

Genotypic and Phenotypic Analysis of Breacanavir (GW640385): An Assessment of the Effects of Specified Resistance-associated Mutations Alone or in Combination With Others in a Survey of 50 Viruses from Protease Inhibitor (PI)-Experienced Patients

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Introduction

Since the introduction of highly active anti-retroviral therapy (HAART) improved control of plasma HIV-1 RNA levels has been associated with reduced levels of mortality in HIV-1 infected patients [Palella et al, 1998]. A common component of HAART regimens is a protease inhibitor (PI). One of the problems with currently available PIs is the emergence of resistance due to the high mutation rate which arises because of the low fidelity of HIV reverse transcriptase (RT). PI resistance usually develops from the accumulation of multiple mutations. These mutations include primary mutations that are associated with reduced susceptibility to the PI in addition to secondary or compensatory mutations that may further reduce drug susceptibility and/or enhance viral replicability.

Previous studies have shown that there is a high incidence of resistant viruses in patients who have received multiple treatment regimens. There is therefore an unmet medical need for an effective PI against highly resistant viruses that have accumulated multiple mutations that confer cross-resistance to the currently available PIs. As a result, a new generation of compounds have been structurally designed with high potency against the HIV-1 aspartyl proteinase ('protease', PRO), the most potent of which is breacanavir* (BCV, GW640385) [Hazen et al, 2003].

In order to assess whether this potency may be observed in treatment-experienced subjects, viruses displaying PI cross-resistance were selected and had a median of 6 PI resistance-associated mutations (PI R-AMs) (range 1-9). Resistance-associated mutations in PRO coding region were available from Monogram, Inc., South San Francisco, CA. Phenotypic susceptibility to aprenavir (APV), lopinavir (LPV), indinavir (IDV), atazanavir (ATV) and breacanavir (BCV) were determined. The 50% inhibitory concentration (IC₅₀) and fold change (FC) based on fold increase of IC₅₀ observed with these resistant viruses compared to a standard wild-type laboratory recombinant virus were determined for these marketed PIs and BCV using the PhenoSense™ assay.

*USAN approved only

Methods

- A total of 50 viruses were studied. 43 Viruses were selected based on the presence of protease (PRO) mutations (median of 6, major: 2; minor: 4) at residues 32 (n=5), 33 (n=7), 46 (n=27), 47 (n=2), 50 (V:n=2; L:n=5), 54 (n=25), 82 (n=21), 84 (n=10), and 90 (n=17) (Table 1). Isolates with D30N were excluded since BCV retains susceptibility to this mutation. Viruses were selected so that single, double, triple and multiple mutations of different combinations were included. A further 7 viruses were made available for analysis by Monogram, Inc., South San Francisco, CA., 5 contained the I50L mutation and there were two with the I50V mutation.
- Sequence analysis was performed at Monogram, Inc., South San Francisco, CA., by a thermocycling method using fluorescent dye labelled dideoxynucleotide chain terminator chemistry. Resistance-associated mutations were classified based on the IAS USA resistance table [Johnson et al, 2005].
- All assays were performed by scientists at Monogram, Inc., South San Francisco, CA [Petropoulos, 2000]. The mean percent inhibition for each drug concentration was determined and used to calculate the IC₅₀. The FC in drug susceptibility was determined by comparing the IC₅₀ for the subject virus to the IC₅₀ for the drug-sensitive reference virus containing the PRO and RT sequences of the NL4-3 strain of HIV-1.

Results

- Fifty recombinant viruses from treatment-experienced patients were assayed with BCV, APV, ATV, IDV and LPV. These viruses had a median of 6 PI R-AMs indicative of a moderately to highly resistant group of viruses. The most prevalent primary PI mutations included L33F (7/50), M46I/L (27/50), V82A/T/F/S (21/50), I84V (10/50) and L90M (17/50). Two of the available viruses had I50V and five I50L. Secondary mutations observed included V32I (5/50), I47V (2/50), I54V/L/M (25/50) (Table 1 and Table 2).

