Resistance Pathways for Potent and Broadly Active HIV-1 Maturation Inhibitors

Emiko Urano[1], Sherimay Ablan[1], Nishani Kuruppu[1], Justin Kaplan[1], David E. Martin[2], T. J. Nitz[2], Carl T. Wild[2], and Eric O. Freed[1]

[1]HIV Dynamics and Replication Program, National Cancer Institute, Frederick, MD

[2]DFH Pharma, Inc. Gaithersburg, MD

Abstract

Background: A betulinic acid-based compound, bevirimat (BVM), the first-in-class HIV-1 maturation inhibitor, acts by blocking a late step in protease-mediated Gag processing: the cleavage of the capsid-spacer peptide 1 (CA-SP1) intermediate to mature CA. BVM was shown to be safe and effective in reducing viral loads in HIV-1-infected patients. However, single-amino-acid polymorphisms in the SP1 region of Gag reduced HIV-1 susceptibility to BVM in patients, leading to the discontinuation of BVM's clinical development.

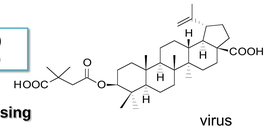
Methods: We carried out an extensive medicinal chemistry campaign to identify BVM derivatives that demonstrate increased potency against consensus clade B strains of HIV-1 and are active against primary isolates with polymorphisms in SP1. Compound activity was tested in assays that measure CA-SP1 processing and virus replication kinetics. Selection experiments were performed to identify mutations that confer resistance to these novel compounds and a variety of virological, structural, and molecular approaches were applied to elucidate the mechanism of resistance for each mutant. To evaluate the effect of Gag polymorphisms and resistance mutations on the kinetics of Gag processing, pulse-chase radiolabeling assays were performed.

Results: We identified a set of BVM derivatives that are more potent than BVM against WT HIV-1 and show robust antiviral activity against SP1 polymorphic strains and clinical isolates. The best of these analogs retain significant activity against BVM-resistant mutants. Selection experiments identified an Ala-to-Val mutation at SP1 residue 1 (SP1-A1V). The SP1-A1V mutation was previously identified in our selections for BVM resistance. In addition, we also selected for the mutation CA-P157A, located in the major homology region (MHR) of CA. Remarkably, the P157A mutant was resistant to not only BVM and the second-generation BVM analogs but also to the structurally distinct maturation inhibitor PF-46396. Pulse-chase data demonstrate that CA-SP1 processing kinetics for P157A are similar to those of the WT. Analysis of the HIV-1 database reveals that Ala1 of SP1 and Pro157 of CA are conserved in ~99.95% of available sequences.

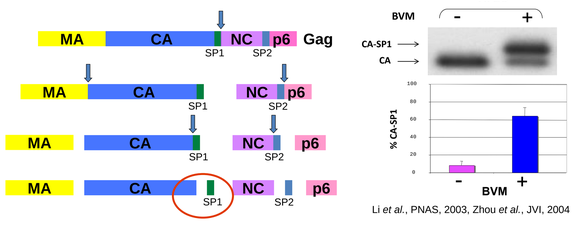
Conclusions: This study identifies a panel of BVM derivatives that display marked improvements relative to BVM in antiviral potency and breadth of activity. The characterization of resistant mutants provides novel insights into the structure of the maturation inhibitor-binding site and the role of SP1 and the CA MHR in virus assembly and maturation. In addition, mutations that confer resistance to these novel compounds arose at highly conserved residues, suggesting a high genetic barrier to resistance. The identification of BVM analogs that are highly potent against a wide range of HIV-1 isolates containing polymorphic SP1 sequences supports ongoing clinical development of this class of inhibitors.

