High *In Vitro* Resistance Barrier for the Bictegravir + Lenacapavir Combination

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Conclusions

- *In vitro*, the combination of bictegravir (BIC) + lenacapavir (LEN) demonstrated a high barrier to the emergence of resistance
- Virologic breakthrough was completely inhibited when both BIC and LEN were above the protein-adjusted 95% effective concentration ($PAEC_{95}$)
- BIC demonstrated synergistic anti–HIV-1 activity and no antagonism when combined with LEN
- These *in vitro* data suggest that a combination of BIC/LEN may have a high barrier to resistance and a high degree of forgiveness, supporting the ongoing clinical investigation of a BIC/LEN single tablet regimen (STR)

Plain Language Summary

- Doctors use bictegravir (BIC) and lenacapavir (LEN) to treat people with human immunodeficiency virus (HIV) infection
- Researchers are developing a single tablet that contains both BIC and LEN
- In this study, researchers tested whether HIV-infected cells in a lab could become resistant to BIC and LEN
- Resistance means the virus stops responding to the medicine
- They treated the cells with BIC and/or LEN, then looked for mutations (changes in the virus) that could cause resistance
- The results showed that the combination of BIC and LEN worked well to prevent resistance
- When researchers used BIC and LEN levels similar to those used in people, they found no mutations that could cause resistance in the virus
- These results support ongoing research into the combination of BIC + LEN together to treat people with HIV infection

Introduction

- A combination of BIC and LEN is being developed as an STR for people with HIV who are virologically suppressed on complex regimens[1]
  - BIC is a global guideline-recommended integrase strand-transfer inhibitor (INSTI) with a high barrier to resistance[2-5]
  - LEN is a first-in-class HIV-1 capsid inhibitor, with no documented *de novo* resistance in the absence of prior exposure[2,6,7]
- There is a strong rationale for combining BIC and LEN, based on:
  - Distinct HIV-1 targets with no cross-resistance[1,2]
  - High potency[2]
  - Little to no circulating resistance[8-10]
- The barrier to resistance and forgiveness level for this combination have not been characterized

Objective

- To characterize the *in vitro* barrier to resistance and antiviral drug interaction effects of a BIC + LEN combination

Clinical and *In Vitro* Characteristics of BIC and LEN Dosed Orally Daily

	BIC	LEN
Clinical C_{trough}[1,a]	High: ~28-fold above $PAEC_{95}$	High: ~28-fold above $PAEC_{95}$
Median half-life[2]	17.3 hours	10-12 days
Integrase-DNA dissociation half-life[12]	163 hours	N/A
Antiviral activity[13,14,b]	2-3 log	2-3 log
HIV target[5,7]	Integrase	Capsid
Clinical resistance prevalence[5,7,15]	Low	Low
Clinical resistance[2,5,7,16]	Multiple RAMs required for clinically significant resistance; rare cases of treatment-emergent INSTI-R; no documented naturally occurring RAMs	RAM patterns (Q67H + K70R; M66I) have been observed in clinical studies; no documented naturally occurring RAMs
RAM replicative capacity[7,17]	Moderate	Very low
Barrier to resistance[2,5,7,15]	High	Moderate
Cross-resistance[2,7,13]	Active against CAI-R variants	Active against INSTI-R variants

[a]Mean C_{trough} at steady state[11] of 4540 (BIC) and 108 (LEN) ng/mL are ~28-fold higher than $PAEC_{95}$ values of 162 (BIC) and 3.88 (LEN) ng/mL.
[b]Reduction in viral load.
BIC, bictegravir; CAI-R, capsid assembly inhibitor resistance; C_{trough}, trough plasma concentration; INSTI-R, integrase strand-transfer inhibitor resistance; LEN, lenacapavir; $PAEC_{95}$, protein-adjusted 95% effective concentration; RAM, resistance-associated mutation.

Methods

Virologic Breakthrough

- MT-2 cells were infected in bulk with HIV-1_{IIIB} at a multiplicity of infection (MOI) of ~0.05 and were subsequently exposed to fixed BIC and/or LEN concentrations
  - Antiretroviral concentrations were selected based on trough concentrations at steady state following once-daily maintenance doses of BIC 75 mg + LEN 50 mg as administered during the Phase 2 portion of the ARTISTRY-1 clinical study[11]
- Wells were visually inspected on a light microscope for the development of virus-induced cytopathic effect (CPE) over 35 days
- Viruses from cultures showing CPE were genotyped using Illumina MiSeq (San Diego, CA, USA) next-generation sequencing by Seq-IT (Kaiserslautern, Germany); detection threshold was ≥ 15% frequency
- Resistance-associated mutations (RAMs) in integrase and capsid:
  - INSTI resistance (INSTI-R) substitutions: T66I/A/K, E92Q/G, T97A, F121Y, Y143R/H/C, S147G, Q148H/K/R, N155H/S, R263K
  - Capsid inhibitor resistance (CAI-R) substitutions: L56I, M66I, Q67H/K/N, K70H/N/S/R, N74D/S, A105S/T, T107A/C/N/S

Combined Antiviral Activity

- MT-2 cells were infected in bulk with HIV-1_{IIIB} at an MOI of ~0.01 and were subsequently exposed to pairwise BIC and LEN concentrations
- The development of CPE at Day 5 was assessed using a luminescence assay (Cell TiterGlo; Promega, Madison, WI, USA)
- The combination effect of each tested pair of inhibitors was determined using the MacSynergy II program (University of Michigan, Ann Arbor, MI, USA)
- The calculated combination volume was used to define synergy or antagonism for the combination:
  - Highly synergistic (≥ 100), moderately synergistic (≥ 50 to < 100), additive (≥ -50 to < 50), moderately antagonistic (≥ -100 to < -50), and highly antagonistic (< -100)

