Islatravir and Ulonivirine Create a Combination With a High Barrier to Resistance In Vitro

Tracy L. Diamond, Winnie Ngo, Min Xu, Silveria Rodriguez, Jay A. Grobler, Ernest Asante-Appiah

Merck & Co., Inc., Rahway, NJ, USA

Background

- Islatravir (ISL) is an investigational nucleoside reverse transcriptase translocation inhibitor (NRTTI) that blocks human immunodeficiency virus subtype 1 (HIV-1) reverse transcriptase (RT) by multiple mechanisms, including inhibition of translocation and delayed chain termination[1]
  - ISL has pharmacokinetics suitable for a long-acting oral agent[2]
  - Daily oral ISL in combination with doravirine (DOR), an approved NNRTI, has demonstrated high clinical efficacy in people living with HIV-1 (PLWH)[3,4]
- Ulonivirine (ULO) is an investigational non-nucleoside reverse transcriptase inhibitor (NNRTI) with a virologic profile similar to DOR, but with differentiated pharmacokinetics that support weekly oral dosing[5,6]
- ISL and ULO are being developed in combination for weekly oral treatment of HIV-1 to provide PLWH with an additional treatment option and to improve adherence[7,8]
- The resistance profiles of ISL and ULO as independent compounds have previously been reported[5,9]
- Here, we characterize the barrier to resistance of the combination in vitro

Methods

In vitro resistance selection for ISL or ULO alone or ISL+ULO combinations

- Wild-type (WT) HIV-1 Subtype B (strain=R8) or RT M184I R8 virus was passaged in SupT1 cells
- Passaged WT or RT M184I virus was used to infect MT4-GFP cells (MT4 cells that express green fluorescent protein driven by expression of tat and rev upon infection) in bulk at a multiplicity of infection of 5 in 10% normal human serum (NHS)
- Infected cells were added to plates containing increasing concentrations of compounds (0.125- to 128-fold IC_{50}) as indicated in **Figure 1**
- Cell/supernatant mixture was passaged to the same wells on fresh plates containing uninfected MT4-GFP cells and fresh compound every 3-4 days for 12 passages (every well is an independent experiment)

Figure 1. In vitro resistance selection schema

Sample plate layout

Fold multiple of IC_{50}

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

2-fold titration of compound concentration relative to WT IC_{50} of each compound in MT4-GFP cells with 10% NHS (ISL IC_{50} = 0.8 nM; ULO IC_{50} = 7.5 nM)

↓

Monitor for viral breakthrough (VBT) by counting the # of cells expressing GFP (VBT = ≥100 GFP-positive cells)

↓

Genotype RT at passage 12

Genotypic analysis:

- Evaluation was focused on any changes at positions previously associated with resistance to RT inhibitors: M41, E44, A62, K65, D67, T69, K70, L74, V75, F77, V90, A98, L100, K101, K103, V106, V108, Y115, F116, V118, E138, Q151, V179, Y181, M184, Y188, G190, L210, T215, K219, H221, P225, F227, M230, L234, P236, Y318
- Any changes that were observed as pure (not mixed with WT) at other locations were also included

Antiviral activity of ISL or ULO against emergent substitutions

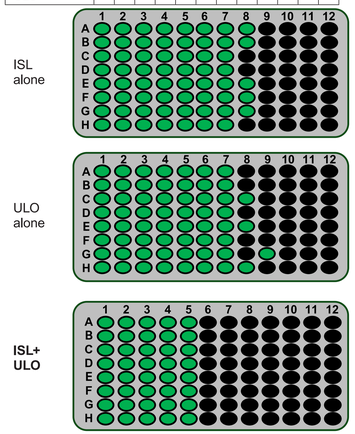
- Provirus clones were generated using WT HIV-1 Subtype B (strain=R8) backbone by site-directed mutagenesis, and virus was generated by transfection
- Potency (IC_{50}) of ISL and ULO against each variant was assessed in a multiple-cycle viral kinetic assay in MT4-GFP cells in growth media containing 100% NHS
- Antiviral activity of emergent variants is reported as fold-change compared to WT

Results

Figure 2. Combination resistance selection study with WT R8 virus

Images of plates are shown with green indicating wells with viral breakthrough (VBT) and black indicating wells without viral breakthrough (VBT) (left panel). RT substitutions observed in resistance selection study at each IC_{50} multiple are also shown (right panel).

