RECENT PROGRESS IN THE DEVELOPMENT OF POTENT AND BROADLY ACTIVE HIV-1 MATURATION INHIBITORS

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Abstract

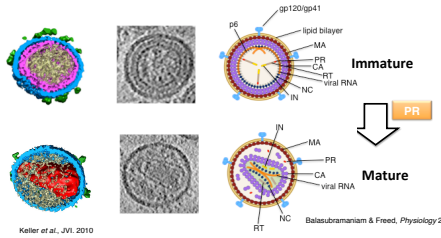
Background: A betulinic acid-based compound, bevirimat (BVM), the first-in-class HIV-1 maturation inhibitor, acts by blocking a late step in protease-mediated Gag processing: the cleavage of the capsid-spacer peptide 1 (CA-SP1) intermediate to mature CA. BVM was shown in multiple clinical trials to be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

Methods: We carried out an extensive medicinal chemistry campaign to develop BVM derivatives that demonstrate not only increased potency against consensus clade B strains of HIV-1 but also that are active against isolates with polymorphisms in SP1 and primary, non-clade B isolates. Compound activity was tested in assays that measure CA-SP1 processing by radioimmunoprecipitation, single-cycle infectivity, cell-cell transmission, and virus replication kinetics. Long-term selection experiments were performed to identify mutations that confer resistance to these novel compounds. Compound binding to Gag was tested directly by using a tritium-labeled derivative. The effects of the compounds on virion morphology were examined at high resolution by cryo-electron tomography.

Results: We identified a set of BVM derivatives that are not only more potent than BVM against WT HIV-1 but also show robust antiviral activity against SP1 polymorphic strains and clade C isolates. These second-generation inhibitors are structurally similar to BVM with C28 heteroatom modifications. The best of these analogs displayed ~3-log improved potency against polymorphic HIV-1 relative to BVM. Selection experiments identified a panel of resistant mutants that contain substitutions in Gag. The resistance mutations were analyzed for their effect on viral assembly, viral fitness, CA-SP1 processing, compound binding, and particle morphology. Cryo-electron tomography analysis demonstrated that HIV-1 maturation inhibitors act not only by blocking CA-SP1 processing but also by stabilizing the immature Gag lattice.

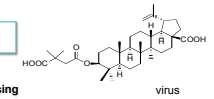
Conclusions: This study identifies a panel of C28 BVM derivatives that display marked improvements relative to BVM in their potency and breadth of activity. Biochemical, virological, and structural analyses performed with these compounds provide novel insights into the target and mechanism of action of HIV-1 maturation inhibitors. The results of this study provide novel insights into the structure of the maturation inhibitor-binding site and the role of SP1 in virus assembly and maturation. Finally, these findings suggest that clinical development of HIV-1 maturation inhibitors should resume.

HIV-1 Virion Maturation

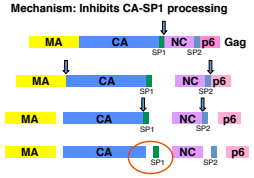


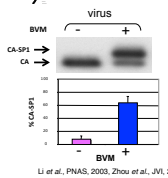
Bevirimat: First-in-class HIV-1 Maturation Inhibitor

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



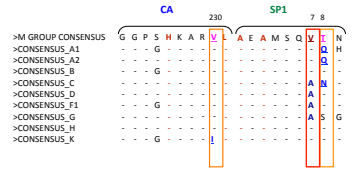
Mechanism: Inhibits CA-SP1 processing





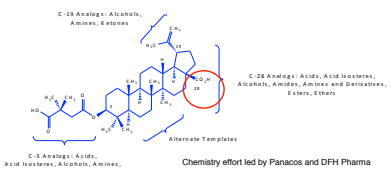
Li et al., PNAS, 2003, Zhou et al., JVI, 2004

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



Aim: To Develop BVM Analogs that More Effectively Inhibit HIV-1 Replication and Display Broader Activity Against Viral Strains Harboring SP1 Polymorphisms

