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Maturation Inhibitor BMS-955176: Integrated Model of Polymorphic Antiviral Responses

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BACKGROUND

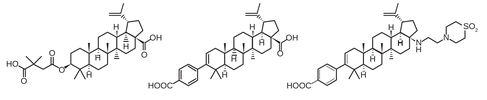
- HIV-1 maturation inhibitors (MIs) disrupt the final step in the HIV-1 protease processing of the Gag polyprotein — the cleavage between capsid (CA) and spacer peptide 1 (SP1) — leading to the production of non-infectious virus.
- BMS-955176 is a second-generation MI currently in Phase IIb, with improved antiviral activity towards Gag polymorphism-containing viruses resistant to the first-generation MI, bevirimat (BVM) (Table 1).[1–3]
- This study was conducted to understand the underlying mechanistic reasons for improvements to polymorphic coverage, using three different MIs (Figure 1).

Table 1: Iterative improvement of MIs against Gag polymorph-containing viruses

Polymorphic SDM	NFV		BVM		BMS-1		BMS-955176	
	EC_{50}	FC	EC_{50}	FC	EC_{50}	FC	EC_{50}	FC
WT	4.3	1.0	10	1.0	16	1.0	1.9	1.0
V362I	4.3	1.0	74	7.2	213	14	4.5	2.4
Q369H	n.d	n.d	7.0	0.7	n.d	n.d	1.9	1.0
V370A	4.1	1.0	552	54	233	15	2.7	1.4
V370M	4.5	1.0	1810	177	>4000	>250	2.8	1.5
ΔV370	5.2	1.2	>10000	>1000	>6000	>375	13	6.8
V370A/ Δ371T	5.7	1.3	1114	109	>10000	>625	6.6	3.5
T371A	n.d.	n.d.	40	3.9	28	1.8	2	1.1
ΔT371	2.0	0.5	77	7.5	292	19	7.3	3.8

EC_{50} values (nM) assayed with the HIV-1 laboratory strain NLRepRlucP373S containing the indicated SDMs; FC = EC_{50} (SDM)/ EC_{50} (WT), data from multiple measurements (N ≥3). The data provided are the mean of at least triplicate values with a mean CV of 83 +/- 50% for all the experiments, and all CV values <200%. BVM, bevirimat; CV, coefficient of variation; n.d., not determined; FC, fold change; NFV, nelfinavir; SDM site directed mutant; WT, wild-type.

Figure 1: Structure of MIs used in this study



EGMI BVM **EGMI BMS-1** **2nd generation MI BMS-955176**

BVM, bevirimat; EGMI, early-generation MI; MI, maturation inhibitor.

METHODS

- Antiviral activities toward wild-type (WT) and Gag polymorph-containing viruses were studied using single- and multiple-cycle replication formats.
- Gag CA/SP1 cleavage kinetics of WT and polymorphic virus-like particles (VLPs) were determined by a novel LC/MS assay.
- A radiolabel binding assay was used to determine VLP/MI affinities and off rates (Table 2).
- Cleavage, binding and antiviral data were used to create an integrated model for calculating rates of CA/SP1 cleavage.

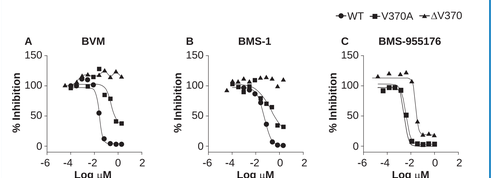
Table 2. Equilibrium binding affinities (K_d values) of BMS-955176 and BVM toward HIV-1 VLPs

VLP	BVM				BMS-955176*				BVM/BMS -955176 K_d ratio
	K_d, nM	SD	N	FC versus WT	K_d, nM	SD	N	FC versus WT	
WT	5.4	2.6	5.0	1.0	3.2	0.6	5	1.0	1.7
V370A	49.2	12.5	5.0	9.1	6.5	2.2	5	2.0	7.5
ΔV370	259	131	5.0	48	33.6	5.9	5	10	7.7

BVM and BMS-955176 equilibrium binding affinities (Kd values) toward HIV-1 VLPs determined by competition displacement of [^{3}H]-BMS-955176* (C20 reduced form of BMS-955176) by other MIs.
BVM, bevirimat; FC, fold change; MI, maturation inhibitor; VLP, virus-like particle; WT, wild-type.

- Assay uses purified VLPs containing the indicated changes in Gag.
- MIs bind reversibly, and binding is displaced by other MIs.
- BMS-955176 binds with greater affinity to HIV-1 Gag than the early-generation maturation inhibitor (EGMI), BVM.
- Representative examples of antiviral inhibition curves in a multiple-cycle replication assay for BVM, BMS-1 and BMS-955176 towards WT, V370A and ΔV370 viruses are shown in Figure 2.
- MPI values were calculated from the plateaus in inhibition at the highest inhibitor concentration, and tabulated and normalized for multiple experiments in Table 3 using the equation MPI= (1 - (signal from the average at the two highest drug concentrations/signal from no drug control)* 100).

