Phenotypic Characterization of Replication-Impaired Lenacapavir-Resistant HIV Clinical Isolates

Poster 00681

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Conclusions

- Here, we show that a novel multicycle (MC) phenotyping assay using a Rev-dependent HIV-1 reporter system, Rev-CEM-Luc/GFP (RevLucGFP) allows evaluation of clinically-relevant HIV-1 capsid (CA) mutations, which were previously shown to have very low replication capacity (RC) and low infectivity[10]
  - Due to their very low RC, characterization of CA mutations such as M66I and others in the standard MC MT-2 cytopathic assay was unsuccessful
- For wildtype (WT) HIV-1 virus, the antiviral activities of approved drugs from major HIV-1 drug classes in the RevLucGFP MC assay were consistent with previously observed data from MT-2 MC assays
- For CA mutants (including post-baseline clinical samples and site-directed mutants [SDMs]), antiviral activity and susceptibility in the RevLucGFP MC assay were consistent with previously observed data from the single cycle (SC) PhenoSense Gag-Pro assay and in MT-2 cells when available
- These findings allow us to better understand phenotypic resistance to lenacapavir (LEN) and assess potential interactions between CA resistance-associated mutations (RAMs)

Plain-Language Summary

Lenacapavir is a long-acting prescription medication that is used in combination with other antiretrovirals to treat HIV-1 infection in adults who have taken HIV-1 medicines before, have resistance to several HIV-1 medicines, and whose current HIV-1 medicines are not working.[1] In some people, the HIV-1 virus may develop mutations (changes in genetic material), making it resistant to lenacapavir, which could prevent lenacapavir from working.[2] Researchers need ways of detecting and studying these HIV-1 virus mutations to better understand how they stop lenacapavir from working effectively. Some HIV-1 virus mutations pose challenges for laboratory study, and existing testing methods often fail to detect them due to the inability of these viruses to infect laboratory cells effectively. Here, we describe a new way of identifying and characterizing specific HIV-1 virus mutations, called the 'Rev-CEM-Luc/GFP multicycle assay' or RevLucGFP.

References: **1.** Sunlenca® Prescribing Information. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/215973s000lbl.pdf (Accessed February 2024). **2.** Link JO, et al. *Nature*. 2020;584:614–8. **3.** Ogbuagu O, et al. *Lancet HIV*. 2023;10:e497–e505. **4.** Gupta SK, et al. *Lancet HIV*. 2023;10:e15–e23. **5.** Segal-Maurer S, et al. *N Engl J Med*. 2022;386:1793–803. **6.** Sun Q, et al. *Viruses*. 2021;13(5):920. **7.** Margot N, et al. Presented at EACS 2023; Presentation PS8 O4. **8.** Hagins D, et al. Presented at CROI 2023; Presentation 522. **9.** Margot N, et al. *J Antimicrob Chemother*. 2022;77(4):989–95. **10.** Margot N, et al. HIV Glasgow 2022; Poster P224 **11.** Wu Y, et al. *Curr HIV Res*. 2007;5(4):394–402.

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Background

- LEN is a potent, first-in-class, long-acting HIV-1 CA inhibitor which primarily inhibits integration via blockade of CA nuclear transport[2]
  - LEN is approved as a twice-yearly subcutaneous treatment option (following oral loading) in combination with other antiretrovirals for the treatment of multidrug-resistant HIV-1 infection in heavily treatment-experienced (HTE) people with HIV-1 (PWH)[1]
- The Phase 2/3 CAPELLA (NCT04150068) and Phase 2 CALIBRATE (NCT04143594) clinical studies were designed to evaluate the efficacy and safety profile of LEN-containing regimens in HTE and treatment-naïve PWH, respectively. High rates of virologic suppression have been demonstrated in both participant groups[3-5]
- Several CA mutations have been identified that are associated with reduced susceptibility to LEN, without affecting susceptibility to other ARV classes[2,6]:
  - *In vitro*, resistance selections (MT-2 cells or peripheral blood mononuclear cells) identified seven mutations at six CA amino acid residues: L56I, M66I, Q67H, K70N, N74D/S, and T107N[2]
  - In clinical studies, CA mutations were identified in 19 out of 258 PWH who received LEN, including M66I, Q67H/K/N, K70H/N/R/S, N74D/H, A105T/S, and T107A/C/N/S[6-9]
  - Previous studies have shown that LEN RAMs were associated with a lack of fully-active ARVs in, or inadequate adherence to, optimized background regimens[7]
- In a previous analysis, low RC was observed for most mutants in the Gag-Pro SC assay, including M66I, which showed a RC of approximately 10% compared with control virus[10]
- Most of these viruses were successfully phenotyped in a SC assay. However, for the majority of CA mutants, RC was too low for phenotyping by the MC MT-2 cytopathic assay
- Thus, there is a need for an optimized MC method to characterize the phenotype of CA mutants with low infectivity rates due to low RC

