Islatravir Selects for HIV-1 Variants in MT4-GFP Cells That Profoundly Reduce Replicative Capacity in Peripheral Blood Mononuclear Cells

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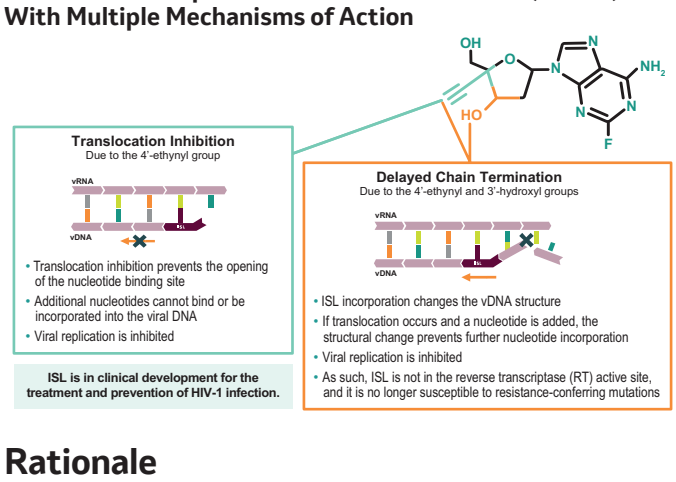
Background

Islatravir (ISL, MK-8591), a First-in-Class Nucleoside Reverse Transcriptase Translocation Inhibitor (NRTTI) With Multiple Mechanisms of Action



ISL is in clinical development for the treatment and prevention of HIV-1 infection.

Rationale

The study was conducted to extend our understanding of resistance pathways for ISL in HIV-1 subtypes

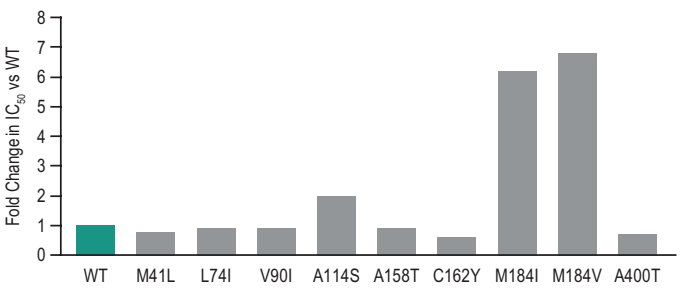
Methods

- Perform viral resistance selection studies with HIV-1 subtype A, B, and C wild-type viruses
  - WT virus was passaged with MT4-GFP [subtype B (R8)] or MT4-GFP/CCR5 [subtype A (92W026) or C (93MW959)] cells biweekly in the presence of increasing concentration of ISL
  - There were 8 replicates each for subtype A, B, and C virus with 10% FBS and 10% NHS, respectively
  - Cultures were monitored for viral breakthrough by measuring the number of GFP-positive cells at each passage, and the RT region of the viral RNA was periodically genotyped by population sequencing
- Phenotype selected variants in MT4-GFP cells and PBMCs
  - Provirus clones were generated by site-directed mutagenesis, and virus was generated by transfection
  - Potency (IC_{50}) of ISL was assessed in a multiple-cycle viral kinetic assay in MT4-GFP cells in 10% or 100% NHS
  - Viral potency in PBMCs (10% NHS) was determined using virus variants expressing GFP
- Assess replicative capacity of variants containing A114S in MT4-GFP cells and PBMCs
  - Replicative capacity was determined by monitoring the number of GFP-positive MT4-GFP cells over time and/or the production of p24 protein in infected cultures

Results

- Resistance was always associated with M184I or M184V across subtypes A, B, and C
- No other substitutions were observed in >2 of 48 selection experiments
  - Substitutions observed in 2 experiments – V90I
  - Substitutions observed in 1 experiment – E36K, E36D, M41L, L74I, V75A, A114S, A158T, C162Y, K166R, G196R, H221Y, A400T

