Phenotypic Analyses of HIV Clinical Isolates With Capsid Substitutions Observed in the PURPOSE 1 and PURPOSE 2 Studies

P012

PURPOSE 1
PURPOSE 2

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Conclusions

- In the Phase 3 PURPOSE 1 and PURPOSE 2 studies, the emergence of breakthrough infections with lenacapavir (LEN) was rare
- The N74D capsid resistance-associated mutation (RAM) showed variable levels of LEN resistance and was associated with low replication capacity (RC), consistent with prior observations
- The infrequency of breakthrough infections, combined with the fitness cost of LEN RAMs, suggest low risk of resistance accumulation in circulating viral populations

Plain Language Summary

- Human immunodeficiency virus (HIV) prevention medication, also known as pre-exposure prophylaxis (PrEP), lowers the chances of someone getting HIV
- Like most viruses, HIV can change over time. When this happens, the virus may stop responding to a medicine. This is called 'resistance'
- Lenacapavir (LEN) is a type of PrEP that is given as an injection twice a year (every 6 months)
- In two clinical studies (PURPOSE 1 and PURPOSE 2), a small number of people got HIV even though they had received LEN. In most of these people, HIV developed a change, called a mutation, in a specific part of the virus' genes called N74D. This study sought to learn more about the N74D mutation
- The researchers found that N74D caused different levels of resistance to LEN. They also found that having N74D made it harder for the virus to grow (replicate)
- In the overall study population, very few people got HIV and developed the N74D mutation. Based on these findings, the researchers concluded that the N74D mutation is unlikely to cause problems with LEN for PrEP in the future

Introduction

- The pivotal Phase 3 PURPOSE 1 (NCT04994509) and PURPOSE 2 (NCT04925752) studies demonstrated that acquisition of HIV while receiving LEN, a first-in-class HIV-1 capsid inhibitor, was rare. This high efficacy and favorable safety profile led to LEN's approval for HIV pre-exposure prophylaxis (PrEP) in multiple countries worldwide[1-3]
- In the PURPOSE 1 primary analysis, no HIV-1 cases were detected in participants receiving LEN during the study.[1] Two participants had unrecognized HIV-1 at baseline, received LEN, and developed the LEN resistance-associated mutation N74D[4,5]
- In the PURPOSE 2 primary analysis, two HIV-1 cases were detected in participants receiving LEN[2] and both developed N74D. Two participants had unrecognized HIV-1 at baseline, received LEN, and developed N74D[5,6]
- N74D-containing viruses through the primary analyses are shown in **Table 1**

Table 1. N74D-Containing Viruses in Participants Receiving LEN in PURPOSE 1 and PURPOSE 2 Through the Primary Analyses

Study	Phase	Participant	Subtype	Timepoint of Resistance Testing	LEN-R	Additional CA Substitutions Tested[a]
PURPOSE 1	Baseline[b]	1	A1	Day 1	-	-
				Day 176	N74D	-
	Baseline[b]	2	D	Day 1	-	L56M L69V
				Day 271	N74D	L56M L69V
PURPOSE 2	Baseline[b]	3	C	Day 1	-	-
				Day 111	N74D	Q67R I73V
	Baseline[b]	4	C	Day 1	-	-
				Day 77	N74D	A105V[c]
	On Study[2,3]	5	C	Week 26	N74D	-
	On Study[2,3]	6	C	Week 13	N74D	-

[a]Additional CA substitutions present either at baseline (not bolded) or that emerged with N74D (bolded and blue) and were phenotyped in this study. [b]Participants who had unrecognized HIV-1 infection at baseline and were dosed with study drug. [c]A105V phenotyped individually, not in the presence of N74D.
CA, capsid-associated; LEN, lenacapavir; R, resistance.

Objective

- To characterize N74D capsid RAMs, including baseline and background capsid sequence diversity, in clinical isolates and site-directed mutants (SDMs)

Methods

- Participant-derived HIV-1 viral *gag* genes, as well as associated SDMs in the participant *gag* or in the *gag* gene of pXXLAI, were synthesized, cloned into pXXLAI DNA plasmids, transfected into 293T cells, and virus harvested
- Fold changes (FCs) in LEN susceptibility and/or RC of viruses relative to wild type through the primary analysis were evaluated using multicycle luciferase reporter assays in T-cell lines and primary peripheral blood mononuclear cells (PBMC), plus the single-cycle PhenoSense Gag-Pro assay (Monogram Biosciences) (**Figure 1**)
- Susceptibility of N74D-containing viruses to bictegravir (BIC) and efavirenz (EFV) was also tested

