Two Distinct Mechanisms of Resistance to Allosteric HIV-1 Integrase Inhibitors

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Introduction

The quinoline-based allosteric HIV-1 integrase (IN) inhibitors (ALLINIs) are promising candidates for clinically useful antiviral agents. Studies using these compounds have highlighted the role of IN in both early and late stages of virus replication. ALLINIs target the clinically unexploited LEDGF/p75 binding site at the IN catalytic core domain (CCD) dimer interface and potently inhibit HIV-1 replication in cell culture. The carboxylic acid of ALLINI hydrogen bonds with one IN subunit, potentially mimicking the interaction with LEDGF/p75. The quinoline-ring, another key structural feature of ALLINIs, engages the other subunit of IN through hydrophobic interactions. Consequently, the quinoline-based ALLINIs inhibit IN-LEDGF/p75 binding and promote aberrant IN multimerization by stabilizing interacting subunits. This multimodal mechanism of action of ALLINIs concordantly results in cooperative inhibition of HIV-1 replication in cell culture.

Selection of viral strains emerging under the selective pressure of the ALLINI BI-D, a potent inhibitor of HIV-1 replication, has revealed mutations at the IN dimer interface near the inhibitor binding site. Here we have investigated A/T124D and H171T IN substitutions that confer resistance to BI-D.

Our findings show that A/T124D and H171T IN substitutions cause BI-D resistance through different mechanisms. A/T124D IN mutation allows IN to resist BI-D induced aberrant multimerization. In contrast, the H171T substitution reduces the ability of BI-D to bind IN at the LEDGF/p75 binding pocket.

Experimental approaches

The EC_{50} of BI-D was determined using WT NL4-3 and NL4-3 with a T124D or H171T IN substitution.

Size exclusion chromatography was used to determine how the A124D or H171T IN mutations affect the ability of BI-D to promote aberrant IN multimerization.

Affinity pull-downs, x-ray crystallography and free energy binding calculations were used to determine the affects of either substitution on the ability of IN to bind LEDGF/p75.

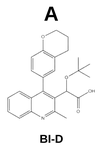
Homogenous time resolved fluorescence (HTRF)-based assays were utilized to determine the affects of the A124D IN substitution on the ability of BI-D to inhibit LEDGF/p75 binding.

Surface plasmon resonance (SPR) was used to measure the ability of A124D and H171T IN mutants to bind BI-D.

Replication capacity of $NL4.3_{T124D_IN}$ and $NL4.3_{H171T_IN}$ were determined relative to WT NL4.3 to compare viral replication capacity.

HIV-1 IN T124D and H171T confer resistance to BI-D

A



BI-D

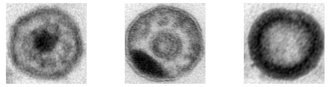
B

	EC_{50} (μM)	Fold Change
WT	0.090 ± 0.03	--
T124D IN	0.604 ± 0.14	6.7
H171T IN	6.110 ± 0.63	67.8

Fig 1. (A) Chemical structure of BI-D; (B) Resistance of NL4.3 T124D and H171T IN substitutions to BI-D.

Virion morphology

A



B

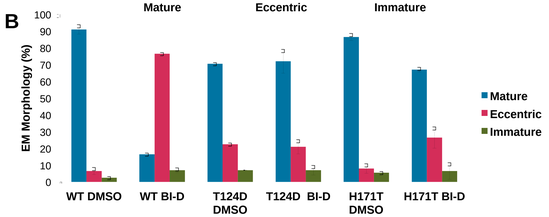


Fig 2. (A) Representative images of mature, eccentric and immature virion morphologies; (B) Quantitation of morphology frequencies with BI-D treatments.