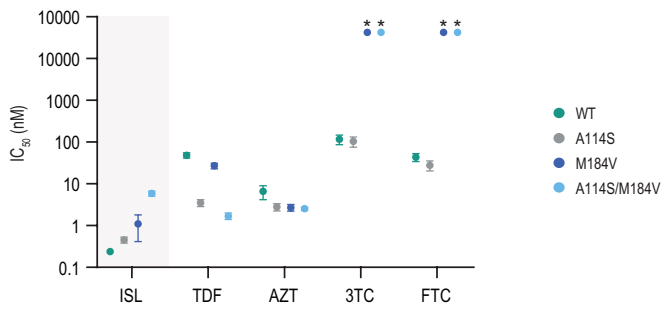
Figure 1. Effect of Single-codon Substitutions on the Susceptibility of HIV-1 Subtype B to ISL in MT4-GFP Cells (100% NHS)



- M184I and M184V were the only single substitutions that conferred >2-fold shift in potency
- Only A114S (observed in 1 selection experiment at passage 38) in combination with M184V augmented resistance conferred by M184V by >2-fold
- A114S is exceedingly rare in clinical isolates
  - 8 out of 139,609 people had A114S detected (Stanford HIV Drug Resistance Database)[1]

Activity Against A114S Variants Differentiates ISL (an NRTTI) From NRTIs

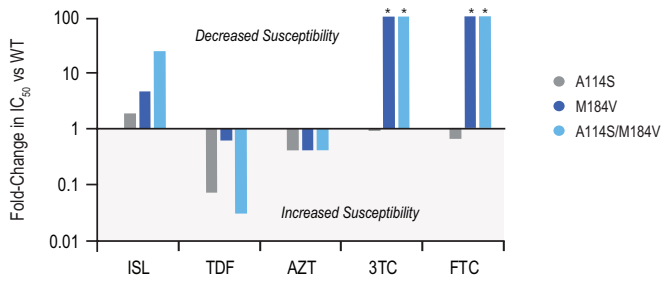
Figure 2A. Potency (IC_{50}) of ISL and NRTIs Against RT Variants in PBMCs



*IC_{50} >42000nM

- ISL displays similar or greater potency against A114S, M184V, and A114S/M184V compared to TDF, AZT, 3TC, and FTC against WT

Figure 2B. Fold Change in IC_{50} From WT for ISL and NRTIs Against RT Variants in PBMCs

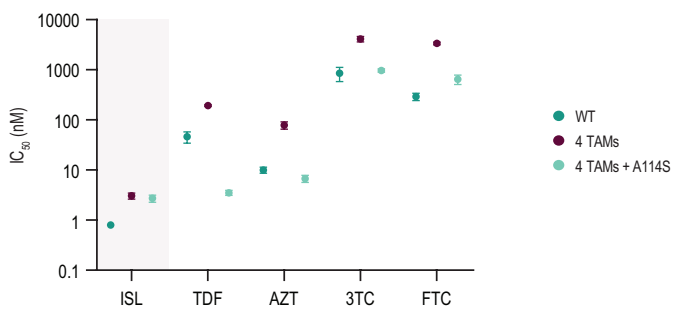


*Fold Change >Max

- In contrast to ISL, A114S maintained or increased susceptibility to the NRTIs

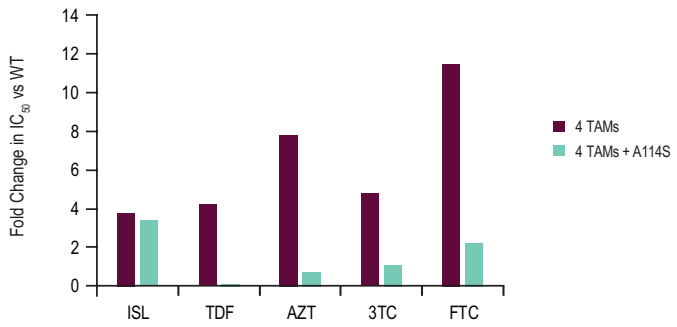
A114S Has Differential Effects on Susceptibility of ISL and NRTIs to Thymidine Analog Mutations (TAM)

Figure 3A. Potency (IC_{50}) of ISL and NRTIs Against Variants Encoding TAMs in MT4-GFP Cells (10% NHS)



