Poster 540

Late Stage Integrase-LEDGF Inhibitors Mode of Action and Acquisition of Resistance

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Abstract

Background:
We and others described a new class of HIV-1 integrase (IN) allosteric inhibitors that bind to the LEDGF binding pocket of IN and inhibit IN-LEDGF interaction. Mutabilis developed very active compounds of this class. Designed to interfere with IN-LEDGF interaction during integration, the major impact of these inhibitors was found on virus maturation, causing a reverse transcription defect in target cells independent of LEDGF and linked to compound-induced IN oligomerization. We wanted to determine whether viral RNA packaging was affected and study IN mutants resistant to these inhibitors and interaction of IN with other cofactors such as Transportin (TNPO3) and VHL-binding protein 1 (VBP1).

Methods:
Virus RNA was isolated, submitted to northern blot and t-RNA primer extension. Interaction of IN with cofactors, or IN oligomerization, were studied by HTRF. Resistance mutations were identified by serial passages with increasing concentration of inhibitors during NL4-3 infection of MT4 cells.

Results:
Wild-type level of HIV-1 genomic RNA was packaged in defective virions in dimeric state, tRNA^{lys3} primer for reverse transcription was properly placed and could be extended. Reverse transcriptase from defective virions was fully active. Several mutations in IN resulted in variable resistance to this class of inhibitors. The most detrimental mutation was T174I with fold change (FC) in EC50 antiretroviral activity over 200. Other mutations were selected, A128T and N222K were the most frequent ones, with much lower FC values around 4-5. These mutants were impaired for interaction with LEDGF to various extents but conserved wt strand transfer activity and IN-IN subunit interaction.. Ternary complexes could be formed between IN LEDGF and TNPO3, and between IN VBP1 and TNPO3. IN was the link between LEDGF and TNPO3. In the IN-VBP1-TNPO3 complex, each protein could interact with its two other partners.

Conclusions:
Inhibition of reverse transcription promoted by IN-LEDGF inhibitors in target cells likely reflects the mislocalization of the components in the aberrant virus particle. Most of the resistance mutations were in the LEDGF-binding pocket, affecting the affinity of the inhibitors for IN. N222K, in the C-ter domain of IN is a notable exception. The genetic barrier to resistance to this first generation of IN-LEDGF allosteric inhibitors is similar to that observed for Nevirapine. We are developing a second generation of inhibitors with improved resistance profile.

IN-LEDGF Allosteric Inhibitors (LEDGINs, ALLINIs, NCINIs, MINIs, INLAIs): consensus on mode of action, not on the name!!!

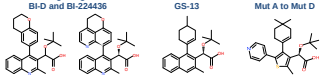
- ✓ bind to the LEDGF binding pocket of HIV-1
- ✓ display dual inhibition of HIV-1 replication with:
  - o weak effect at integration in target cells linked to inhibition of IN-LEDGF interaction
  - o major impact on virus maturation and infectivity at late stage in producer cells linked to IN multimerization promoted by compound binding to IN independently of LEDGF
- ✓ Virus particles produced in the presence of these inhibitors are deformed with the ribonucleoprotein located outside the virus core

References:
Christ et al., Nat. Chem. Biol. 2010; Fenwick et al., 18th CROI Boston 2011; Christ et al., AAC 2012; Jurado et al., PNAS 2013; Le Rouzic et al., 2013; Balakrishnan et al., PLoS One 2013; Sharma et al., PLoS Path. 2014; Gupta et al., JBC 2014 and many others…

Three questions:

1) **Is there a defect in RNA packaging in HIV-1 virions exposed to INLAIs during virus production?**
2) **What are the IN mutations and mechanisms that promote resistance to INLAIs?**
3) **Are there ternary complexes formed between HIV-1 Integrase with IN cellular cofactors LEDGF, Transportin TNPO3 or VBP1 (VHL-binding protein 1)**

Compounds studied



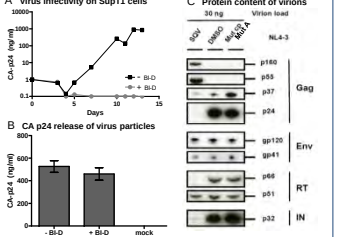
Boehringer Ingelheim BI-D and BI-224436 — **BI-D** WO2009/062285; **BI-224436** WO2012/003497

