Susceptibility of Diverse HIV-1 Patient Isolates to the Maturation Inhibitor, Bevirimat (MPC-4326*), is Determined by Clade-Specific Polymorphisms in Gag CA-SP1

16th Conference on Retroviruses
And Opportunistic Infections
February 8-11, 2009
Montréal, Canada

K Salzwedel[1], F Hamy[2], S Louvel[2], M Sakalian[1], M Reddick[1], C Finnegan[1], D Martin[1], S McCallister[1], T Klimkait[2, 3], and G Allaway[1].
[1]Panacos Pharmaceuticals, Gaithersburg, MD, USA; [2]InPheno AG, Basel, Switzerland; [3]University of Basel, Basel, Switzerland.

M-267
Vijay Baichwal
Myriad Pharmaceuticals Inc.
320 Wakara Way
Salt Lake City UT 84108
vbaichwa@myriad.com
Tel: 801-584-1143
Fax: 801-505-5148
ksalzwedel@panacos.com

Abstract

BACKGROUND: The HIV-1 maturation inhibitor bevirimat (BVM, MPC-4326) binds to Gag and specifically inhibits CA-SP1 processing. Recent clinical studies identified key baseline polymorphisms at Gag positions 369/370/371 in SP1 that correlated with variable patient responses. Polymorphisms at these 3 positions are found in ~30% of patients with clade B virus. Since the clade B consensus sequence at these positions (QVT) differs from that of other clades, we examined the susceptibility of non-clade B isolates to BVM *in vitro* to determine which polymorphisms affect BVM activity in these other clades.

METHODS: A panel of 25 non-clade B viruses was compiled with multiple representatives from each clade with global prevalence >1% worldwide (clades A, C, CRF01_AE, CRF02_AG, D, G). The panel consisted of 10 isolates with known, distinct CA-SP1 genotypes and 15 randomly selected patient plasma samples from Switzerland. The complete Gag-PR region from each isolate was amplified and cloned into a pNL4-3 background. The InPheno replicative *in-vitro* phenotyping assay, deCIPhR (dual-enhancement of Cell Infection to Phenotype Resistance), was used to quantitate susceptibility to BVM. Fold-change (FC) in IC50 was compared to FC values for BVM-treated patient isolates and site-directed mutant controls.

RESULTS: None of the 25 viruses in the test panel contained the wild-type QVT clade B consensus sequence at positions 369-371; nonetheless, 7/25 isolates (28%) were highly susceptible to BVM (FC <2). These included 5 viruses containing the clade A and CRF02_AG consensus sequence, QVQ. 9/25 isolates (36%) had intermediate susceptibility (FC 2-10), and 9/25 isolates (36%) fell into the least susceptible category (FC >10). Of the intermediate/least susceptible viruses, 13/18 contained V370A, V370M, or ΔV370 polymorphisms, all of which are key polymorphisms in clade B.

CONCLUSIONS: Our analysis demonstrates that some, but not all, polymorphisms at Gag 369/370/371 in SP1 reduce the susceptibility of viruses to BVM *in vitro*. Specifically, the T371Q polymorphism that gives rise to the clade A and CRF02_AG consensus sequence, QVQ, appears to have no effect on BVM susceptibility. This suggests that, following more extensive testing, it may be possible to exclude the T371Q polymorphism from a future genotyping algorithm used to identify patients suitable for BVM treatment. Additional phenotyping and genotyping should help to further refine the genotyping algorithm for non-clade B patients.

Background

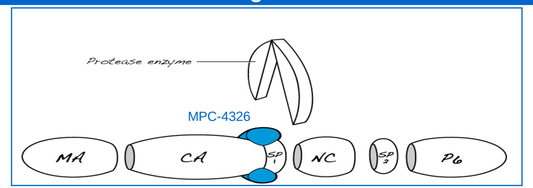


Figure 1: Bevirimat (BVM, MPC-4326) binds to Gag and specifically inhibits CA-SP1 processing

