No Antagonism or Cross-Resistance Observed Between Islatravir and Lenacapavir

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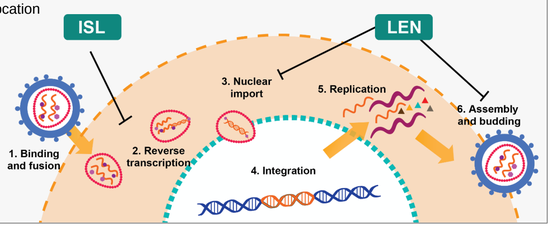
Background

Islatravir (ISL) and Lenacapavir (LEN) target multiple stages of the HIV replication cycle

- ISL is a nucleoside reverse transcriptase translocation inhibitor (NRTTI) that inhibits HIV-1 reverse transcriptase (RT) by multiple mechanisms, including inhibition of translocation and delayed chain termination
- LEN is a novel capsid (CA) inhibitor that inhibits multiple points in the HIV-1 replication cycle, including capsid-mediated nuclear uptake of proviral DNA, virus assembly and release, and capsid core formation
- The combination of ISL and LEN has the potential to be a highly-effective treatment option for HIV-1
- Here, several in vitro studies are described which support the combination of ISL and LEN for the treatment of HIV-1 infection



Methods

Antiviral activity was determined in MT4 cells which express green fluorescent protein upon infection (MT4-GFP cells)

- Antiviral activity of the combination of ISL with LEN was tested across a range of concentrations using wild-type (WT) R8 (subtype B) virus. Cytotoxicity was also assessed in the same cells using the CellTiter-Glo® assay (Promega). Analysis of the combined antiviral activities and cytotoxicity was conducted using the MacSynergy II 3-dimensional model for statistical evaluation of drug-drug interactions
- The effects of substitutions in RT and CA on antiviral activity of ISL and LEN was tested using WT R8 virus or resistance-associated variants prepared by site-directed mutagenesis. Fold-change compared to WT was used to assess antiviral activity across compounds and variants

Static resistance selection method for ISL/LEN combinations

- WT R8 virus or RT M184I R8 virus was passaged in SupT1 cells
- The resultant virus was used to infect MT4-GFP cells in bulk at a multiplicity of infection of 5 in 10% normal human serum (NHS)
- Infected cells were added to plates containing compounds titrated in DMSO
- Cell/supernatant mixture was passaged to fresh plates containing uninfected MT4-GFP cells and fresh compound every 3-4 days for 12 passages

Sample plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	128-fold	64-fold	32-fold	16-fold	8-fold	4-fold	2-fold	1-fold	0.5-fold	0.25-fold	0.125-fold	No compound
B	128-fold	64-fold	32-fold	16-fold	8-fold	4-fold	2-fold	1-fold	0.5-fold	0.25-fold	0.125-fold	No compound
C	128-fold	64-fold	32-fold	16-fold	8-fold	4-fold	2-fold	1-fold	0.5-fold	0.25-fold	0.125-fold	No compound
D	128-fold	64-fold	32-fold	16-fold	8-fold	4-fold	2-fold	1-fold	0.5-fold	0.25-fold	0.125-fold	No compound
E	128-fold	64-fold	32-fold	16-fold	8-fold	4-fold	2-fold	1-fold	0.5-fold	0.25-fold	0.125-fold	No compound
F	128-fold	64-fold	32-fold	16-fold	8-fold	4-fold	2-fold	1-fold	0.5-fold	0.25-fold	0.125-fold	No compound
G	128-fold	64-fold	32-fold	16-fold	8-fold	4-fold	2-fold	1-fold	0.5-fold	0.25-fold	0.125-fold	No compound
H	128-fold	64-fold	32-fold	16-fold	8-fold	4-fold	2-fold	1-fold	0.5-fold	0.25-fold	0.125-fold	No compound

8 replicates for every concentration

2-fold titration of compound concentration relative to IC_{50} of each compound in MT4-GFP cells with 10% NHS

↓

Monitor for breakthrough by counting the # of cells expressing GFP

↓

Genotype RT and CA at passage 12

Results

ISL with LEN demonstrated additive inhibition of HIV-1 replication with no evidence of antagonism