Gilead Sciences GS-13 — **GS-13** WO2012/003497

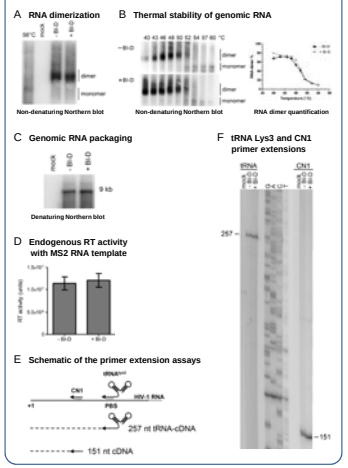
Mutabilis Mut A to Mut D — **Mut A** WO2014/053666

Part I- HIV-1 NL4-3 produced in the presence of INLAIs

Infectivity defect (A) with normal p24 release (B), normal Gag maturation and protein content (C)



After BI-D treatment, no defect in genomic RNA dimerization (A) and thermal stability (B), RNA packaging (C), endogenous RT activity (D) and t-RNA Lys3 priming of RT (E-F)



Part II- Studies on IN polymorphism and mutations that may promote resistance to Integrase-LEDGF Allosteric Inhibitors (INLAIs)

INLAIs IC50 IN-LEDGF interaction, AC50 IN-IN multimerization (A) EC50 ARV activity, Cytotoxicity, and Selectivity Index (B)

A

Compound	IC50 (nM) CCD/RD	IC50 (nM) IN/LEDGF	AC50 (nM) IN-IN (NL4-3)
Mut A	67	89	46
Mut B	32	12	16
Mut C	82	54	22
Mut D	420	180	34
BI-D	66	49	36
BI-224436	77	68	25
GS-13	370	160	77

B

Compound	EC50 (nM) HXB2	EC50 (nM) NL4-3	CC50 (µM) (MT4)	Selectivity Index (MT4)
076	2	3	38	14615
Mut A	12	31	42	1355
Mut B	2	3	44	13750
Mut C	7	15	75	5000
Mut D	18	45	140	3111
BI-D	42	190	150	789
BI-224436	20	45	200	4444
GS-13	7	19	18	1055

- Activity on IN-IN > IN-LEDGF
- Correlation with ARV activity
- Mut B most potent compound

- ARV activity in nanomolar range
- High Selectivity index
- Mut B most potent ARV activity, equivalent to DTG

ARV activity of INLAIs on 124/125 polymorphic viruses (A) and on resistant viruses (B)

A

Compound	EC50 (nM) NL4-3	Polymorphism 124/125 AA	TT	AT	TA	EC50 NL4-3/MT4 (Fold shift / wt) NA	NT	GA	ST
EVG	2	1	1	1	0.7	1	1	1	0.4
Mut A	31	48	1	0.3	52	31	1	ND	ND
Mut B	3	1	1	1	1	2	1	3	1
Mut C	15	0.4	1	0.6	2	2	1	1	1
Mut D	45	0.6	1	0.8	1	1	1	2	1
BI-D	190	0.4	1	0.4	0.5	2	1	1	1
BI-224436	45	0.6	1	0.7	0.8	0.6	1	1	1
GS-13	19	0.8	1	1	1	5	2	ND	ND

Fold shift color code: 0-1 | 1-5 | 5-10 | 10-50 | >50

All compounds except Mut A conserved full activity against all polymorphisms.
Mut A does not tolerate an A residue at position 125

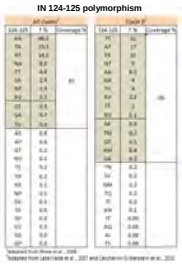
B

Compound	EC50 (nM) NL4-3	Resistance mutations EC50 NL4-3/MT4 (Fold shift / wt) A128T	Y99H	L102F	H171Q	H171T	T174I	N222K
EVG	2	1	1	1	1	1	1	1
Mut A	31	11	16	19	4	25	1600	ND
Mut B	3	2	4	244	5	69	313	3
Mut C	15	5	2	123	1	12	12	3
Mut D	45	7	2	13	1	7	15	2
BI-D	190	1	4	35	ND	25	225	ND
BI-224436	45	5	2	41	4	38	544	2
GS-13	19	26	11	51	5	22	688	ND