Bevirimat: First-in-class HIV-1 Maturation Inhibitor

Bevirimat (BVM, PA-457, DSB)
(dimethylsuccinyl betulinic acid)



Mechanism: Inhibits CA-SP1 processing



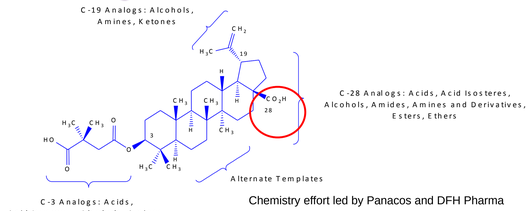
Li *et al.*, PNAS, 2003, Zhou *et al.*, JVI, 2004

Polymorphisms in SP1 (e.g., V7A) Reduced HIV-1 Susceptibility to BVM and Were Associated with a Lack of Response in Patients

	CA								SP1									
								230								7	8	
>M GROUP CONSENSUS	G	G	P	S	H	K	A	R	V	L	A	E	A	M	S	Q	V	T	N
>CONSENSUS_A1	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	Q	H
>CONSENSUS_A2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Q	-
>CONSENSUS_B	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
>CONSENSUS_C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	N	-
>CONSENSUS_D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-
>CONSENSUS_F1	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-
>CONSENSUS_G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	S	G
>CONSENSUS_H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
>CONSENSUS_K	-	-	-	G	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-

The summary of consensus sequences.

Second-Generation Maturation Inhibitor Program to Overcome V7A Restriction

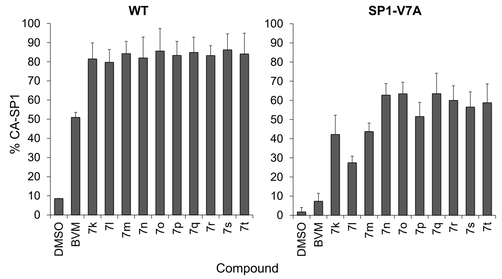


Chemistry effort led by Panacos and DFH Pharma

~500 Analogs Synthesized

Identification of Second-Generation Maturation Inhibitors

Screening : CA-SP1 accumulation 100 nM



HeLa cells were transfected with WT (pNL4-3) or polymorphic (pNL4-3 SP1-V7A) HIV-1 molecular clones. At 24 hour posttransfection, HIV-1-expressing cells were labeled with [35S]Met/Cys for 2-3 h. Maturation inhibitors were maintained throughout the transfection and labeling. Viruses were collected by centrifugation. CA and CA-SP1 proteins were separated on 13.5-15% acrylamide gels by SDS-PAGE, exposed to a phosphorimager plate and quantified by Quantity One software.

Second-Generation BVM Analogs Show Low-nM Antiviral Activity

Compound	WT IC_{50}	SP1-V7A IC_{50}
BVM	136 ± 30 nM	>500
7k	3 ± 1	145 ± 29
7l	14 ± 7	201 ± 44
7m	3 ± 0.3	9 ± 2
7n	7 ± 3	14 ± 4
7o	9 ± 3	22 ± 5
7p	3 ± 0.4	26 ± 6
7q	5 ± 1	22 ± 7
7r	5 ± 3	10 ± 3
7s	21 ± 19	8 ± 2
7t	2 ± 0.5	19 ± 6

The IC50s were calculated using MT4 cell line with WT or SP1-V7A. HIV-infected MT-4 cells were incubated with a serial dilution of compounds. At 4 days post infection, the culture supernatants were subjected to RT assay to examine the viral replication. The 50% inhibitory concentrations were defined as drug concentrations that reduced RT levels by 50% relative to no drug (DMSO) controls using GraphPad Prism 6 software.

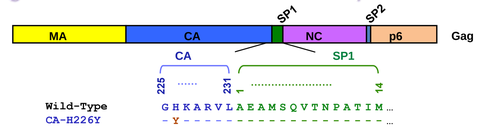
Second-Generation BVM Analogs Are Active Against Primary Isolates in PBMC

		IC_{50}: PBMC	
		BVM	Compound 7r
NL4-3	Subtype B	4.8 nM	5.2 nM
92UG031	Subtype A	2829.0	>1000.0
93IN101	Subtype C	2651.0	66.6
99UGA07412MI	Subtype D	15.4	14.5
CMU02	Subtype E	3327.0	95.6
93BR029	Subtype F	6.9	12.9
JV1083	Subtype G	4188.0	221.0
YBF30	Group N	1489.0	43.4
BCF02	Group O	444.0	6.9