Differential multimerization of WT, A124D and H171T INs in the presence of BI-D

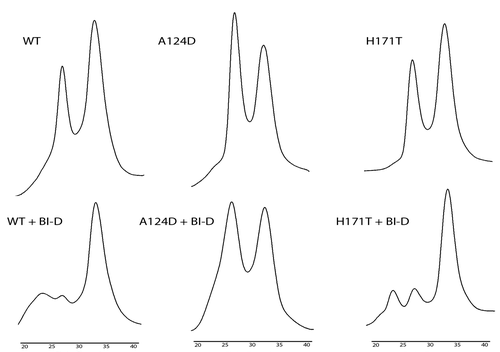


Fig 3. Size exclusion chromatography demonstrating that BI-D induces higher order oligomerization of WT and H171T INs but not A124D IN.

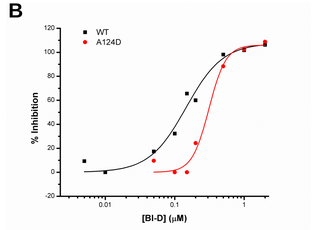
A124D and H171T differently affect IN-LEDGF/p75 binding

A

IN-LEDGF/p75 Binding

	Kd (μM)	Fold Change
WT IN	0.108 ± 0.009	--
A124D IN	0.104 ± 0.015	0.96
H171T IN	1.056 ± 0.011	9.78

B

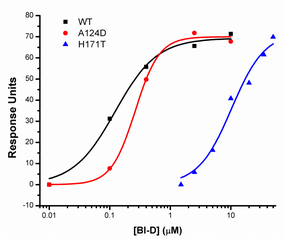


BI-D Inhibition of IN-LEDGF/p75 Binding

	IC_{50} (μM)
WT IN	0.145 ± 0.02
A124D IN	0.311 ± 0.04
H171T IN	ND

Fig 4. (A) Apparent K_d of IN-LEDGF/p75 binding with WT, A124D and H171T INs. (B) The inhibition of LEDGF/p75 binding by BI-D. At relative concentrations, H171T did not bind LEDGF/p75.

BI-D binding to IN



BI-D Inhibition of IN-LEDGF/p75 Binding

	K_D (μM)	Fold Change
WT IN CCD	0.123 ± 0.3	--
A124D IN CCD	0.265 ± 0.2	2.2
H171T IN CCD	10.29 ± 2.3	83.7

Fig 5. BI-D binding to WT, A124D and H171T IN determined by SPR.

Free energy calculations

Receptor	ΔG_{bind} (calculated)	ΔG_{bind} (experimental)
H171-doubly-protonated	-11.19	-10.98
H171-Nd-protonated	-10.12	
H171-Ne-protonated	-5.9	
H171T mutant	-6.68	NA

Fig 6. Free energy calculations of the doubly protonated form and the N_d-protonated tautomer states of H171 and H171T.

Similar binding orientations of BI-D to WT and H171T IN CCDs

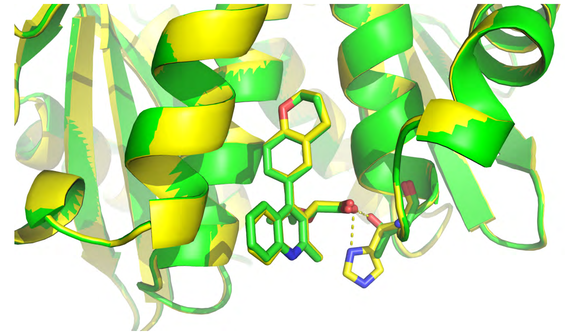


Fig 7. Crystal Structure overlay of BI-D bound to WT (yellow) or H171T (green) IN CCD dimer.

Conclusions

Here we show that the reported IN resistance mutations to BI-D, A/T124D and H171T exhibit two distinct mechanisms of resistance.

A124D IN substitution overcomes BI-D inhibition by reducing the ability of BI-D to promote aberrant IN multimerization. This substitution did not significantly affect BI-D binding to A124D IN nor BI-D inhibition of A124D IN-LEDGF/p75 binding. However, viruses with the A/T124D mutation did have significantly reduced replication capacity due to increased aberrant core morphology in mature virions in the absence of BI-D.

Conversely, the H171T substitution adversely affects BI-D binding to H171T IN and concomitantly compromises IN-LEDGF/p75 binding. Additionally, the free energy of binding of BI-D to H171T IN was significantly reduced compared to WT IN.

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