Figure 2: MI dose-responses in a multiple cycle antiviral assay

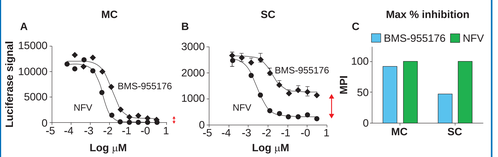


Units are in *Renilla* luciferase RLUs. In cases where there is no curve and thus no plateau *per se*, the same calculation method was employed using the two values at the highest compound concentrations.
BVM, bevirimat; MPI, maximal percent inhibition; RLU, relative light unit; WT, wild-type.

RESULTS

- In antiviral assays, EGMIs do not achieve maximal percent inhibition (100% MPI) of certain Gag-polymorphic viruses, even at saturating concentrations, resulting in low levels of breakthrough virus (partial antagonism) (Table 3).
- BMS-955176 achieves higher MPIs against these polymorph-containing viruses, which is correlated with lower FC-EC_{50} values (Figure 3).
- Mechanistic studies show that more rapid CA/SP1 cleavage of polymorphic VLPs (2–10-fold versus WT) shows some positive correlation with reduced viral sensitivity to EGMIs (Table 4, Figure 4).
- In addition, *in vitro* inhibition of WT CA/SP1 cleavage by BMS-955176 persists for longer compared with BVM (Tables 1 and 4, Figure 4).

Figure 3: BMS-955176 inhibition of ΔV370 virus in two antiviral formats



Dose–response curves for BMS-955176 (diamonds) and NFV control (circles) in the A) MC and B) SC assay toward the ΔV370 virus; vertical double-headed arrows highlight differences in residual signal between the two compounds in the two antiviral formats, C) comparison of MPI data for A and B shown as MPI of luciferase signal. Data shown are an average of three experiments. MC, multiple cycle; MPI, maximal percent inhibition; NFV, nelfinavir, a HIV protease inhibitor; SC, single cycle.

- Single-cycle format is more demanding in terms of driving to complete inhibition (Figure 3).

Table 3: Maximal percent inhibition in multiple-cycle (MC) and single-cycle (SC) assays

	MC assay									SC assay								
	WT			V370A			ΔV370			WT			V370A			DV370		
MI	MPI	SD	N	MPI	SD	N	MPI	SD	N	MPI	SD	N	MPI	SD	N	MPI	SD	N
BVM	98.4	1.0	8	65.4	3.8	4	9.2	6.3	4	82.3	2.7	15	19.0	3.5	3	-25.6	30.9	12
BMS-1	97.0	0.5	4	63.2	4.7	3	3.7	2.9	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BMS-955176	100	0.4	11	98.2	1.3	6	91.9	4.4	6	93.0	2.5	26	76.5	3.0	5	45.9	7.4	30
NFV	100	0.2	10	99.9	0.1	5	100	0.1	5	99.3	0.4	21	99.0	0.6	4	99.1	0.9	26

BVM, bevirimat; MPI, maximal percent inhibition, MC, multiple cycle; n.d., not determined; NFV, nelfinavir; SC, single cycle; SD, standard deviation; WT, wild-type.

- In both antiviral formats BVM and BMS-1 exhibit pronounced incomplete antiviral inhibition (MPI <100%) toward polymorphs, even at saturating drug concentrations.
- BMS-966176 exhibits >MPI than EGMIs in MC and SC assays against *Gag* polymorph-containing viruses (Table 3).
- Incomplete inhibition is more pronounced in SC antiviral assay (Table 3).

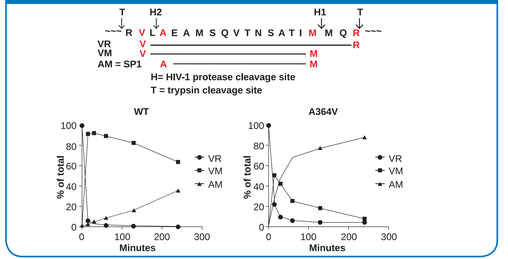
Table 4: Rates of cleavage of WT and Gag polymorphic VLPs at CA/SP1 by HIV-1 protease in vitro

Virus	Rate of CA/SP1 cleavage		FC–EC_{50} (antiviral data)	
	$k_{clv,\ obs}$ min^{-1a}	Rel $k_{cl,obs}$ (versus WT)[a]	BVM	BMS-955176
WT	0.002	1	1	1
V370A/ΔT371	0.003	2.2	109	3.5
ΔV370	0.004	2.7	>1000	6.8
V362I	0.004	2.9	7.2	2.4
V370A	0.005	3	54	1.4
A364V	0.014	9.7	>1000	759

[a] [HIV-1 protease] =270 nM, 22º C. FC data from Table 1; FC = EC_{50} SDM/EC_{50} WT; WT = NLRepRlucP373S; BVM, bevirimat; SDM, site directed mutant; VLP, virus-like particle; WT, wild-type.

- CA/SP1 cleavage rates are a function of the *Gag* polymorph; faster rates may result in reduced MI sensitivity, depending on MI[1] but rate of cleavage is not the only controlling factor in MI efficacy (Table 4, Figure 4).

