Investigating the Mechanism of a Unique Human Immunodeficiency Virus-1 (HIV-1) Entry Inhibitor, MF275

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1. Abstract

Background: HIV-1 entry into cells is mediated by sequential binding of target cell CD4 and CCR5 or CXCR4 to the metastable envelope (Env) trimer of gp120-gp41 heterodimers. We determined that MF275, a single diastereomer of the small molecule entry inhibitor PF-68742, is necessary and sufficient to inhibit entry of a subset of HIV-1 strains. We investigated the mechanism of MF275.

Methods: Recombinant luciferase-expressing HIV-1 pseudotyped by wild-type (WT) or mutant HIV-1 Envs was incubated with MF275, other entry inhibitors, and/or antibodies. The virus-inhibitor mixture was added to CD4+ CCR5+ or CD4- CCR5+ target cells and luciferase activity measured.

Results: Unlike other entry inhibitors, MF275 not only reversibly inhibited the infection of CD4+ CCR5+ cells by some HIV-1 strains, but also irreversibly enhanced the infection of CD4- CCR5+ cells by others. In both cases, the strain susceptibility profiles were unique from those of CD4-mimetics, BMS-378806, and maraviroc. Furthermore, MF275 activity was not affected by mutations conferring resistance to other entry inhibitors and vice versa. In line with its activating activity, MF275 sensitized susceptible Envs to neutralization by a variety of broadly neutralizing antibodies against different epitopes. Changes in the gp120 C5 and gp41 fusion peptide (FP) and disulfide loop (DSL) regions conferred resistance to MF275 inhibition but not activation. Furthermore, sensitivity to other entry inhibitors in the presence of MF275 indicated that inhibition and activation target different conformational intermediates along the entry pathway, with the former targeting the prehairpin intermediate.

Conclusion: MF275 is unique among HIV-1 entry inhibitors. Depending on the conformation of the target Env, which appears related to the gp120-gp41 interface, MF275 mediates inhibition or activation via distinct mechanisms. Further characterization of the MF275 mechanisms and binding site/s will advance understanding of the HIV-1 entry pathway as well as assist optimization of its clinical utility as an antiretroviral in multi-class drug resistance and potentially as an adjunct to vaccines.

2. Introduction

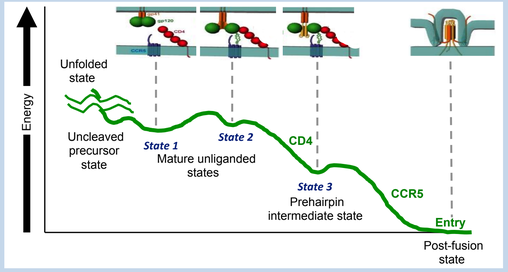


Figure 1. The HIV-1 entry pathway.

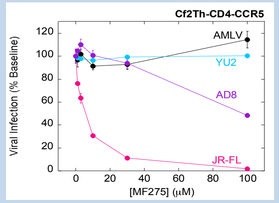
- Existing classes of entry inhibitors:
  1. CD4-mimetics (e.g. (+) (R,R) BNM-III-170)
  2. Inhibitors of CD4-induced conformational changes (e.g. BMS-378806)
  3. Chemokine receptor antagonists (e.g. maraviroc)
  4. Fusion inhibitors (e.g. enfuvirtide)
- Murray *et al.* (2010) *J. Virol.* (**1**)
  - PF-68742 is a novel small molecule inhibitor of HIV-1 entry
  - Resistance conferred by mutations in gp120 C5, gp41 FP and DSL regions, which constitute a putative gp120-gp41 interface

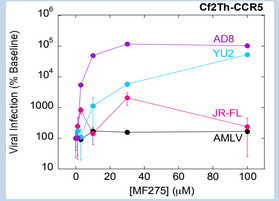
3. Methods

Recombinant luciferase-expressing HIV-1 pseudotyped by WT or mutant Envs was incubated with increasing concentrations of entry inhibitor and/or antibody. The virus-inhibitor mixture was then added to CD4+ CCR5+, CD4+ CXCR4+, or CD4- CCR5+ target cells, and luciferase activity measured 48 to 72 hours later. The level of infection relative to that in the absence of compound is reported. The means and standard deviations from triplicate samples within a typical experiment are shown.

4. Results

I. A single PF-68742 diastereomer, MF275 inhibits CD4-dependent and activates CD4-independent infection

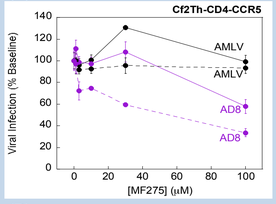


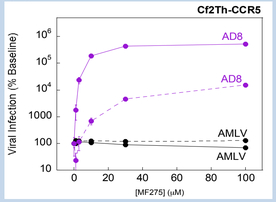


Env	Clade	IC50 (μM)
AMLV	N/A	>100
191084 B7-19	A	75.9 ± 24.1
BG505	A	74.2 ± 25.2
AD8	B	>100
BB1012	B	>100
JR-FL	B	14.5 ± 2.7
YU2	B	>100
C1086	C	76.5 ± 23.5
C5	C	91.1 ± 4.5
ce0393	C	>100
ZM109F	C	75.0 ± 15.7
3016	D	98.6 ± 1.4

Figure 3. MF275 reversibly inhibits CD4-dependent infection and irreversibly activates CD4-independent infection with inversely related susceptibility patterns unique among HIV-1 entry inhibitors. Effects of washout not shown.