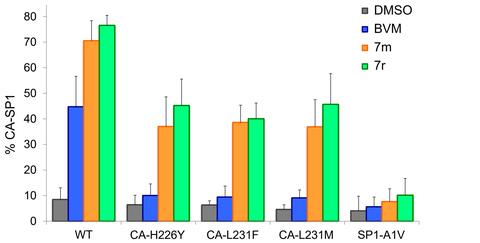
		CA	SP1
NL4-3 WT	Subtype B	HKARVLAEAMS	QVTN--PATIM
92UG031	Subtype A	****T*******	AQH**T-N**

The IC50s were calculated using PBMC with multi-clade HIV-isolates. HIV-infected cells were incubated with a serial dilution of compounds. At 6 days postinfection, the culture supernantants were subjected to p24 ELISA assay to examine the viral replication. The 50% inhibitory concentrations were defined as drug concentrations that reduced p24 antigen values by 50% relative to no drug (DMSO) control. Amino acid sequences of the CA-SP1 boundary region from NL4-3 and Subtype A HIV-1 isolate tested in above Table.

Second-Generation BVM Analogs Are Active Against BVM-Resistant Mutants except SP1-A1V

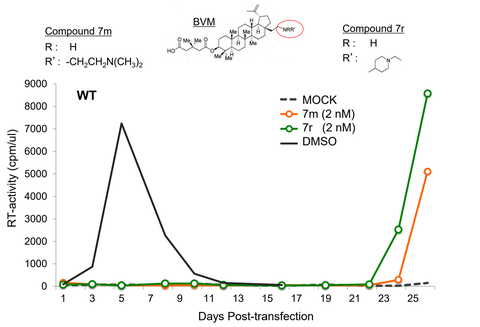


Adamson *et al.*, JVI. 2006



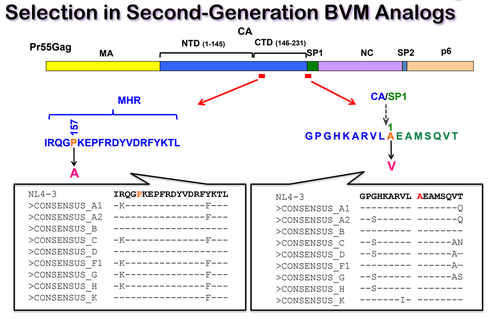
HeLa cells were transfected with WT or BVM-resistant mutants. At 24 hour posttransfection, HIV-1-expressing cells were labeled with [35S]Met/Cys for 2-3 h. Maturation inhibitors were maintained throughout the transfection and labeling. Viruses were collected by centrifugation. CA and CA-SP1 proteins were separated on 13.5-15% acrylamide gels by SDS-PAGE, exposed to a phosphorimager plate and quantified % of CA-SP1 accumulation.

Second-Generation BVM Analogs Potently Inhibit HIV-1 Replication



Jurkat cells were transfected with WT NL4-3 by DEAE/dextran and cells were cultured in the presence of the compounds. The culture supernantants were collected every 2-3 days and were subjected to RT assay to examine the viral replication.

Mutations in CA and SP1 Were Acquired During Selection in Second-Generation BVM Analogs



The position of the amino acid changes in Gag. Both amino acid CA-P157 and SP1-A1 are highly conserved across all clades of HIV-1.