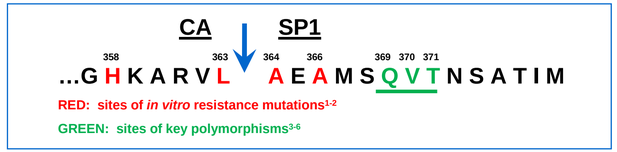


Figure 2: Determinants of bevirimat response to clade B virus

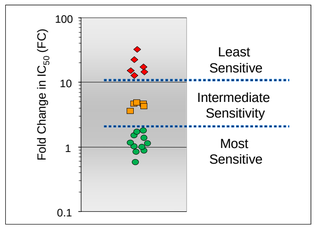
Polymorphisms at Gag 369, 370, 371 correlate with reduced *in vitro* susceptibility of clade B patient isolates to bevirimat

Table 1: Phenotypes of clade B patient isolates correlate with polymorphisms at Gag 369, 370, and 371

		InPheno deCIPhR™ GAG+PR Phenotype Assay
Patient ID	**CA-SP1 Sequence**	**Fold Change in IC_{50}**
Wild-Type	...GHKARVL-AEAMS**QVT**NSATIM	1
129	...GHKARVL-AEAMS**QVT**SSATMM	0.57
180	...GHKARVL-AEAMS**QVT**NPPTIM	0.84
6	...GHKARVL-AEAMS**QVT**NSATIM	0.87
126	...GHKARVL-AEAMS**QVT**GSAAVM	1.02
1	...GHKARVL-AEAMS**QVT**NSATVM	1.14
182	...GHKARVL-AEAMS**QVT**NPATIM	1.16
14	...GHKARVL-AEAMS**QVT**PSATVM	1.51
16	...GHKARVL-AEAMS**QVT**NPSNIM	1.69
3	...SHKARIL-AEAMS**QVT**GPANIM	1.79
127a	...GHKARVL-AEAMS**QMT**NSATAM	3.6
127b	...GHKARVL-AEAMS**QMT**NSATAM	4.25
4	...GHKARVL-AEAMS**QMT**NPATIM	4.6
24	...SHKARVL-AEAMS**QV**-NPTNIM	4.61
10	...NHKARIL-AEAM**CHV**TNSATVM	4.85
12	...GHKARVL-AEAMS**QMT**NSATTM	12.8
8	...SHKARVL-AEAM**CQA**-NSTTVM	14.5
125a	...GHKARVL-AEAMS**QAT**ASNVIM	15.3
15	...GHKARVL-AEAMS**QA**-NSSSIM	17.4
125b	...GHKARVL-AEAMS**QAT**ASNVIM	22.2
30	...GHKARVL-AEAMS**QAT**NSAAIM	32.3

Figure 2: Clade B patient isolates cluster into 3 phenotypic groups:

- **GREEN** = most sensitive (FC < 2)
- **ORANGE** = intermediate sensitivity (FC = 2-10)
- **RED** = least sensitive (FC > 10)



Site-directed mutagenesis demonstrates that some, but not all, changes at 369-371 are sufficient to reduce susceptibility to bevirimat

Table 2: Site-directed mutagenesis at 369, 370, 371

Mutant	**Replication Capacity**	**Fold Change in IC_{50}**	**CA-SP1 Sequence**
Wild-Type	100	1.0	...GHKARVL-AEAMS**QVT**NPATIM
T371A	100	1.8	...GHKARVL-AEAMS**QVA**NPATIM
Q369A	89	1.9	...GHKARVL-AEAMS**AVT**NPATIM
Q369H	131	3.2	...GHKARVL-AEAMS**HVT**NPATIM
ΔT371	100	18	...GHKARVL-AEAMS**QV**-NPATIM
ΔQ369	37	23	...GHKARVL-AEAMS-**VT**NPATIM
ΔV370	53	27	...GHKARVL-AEAMS**Q**-TNPATIM
V370M	87	42	...GHKARVL-AEAMS**QMT**NPATIM
V370A	55	54	...GHKARVL-AEAMS**QA**TNPATIM
L363M*	80	10	...GHKARV**M**-AEAMS**QVT**NPATIM
A364V*	95	52	...GHKARVL-**V**EAMS**QVT**NPATIM

* Resistance mutations identified by *in vitro* selection[1-2]

The Gag 369-371 consensus sequence differs in non-subtype B virus clades

Table 3: Consensus sequence at 369, 370, 371 for different virus clades

	Gag consensus sequence*		
Clade	**369**	**370**	**371**
B	Q	V	T
A	Q	V	Q
CRF01_AE	Q	A	Q
CRF02_AG	Q	V	Q
C	Q	A	N
D	Q	A	T

* Los Alamos HIV sequence database[6]