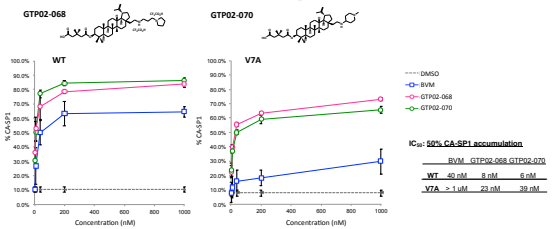
Second-Generation Maturation Inhibitor Program to Overcome V7A Restriction



Chemistry effort led by Panacos and DFH Pharma

~500 Analogs Synthesized

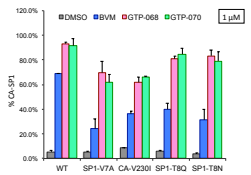
BVM Analogs Display Significant Improvements in Potency and Breadth of Activity



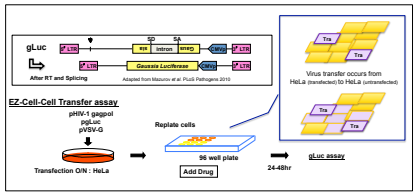
IC_{50}: 50% CA-SP1 accumulation

	BVM	GTP02-068	GTP02-070
WT	40 nM	8 nM	6 nM
V7A	> 1 uM	23 nM	39 nM

BVM Analogs Display Potent Activity Against CA-SP1 Polymorphic Viruses

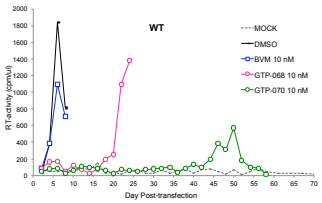


Application of a Cell-to-Cell Transfer Assay to Screen Inhibitors

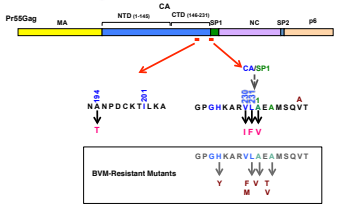


Schematic representation of EZ-Cell-Cell Transfer Assay. The gLuc Transfer vector is depicted at the top. This vector is transfected into HeLa cells along with Gag-Pol expression vector and a VSV-G expression vector. Cells are treated with varying concentrations of maturation inhibitor. Virus is transferred from transfected producer cells (Tra) to neighboring untransfected cells (purple). Transfer of vector from producer to target cell give rise to Gaussia luciferase (gLuc) expression. Inhibition of cell-cell transfer in the presence of maturation inhibitor results in a reduction in gLuc signal.

Second-Generation BVM Analogs Potently Inhibit HIV-1 Replication



Mutations in CA and SP1 Were Acquired During Selection in GTP02-068/-070



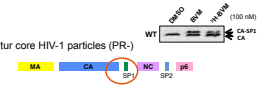
Conclusion

- C-28 alkyl amine derivatives of BVM have been identified that exhibit potent activity against both WT and polymorphic HIV-1 isolates
- These compounds also maintain the favorable metabolic profile observed with BVM, and exhibit significantly reduced protein binding and enhanced solubility
- We are currently selecting for drug resistance and testing effects on primary isolates and additional Gag polymorphs
- GTP-068 and GTP-070, like BVM, stabilize the immature Gag lattice and disrupt maturation.
- Efforts underway to define the structure of the maturation inhibitor binding pocket to enable the rational design of additional chemical classes of inhibitors
- These results with "second-generation" BVM derivatives argue that clinical development of HIV-1 maturation inhibitors should resume