Figure 1. Phenotyping Assays

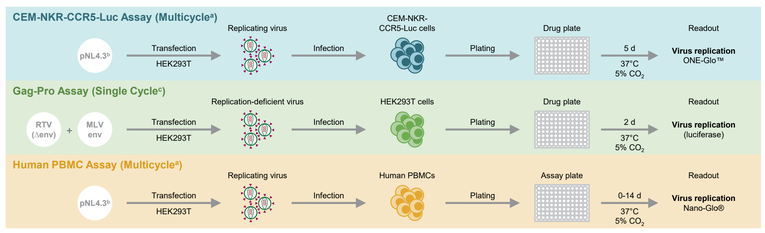


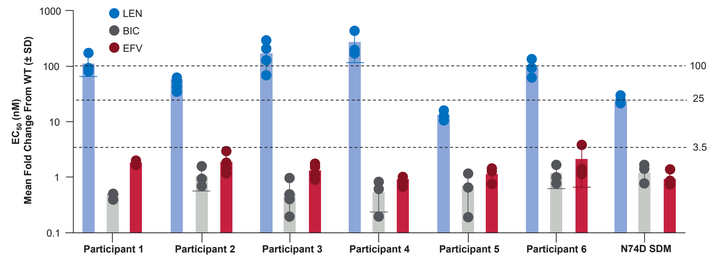
Figure reproduced with permission from Margot et al.[7]
[a]Secreted Nano luciferase (sNLuc-IRES-Nef) was quantified using reagents from Promega Corporation, Madison, WI, US. [b]Contains C-C motif chemokine receptor 5 (CCR5)-tropic JRFL envelope and sNLuc-IRES-Nef genes. [c]NL4.3-based resistance test vector (RTV) *env*-deleted; luciferase reporter gene in place of *env*.
PBMC, peripheral blood mononuclear cells; HEK, human embryonic kidney; MLV, murine leukemia virus.

Results

LEN-Resistant HIV-1 Viruses

- Among N74D-containing viruses tested, a wide range of LEN-resistance levels was observed in multicycle luciferase reporter assays (FC range: 14-282; **Figure 2**)
  - N74D-containing viruses maintained susceptibility to BIC and EFV, suggesting susceptibility to other drug classes is not impacted by the N74D mutation
- These results were similar to the results from the single-cycle assay (**Table 2**)
- No resistance to LEN, BIC, or EFV was observed in participant-derived baseline samples tested, indicating absence of baseline resistance prior to N74D acquisition for Participants 1-4 (**Figure 3**)

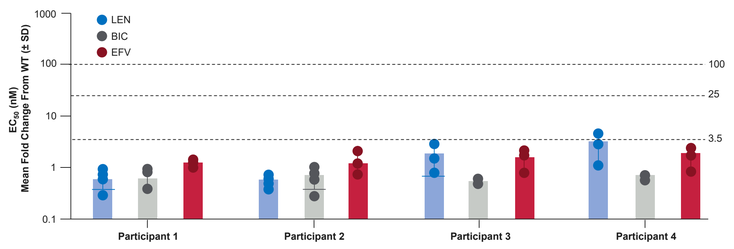
Figure 2. Fold Resistance of N74D-Containing Participant-Derived Viruses from PURPOSE 1 and PURPOSE 2



BIC, bictegravir; EC_{50}, half maximal effective concentration; EFV, efavirenz; LEN, lenacapavir; SD, standard deviation; SDM, site-directed mutant; WT, wild type.

Results (cont.)

Figure 3. Fold Resistance of Baseline Participant-Derived Viruses From PURPOSE 1 and PURPOSE 2



BIC, bictegravir; EC_{50}, half maximal effective concentration; EFV, efavirenz; LEN, lenacapavir; SD, standard deviation; WT, wild type.

Table 2. Phenotypic Susceptibility of Participant-Derived Viruses From PURPOSE 1 and PURPOSE 2 in PhenoSense Gag-Pro Assay