References:
1.Li *et al.* (2003) *PNAS* 100:13555-60
2.Adamson *et al.* (2006) *J. Virol.* 80:10957-71
3.Salzwedel *et al.*, XVII International HIV Drug Resistance Workshop, June 10-14, 2008
4.McCallister *et al.*, XVII International HIV Drug Resistance Workshop, June 10-14, 2008
5.Hamy *et al.*, XVII International HIV Drug Resistance Workshop, June 10-14, 2008
6.Van Baelen *et al.*, XVII International HIV Drug Resistance Workshop, June 10-14, 2008

*MPC-4326 (Bevirimat dimeglumine) is being developed by Myriad Pharmaceuticals, Inc.
Poster presented by Vijay Baichwal
For information please contact Patrick Burke at pburke@myriad.com

Many polymorphisms common in non-clade B isolates are susceptible to bevirimat

Table 4: Phenotype of selected non-clade B patient isolates

HIV-1 Isolate	**Replication Capacity (% of WT)**	**Fold Change in IC_{50}**	**CA-SP1 Sequence**
CLADE B consensus			
Wild-Type	100	1	...GHKARVL-AEAMS**QVT**NSATIM
CLADE A			
92UG029	22	14.3	...SHKARIL-AEAMS**QAQ**HTNIM
93UG059	14	1.9	...GHKARVL-AEAMS**QVQ**QTSIM
IP8017128	22	3.4	...SHKARVL-AEAMS**QVQ**HTNIM
IP7023695	60	2.5	...SHKARVL-AEAMS**QAQ**QGNIM
IP7019098	18	3.9	...GHKARVL-AEAMS**QAQ**QTAIM
IP7027576	14	1.9	...GHKARVL-AEAMS**QVQ**PTNVM
CRF01_AE			
CMUO6	45	9.3	...SHKARVL-AEAMS**HAS**GNTIM
IP8012601	52	0.9	...SHKARVL-AEAMS**SAQ**QANIM
IP8012990	24	1	...SHKARVL-AEAMS**QVQ**QPGIM
CRF02_AG			
IP8012523	70	0.9	...GHKARVL-AEAMS**QVQ**QTNVM
IP8011282	34	2	...SHKARVL-AEAMS**QAQ**QASVM
IP8011210	38	2.7	...SHKARVL-AEAMS**QAQ**QTTIM
IP8012907	39	0.8	...GHKARVL-AEAMS**QVQ**QTNIM
CLADE C			
ZB18	85	19.7	...SHKARVL-AEAMS**QAN**NTNILM
92BR025	11	11.7	...GHKARVL-AEAMS**KVN**NTNIM
98BR004	29	4.5	...GHKARVL-AEAMS**QVN**NTNIM
98CN006	34	13.9	...SHKARVL-AEAMS**QTN**STILM
98TZ013	73	13.7	...SHKARVL-AEAMS**QTT**STNILM
IP8013036	33	3.6	...SHKARVL-AEAMS**QAN**NSNVM
CLADE D			
92UG021	68	17.7	...GHXARVL-AEAMS**QAT**ANATIM
IP7025314	25	1.6	...GHKARVL-AEAMS**QAN**SAIM
IP7014712	30	8.7	...GHKARIL-AEAMS**QMN**SATVM
CLADE G			
BCF-DIOUM	33	4.7	...SHKARVL-AEAMS**QAS**GNTIM
IP7005281	52	3.1	...GHKARVL-AEAMS**QVS**GAAIM
IP7011053	8	25.8	...GHKARVL -AEAMS**QVS**GASAAIM

- All of the 25 non-clade B viruses selected contained polymorphisms that differ from the clade B consensus sequence, QVT, at positions 369-371 in Gag
- Despite this, 7/25 isolates (28%) were highly susceptible to BVM (FC < 2), and an additional 9/25 isolates (36%) had intermediate susceptibility (FC = 2-10)
- The clade A and CRF02_AG consensus sequence, QVQ, was particularly sensitive to BVM, with FC < 3.5 for all 6 isolates
- The current clade B patient genotype screening algorithm appears to be too stringent for use in evaluating patients with non-clade B virus and we are continuing to refine the clade B algorithm as we collect new data
- Additional *in vitro* phenotyping studies will help to further refine the genotyping algorithm to permit the identification of patients with non-clade B viruses that are suitable for treatment with BVM