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Phenotypic Susceptibility to Bevirimat Among HIV-Infected Patient Isolates Without Prior Exposure to Bevirimat

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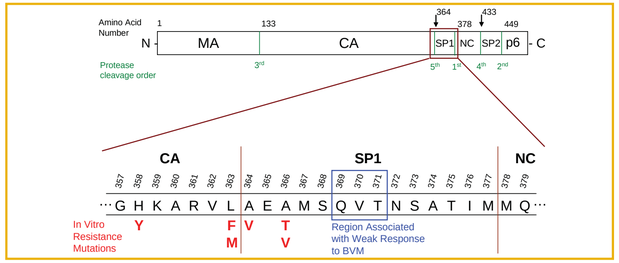
Introduction

- Bevirimat (BVM or PA-457) belongs to a novel class of HIV-1 maturation inhibitors.
- BVM inhibits the last step in the cleavage of the Gag polyprotein into capsid (CA) and SP1, leading to a defective virus
- The mutations H358Y and L363F/M in CA, and A364V and A366T/V in SP1, were selected through *in vitro* resistance selection experiments with BVM[1]
- Results from a phase II trial in treatment-experienced patients where BVM was added to background antiretroviral regimen showed that 24/46 (52%) patients treated with BVM showed a weak viral load response (< 0.5 $\log_{10}$) which correlated with the presence of polymorphisms in SP1 (residues Q369, V370 and T371)[2]
  - Baseline analyses confirmed phenotypic resistance (>100-fold) to BVM in multiple patients[3]

Figure 1. HIV-1 Gag Polyprotein Schema



Objective

- Investigate phenotypic susceptibility to BVM in HIV-1 from subjects who have not been treated with BVM

Methods

- Stored HIV-1 subtype B samples from 31 patients from past Gilead clinical studies were analyzed
  - 21 from treatment-experienced patients with or without PI resistance mutations (18 and 3, respectively) from study GS-99-907 (11 samples collected from Feb-00 to Jul-00) or GS-US-183-0105 (10 samples collected from Mar-06 to May-06)
  - 10 from treatment-naïve patients without PI resistance mutations from study GS-01-934 (collected from Aug-03 to Sep-03)
- The entire HIV-1 Gag and Protease (PR) genes were amplified by PCR (Phusion™, New England Biolabs, Ipswitch, MA, USA) and subcloned into Zero Blunt TOPO (Invitrogen, Carlsbad, CA, USA)
- After restriction enzyme digestion, the fragments were ligated into the wild-type pXXLAI viral DNA construct (SfoI restriction site upstream of Gag and XmaI site downstream of PR)
- Polyclonal mutant plasmid stocks were generated directly by transformation of XL-10 cells (Stratagene, Cedar Creek, TX, USA)
- The entire Gag-PR region of the plasmids was sequenced (Elim Biopharmaceuticals, Hayward, CA, USA)
- Recombinant pXXLAI virus plasmids (wild-type or mutant) were transfected into 293T cells (TransIT®-293; Mirus, Madison, WI, USA) and virus supernatants were harvested after 48 hours
- Drug susceptibilities (BVM, tenofovir, lopinavir, atazanavir) were determined in 5-day XTT assays in MT-2 cells
- Gag-PR DNA fragments for the site-directed mutant viruses (V362I/wild-type or SP1 reversion) were obtained by PCR. Viruses were generated as outlined above, except that they were made from individual clones

Results

Table 1. Phenotypic Susceptibility and Genotypic Data for the 31 Patient HIV Isolates

