rev* **40**:1-47.
- 405 17. **Le Tiec, C., A. Barrail, C. Goujard, and A. M. Taburet.** 2005. Clinical
406 pharmacokinetics and summary of efficacy and tolerability of atazanavir. *Clin*
407 *Pharmacokinet* **44**:1035-50.
- 408 18. **Li, J. H., J. Q. Xu, X. M. Cao, L. Ni, Y. Li, Y. Y. Zhuang, and J. B. Gong.** 2002.
409 Influence of the ORM1 phenotypes on serum unbound concentration and protein binding
410 of quinidine. *Clin Chim Acta* **317**:85-92.
- 411 19. **Li, J. H., J. Q. Xu, Y. Li, Y. Y. Zhuang, and J. B. Gong.** 1999. Genetic polymorphisms
412 of orosomucoid on the Han population in Nanjing of China. *Clin Chim Acta* **288**:161-8.
- 413 20. **McCollam, P. L., M. A. Crouch, and P. Arnaud.** 1998. Caucasian versus African-
414 American differences in orosomucoid: potential implications for therapy.
415 *Pharmacotherapy* **18**:620-6.
- 416 21. **Oie, S., M. A. Jacobson, and D. I. Abrams.** 1993. Alpha 1-acid glycoprotein levels in
417 AIDS patients before and after short-term treatment with zidovudine (ZDV). *J Acquir*
418 *Immune Defic Syndr* **6**:531-3.
- 419 22. **Piketty, C., L. Gerard, C. Chazallon, A. G. Marcelin, F. Clavel, A. M. Taburet, V.**
420 **Calvez, I. Madelaine-Chambrin, J. M. Molina, J. P. Aboulker, and P. M. Girard.**
421 2006. Salvage therapy with atazanavir/ritonavir combined to tenofovir in HIV-infected
422 patients with multiple treatment failures: randomized ANRS 107 trial. *Antivir Ther*
423 **11**:213-21.
- 424 23. **Taburet, A. M., C. Piketty, C. Chazallon, I. Vincent, L. Gerard, V. Calvez, F. Clavel,**
425 **J. P. Aboulker, and P. M. Girard.** 2004. Interactions between atazanavir-ritonavir and

426 tenofovir in heavily pretreated human immunodeficiency virus-infected patients.
427 Antimicrob Agents Chemother **48**:2091-6.
428 24. **Wright, J. D., F. D. Boudinot, and M. R. Ujhelyi.** 1996. Measurement and analysis of
429 unbound drug concentrations. Clin Pharmacokinet **30**:445-62.

430

431 **Figures legends**

432 Figure 1: Bound atazanavir concentrations versus unbound atazanavir concentrations in solutions
433 of orosomucoid (solid circles) and albumin (open circles). The curve is an ordinary least-square
434 fit of the Emax model to the data using WinNonLin.

435
436 Figure 2: Observed atazanavir concentrations (solid circles in patients with the ORM1*S
437 phenotype and open circles in patients without the ORM1*S phenotype) and predicted population
438 concentrations for median ALAT (29 IU/L) and median orosomucoid (1.0 g/L) in patients with
439 (continuous line) or without (dashed line) the ORM1*S phenotype versus time, at week 6.

440 **Tables**

441

442 Table 1: Characteristics of the 51 patients included in the population pharmacokinetic analysis

443

	Median	min-max
Age (years)	41	29-62
Week 6 weight (kg)	65.6	45.0-105.6
Week 6 body mass index (kg/m ²)	21.3	17.0-33.6
Baseline ALAT level (IU/L)	29	5-114
Baseline ASAT level (IU/L)	29	16-149
Week 6 ALAT (IU/L)	38	7-175
Week 6 creatinine clearance (mL/min)	87	23-178
Week 6 albumin (g/L)	40.4	31.2-47.7
Week 6 orosomuroid (g/L)	1.0	0.6-1.5