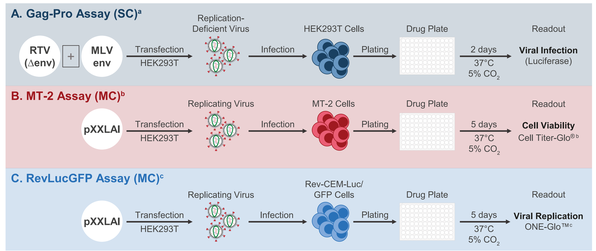
Objectives

- To characterize HIV-1 CA mutants with low infectivity via a novel MC phenotyping assay using a sensitive Rev-dependent HIV-1 reporter-controlled cell line

Methods

- Genetic fragments of HIV-1 Gag-Protease from baseline and post-baseline plasma samples were obtained from CAPELLA and CALIBRATE clinical studies (n=21)
- Clinical samples and corresponding SDMs (n=15) were created and cloned into pXXLAI HIV-1 molecular clones and transfected into the HEK293T cell line
- The resulting viruses were evaluated via the PhenoSense SC Gag-Pro assay (**Figure 1A**) and two different MC infection formats: MT-2 and RevLucGFP (Rev-dependent HIV-1 reporter)[11] cell lines (**Figures 1B and 1C**), with readouts of cell viability or viral replication, respectively
- RevLucGFP cell line was purchased from Virongy Biosciences (VA, USA)[11] and HIV-1 drugs were synthesized at Gilead Sciences, Inc.
- For RevLucGFP, optimal assay conditions, including cell seeding density and growth, multiplicity of infection, and assay duration, were determined
- Antiviral assay outputs included fold change (FC) in LEN susceptibility and RC (Gag-Pro assay)

Figure 1. Phenotyping Assays



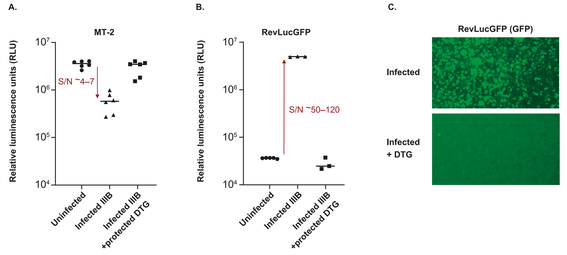
[a]NL4.3-based RTV to env-deleted with luciferase reporter gene in place of env; [b]Promega Cell Titer-Glo measures adenosine triphosphate, to estimate the number of live cells; [c]Promega ONE-Glo detects firefly luciferase reporter gene expression in mammalian cells. HEK, human embryonic kidney; MC, multicycle; MLV, murine leukemia virus; RTV, resistance test vector; SC, single cycle.

Results

- Evaluation of drug inhibition in MT-2 and RevLucGFP cells was carried out by luciferase assays and microscopy. Signal/noise range (maximum/minimum cell survival) was 4–7 for the MT-2 assay, which relies on viral cytopathicity for signal detection (**Figure 2A**)
- The signal/noise range (maximum/minimum infectivity) was higher with the RevLucGFP assay, at 50–120. Low background luminescence was observed from uninfected RevLucGFP cells, comparable to infected but fully protected cells (**Figures 2B and 2C**)

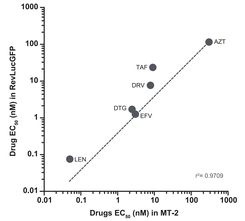
Results

Figure 2. Characterization of RevLucGFP Cells



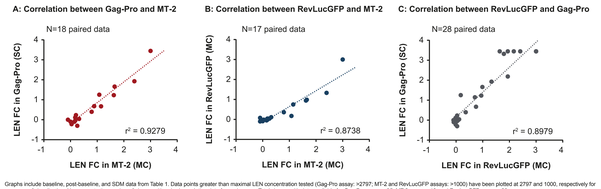
DIC, differential interference contrast; DTG, dolutegravir; GFP, green fluorescent protein; S/N, signal to noise

Figure 3. Correlation of EC_{90} Between RevLucGFP and MT-2 Cells



RevLucGFP: N=24; MT-2 cells, N=30.
AZT, azidothymidine; DRV, darunavir; DTG, dolutegravir; EC_{90}, half-maximal effective concentration; EFV, efavirenz; LEN, lenacapavir; TAF, tenofovir alafenamide.

Figure 4. Correlation Between Assays for Baseline and CA Mutants



Graphs include baseline, post-baseline, and SDM data from Table 1. Data points greater than maximal LEN concentration tested (Gag-Pro assay: >2797; MT-2 and RevLucGFP assays: >1000) have been plotted at 2797 and 1000, respectively for purposes of visualization. N represents the number of viruses with paired correlation data for each assay. Total viruses with data available for Gag-Pro assay = 35, MT-2 assay = 18, and RevLucGFP assay = 28.
CA, capsid; FC, fold change; LEN, lenacapavir; MC, multicycle; SC, single cycle; SDM, site-directed mutant.

