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Antiviral Activity of Lenacapavir Against HIV-2 Isolates

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Introduction

- Lenacapavir (LEN) is a first-in-class, multistage inhibitor of HIV-1 capsid function in clinical development that was recently approved in Canada, the European Union, and the US for use in adults with multidrug-resistant HIV-1 infection[1-3]
- LEN is highly potent against HIV-1 in vitro and maintains wild-type activity across HIV-1 isolates with resistance to all existing drug classes[2,4]
- In clinical trials, LEN has shown high levels of efficacy in people with HIV-1 who are treatment-naïve or -experienced[5-7]; however, a comprehensive characterization of the antiviral activity of LEN against HIV-2 is lacking
- HIV-2 is endemic in West Africa and is found in other regions with socioeconomic ties to West African countries
  - HIV-2 is intrinsically resistant to many antiretroviral (ARV) drugs used for HIV-1 treatment
  - High rates of multiclass drug resistance have been reported in people living with HIV-2 (PLWH2) who have received ARV therapy; many such people have few fully active treatment options[8-10]
- The availability of a new, HIV-2-active ARV class could provide a much-needed option for second-line or salvage therapy and potentially improve ARV therapy outcomes in PLWH2

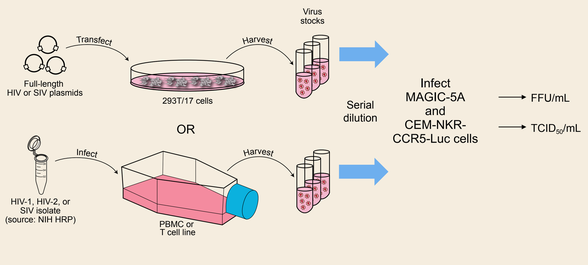
Objective

- To study the activity of LEN against a panel of HIV-2 isolates with or without resistance to existing drug classes

Methods

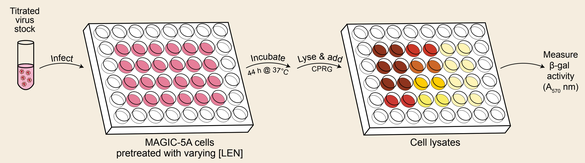
- The activity of LEN against HIV-1 and -2 isolates from ARV-naïve individuals was directly compared in 2 different assays: single-cycle infections of HeLa-CD4-CCR5-LTR-β-galactosidase (MAGIC-5A) indicator cells and multicycle infections of an immortalized T-cell line (CEM-NKR-CCR5-Luc)[11]
- Drug-resistant HIV-2 variants with mutations in reverse transcriptase (RT) and integrase (IN) were tested for resistance to LEN in the single-cycle assay
- Simian immunodeficiency virus of macaques (SIV_{mac}) 251 was tested for LEN susceptibility in both single- and multicycle assays
- Sooty mangabey SIV (SIV_{sm}) E660, SIV_{mac} 239, and African green monkey SIV (SIV_{agm}) sab2 were also tested in the single-cycle assay

Virus Production and Titration



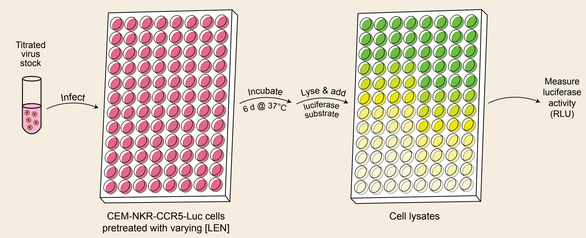
FFU = focus-forming units; NIH HRP = National Institutes of Health HIV Research Program; PBMC = peripheral blood mononuclear cells; $TCID_{50}$ = 50% tissue culture infectious dose.

Single-Cycle Drug Susceptibility Assays



Infections initiated with 1500 FFU of HIV or SIV/well (multiplicity of infection [MOI] = 0.05). β-gal = β-galactosidase. CPRG = chlorophenol red β-D-galactopyranoside.

Multicycle Drug Susceptibility Assays



Infections initiated with titers that yielded scorable luciferase levels after 6 d of culture while minimizing virus-induced cytopathic effects (MOI = 0.003-0.01 $TCID_{50}$/target cell); lysis buffer and substrate from Bright-Glo™ Luciferase Assay System (Promega Corporation, Madison, WI).