Table 1. Combination antiviral activity and cytotoxicity results for ISL with LEN

Combination	Synergy/antagonism volume (nM²%; n=4)	
	Antiviral activity	Cytotoxicity
ISL + LEN	0.31/-4.69	12.93/0

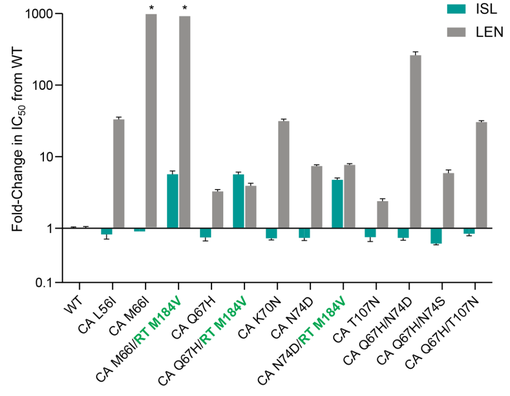
Synergy/antagonism volumes are 95% confidence values and can be interpreted as follows: Synergistic >50, Additive -50 to 50, Antagonistic <-50.

ISL exhibited potent (nM) antiviral activity against published LEN resistance-associated variants[a] with or without M184V-containing RT

Figure 1. Antiviral activity of ISL and LEN against WT R8 virus and viruses encoding published LEN resistance-associated mutations in 100% NHS

Activity of ISL and LEN against WT R8 virus (left panel) and fold-change in IC_{50} of ISL and LEN against variants compared to WT (right panel) is shown.

WT IC_{50} ± standard deviation (nM)	
ISL	LEN
1.06 ±0.21 (n=40)	2.39 ± 0.54 (n=29)



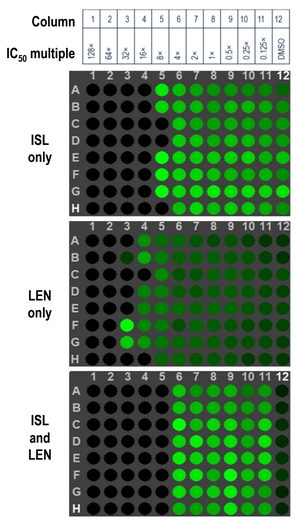
*Fold-Changes for CA M66I and CA M66I/RT M184V were >1,758.0- and >917.3-fold, respectively.

[a]Link et al. (2020) Clinical targeting of HIV capsid protein with a long-acting small molecule. *Nature* 584, 614-618.

ISL/LEN combination more effectively suppressed viral breakthrough and demonstrated a higher barrier to the emergence of resistance than either compound on its own

Figure 2. Combination resistance selection experiment with WT R8 virus

Images of plates are shown with **green** indicating wells containing GFP-expressing cells and black wells indicating cells without breakthrough viral replication (left panel). RT and CA substitutions observed in resistance selection experiments at each IC_{50} multiple are also shown (right panel).