III. MF275 inhibition and activation target different intermediates along the entry pathway





Inhibitor	Potentiation of MF275 inhibition		Location relative to MF275 target intermediate
	*Added **before** MF275*	*Added **after** MF275*	
BMS-378806	+	-	Upstream
Maraviroc	+	-	Upstream
Enfuvirtide	+	+	Similar

Concentrations of second inhibitor
—— None
-- -- Subinhibitory

Figure 5. (A) MF275 inhibition targets an intermediate parallel to that targeted by enfuvirtide; (B) MF275 activation requires State 1 → State 2 transition, CCR5 binding, and 6-helix bundle (6HB) formation.

II. The MF275 binding site and mechanism are unique

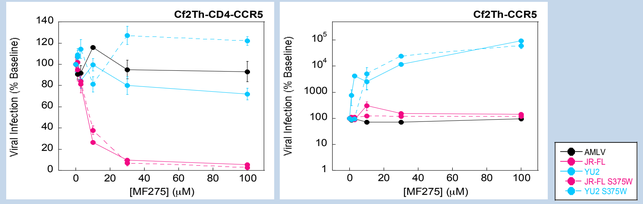


Figure 4. MF275 inhibition and activation are not affected by the S375W mutation that abrogates CD4-mimetic activity.

- Binding of radiolabelled PF-68742 to soluble gp120 is not affected by the S375W mutation (not shown)
- MF275 inhibition and activation are not affected by mutations altering the State 1 ⇔ State 2 equilibrium (not shown)

IV. MF275 inhibition and activation are mediated by two different mechanisms involving the gp120-gp41 interface

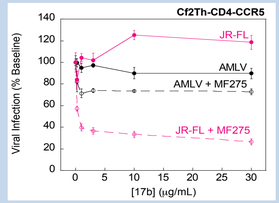


Figure 6. MF275 sensitizes Envs to broadly neutralizing antibodies by inducing exposure of the target epitopes. Other Envs (YU2, AD8) and antibodies (19b, 4e10) not shown.

	Ab	Epitope
1	sCD4	CD4bs
2	VRC01	CD4bs
3	17b	CD4i
4	PG9	V1/V2/V3
5	19b	V3
6	4e10	MPER
7	35O22	Gp120-gp41, Glycan
8	2G12	Glycan
9	VRC34	FP
10	PGT151	Gp120-gp41

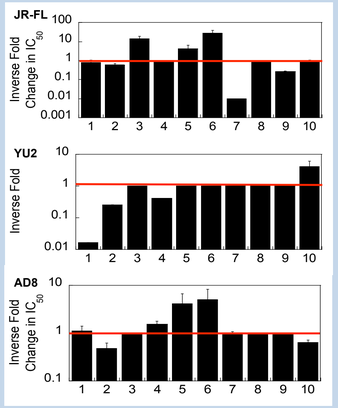
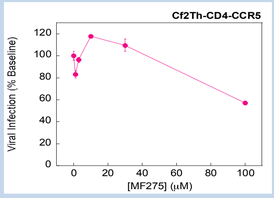


Figure 7. MF275 inhibition and activation are associated with distinct conformational changes between Envs. Y-axis = inverse of fold change in IC50 of antibody in the presence of MF275. Similar results were obtained with CD4-independent infection (not shown).

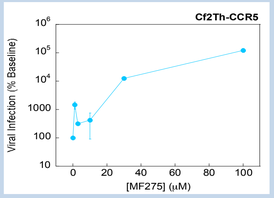
- Gp120-gp41 interface mutations alter Env sensitivity to VRC34, 4e10, enfuvirtide, and BMS-378806 (not shown)
- MF275 induces a different set of conformational changes in gp120-gp41 interface mutants compared to WT (not shown)

Figure 8. Gp120-gp41 interface mutations do not affect binding. Gp120 C5, gp41 FP (T529A shown here) and DSL mutations are resistant to MF275 inhibition but not activation.

Inhibition (JR-FL)



Activation (YU2)



5. Conclusions

- Characterized potency, breadth, and conformational changes of a PF-68742 diastereomer, MF275 → unique among HIV-1 entry inhibitors
- MF275 mediates reversible inhibition and irreversible activation via different mechanisms associated with distinct conformational changes
- Whether it contributes to the MF275 binding site, the putative gp120 C5-gp41 DSL interface appears to play an important role in the dichotomy between its inhibitory and activating mechanisms

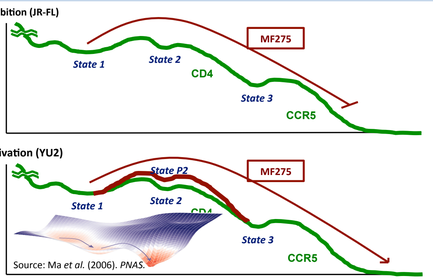


Figure 9. Proposed mechanisms for MF275 inhibition of CD4-dependent infection and activation of CD4-independent infection.

6. Future Directions & Significance

Future directions:
- Binding assay with WT and mutant soluble gp120, AD8-SOSIP
- SmFRET analysis of effects of MF275 on the State 1 ⇔ State 2 ⇔ State 3 equilibrium

Scientific significance:
- Better understanding of entry pathway as an energy landscape with balance between entry and inhibition
- Functional significance of gp120-gp41 interface

Clinical significance:
- Structure-activity optimization
- Novel class of ART: multi-class resistance, vaccine adjunct (**2**)

7. Acknowledgements

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8. Selected References

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