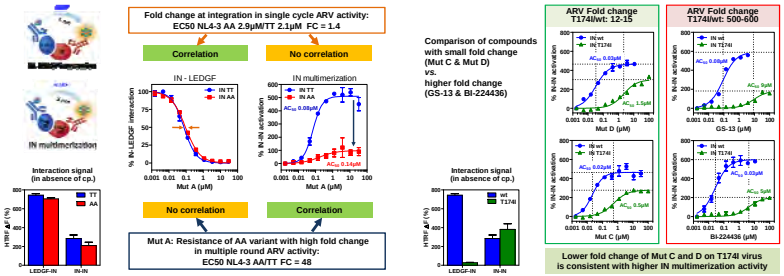
Fold shift color code: 0-4 | 4-10 | 10-20 | 20-50 | >50

Most detrimental mutations: T174 I> L102F> H171T
Mut D and Mut C: improved resistance profile
EVG and INSTIs: fully conserved activity

IN 124-125 polymorphism and resistance mutations that may affect binding of INLAIs to the LEDGF-binding pocket of IN

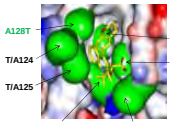


Resistance of the 124/125 AA virus to Mut A correlates with an IN-multimerization defect and not with effect on IN-LEDGF inhibition



Resistance mechanism linked to T174I virus: Impaired binding affinity strongly impacts IN multimerization

INLAI-resistance mutations in the compound-binding site



INLAI-resistance mutation in CTD N222K

Acknowledgments for Supports

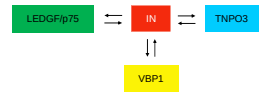
EF2 grant agreement 305127

Conclusions

1) **In virus produced in the presence of INLAI there was no defect in packaging of the genomic RNA, nor in tRNA^{lys3} priming.**
2) **Polymorphic positions 124/125 in HIV-1 IN sequence may affect the activity of some INLAI compound, mostly by IN-multimerization defect not by IN-LEDGF interaction.**
3) **The most detrimental resistance mutation T174I affects strongly both compound binding affinity and the extent of IN multimerization.**
4) **The most detrimental INLAI-resistant viruses are defective in replication capacity.**
5) **There was no antagonism in vitro of INLAI compounds with other classes of ARV drugs.**
6) **IN/p75/TNPO3 form a ternary complex with IN being the link between p75 and TNPO3.**
7) **IN/VBP1/TNPO3 form a ternary complex each protein interacts with the two others.**
8) **No ternary complex between IN/p75/VBP1. p75 outcompetes VBP1 for binding to IN.**

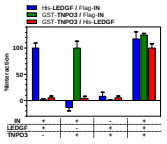
Part III- HIV-1 IN and cellular cofactors ternary complexes

IN / LEDGF(p75) / Transportin (TNPO3) / VBP1 (VHL-binding protein) "Menage a trois"



LEDGFp75 / TNPO3 / VBP1 have been found to interact with HIV-1 Integrase in yeast two-hybrid screen (collaboration with JC. Rain Hybrigenics)

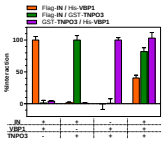
Ternary complex LEDGF(p75) / IN / Transportin (TNPO3) detected by HTRF



In this ternary complex, IN interacts with TNPO3 and p75, but no interaction detected between p75 and TNPO3



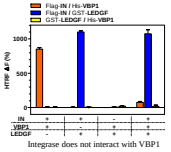
Ternary complex VBP1 / IN / Transportin (TNPO3) detected by HTRF



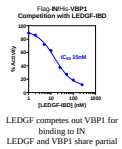
In this ternary complex each protein interacts with the two other partners



No ternary complex between Integrase / LEDGF / VBP1



Integrase does not interact with VBP1 in the presence of LEDGF



LEDGF competes out VBP1 for binding to IN
LEDGF and VBP1 share partial binding site on IN

