European Medicines Agency

Product name: **REYATAZ**
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SCIENTIFIC DISCUSSION

Detailed Description of Reyataz antiviral activity and resistance data
in vitro and *DE Novo* resistance

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Antiviral activity *in vitro*

The susceptibility of atazanavir was examined against single clinical isolates from subtypes A to G of the HIV-1 M group, as well as one primary strain from group O. The anti HIV activities of atazanavir were performed using PBMC cells infected with these clinical isolates. Atazanavir exhibited potent inhibitory activity against all 8 primary isolates tested, with EC_{50} values ranging from 0.4 to 6.9 nM. The anti-HIV activities of atazanavir were also measured against 4 isolates of HIV-2, with EC_{50} values ranging from 1.9 to 3.2 nM.

A further analysis of clinical isolates was performed using recombinant viruses generated from virus from individuals involved in atazanavir clinical studies AI424007, AI424008 and AI424034. Subtype B was the most prevalent subtype, representing 174 (74%) of the isolates evaluated. The range of atazanavir susceptibilities for subtype B isolates was 0.3 to 6.5. The non-B subtypes had susceptibilities ranging from 0.4 to 1.8, well within the range of protease susceptibilities observed for subtype B isolates (Table 1). These results indicate that atazanavir susceptibility is retained not only among B subtype viruses, but also viruses of the other subtypes.

Table 1: Activity against wild-type virus*

Parameters		**HIV-1 (group M)**	**HIV-1 (group O)**	**HIV-2**
EC_{50} range (nM)		0.4 - 6.5	6.9	1.9 - 32
Serum adjusted (ng/ml)	**EC_{90}**	14 (Estimated)		

Types of isolates (group M)	**N (clin/lab)**	**EC_{50} FC vs WT (range)**
Subtype B	174	0.3 - 6.5
Non-B	52	0.5 - 1.8
CRF	15	0.4 - 1.2

* Cell lines: MT-2; CEM-SS; Macrophages; PBMCs; 293 cells; FC = fold change; WT = wild type

***In vitro* resistance**

In vitro selection of resistance from WT HIV-1 virus

Three HIV-1 strains (RF, LAI, NL4-3) were passaged in MT-2 cells in the presence of increasing concentrations of atazanavir. Breakthrough virus was first observed by viral-induced cytopathic effect that was subsequently confirmed by drug susceptibility analysis. Table 2.1 provides more detailed information concerning the *in vitro* selection of resistance from WT HIV-1 virus.

Table 2.1: Mutations selected in vitro in cell lines1 in the presence of atazanavir

Virus Strain: RF	**Mutations at codons**			
	N88S	**M46I, N88S**	**V32I, M46I, A71, N88S**	**V32I, L33F,M46I, A71, I84V, N88S**
Selection Time (months)	1	2.4	3.5	4.8
Drug Concentration at Selection Time (nM)	25	100	225	500
IC_{50} Fold Change[2] vs BL	4	6	12	183

Virus Strain: LAI	Mutations at codons	
	L10Y/F, I50L, A71V, N88S	L10Y/F, I50L, L63P, A71V, N88S
Selection Time (months)	2.6	4.7
Drug Concentration at Selection Time (nM)	28	500
IC_{50} Fold Change[2] vs BL	36	93

Virus Strain: NL4-3	Mutations at codons	
	V32I, M46I, I84V	V32I, M46I, I84V, L89M
Selection Time (months)	3.9	4.6
Drug Concentration at Selection Time (nM)	40	200
IC_{50} Fold Change[2] vs BL	6	96

[1] MT-2 cells; [2] Using a reverse transcriptase assay; BL = baseline

Following 1 month of passage in drug concentrations up to 25 nM, the RF strain of HIV-1 showed a 4-fold decrease in susceptibility to atazanavir (Table 2.1). This virus contained a single N88S substitution in the protease gene. Additional substitutions accumulated with time and increased atazanavir concentrations as shown. LAI and NL4-3 viruses with reduced susceptibility to atazanavir took longer to break through, but viral variants did appear with prolonged drug selection. The LAI viruses that emerged at 2.6 and 4.7 months displayed 36- and 93-fold decreases in susceptibility to atazanavir, respectively. The NL4-3 viruses selected at 3.9 and 6 months post initiation of treatment displayed 4.6- and 96-fold reductions in susceptibility to atazanavir, respectively (Table 2.1).

In vitro cross resistance of clinical isolates resistant to other drugs

A panel of 950 HIV-1 recombinant clinical isolates were profiled for their susceptibility to amprenavir, nelfinavir, ritonavir, saquinavir, lopinavir and atazanavir. The *in vitro* cross-resistance to clinical isolates resistant to other compounds of same drug class is summarised in Table 2.2.