- Representative drugs belonging to the major classes of HIV-1 inhibitors, including capsid inhibitor (CAI), integrase strand transfer inhibitor (INSTI), nucleoside or non-nucleoside reverse transcriptase inhibitor (NRTI and NNRTI), and protease inhibitor (PI), were tested. A positive correlation across drug classes was observed between WT half-maximal effective concentration (EC_{50}) values in MT-2 and RevLucGFP assays (**Figure 3**)
- Eleven mutants that were non-infectious in MT-2 assays, with very low RC, were successfully phenotyped in RevLucGFP cells. These included clinical isolates containing M66I in various genetic contexts and combinations of LEN RAMs with FC ranging from 43.5 to >1000 (**Table 1**)
- All CA mutants resistant to LEN retained sensitivity to other main HIV-1 drug classes, including INSTIs (bictegravir and dolutegravir), NRTI (tenofovir alafenamide), and NNRTI (efavirenz); data not shown
- Good correlations were observed between the assays for baseline and CA mutants (**Figure 4**). Most viruses were successfully phenotyped with the Gag-Pro assay as it is a SC assay, whereas the MT-2 MC assay had the lowest number of phenotyped viruses due to the lack of virally-induced cytopathic effect and low RC. The RevLucGFP assay enabled the phenotyping of more CA mutants than the MT-2 assay

Table 1. LEN Phenotypic Resistance, Fold Change, and Replication Capacity

Sample ID		LEN Resistance Mutations						LEN FC[a]			RC, %[b]
		M66	Q67	K70	N74	A105	T107	Gag-Pro[c] SC	MT-2 MC	RevLucGFP MC	Gag-Pro[c]
1	BL							1.0	0.8	0.8	163
	W54		H					4.7	14	5.6	49
	SDM		H					4.8	7.7	N/A	58
2	BL							0.8	1.0	0.82	98
	W10			H				85	249	21.4	37
	SDM			H				154	NI	87	10
3	BL							0.8	1.4	0.9	93
	W26	I						2098	NI	62	12
	SDM	I						>>	NI	N/A	1.5
4	BL							0.9	1.5	1.1	10
	W26	I				T		>>	NI	87	1.2
	SDM1	I						>>	NI	N/A	1.5
	SDM2					T		AF	NI	NI	AF
5	BL							0.6	1.1	0.97	8.5
	W4	I		S				AF	NI	NI	AF
	SDM1	I						>>	NI	N/A	1.5
	SDM2			S				AF	NI	NI	AF
6	BL							1.1	2.1	1.3	103
	W4	I					S	>>	NI	43.5	24
	SDM1	I						>>	NI	N/A	1.5
	SDM2						S	1.0	NI	NI	AF
7	BL						S	1.7	1.6	1.1	293
	W4		H	R			S	46	45	9.7	109
	SDM1		H					4.8	7.7	N/A	58
	SDM2			R				1.2	NI	NI	9.7
	SDM3						S	1.0	NI	NI	AF
	SDM4		H	R				15	NI	NI	AF
8	BL							1.3	1.5	0.96	146
	W10		H	R				18	12	1.5	63
	SDM1		H					4.8	7.7	N/A	58
	SDM2			R				1.2	NI	NI	9.7
	SDM3		H	R				15	NI	NI	AF
9	BL							0.5	1.8	1.1	22
	W4	I	H	R				>>	NI	146	3.1
	SDM1	I						>>	NI	N/A	1.5
	SDM2		H					4.8	7.7	N/A	58
	SDM3			R				1.2	NI	NI	9.7
	SDM4	I	H					1594	NI	N/A	AF
	SDM5		H	R				15	NI	NI	AF
10	BL							1.0	0.8	1.2	74
	W10	I			D	T		>>	NI	>>	16
	W22	I	H		D	T		>>	>>	>>	13
	#1	I						>>	NI	>>	0.6
	#2				D			17	42	8.8	49
	#3					T		2.5	6.2	2.3	57
	#4	I			D			>>	NI	>>	2.5
	#5	I				T		>>	NI	256	3.7
	#6				D	T		14	NI	5.1	17
	#7	I	H		D			>>	NI	NI	0.6
	#8	I	H			T		>>	NI	>>	0.6

Red text indicates non-infectious values in the MT-2 MC assay; green text indicates values in the RevLucGFP MC assay that were non-infectious in the MT-2 MC assay. SDMs were made in pXXLAI WT background except for SDMs for Participant 10, which were made using the BL isolate genetic background. [a]FC in EC_{90} compared with WT control (MT-2 and Gag-Pro); [b]Expressed as % of WT control (drug-sensitive reference strain [CNDO] containing PR and RT sequences from HIV-1 strain NL4-3); [c]PhenoSense Gag-Pro assay (Monogram).
>>, greater than maximal LEN concentration tested (Gag-Pro assay: >2797; MT-2 and RevLucGFP assays: >1000); AF, assay failure; BL, baseline; EC_{90}, half-maximal effective concentration; FC, fold change; LEN, lenacapavir; MC, multicycle; N/A, not available; NI, non-infectious in assay; RC, replication capacity; SC, single cycle; SDM, site-directed mutants; W, week; WT, wild type.