Results

Susceptibility of HIV-1, HIV-2, and SIV Isolates to LEN: Single-Cycle Assay

Virus	Isolate	Group/Subtype	IC_{50}, nM[a]	No. of Assays
HIV-1	92UG029	M/A	0.19 ± 0.067	3
	NL4-3	M/B	0.15 ± 0.067	10
	89.6	M/B	0.22 ± 0.079	3
	LAI	M/B	0.14 ± 0.036	3
	MJ4	M/C	0.31 ± 0.18	4
	92UG001	M/D	0.23 ± 0.066	3
	94UG114.1.6	M/D	0.20 ± 0.064	3
	93BR020	M/F	0.31 ± 0.15	3
	MVP5180-91	O	0.19 ± 0.080	3
	BCF01	O	0.14 ± 0.031	3
HIV-2	ROD9	A	2.3 ± 0.98	5
	ST	A	1.6 ± 0.33	3
	7924A	A	2.2 ± 0.85	3
	MVP15132	A	2.8 ± 0.93	3
	60415K	A	2.7 ± 0.33	3
	CBL-20	A	2.4 ± 0.24	3
	CBL-23	A	1.7 ± 0.80	3
	CDC77618	A	2.6 ± 0.40	3
	EHO	B	2.5 ± 0.42	3
	CDC310072	B	2.5 ± 0.44	3
	CDC310319	B	3.2 ± 0.51	3
	7312A	CRF01_AB	1.1 ± 0.10	3
SIV	sm E660	–	0.85 ± 0.11	3
	mac239 SpX	–	0.81 ± 0.37	3
	mac251	–	1.1 ± 0.077	3
	agm sab2	–	0.61 ± 0.14	3

[a]Single-cycle infections of MAGIC-5A cells. IC_{50} = half-maximal inhibitory concentration.

- In the single-cycle assay, LEN inhibited HIV-1 with a mean IC_{50} of 210 pM (range 140-310 [n = 10 isolates])
- In comparison, the mean IC_{50} for HIV-2 was 2.3 nM (range 1.1-3.2 [n = 12 isolates]), indicating an average 11-fold decrease in the activity of LEN against HIV-2 vs -1
- SIV isolates from SIV_{sm}, as well as SIV_{agm} sab2, showed susceptibilities to LEN that were similar to HIV-2

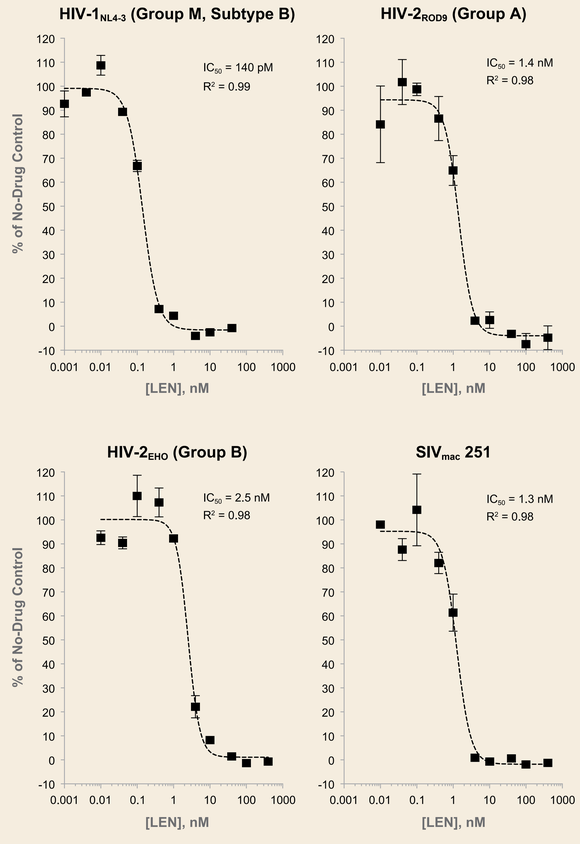
Susceptibility of HIV-1, HIV-2, and SIV Isolates to LEN: Multicycle Assay

Virus	Isolate	Group/Subtype	IC_{50}, nM[a]	No. of Assays
HIV-1	92UG029	M/A	0.20 ± 0.068	2
	Q23-17	M/A	0.12 ± 0.038	2
	NL4-3	M/B	0.12 ± 0.071	2
	LAI	M/B	0.12 ± 0.037	2
	93BR020	M/F	0.17 ± 0.094	3
	BCF01	O	0.074 ± 0.0051	2
HIV-2	ROD9	A	3.1 ± 0.36	2
	ST	A	1.3 ± 0.32	2
	MVP15132	A	1.2	1
	60415K	A	2.6 ± 0.87	2
	CDC77618	A	1.2 ± 0.018	2
	CDC310072	B	2.8 ± 1.9	2
	CDC310319	B	3.4 ± 1.9	3
	7312A	CRF01_AB	1.1 ± 0.16	2
SIV	mac251	–	1.3 ± 0.57	2

[a]Multicycle infections of CEM-NKR-CCR5-Luc cells.