Compound	IC_{50} multiple in each column									
ISL and/or ULO	DMSO	0.125x	0.25x	0.5x	1x	2x	4x	8x	16x	32x	64x	128x



Selection compounds	IC_{50} multiple	RT substitutions observed
ISL	8-fold	**M184I**
	4-fold	**M184I**
	2-fold	**M184I,** M184MIV
	1-fold	**M184MI, M184I**
	0.5-fold	**M184MI, M184I, None,** D67D/N
	0.25-fold	**M184MI, None**
	0.125-fold	**None**
ULO	16-fold	**V106A**
	8-fold	**V106A, F227F/L, M230M/L**
	4-fold	**V106A, V106V/A, V108I, V108V/I, Y188Y/L, M230M/L,** V90I, F116F/S, K219N, M230L, P236P/L, Y318F
	2-fold	**V106A, V106V/A, V108I, Y188Y/L,** A62A/V, H221H/Y
	1-fold	**V106V/A, V108V/I, E399E/G**
	0.5-fold	**V106V/A, None**
	0.25-fold	**None**
	0.125-fold	**None**
ISL+ULO	1-fold	**V108V/I, M184I, V189V/I,** E138K
	0.5-fold	**V108V/I, M184M/I, V189V/I**
	0.25-fold	**None**
	0.125-fold	**None**

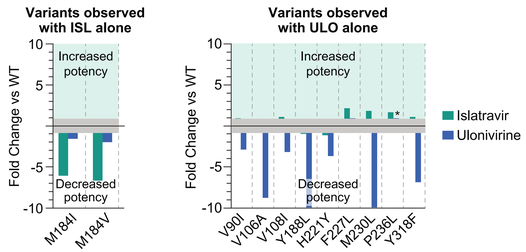
Substitutions are depicted by the single letter amino acid (AA) code within the reference sequence followed by the AA position number and then the single letter AA codes predicted by the sequencing analysis. Multiple AA codes are shown if a mixture was observed in sequencing. Substitutions shown in **bold font** represent those seen in more than one well across all IC_{50} multiples on each plate. Those in plain font were seen in 1 of 96 wells on the plate.

- ISL+ULO combination suppressed VBT at 8-fold lower concentrations of each compound compared to either ISL or ULO alone; there was no VBT above **1xIC_{50}** with the combination
- With ISL or ULO alone, M184I and V106A, respectively, were most common substitutions observed
- With ISL+ULO, V106A was not observed and V108I in combination with M184I was observed most frequently

Figure 3. Antiviral activity of ISL and ULO against variants encoding single substitutions observed in resistance selection studies with either ISL or ULO alone

IC_{50} against WT virus in 100% NHS (mean ± standard deviation)

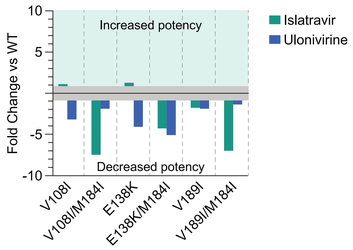
ISL	ULO
1.06 ± 0.21 nM (n = 40)	51.30 ± 29.71 nM (n = 63)



*ULO data for P236L is from Monogram PhenoSense®.

- ISL and ULO maintained potent activity against M184I (6.2- and 1.7-fold decreased vs WT) and M184V (6.8- and 2.1-fold decreased vs WT) variants
- ISL retained WT potency (2.3-fold increased to 1.3-fold decreased vs WT) against variants and from ULO alone selection studies
- ULO potency was reduced against most variants from ULO alone selection studies

Figure 4. Antiviral activity of ISL and ULO against emergent substitutions from ISL+ULO combination resistance selection studies

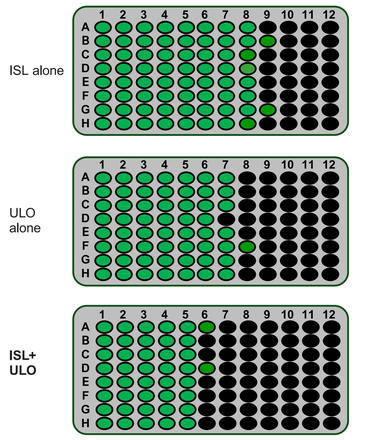


- ISL or ULO potency was not reduced by >10-fold against any of the variants selected in ISL+ULO combination resistance selection studies (impact of mutations ranged from 1.4-fold increase to 7.6-fold decrease in potency vs WT for both compounds)
- V189I has no impact on susceptibility to ULO with or without M184I (1.5- and 2.0-fold vs WT, respectively)
- ULO associated mutations V108I, E138K, and V189I did not alter ISL potency compared to what was expected with or without M184I (all within 2-fold of WT)
- The addition of M184I did not alter ULO potency compared to what was observed for each variant without M184I (all within 2-fold of WT)

Figure 5. Combination resistance selection study with virus bearing preexisting M184I substitution in RT

Images of plates are shown with green indicating wells with VBT and black indicating wells without VBT (left panel). RT substitutions observed in resistance selection studies at each IC_{50} multiple are also shown (right panel).