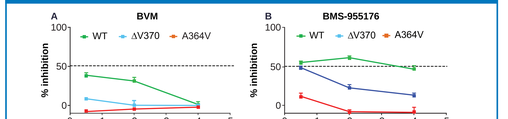
Figure 4: Rates of cleavage of WT and Gag polymorphic VLPs at CA/SP1 by HIV-1 protease in vitro



[HIV-1 protease] = 270 nM, 22°C. FC data from Table 1; FC = EC_{50} SDM/EC_{50} WT.
SDM, site-directed mutant; WT, wild-type (NLRepRlucP373S).

- Persistent inhibition of *in vitro* CA/SP1 cleavage depends on genotype and MI[1] (Figure 5).
- Persistence: WT > ΔV370 > A364V.

Figure 5. Inhibition of HIV-1 protease-mediated CA/SP1 cleavage by MIs



A) BVM, B) BMS-955176: Time-dependent inhibition of CA/SP1 cleavage of WT, A364V and ΔV370 VLPs *in vitro* as monitored by LC/MS analysis; values are an average of nine replicates; bars are SEM.
BVM, bevirimat; SEM, standard error of the mean; VLP, virus-like particle; WT, wild-type.

- Innate CA/SP1 cleavage rates and MI-specific Gag VLP affinities allow for modeling the kinetics of CA/SP1 MI cleavage inhibition using the scheme shown in Figure 6.

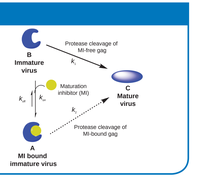
Figure 6: Scheme for inhibition of HIV-1 infectivity by MIs

Immature virus B is in equilibrium for binding to MI to produce MI-bound form A.

Model 1: HIV protease can only cleave immature virus (form B) at CA/SP1 in the absence of MIs with innate cleavage rate constant (k_f), a function of Gag polymorph.

Model 2: HIV-1 protease may also cleave CA/SP1 in MI-bound form A via cleavage rate constant (k_c) to produce mature virus form C, providing an escape mechanism for the formation of mature virus in the presence of MIs.

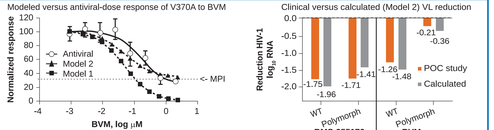
Rate constant (k_c) is a function of both MI and Gag polymorph, and is derived from inclusion of MPI values from multiple cycle antiviral MPI values (Table 3).



MI, maturation inhibitor

- Biochemical (CA/SP1 cleavage rates, MI-specific Gag affinities) and virologic (MPI) data were combined to create an integrated kinetic model to calculate rate reductions of CA/SP1 cleavage by MIs (Figure 7).
- These predictions are in accord with both preclinical antiviral potency and clinical viral load reductions as a function of MI and *Gag* polymorph.

Figure 7: Modeled versus observed antiviral responses



A) Antiviral dose–response curve for inhibition of HIV-1 Gag V370A by BVM (mean of 3 experiments, data normalized to no response = 100% activity (open circles, solid line); normalized calculated rates of CA/SP1 cleavage ($k_{cl,obs}$) are shown for Model 1 (closed squares, dotted line) and Model 2 (closed triangles, dotted line).

B) Model 2 reduction in rate of CA/SP1 cleavage of WT or V370A (surrogate for polymorphic viruses) at 300 nM MI, versus observed reduction in HIV-1 log_{10} copies/mL RNA in proof of concept (POC) studies; BMS-955176 polymorphic clinical subtype B = changes at positions Gag 362, 369, 370 or 371; BVM polymorphic clinical = changes at positions Gag 369, 370 or 371; BMS-955176 clinical POC study, 40 mg maximal median HIV-1 log_{10} RNA reduction (Study AI468002);[4] BVM clinical POC study, 250–400 mg.[2]

BVM, bevirimat; MPI, maximal percent inhibition; MI, maturation inhibitor; VL, viral load; WT, wild-type.

CONCLUSIONS

- BMS-955176 is a second-generation MI currently in Phase IIb with potency towards Gag polymorphic viruses resistant to the first-generation MI, BVM.[1,5]
- A combination of antiviral and biochemical approaches were used to understand the underlying mechanism for these spectrum differences.
- EGMIs, such as BVM and BMS-1, exhibit incomplete antiviral activity, even at saturating drug concentrations.
- BMS-955176 exhibits greater ability to maximally inhibit polymorph-containing variants, in part due to higher affinity for its Gag target.
- These data were integrated into a semi-quantitative kinetic model, which posits that, depending on MI and polymorph, some degree of HIV-mediated protease cleavage may still occur on Gag/MI complexes.
- Model outputs are in accord with *in vitro* antiviral observations and correlate with observed *in vivo* MI efficacies for both MIs.
- These findings support the further clinical development of BMS-955176 and may be useful to help guide development of newer MIs, with potential applicability to other virus systems and inhibitors.

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