- ISL remained comparable or more potent than the NRTIs against 4 TAMs

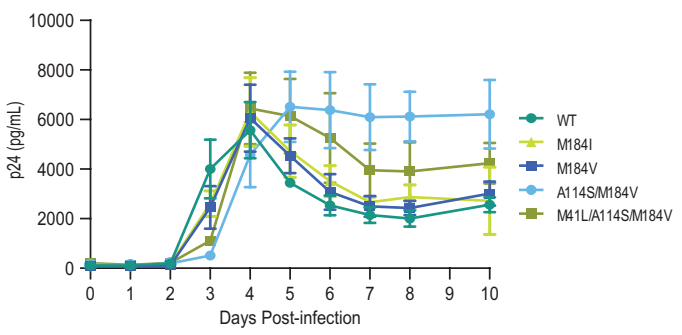
Figure 3B. Fold Change in IC_{50} from WT for ISL and NRTIs Against Variants Encoding TAMs in MT4-GFP Cells (10% NHS)



- A combination of 4 TAMs had a modest impact (<4-fold) on ISL potency
- A combination of 4 TAMs conferred >4-fold potency reductions to the tested NRTIs
- A114S mitigated resistance to NRTIs caused by 4 TAMs but did not impact susceptibility to ISL

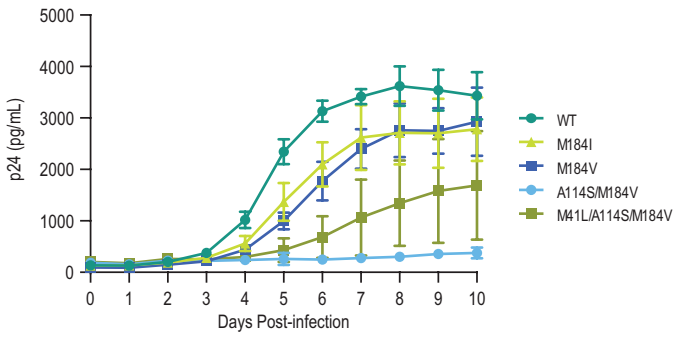
A114S-Containing Viruses With Reduced Susceptibility to ISL Have Profound Replicative Capacity Defects in PBMCs

Figure 4A. Replicative Capacity of Viruses in MT4-GFP Cells



- All viruses replicated to some extent in MT4-GFP cells as monitored by GFP (data not shown) and p24

Figure 4B. Replicative Capacity of Viruses in PBMCs



HIV-1 Variant	R_0 (10^{slope})
WT	2.30
M184I	1.94
M184V	1.91
A114S/M184V	1.08
M41L/A114S/M184V	1.48

Linear regression was performed during the phase with the largest increase in p24 for each virus to determine the R_0 value for each virus.

- No replication was observed for A114S/M184V in PBMCs (R_0 = 1.08)
- M41L/A114S/M184V had decreased replicative capacity in PBMCs (~36% of WT)

Conclusions

- In resistance selection studies, M184I and M184V were the most common substitutions to emerge across multiple HIV subtypes
- With the exception of A114S, other substitutions observed during these studies had minimal effects on viral susceptibility alone or in combination with M184 substitutions
- A114S augmented resistance conferred by M184V but had minimal effect (2-fold) on its own
- Virus containing A114S in combination with M184V did not replicate in PBMCs
- The differential impact of A114S on ISL vs NRTIs is consistent with its distinct mechanism of action

References

1. Rhee SY, et al. *Nucleic Acids Res.* 2003;31(1):298-303.
2. Grobler J, et al. Conference on Retroviruses and Opportunistic Infections 2019.
3. Rudd DJ, et al. Conference on Retroviruses and Opportunistic Infections 2020.

Abbreviations

ISL, islatravir; FBS, fetal bovine serum; GFP, green fluorescent protein; NHS, normal human serum; PBMC, peripheral blood mononuclear cells; WT, wild-type; AZT, zidovudine; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine; FTC, emtricitabine; R_0, replicative ratio; 4 TAMs, D67N/K70R/T215F/K219Q

https://bit.ly/3lMyghv

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