Table 1. Primary PI mutations identified in the 50 viruses

Mutation	Count N=50	Percentage with mutation
L33F	7	14
M46I/L	27	54
I50V	2	4
I50L	5	10
82A/T/F/S	21	42
I84V	10	20
L90M	17	34

Table 2. Secondary PI mutations identified in the 50 viruses

Mutation	Count N=50	Percentage with mutation
L10I/F/V	27	54
K20R/M	12	24
L24I	5	10
V32I	5	10
M36I/L	21	42
I47V	2	4
F53L	5	10
I54V/L/M	25	50
L63P	35	70
A71V/T/I	22	44
G73S/A	3	6
V77I	13	26
N88D/S	5	10

Table 3. Range of FC based on IC₅₀ relative to wildtype

FC category	BCV	ATV	APV	IDV	LPV/r
R <2.5	39 (78%)	17 (34%)	22 (44%)	22 (44%)	21 (42%)
2.5 ≤ R ≤ 5	8 (16%)	8 (16%)	9 (18%)	7 (14%)	7 (14%)
5 ≤ R ≤ 10	3 (6%)	5 (10%)	9 (18%)	8 (16%)	6 (12%)
R >10	0	20 (40%)	10 (20%)	13 (26%)	16 (32%)

Table 4. IC₅₀ summary statistics (nM)

	MEAN	STD	MIN	MAX	MEDIAN	Q1	Q3
BCV	0.4	0.4	0.1	2.1	0.3	0.2	0.4
ATV	21.0	29.6	0.8	176.5	9.1	3.2	24.8
APV	73.9	90.5	1.2	410.1	43.3	11.1	109.1
IDV	78.1	108.3	3.2	571.1	35.9	13.0	101.4
LPV/r	39.7	57.1	0.7	249.3	13.7	3.7	55.4

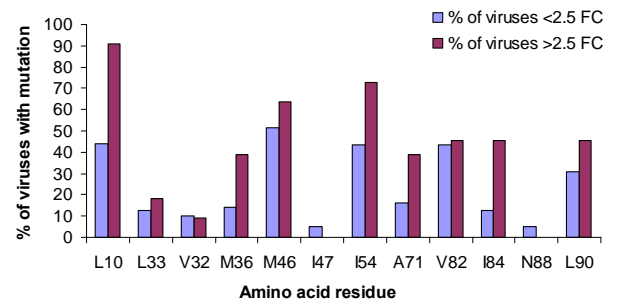
- BCV was the most potent of the PIs analysed [median IC₅₀ across all mutant viruses: 0.3 nM] (Table 4).

Table 5. FC based on IC₅₀ relative to wildtype: summary statistics

- The FCs for BCV, APV, LPV, IDV and ATV were 1.8, 6.3, 13, 8.0 and 11 respectively. The range for BCV was 0.28-8.9. The greatest FC observed was 95-fold for ATV (Table 5).

	MEAN	STD	MIN	MAX	MEDIAN	Q1	Q3
BCV	1.76	1.75	0.28	8.89	1.19	0.74	1.85
ATV	11.28	15.94	0.42	95.00	4.87	1.73	13.00
APV	6.30	7.71	0.10	35.00	3.71	0.96	9.35
IDV	8.03	11.14	0.33	59.00	3.70	1.34	10.56
LPV/r	12.56	18.04	0.21	79.00	4.30	1.16	17.25

Figure 1. BCV - Frequency of mutations in virus with <2.5 fold (N=39) or >2.5 fold (N=11) resistance



- There was a higher prevalence of the major PI mutation I84V (5/11 vs 5/39) in viruses with >2.5-FC to BCV compared to those with <2.5-FC. At >2.5-FC, I84V was found with I54V/L/M and I10I/F but these were also present in some viruses at <2.5-FC (Figure 1).
- There were more secondary PI mutations L10I/F/V/R (10/11 vs 17/39), M36I (6/11 vs 16/39), I54L/M/V (8/11 vs 17/39) and A71V/T (6/11 vs 16/39) in viruses with >2.5-FC to BCV than those with <2.5-FC (Figure 1).

Table 6. Mean FC according to presence of primary mutation

Mutation ¹	Mean FC				
	BCV	ATV	APV	IDV	LPV/r
33F (n=6)	1.36	12.50	7.80	3.17	5.76
46I (n=15)	2.22	15.82	8.60	16.37	23.71
46L (N=7)	2.13	13.20	7.55	13.09	25.61
I50L (N=5)	0.45	15.85	1.06	0.71	0.67
I50V (N=2)	3.32	0.47	15.50	0.78	4.45
82A/S/F (N=21)	1.65	16.49	5.57	13.15	22.78
84V (N=10)	3.77	19.11	13.61	11.10	21.04
90M (N=17)	1.90	16.88	6.76	14.85	17.84

- Viruses with I50V (2/2) showed >2.5-FC to BCV, APV and LPV, while viruses with I50L showed >2.5-FC with ATV only (Table 6).
- Viruses with I84V showed mean FC >2.5 to all PIs tested (Table 6).