Acknowledgments

Hetero Research Foundation

NCI-CBL
Gary Pauly
Joel Schneider

Southern Research Institute

South Asian University
Ritu Gaur

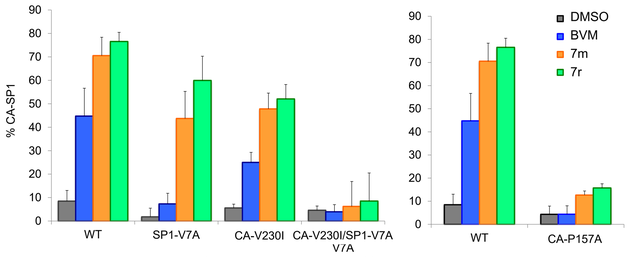
NIAMS-NIH
Juan Fontana
Alasdair Steven

University of Delaware
Tatyana Polenova

NCI-HIV DRP Retrovirus assembly Section
Siddhartha Datta
Alan Rein

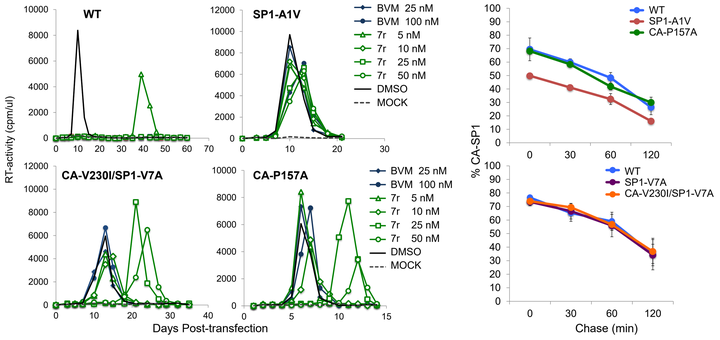
University of Missouri
Anna Gres
Karen Kirby
Stefan Sarafianos

The CA-P157A Mutation And Double Mutations CA-V230I/SP1-V7A Confer Resistance to BVM Analogs



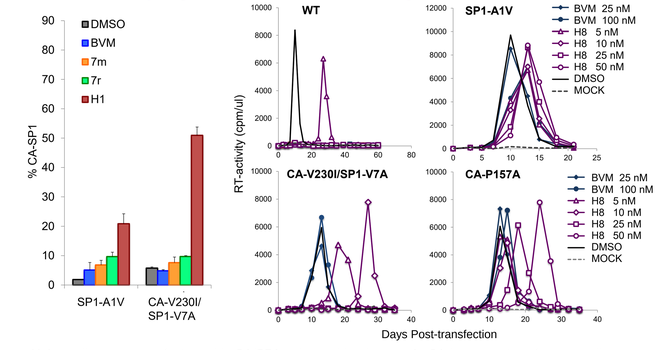
HeLa cells were transfected with WT or CA/SP1-polymorphic viruses or CA-P157A HIV-1 molecular clones. At 24 hour posttransfection, cells were labeled with [35S]Met/Cys. Maturation inhibitors were maintained throughout the transfection and labeling. Virion-associated CA-SP1 was analyzed.

The CA-P157A Mutation Confers Partial Resistance to All BVM Analogs in Replication Assays



Jurkat cells were transfected with WT or HIV CA/SP1 mutants by DEAE/dextran and cells were cultured in the presence of the compounds. HIV-1 replication was monitored by RT activity in culture supernatant. (Left). Cells transfected with the WT or HIV CA/SP1 mutants were pulse labeled and chased for the indicated times. Cell lysates were immunoprecipitated with HIV-Ig, and the extent of processing of CA-SP1 to CA was analyzed (Right).

Recently Synthesized BVM Analogs Display Significant Activity Against BVM/BVM Analog-resistant Mutants



HeLa cells were transfected with CA/SP1-polymorphic viruses. At 24 hour posttransfection, cells were labeled with [35S]Met/Cys, virion associated CA-SP1 was analyzed. Jurkat cells were transfected with WT or HIV CA/SP1 mutants by DEAE/dextran and cells were cultured in the presence of the compounds. HIV-1 replication was monitored by RT activity in culture supernatant. (Right).

Conclusion

- A set of BVM derivatives have been identified that exhibit low-nM activity across major clades of HIV-1; these compounds are active against SP1 polymorphic viruses that display reduced susceptibility to BVM
- These second-generation maturation inhibitors also retain some activity against HIV-1 isolates selected for resistance to BVM
- Selection for resistance to the second-generation BVM analogs gave rise to a mutation in a highly conserved position in the CA major homology region (MHR), and at the CA-SP1 cleavage site.
- The level of CA-SP1 accumulation was significantly reduced in the presence of BVM and BVM analogs against CA-P157A mutant, however, the extent of CA-SP1 processing of CA-P157A was similar to that of WT
- The CA-P157A mutant was able to replicate in the Jurkat T-cell line, and conferred partial resistance to all BVM analogs.
- **Efforts are underway to define the structure of the maturation inhibitor binding pocket to facilitate the rational design of additional maturation inhibitors and to better understand the function of the CA-SP1 region of Gag in virus maturation**