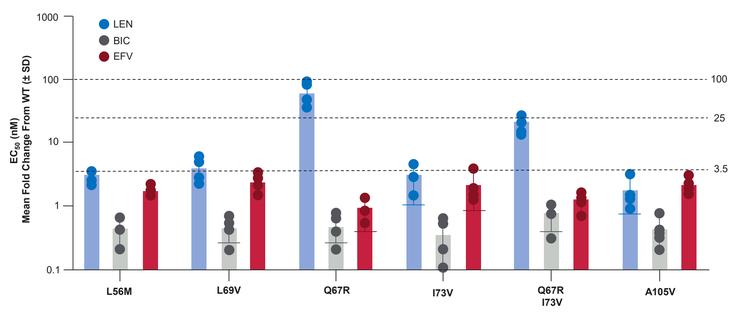
Study	Participant	Timepoint of Resistance Testing	LEN-R	Additional CA Substitutions Tested[a]	LEN FC	
					CEM (Multicycle[b])	Gag Pro (Single Cycle)
PURPOSE 1	1	Day 1	-	-	0.6	1.7
		Day 176	N74D	-	119.7	161.0
	2	Day 1	-	L56M L69V	0.6	1.4
		Day 271	N74D	L56M L69V	48.6	84.0
PURPOSE 2	3	Day 1	-	-	1.8	1.9
		Day 111	N74D	Q67R I73V	181.3	AF
	4	Day 1	-	-	2.8	1.9
		Day 77	N74D	-	282.2	398.0
	5	Week 26	N74D	-	13.7	51.0
	6	Week 13	N74D	-	100.2	63.0

[a]Additional CA substitutions present either at baseline (not bolded) or that emerged with N74D and were phenotyped (bolded and blue). [b]Data from Figures 2 and 3 for comparison.
AF, assay failure; CA, capsid-associated; CEM, CEM-Nkr-CCR5-Luc cells; FC, fold change; LEN, lenacapavir; R, resistance; WT, wild type.

Unique Variants Within Region of LEN Binding Pocket

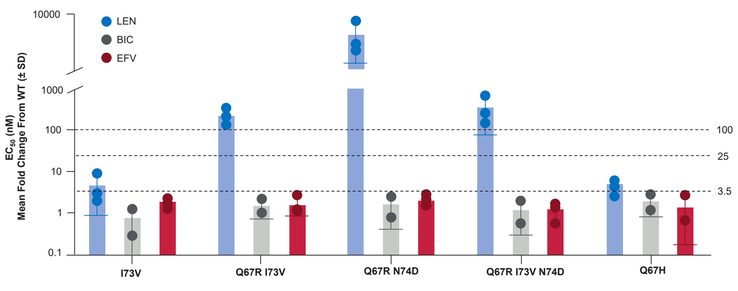
- The additional CA mutations observed in participants were evaluated in the absence of N74D (**Figure 4**)
  - L56M and L69V were capsid polymorphisms within the LEN binding pocket that have not been previously characterized in non-B virus subtypes (Participant 2)
  - In one participant with unrecognized HIV at baseline who received LEN (Participant 3), emergence of Q67R and I73V capsid substitutions alongside N74D was observed
  - The A105V mutation emerged alongside N74D (Participant 4)
  - The L56M, L69V, I73V, and A105V mutations had little-to-no impact on the susceptibility to LEN (FC range: 1.6-3.9; **Figure 4**)
- SDMs containing Q67R ± I73V and N74D were associated with LEN FCs of > 200 in multicycle assays (**Figure 5**) compared to a LEN FC of 25.8 for a single N74D SDM suggesting that Q67R increases the resistance of N74D to LEN
- The Q67R single SDM was noninfectious in T-cell lines, similar to what has been observed for the Q67K SDM[8]

Figure 4. Fold Resistance of Additional CA Substitutions in the Absence of N74D



L56M and L69V were tested in Participant 2; Q67R, I73V and Q67R I73V in Participant 3; and A105V in Participant 4.
BIC, bictegravir; EC_{50}, half maximal effective concentration; EFV, efavirenz; LEN, lenacapavir; SD, standard deviation; WT, wild type.