Table 2.2: Cross-resistance to atazanavir of clinical isolates resistant to other PIs1

Resistance profile	APV	NFV	RTV	SQV
No. of isolates tested	2	121	27	7
No. of isolates susceptible	2	103	26	7
No. of isolates resistant	0	18	1	0
Fold Change of resistant isolates vs WT[1]	NA	3-14	3.3	NA

Resistance profile	APV/ LPV	HFV/ SQV	IDV/ NFV	APV/ RTV	IDV/ RTV	NFV/ RTV	LPV/ RTV	RTV/ SQV
No. of isolates tested	1	5	3	5	3	30	2	8
No. of isolates susceptible	1	2	1	5	2	25	2	8
No. of isolates resistant	0	3	2	0	1	5	0	0
Fold Change of resistant isolates vs WT[1]	NA	4-7	5-6	NA	4.0	3-11	NA	NA

Resistance profile	NFV/ RTV/ SQV	NFV/ RTV/ LPV	APV/ NFV/ RTV	APV/ LPV/ RTV	IDV/ NFV/ SQV	IDV/ NFV/ RTV	APV/ RTV/ SQV	LPV/ NFV/ SQV
No. of isolates tested	28	32	2	5	1	21	9	1
No. of isolates susceptible	7	13	1	5	0	6	1	1
No. of isolates resistant	21	19	1	0	1	15	8	0
Fold Change of resistant isolates vs WT[1]	3-36	3-9	4.3	NA	5.4	4-12	3-12	NA

Resistance profile	NFV/ LPV/ RTV/ SQV	APV/ SQV/ RTV/ LPV	APV/ IDV/ NFV/ RTV	IDV/ NFV/ RTV/ SQV	APV/ NFV/ LPV/ RTV	LPV/ NFV/ RTV/ SQV
No. of isolates tested	25	1	18	10	17	25
No. of isolates susceptible	5	0	6	0	4	0
No. of isolates resistant	20	1	12	10	13	25
Fold Change of resistant isolates vs WT[1]	3-499	11	3-12	3-82	3-18	5-41

Resistance profile	APV/ NFV/ LFV/ RTV/ SQV	APV/ IDV/ NFV/ RTV/ SQV
No. of isolates tested	111	31
No. of isolates susceptible	5	2
No. of isolates resistant	106	29
Fold Change of resistant isolates vs WT[1]	3-362	4-41

[1] Using either ViroLogic (now Monogram BioSciences) PhenoSense or Virco's Antivirogram assays
NA = Not applicable; WT = wildtype

DE NOVO MUTATIONS IN PATIENTS FAILING ATAZANAVIR/RITONAVIR

De novo mutations in treatment experienced patients failing atazanavir/ritonavir

De novo mutations in treatment-experienced patients failing therapy are presented in Table 3.1 by frequency (i.e. > 20 % or 10% to 20%) followed by a short description of correlation observed between specific mutations and change in phenotypic sensitivity.

Table 3.1: *De novo* substitutions in treatment experienced patients failing therapy with atazanavir + ritonavir (Study AI424045, 48 weeks)

Frequency	*de novo* PI substitutions (n = 35)[a, b]
> 20%	M36, M46, I54, A71, V82
10% - 20%	L10, I15, K20, V32, E35, S37, F53, I62, G73, I84, L90

[a] Number of patients with paired genotypes classified as virological failures (HIV RNA ≥ 400 copies/ml).
[b] Ten patients had baseline phenotypic resistance to atazanavir + ritonavir (fold change [FC] > 5.2). FC susceptibility in cell culture relative to the wild-type reference was assayed using PhenoSense (Monogram Biosciences, South San Francisco, California, USA).

Viral resistance profiles were consistent with prior resistance assessments of antiretroviral-experienced atazanavir-treated patients who subsequently had virologic failure. Baseline phenotypic resistance to atazanavir, ritonavir and lopinavir were low in both treatment regimens. Few on-study substitutions occurred in viral strains from these subjects with virologic failure. *De novo* mutations emerged at a frequency of > 20% in a minority of virologic failure subjects. Five substitutions emerged de novo in > 20% of subjects and none of these substitutions are specific to atazanavir. A greater number of de novo substitutions were present in 10% to 20% of subjects. Similar to the more frequently observed substitutions, none are specific to atazanavir. These new substitutions may reflect re-emergence of archived resistance on atazanavir + ritonavir in Study AI424045 treatment-experienced population.

De novo mutations in treatment naïve patients failing atazanavir/ritonavir

In clinical trials of antiretroviral treatment naïve patients treated with unboosted atazanavir, the I50L substitution, sometimes in combination with an A71V change, is the signature resistance substitution for atazanavir. Resistance levels to atazanavir ranged from 3.5- to 29-fold without evidence of phenotypic cross resistance to other PIs. In clinical trials of antiretroviral treatment naive patients treated with boosted atazanavir, the I50L substitution did not emerge in any patient without baseline PI substitutions. The N88S substitution has been rarely observed in patients with virologic failure on atazanavir (with or without ritonavir). While it may contribute to decreased susceptibility to atazanavir when it occurs with other protease substitutions, in clinical studies N88S by itself does not always lead to phenotypic resistance to atazanavir or have a consistent impact on clinical efficacy.

Table 3.2. *De novo* substitutions in treatment naive patients failing therapy with atazanavir + ritonavir (Study 138, 96 weeks)

Frequency	de novo PI substitution (n=26)[a]
>20%	none
10-20%	none

[a] Number of patients with paired genotypes classified as virological failures (HIV RNA ≥400 copies/ml.

The M184I/V substitution emerged in 5/26 atazanavir/ritonavir and 7/26 lopinavir/ritonavir virologic failure patients, respectively.