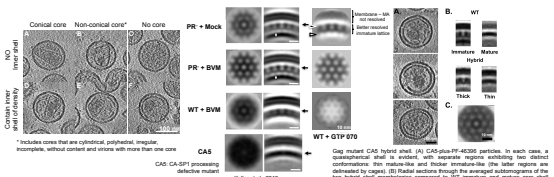
Inhibitor Binding Assay Using ^{3}H-BVM

- BVM is able to block PR-mediated CA-SP1 processing in the context of in vitro-assembled Gag but does not block processing in non-assembled-monomeric Gag
  (Li F *et al.* Proc Natl Acad Sci U S A. 2005, Sakalian *et al.* J Virol. 2006)
- Direct binding of ^{3}H-BVM to immature but not mature HIV-1 particles has been detected
  (Zhou *et al.* J Biol Chem. 2005, Zhou *et al.* J Virol. 2006)
- A recent study using photoactivatable BVM derivatives observed direct cross-linking of the compound to residues in the vicinity of the CA-SP1 cleavage site
  (Nguyen *et al.* Retrovirology. 2011)

Our Strategy
- ^{3}H-BVM
- Purified immatur core HIV-1 particles (PR-)



Cryo-EM Analysis and Subtomogram Averaging of GTP-070-treated Immature Virions



* Includes cores that are cylindrical, polyhedral, irregular, incomplete, without content and virions with more than one core

	NO inner shell			Contain inner shell of density				
	Conical core	Non conical core*	No core	Conical core	Non conical core*	No core	Immature	Total (n)
wt	90	16	3	0	0	1	1	100
wt + GTP 068	6	13	20	8	13	39	1	79
wt + GTP 070	1	16	13	6	9	55	0	78

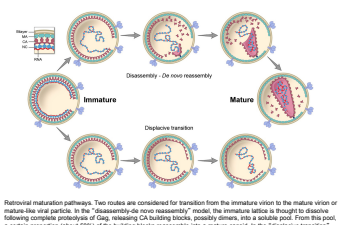
Gag mutant CA5 hybrid shell. (A) CA5-plus-PF-46396 particles. In each case, a quasihexagonal shell is evident, with separate regions exhibiting two distinct conformations: thin mature-like and thicker immature-like (the latter regions are delineated by cages). (B) Radial sections through the averaged subtomograms of the two hybrid shell morphologies compared to WT immature and mature core shell accentuate the difference in CA layer thickness between the two regions. This implies a change of CA organization/conforma- tion. (C) Spherical section through the averaged "thick" CA5 lattice.

WT + PF46396

PF46396: was found by Pfizer to have a similar mode of action to that of BVM

Keller et al., 2013

Our Model of Maturation Pathway



Retroviral maturation pathways. Two routes are considered for transition from the immature virion to the mature virion or mature-like viral particle. In the "disassembly-de novo reassembly" model, the immature lattice is thought to dissolve following complete proteolysis of Gag, releasing CA building blocks, possibly dimers, into a soluble pool. From this pool, a certain proportion (about 50%) of the building blocks reassemble into a mature capsid. In the "displacive transition" model, as appears to occur with the CA5 mutant, the immature-like CA-SP1 shell remains intact but patches of it undergo an in situ remodeling to a thinner mature-like state. Eventually, these patches merge. In a version of this model that would produce conical cores, a portion of initially spherical shell would have to be pinched off, coupled with the lattice maturation event. Our data support the "disassembly-reassembly" model as the likely path to productive core formation and support the idea that cleavage of SP1/SP1 from CA serves primarily to release CA from an immature-like lattice into the soluble pool.

Future Directions

To develop more potent maturation inhibitors
- Continue screening to identify more second-generation maturation inhibitor candidates.
- Evaluate the stability and inhibitory potency of these compounds *in vivo* (animal model).
- Evaluate the breadth of antiviral activity against diverse HIV-1 isolates.

To define the structure of the maturation inhibitor binding pocket
- Characterize resistance mutations that arise during propagation of HIV-1 in the presence of second-generation maturation inhibitors.
- Establish assays to measure binding activity of maturation inhibitors to HIV-1 Gag (Mass spec, SPR, ^{3}H-labeling, cryo-electron tomography).
- Solve the structure of assembled Gag bound to maturation inhibitors (ss NMR).