444 *ALAT: alanine aminotransferase*

445 *ASAT: aspartate aminotransferase*

446

447 Table 2: Population pharmacokinetic parameters of atazanavir (estimates and relative standard
 448 errors of estimation) for the basic model and for the final model
 449

Parameters	Basic model		Final model	
	Estimate	RSE (%)	Estimate	RSE (%)
ka (h ⁻¹)	0.75	7.6	0.73	10.7
Cl/F (L/h)	7.8	9.0	13.3	13.2
$\beta^{ALAT}_{Cl/F}$	-	-	0.86	35.0
$\beta^{AAG}_{Cl/F}$	-	-	4.3	46.9
V/F (L)	78.2	10.5	79.7	10.5
$\beta^{ORM1*S}_{V/F}$	-	-	1.9	32.8
ω_{ka} (%)	138.7	21.0 ^a	139.3	26.2 ^a
$\omega_{Cl/F}$ (%)	43.4	54.4 ^a	26.7	27.8 ^a
$\omega_{V/F}$ (%)	31.1	64.8 ^a	27.0	102.2 ^a
σ (%)	18.3	37.2 ^a	19.9	50.3 ^a

450 ^a RSE for ω^2_{ka} , $\omega^2_{V/F}$, $\omega^2_{Cl/F}$ and σ^2

451 *ka*: absorption rate constant, *Cl/F*: apparent clearance, *V/F*: apparent volume of distribution

452 $\beta^{ALAT}_{Cl/F}$: facteur associated with ALAT concentrations on atazanavir *Cl/F*

453 $\beta^{AAG}_{Cl/F}$: facteur associated with AAG concentrations on atazanavir *Cl/F*

454 $\beta^{ORM1*S}_{V/F}$: facteur associated with *ORM1*S* phenotype on atazanavir *V/F*

455 ω_{ka} , $\omega_{Cl/F}$, $\omega_{V/F}$: inter individual variabilities for *ka*, *Cl/F* and *V/F* respectively

456 σ : residual error

457

458 Figure 1

459

460

461

462

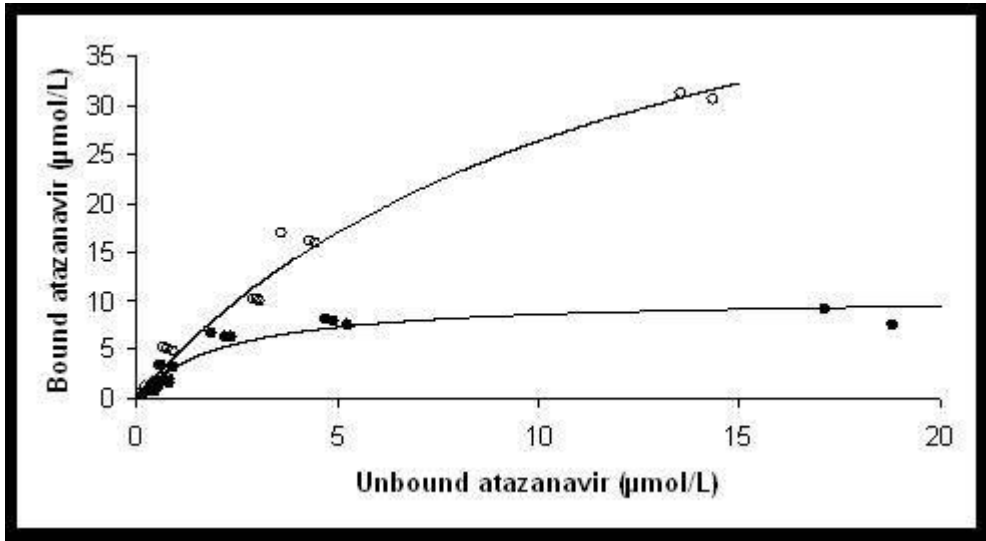
463

464

465

466

467



468 Figure 2

469