BVM FC[a]	Prior ARV[b]	Isolate ID	LPV FC[c]	ATV FC[c]	Capsid[d] 357 G	358 H	359 K	360 A	361 R	362 V	363 L	SP1[e] 364 A	365 E	366 A	367 M	368 S	369 Q	370 V	371 T	372 N	373 S	374 A	375 T	376 I	377 M
0.7	Exp.	22	0.9	1.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.9	Naïve	32	0.5	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	I/V	-	G	-	-	-	-	-
1	Exp.	7	4.1	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.2	Exp.	8	28.7	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.2	Exp.	21	0.6	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	V	-
1.3	Exp.	26	46.5	571	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	P	-	N	-	-
1.3	Exp.	28	99.3	14.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.8	Naïve	36	1.1	1.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Q	-	-	N	A	-	-
2.1	Exp.	20	13.3	51.9	S	-	-	-	-	-	-	-	-	-	-	-	H	-	-	-	-	-	-	V	-
2.2	Exp.	5	12.1	63.2	-	-	-	-	-	-	-	-	-	-	-	-	H/Q	-	-	-	-	-	N	-	-
4.6	Naïve	37	0.8	0.9	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	V	-
4.7	Exp.	9	16.5	36.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	V	-
5.2	Naïve	30	0.7	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Exp.	25	58.9	184	-	-	-	-	-	-	-	-	-	-	-	-	-	-	del	-	P	-	-	-	-
36	Exp.	27	29.2	52.1	S	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-
>85	Exp.	12	1.1	1.2	S	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
>100	Naïve	39	0.8	1.4	S	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-
>100	Exp.	23	203	521	S	-	-	-	-	I	-	-	-	-	-	-	-	A	del	-	-	-	A	-	-
>100	Exp.	24	69.0	590	-	-	-	-	-	I	-	-	-	-	-	-	-	A	del	T	-	-	A	-	-
>100	Exp.	1	22.1	62.9	-	-	-	-	-	-	-	-	-	-	-	C	-	A	del	-	-	N	-	-	-
>100	Exp.	2	15.5	10.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	G	A	-	-
>100	Exp.	3	13.0	15.1	S	-	-	-	-	-	-	-	-	-	-	-	-	A	del	H/N	-	-	-	-	-
>100	Exp.	4	15.2	13.3	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	T	N	-	-
>100	Exp.	6	3.4	29.2	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	A	T	-	-	-
>100	Exp.	10	15.3	8.5	-	-	-	-	-	-	-	-	-	-	-	-	-	I	del	-	P/S	-	-	V	-
>100	Exp.	29	89.3	3.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-
>100	Naïve	31	1.1	1.9	-	-	-	-	-	-	-	-	-	-	-	-	-	A	S	A/T	-	N/T	-	V/I	-
>100	Naïve	33	1.6	1.8	-	-	-	-	-	-	-	-	-	-	-	G	-	M	-	-	T	-	-	-	-
>100	Naïve	34	1.4	1.6	S	-	-	-	-	-	-	-	-	-	-	-	-	A	-	T	P	-	A	-	-
>100	Naïve	35	1.0	0.9	S	-	-	-	-	-	-	-	-	-	-	-	-	A	Q	S	-	-	N	M	-
>100	Naïve	38	0.9	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	A/V	-	S/G	X[f]	X[g]	A/T	M/I	-

a. BVM fold change compared to wild-type EC_{50} of 34 nM. Average of at least 3 independent experiments.
b. Prior antiretroviral (ARV) experienced or naïve
c. Lopinavir (LPV) and atazanavir (ATV) fold change compared to wild-type EC_{50} of 24.4 nM and 4.8 nM, respectively. Average of at least 2 independent experiments
d. The last 7 amino acids of the Gag-Capsid sequences are shown
e. The complete amino acid sequences of Gag-SP1 are shown (14 residues)
f. A complex S373P/T/Q/K amino acid mixture was found at that site
g. A complex A374A/G/T/S amino acid mixture was found at that site

Sites of In Vitro Selected Resistance Mutations[1]
Region Previously Identified with BVM Resistance[2]
BVM FC <10

- 15/31 patient viruses analyzed showed significantly reduced susceptibility to BVM (>100-fold) without prior BVM exposure
- 18/21 isolates with prior ARV experience had at least 3 PI-R mutations (D30N, V32I, L33F, M46I/L, I47V, G48V, I50V, I54M/V/L, L76V, V82A/F/L/T, I84V, L90M). 17/18 had FC >15 for either LPV or ATV or both. Isolate #7 (M46I, V82F, L90M) had FC = 4.1 for LPV
- The remaining 13 isolates had no PI-R mutations and were within 2.1-fold of wild-type for LPV and ATV (overall range of 0.5 to 2.1)
- All 31 isolates were within 1.9-fold of wild-type for TFV (0.3 to 1.9), consistent with only wild-type RT being included in the recombinant virus isolates