Figure 5. Impact of Q67 and I73 Site-Directed Mutants on LEN Potency

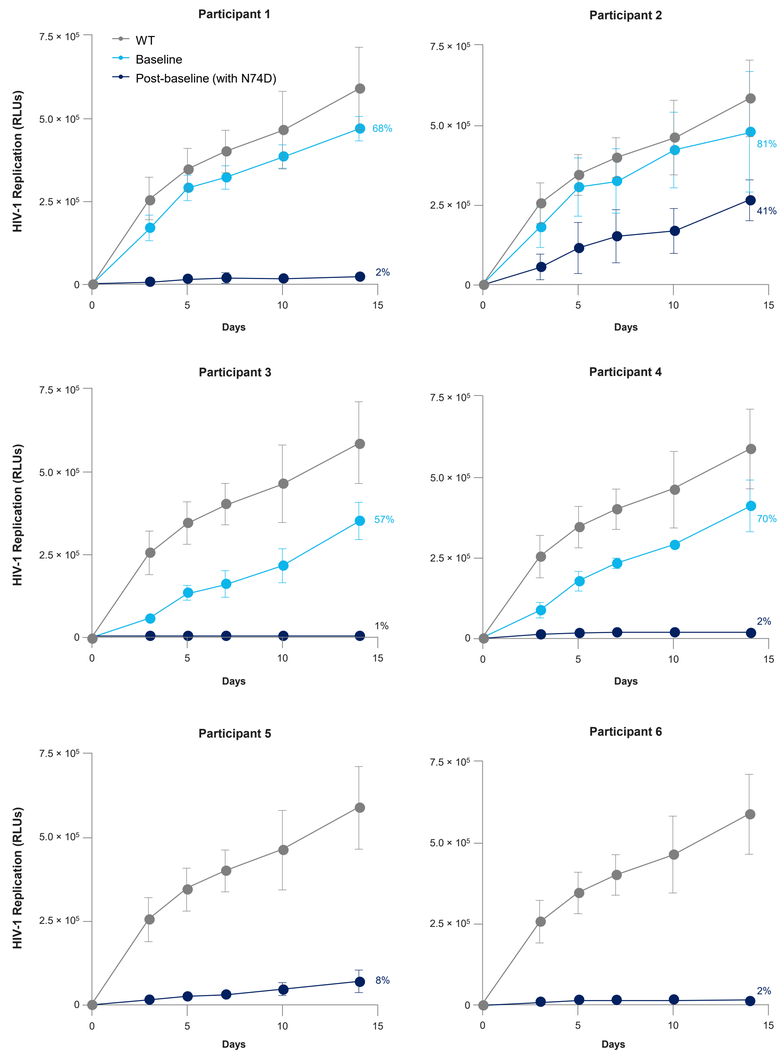


All testing was performed using a site-directed mutant in pXXLAI.
BIC, bictegravir; EC_{50}, half maximal effective concentration; EFV, efavirenz; LEN, lenacapavir; SD, standard deviation; WT, wild type.

Replication Capacity of LEN RAMs

- The RC of all participant-derived isolates with N74D was low (1-41% of wild type) in PBMC assays, demonstrating a significant decrease in viral fitness (**Figure 6**)

Figure 6. Replication Capacity of LEN-resistant N74D Participant-Derived Viruses From PURPOSE 1 and PURPOSE 2



Participants 1-4 had unrecognized HIV-1 at baseline; Participants 5 and 6 acquired HIV-1 during the study. Percentages shown are percentage of WT at Day 14.
LEN, lenacapavir; RLU, relative light unit; WT, wild type.

Prevalence of Circulating I73V and Q67R

- The I73 and Q67 residues are highly conserved, and analysis of the LANL database demonstrated that the prevalence of the I73V and Q67R substitutions was very low (**Table 3**)[9,10]

Table 3. Circulating Levels of I73V and Q67R are Low

Los Alamos National Laboratory Database[11]

HIV Subtype (N = 11,652)	I73V		Q67R	
	Count	Prevalence (%)	Count	Prevalence (%)
A1 (n = 632)	0	0.00	1	0.16
AD (n = 32)	0	0.00	0	0.00
AE (n = 2589)	0	0.00	1	0.04
B (n = 5290)	1	0.02	3	0.06
BF (n = 129)	0	0.00	0	0.00
C (n = 2469)	1	0.04	0	0.00
D (n = 337)	0	0.00	0	0.00
F1/F2 (n = 175)	0	0.00	0	0.00

References: **1.** Bekker LG, et al. *N Engl J Med.* 2024;391:1179-92. **2.** Kelley CF, et al. *N Engl J Med.* 2025;392:1261-76. **3.** Cox S, et al. *J Infect Dis.* 2026;233:e203-11. **4.** Ndlovu N, et al. Oral 128 presented at: CROI; Feb. 22-25, 2026; Denver, CO. **5.** Cox S, et al. Oral 130 presented at: CROI; Feb. 22-25, 2026; Denver, CO. **6.** Cantos VD, et al. Oral 129 presented at: CROI; Feb. 22-25, 2026; Denver, CO. **7.** Margot N, et al. Poster P224 presented at: HIV Glasgow; Oct. 23-26, 2022; Glasgow, UK. **8.** Pennetzdorfer N, et al. *Sci Transl Med.* 2026;18:eaea0947. **9.** Omoding D, et al. *J Antimicrob Chemother.* 2025; 80:955-61. **10.** Chen N, et al. *Open Forum Infect Dis.* 2025;12:ofaf028. **11.** HIV Los Alamos National Laboratory. http://www.hiv.lanl.gov (accessed Oct. 20, 2026).

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