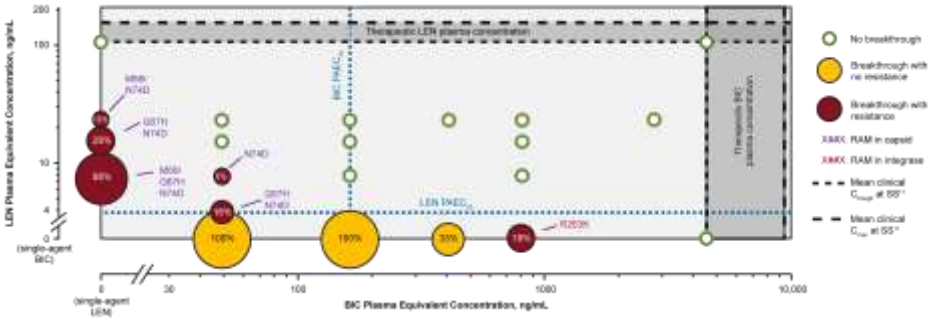
Results

Clinical and *In Vitro* Drug Concentrations

	BIC	LEN
Human serum shift[13,18]	44.0	17.4
EC_{95}, nM[13,18]	8.3	0.23
$PAEC_{95}$, nM	361	4.00
$PAEC_{95}$, ng/mL	162	3.88
Mean clinical C_{trough} at SS, ng/mL[11]	4540	108
Mean clinical C_{trough} at SS, nM[a]	10,102	112
Cell culture equivalent (C_{trough}), nM	230	6.41
Cell culture concentration range, nM	2.5-230	0.12-6.41

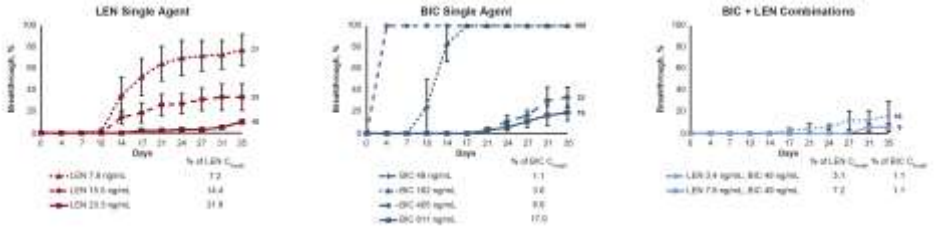
[a]Molecular weight (g/mol) for bictegravir is 449.4 and lenacapavir is 968.3
BIC, bictegravir; C_{trough}, trough plasma concentration; EC_{95}, 95% effective concentration; LEN, lenacapavir; $PAEC_{95}$, protein-adjusted 95% effective concentration; SS, steady state.

Breakthrough Frequency and Emergent Resistance for Breakthrough Resistance Selections



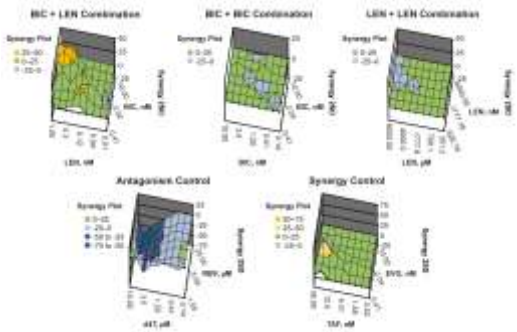
Area of circles are proportional to amount of breakthrough over 35 days.
BIC, bictegravir; C_{max}, maximum plasma concentration; C_{trough}, trough plasma concentration; LEN, lenacapavir; $PAEC_{95}$, protein-adjusted 95% effective concentration; RAM, resistance-associated mutation; SS, steady state.

- Cells exposed to BIC or LEN alone showed a dose-dependent breakthrough frequency
  - Emergent RAMs were seen in capsid (M66I, Q67H, and N74D) and integrase (R263K) at concentrations 5- to 14-fold below clinical trough plasma concentration (C_{trough})
- When BIC and LEN were combined at various concentrations:
  - Breakthrough was greatly decreased at subtherapeutic concentrations tested; breakthrough was completely inhibited at levels of BIC + LEN above $PAEC_{95}$



BIC and LEN concentrations are equivalent clinical concentrations; values are means from 2-4 independent experiments; error bars denote standard error.
BIC, bictegravir; C_{trough}, trough plasma concentration; LEN, lenacapavir.

In Vitro Combination Antiviral Activity



2SD, 2 standard deviations; BIC, bictegravir; d4T, stavudine; EVG, elvitegravir; LEN, lenacapavir; RBV, ribavirin; TAF, tenofovir alafenamide.

In Vitro Combination Antiviral Activity

In Vitro Drug Combination	Synergy/Antagonism Volumes, $\mu M^2 \cdot \%^a$		Combination Effect
	Mean Synergy ± SD	Mean Antagonism ± SD	
BIC + LEN	148 ± 13	-14 ± 10	Highly synergistic
LEN + LEN *Additivity control*	18 ± 18	-16 ± 6	Additive
BIC + BIC *Additivity control*	5 ± 5	-10 ± 10	Additive
TAF + EVG *Synergy control*	201 ± 42	-20 ± 19	Highly synergistic
RBV + d4T *Antagonism control*	5 ± 5	-902 ± 299	Highly antagonistic

[a]Data represent the mean of two independent experiments performed in triplicate.
BIC, bictegravir; d4T, stavudine; EVG, elvitegravir; LEN, lenacapavir; RBV, ribavirin; TAF, tenofovir alafenamide.

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