Table 2A. Summary of Phenotypic Susceptibility to BVM by Treatment Experience

BVM Fold Change from Wild-type	ARV-Exp. (n=21)[a]	ARV-Naïve (n=10)[a]	All Isolates (n=31)[a]
>100	9 (43%)	6 (60%)	15 (48%)
10 to 100	3 (14%)	0	3 (10%)
<10	9 (43%)	4 (40%)	13 (42%)

a. Numbers of isolates and percents for each category are shown

Table 2B. Summary of Phenotypic Susceptibility to BVM by PI Resistance

BVM Fold Change from Wild-type	Isolates with PI Resistance (n=18)[a,b]	Isolates without PI Resistance (n=13)[b]	All Isolates (n=31)[b]
>100	9 (50%)	6 (46%)	15 (48%)
10 to 100	2 (11%)	1 (8%)	3 (10%)
<10	7 (39%)	6 (46%)	13 (42%)

a. These 18 patient isolates had at least 3 of the PI-R mutations: D30N, V32I, L33F, M46I/L, I47V, G48V, I50V, I54M/V/L, L76V, V82A/F/L/T, I84V, L90M
b. Numbers of isolates and percents are shown

- Bevirimat susceptibility did not correlate with treatment-experience or PI resistance

Table 3. Phenotypic Susceptibility of Site-Directed Mutants to Bevirimat

Isolate ID[a]	BVM FC[b]	Capsid[c] 357 G	358 H	359 K	360 A	361 R	362 V	363 L	SP1[d] 364 A	365 E	366 A	367 M	368 S	369 Q	370 V	371 T	372 N	373 S	374 A	375 T	376 I	377 M
1	>100	-	-	-	-	-	-	-	-	-	-	-	C	-	A	del	-	-	N	-	-	-
SDM-1 (wt SP1)	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	>100	S	-	-	-	-	-	-	-	-	-	-	-	-	A	del	H/N	-	-	-	-	-
SDM-3 (wt SP1)	0.7	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	>100	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	A	T	-	-	-
SDM-6a (wt SP1)	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SDM-6b (wt V370)	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-
SDM-6c (wt S373/ A374)	>100	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-
7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SDM-7 (V362I)	>100	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SDM-8 (V362I)	>100	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	>85	S	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SDM-12 (wt V362)	0.7	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LAI	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SDM-LAI (V362I)	>100	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a. Site-directed mutants created by PCR with specific changes in SP1 (isolates 1, 3, and 6) or capsid (V362I; isolates 7, 8, 12 and LAI lab strain) as shown. All other PR and Gag sequence were identical to the patient isolate or lab strain
b. Bevirimat (BVM) fold change compared to wild-type LAI. Average of at least 3 independent experiments
c. The last 7 amino acids of the Gag-Capsid sequences are shown
d. The complete amino acid sequences of Gag-SP1 are shown (14 residues)

- Reversion of the 14 amino acid SP1 sequence to wild-type SP1 restored BVM susceptibility
- Reversion or addition of V362I in capsid or V370A in SP1 changed BVM susceptibility by >85-fold

Conclusions

- **Nearly half of the patient viruses analyzed (15/31, 48%) showed significantly reduced susceptibility to BVM (>100-fold) without prior BVM exposure**
- **The presence of reduced susceptibility to BVM did not correlate with ARV treatment experience or protease inhibitor resistance**
- **Reduced susceptibility to BVM was strongly associated with genetic polymorphisms in SP1 and the capsid junction**
  - **Not all SP1 polymorphisms lead to reduced BVM susceptibility**
  - **The SP1 mutation V370A and the previously undescribed capsid mutation V362I conferred high-level resistance to BVM**
- **None of the known in vitro-selected resistance mutations to BVM were observed in the panel**
- **These genotypic and phenotypic data extend the existing dataset and help in building a predictive algorithm for susceptibility to BVM**

References

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