- In the multicycle assay, a comparable difference in LEN activity between HIV-1 and -2 was noted, with mean IC_{50} values of 130 pM for HIV-1 (range 74-200 [n = 6 isolates]) and 2.1 nM for HIV-2 (range 1.1-3.4 [n = 8 isolates])

Representative LEN Dose-Response Curves



Data are from single-cycle assay; *data points* indicate amounts of β-galactosidase activity produced in LEN-treated cultures relative to solvent-only (ie, no-drug) control cultures; each *point* is mean of 2 cultures that were maintained in parallel; IC_{50} and R^2 values were calculated using 4-factor regression model in Prism v6.0h (GraphPad Software, Boston, MA); *error bars* indicate standard deviations (SDs).

Activity of LEN Against Drug-Resistant HIV-2 Mutants

Virus	Mutated Clone	Region	Mutation	Fold IC_{50}, nM[a]	Difference[b]
HIV-2	pROD9	None	None	1.3 ± 0.23	–
HIV-2	pROD9	RT	K65R+Q151M+M184V	1.0 ± 0.015	0.79
		RT[c]	K65R+N69S+V111I+Q151M+M184V	0.96 ± 0.0053	0.73
		IN	I84V+Q91R+T97A+Y143C	1.2 ± 0.048	0.93
		IN	G140S+Q148H	1.2 ± 0.17	1
		IN	R263K	1.6 ± 0.24	1.2
		IN	231ins SREGK[d]	1.2 ± 0.48	0.92
HIV-1	pNL4-3	None	None	0.10 ± 0.0056	0.08

[a]Single-cycle infections of MAGIC-5A cells; IC_{50} values are means ± SDs of 2 independent determinations; [b]Relative to IC_{50} for wild-type HIV-2_{ROD9}; [c]This clone contains patient-derived RT insert; additional amino acid differences relative to pROD9 RT were as follows: K30R, K64R, P126Q, V135T, V167I, K176P, I180L, F214L, H228Q, I251V, L270I, and K277R; [d]Insertion of 5 amino acids (serine-arginine-glutamic acid-glycine-lysine) at codon 231 of IN.

- Presence of drug resistance mutations in HIV-2 RT or IN had no effect on LEN activity (fold-change in LEN IC_{50}: 0.73-1.2 relative to wild-type HIV-2_{ROD9})

Summary: Activity of LEN Against HIV-1 and HIV-2 Isolates

Symbols indicate mean IC_{50} values observed for individual isolates of HIV-1 and -2; *P* values calculated via analysis of variance with Tukey's post-test.

Conclusions

- In this study, LEN was active against HIV-2 isolates with low-nanomolar activity
- LEN potency against HIV-2 was reduced 11- to 16-fold in comparison to HIV-1 regardless of presence of drug resistance mutations in HIV-2 RT or IN
- As a result of this difference in potency, treating PLWH2 with a LEN-based regimen could require careful monitoring to assess virologic responsiveness
- These data provide information on the potential clinical utility of LEN in PLWH2 for whom treatment options are limited

References: **1.** Bester S, et al. Science 2020;370:360-4; **2.** Link J, et al. Nature 2020;584:614-8; **3.** Paik L. Drugs 2022;82:1499-504; 4. Margot N, et al. Antimicrob Agents Chemother 2021;65:e02057-20; **5.** Margot N, et al. J Antimicrob Chemother 2022;77:989-95; **6.** Margot N, et al. J Infect Dis 2022;226:1985-91; **7.** Segal-Maurer S, et al. N Engl J Med 2022;386:1793-803; **8.** Berzow D, et al. Clin Infect Dis 2021;72:503-9; **9.** Gottlieb G, et al. Lancet HIV 2018;5:e390-9; **10.** Visseaux B, et al. Infect Genet Evol 2016;46:233-40; **11.** Smith RA, et al J Infect Dis 2022;226:497-509.

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