Compound	IC_{50} multiple in each column											
ISL and/or ULO	DMSO	0.125x	0.25x	0.5x	1x	2x	4x	8x	16x	32x	64x	128x



Selection compounds	IC_{50} multiple	RT substitutions observed
ISL	16-fold	**None**
	8-fold	**V118V/I, None**
	4-fold	**None**
	2-fold	**None**
	1-fold	**None**
	0.5-fold	**None**
	0.25-fold	**None**
	0.125-fold	**None**
ULO	8-fold	**M230I**
	4-fold	**E138K, M230I, M230L, Y318F**
	2-fold	**E138K, E138E/K, M230M/I, M230M/L**
	1-fold	**E138E/K, M230M/L, Y318Y/F,** V189V/I
	0.5-fold	**None**
	0.25-fold	**None**
	0.125-fold	**None**
ISL+ULO	2-fold	**None**
	1-fold	**E138E/K,** H221H/Y, Y318Y/F
	0.5-fold	**None**
	0.25-fold	**None**
	0.125-fold	**None**

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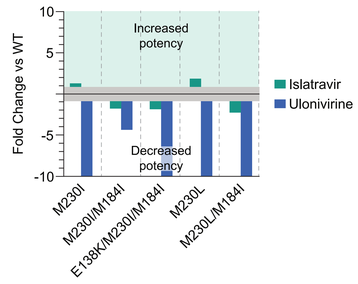
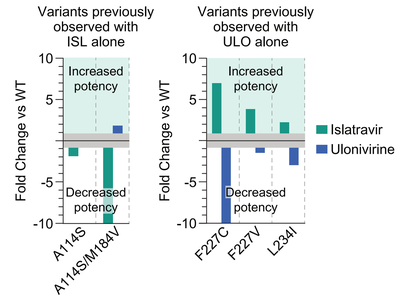
- ISL+ULO combination suppressed VBT at 4- to 8-fold lower concentrations of each compound compared to either ISL or ULO alone; there was VBT in only 2 wells with compound concentrations above 1xIC_{50} with the combination
- There was no reversion of M184I observed in any wells across the 3 plates
- With ISL alone, no additional pure (without WT) substitutions were observed
- With ULO alone, E138K, M230I/L, and Y318F emerged instead of V106A or V108I
- With ISL+ULO, E138K/M184I was most prevalent

Figure 6. Antiviral activity of ISL and ULO against novel variants from resistance selection studies starting with M184I virus



- ISL is 1.5- and 2.0-fold more potent against M230I and M230L variants and has potency reduced ≤2.4-fold against all variants containing M184I with M230I or M230L vs WT
- ULO has reduced potency >10-fold against all variants with M230I or M230L vs WT; potency was increased 2-3 fold for M230I/M184I and M230L/M184I vs. M230I or M230L alone
- The largest reduction in ULO potency was observed for the E138K/M230I/M184I variant (40.9-fold reduced ULO potency vs WT); ISL remained potent against this variant (potency was reduced 2.0-fold vs WT)

Figure 7. Antiviral activity of ISL and ULO against variants detected in previous ISL and ULO alone in vitro resistance selection studies



- The A114S/M184V variant previously observed in ISL in vitro selection studies[9] showed enhanced susceptibility to ULO (2.0-fold increased potency vs WT)
- The F227C, F227V, and L234I variants previously observed in ULO in vitro selection studies[5] showed enhanced susceptibility to ISL (2.4- to 7.1-fold increased potency vs WT)

Conclusions

- ISL+ULO combination more effectively suppressed viral breakthrough and demonstrated a higher barrier to the emergence of resistance than either compound on its own
- The compounds demonstrated complementary resistance profiles which support further clinical development of ISL/ULO as a once-weekly oral treatment for HIV-1

References

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