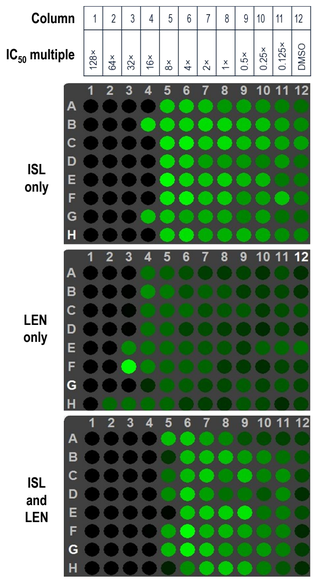
Selection with WT R8 virus

Selection compounds	IC_{50} multiple	RT substitutions observed	CA substitutions observed
ISL	8-fold	**M184I**	**None**
	4-fold	**M184I**	**H87HQ, None**
	2-fold	**M184I,** M184MIV	**H87HQ, None**
	1-fold	**M184MI, M184I**	**None**
	0.5-fold	D67DN, **M184MI, M184I, None**	**H87HQ, None**
	0.25-fold	**M184MI, None**	**H87HQ, None**
	0.125-fold	**None**	**None**
LEN	32-fold	**None**[b]	T54TN[a], **M66I**, N74D[a]
	16-fold	**None**[b]	A14AT[a], Q67H[a], H87Q[a], **H87HQ**
	8-fold	**None**[b]	**H87HQ**
	4-fold	**None**	**None**
	2-fold	**None**	**H87HQ**
	1-fold	**None**	**None**
	0.5-fold	**None**	**None**
	0.25-fold	**None**	**None**
	0.125-fold	**None**	**None**
ISL/LEN	4-fold	**M184I, M184MIV,** E203EK	**H87HQ, None**
	2-fold	**M184I, M184MIV**	**H87HQ, None**
	1-fold	**M184MI**	**None**
	0.5-fold	**None**	**None**
	0.25-fold	**None**	**None**
	0.125-fold	**None**	**None**

Substitutions are depicted by the single letter amino acid (AA) code within the reference sequence followed by the AA position number and then the single letter AA codes predicted by the sequencing analysis. Multiple AA codes are shown if a mixture was observed in sequencing.
Substitutions shown in bold font represent those seen in more than one well across all IC_{50} multiples on each plate.
[a]T54TN was observed with N74D in a single well. A14AT was observed in a single well with H87HQ. H87Q was observed in a single well with Q67H.
[b]E28K, R277K, M357T, and N418S RT substitutions were observed in a few wells in the LEN alone selection experiment in the same wells as M66I or Q67H CA substitutions and are not considered relevant to LEN.

Figure 3. Combination resistance selection experiment with RT M184I R8 virus

Images of plates are shown with **green** indicating wells containing GFP-expressing cells and black wells indicating cells without breakthrough viral replication (left panel). There was no reversion of M184I observed and no additional ISL resistance-associated substitutions were observed. CA substitutions observed in resistance selection experiments at each IC_{50} multiple are also shown (right panel).



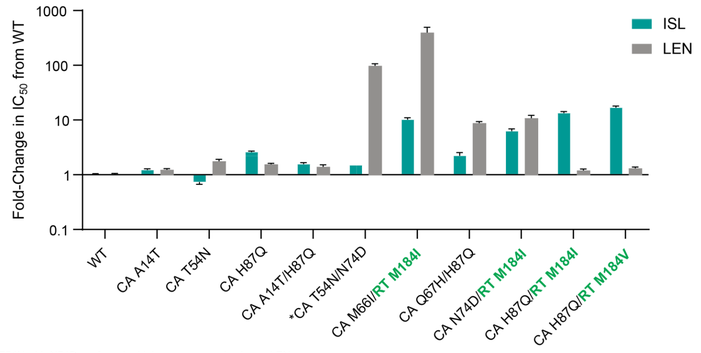
Selection with RT M184I R8 virus

Selection compounds	IC_{50} multiple	CA substitutions observed
ISL	16-fold	**None**
	8-fold	**None**
	4-fold	**None**
	2-fold	**None**
	1-fold	**None**
	0.5-fold	**None**
	0.25-fold	**None**
	0.125-fold	**None**
LEN	64-fold	**M66I**
	32-fold	**M66I, N74D**
	16-fold	**N74ND, None**
	8-fold	**None**
	4-fold	**None**
	2-fold	**None**
	1-fold	**None**
	0.5-fold	**None**
	0.25-fold	**None**
	0.125-fold	**None**
ISL/LEN	8-fold	**None**
	4-fold	**None**
	2-fold	**None**
	1-fold	**None**
	0.5-fold	**None**
	0.25-fold	**None**
	0.125-fold	**None**

Substitutions are depicted by the single letter amino acid (AA) code within the reference sequence followed by the AA position number and then the single letter AA codes predicted by the sequencing analysis. Multiple AA codes are shown if a mixture was observed in sequencing.
Substitutions shown in bold represent those seen in more than one well across all IC_{50} multiples on each plate.

No novel single mutation substantially impacted the potency of ISL or LEN

Figure 4. Antiviral activity of ISL and LEN against emergent variants from combination resistance selection experiments in 100% NHS



*T54N with N74D conferred a higher potency reduction to LEN.

Conclusion

Additive antiviral activity, a lack of antagonism or cross-resistance, and a high barrier to the emergence of resistance suggest that ISL and LEN can make an effective 2-drug combination for the treatment of HIV.

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