Table 7. Viruses with FC > 2.5 to BCV : FC for all compounds tested and summary of genotypes

Subject ID	Timepoint	FC					Mutation summary*
		BCV	ATV	APV	IDV	LPV/r	
1	DAY 1	2.6	12	8.9	11	7.9	L10I, K20R, V32I, M36I, M46I, A71A/V, V82A
2	WEEK 24	2.6	0.52	12	0.89	4.9	M46I, I50V
3	DAY1	3.1	95	9.5	59	64	L10I/V, K20X, M36I, M46I, I54V, V82A, L90M
4	WEEK 16	3.2	23	27	5.5	7.4	L10F, K20V, L33F, M36L, I54M, L90M
5	DAY1	3.2	11	11	36	54	L10I, M46I, I54V, V82F, L90M
6	DAY1	3.8	30	8.1	29	40	L10I, K20V, M36I, M46I, I54V, I84V, L90M
7	WEEK 48	4.0	0.42	19	0.66	4.0	L10I, M36I, M46L, I50V
8	WEEK 16	4.6	15	25	6.49	12	L10F, I54M, I84V
9	WEEK 32	5.8	22	10	9.2	9.3	L10L/F, I54L, I84V, L90M
10	WEEK 24	7.0	13	23	3.6	9.7	L10I, M36I, I54L, I84V
11	WEEK 16	8.9	35	35	17	79	L10I, K20R, L24I, L33L/F, M36I, M46L, I54V, V82A, I84V
Mean FC		4.4	23	17	16	26	

* IAS USA resistance mutations only

- Viruses with >2.5-FC to BCV had between 2 and 9 PI mutations (Table 7).
- There was a mean of 6 and 8 PI mutations in viruses above and below 2.5-FC with BCV respectively.
- Multiple PI mutations were present in viruses with both above and below 2.5-FC for BCV.

Discussion

- BCV was the most potent compound tested against these 50 resistant viruses with a median IC₅₀ of 0.3 nM.
- Seventy-eight percent of viruses had a mean FC to BCV of <2.5 which compared to 34%, 44%, 44% and 42% for ATV, APV, IDV and LPV respectively. Similarly, no viruses had FC to BCV >10 compared to 40%, 20%, 26% and 32% for ATV, APV, IDV and LPV respectively.
- PI resistance mutations were compared to the fold change in BCV IC₅₀ to determine whether a pattern of mutational changes could be linked with decreasing susceptibility to BCV. There was no obvious difference in mutation pattern between viruses with a FC <2.5 and those with a FC >2.5. However, there is some increased frequency in mutations L10I/F/V, M36I/L, I54L/M/V, A71V/T/I, I84V and L90M in viruses with FC >2.5 than in viruses with FC <2.5. The presence of PI resistance mutations in combination made it difficult to attribute the small changes in sensitivity to any one mutation.
- A previous study (Hazen et al, 2003) of 55 highly resistant isolates also showed a good capacity to retain potency against viruses with multiple mutations. That study included viruses with highly complex patterns of PI R-AMs and so it was not possible to identify conclusively individual key resistance mutations. In the present study, the viruses were chosen in part because of their less complex mutational patterns, but as a result overall modest FC relative to wild-type viruses were observed with BCV and modest to high FCs for the other PIs. For this reason, it was again difficult to ascribe mutations to BCV resistance.
- It would appear that combined mutations are likely required for resistance to BCV. Therefore the identification of a series of relevant mutations based on relative associated levels of resistance might be beneficial. In order to establish this, further analyses, including decision tree analysis, will be performed.

Conclusion

- BCV showed superior in vitro potency and lower FC in PI-resistant viruses compared with the other PIs tested.
- Multiple resistance-associated mutations are likely required to confer cross-resistance to BCV.
- No clearly defined mutational pattern linked to reduced susceptibility to BCV was identified with this limited dataset, larger datasets will be examined and further analytical methods applied.

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