HIV-1 Susceptibility to Lenacapavir is Not Affected by Capsid Protein Polymorphisms T107A and T107S

Poster 581

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Conclusions

- HIV-1 viruses harboring capsid protein (CA) polymorphisms T107A and T107S showed full susceptibility to lenacapavir (LEN), in contrast to viruses with T107N which showed low-level resistance
- Structural analyses do not suggest steric interference with T107A/S substitutions, whereas the T107N mutant shows steric interference in the LEN binding site, consistent with *in-vitro* findings
- These data indicate that the presence of the T107A and T107S polymorphisms alone in HIV-1 sequences from people with HIV-1 (PWH) are unlikely to affect the effectiveness of LEN in people being treated for HIV-1

Plain Language Summary

- The genetic information of HIV (its 'DNA') is surrounded by a protein shell called capsid, which protects HIV from the cellular environment as HIV enters into a cell (a process called infection)
- Lenacapavir is a medicine that treats or prevents HIV infection in people by attaching itself to the capsid and blocking its function, leading to the death of the virus
- In rare cases, HIV can change (mutate) in such a way that it is harder for lenacapavir to attach to the capsid and kill the virus (a process called drug-resistance)
- In this study, we tested viruses with different changes (mutations) in the capsid protein to learn whether the mutations could reduce how well lenacapavir works and used computer models to understand why certain mutations make HIV more resistant to lenacapavir
- We found that some of the mutations we studied did not affect how lenacapavir works, while others had effects that made it harder for lenacapavir to treat the infection

References: **1.** SUNLENCA® Prescribing Information. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/215973s000lbl.pdf (accessed January 2026). **2.** YEZTUGO® Prescribing Information. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2025/220020s000lbl.pdf (accessed January 2026). **3.** Link JO, et al. *Nature.* 2020;584:614-18. **4.** Ogbuagu O, et al. *Clin Infect Dis*. 2025;80:566–74. **5.** Margot N, et al. *J Antimicrob Chemother*. 2022;77:989–95. **6.** Tao K, et al. *Viruses*. 2023;15:992. **7.** Demirdjian S, et al. *J Med Virol*. 2025:e70340. **8.** Meng EC, et al. *Protein Sci*. 2023;32:e4792. **9.** Shapovalov MV and Dunbrack RL Jr. *Structure*. 2011;19:844–58.

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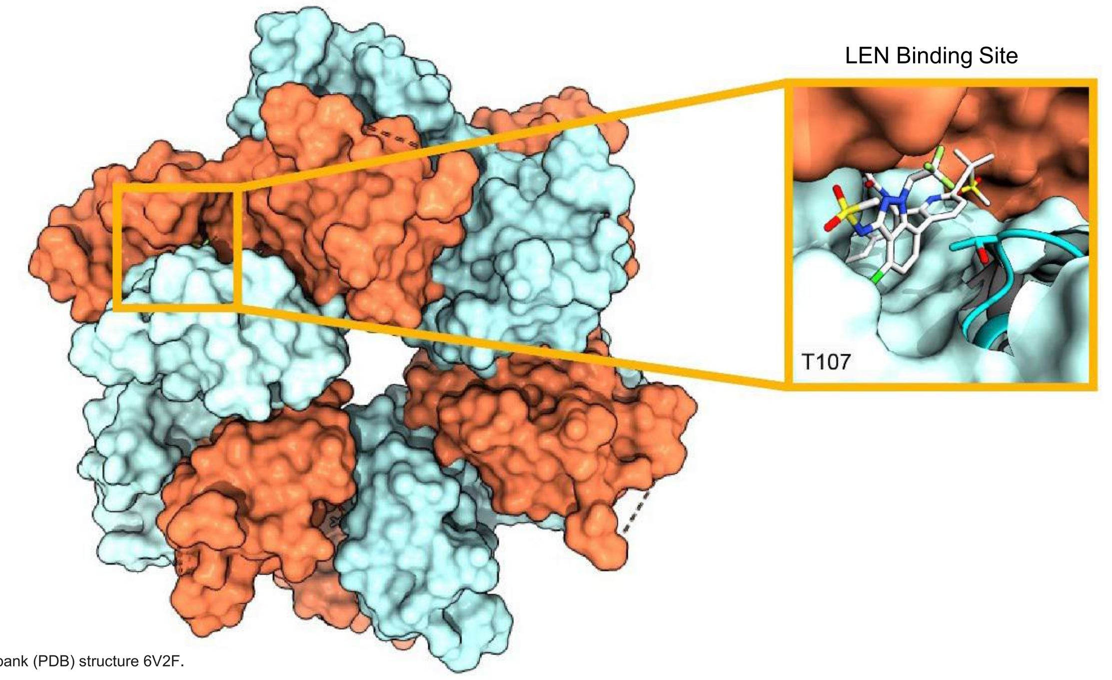
Author Disclosures:
Nicolas Margot, Vidula Naik, Lisa Selzer, Nina Pennetzdorfer, and Christian Callebaut are all employees and shareholders of Gilead Sciences, Inc.

Background

- LEN, the first-in-class twice-yearly HIV-1 CA inhibitor, is approved for the treatment of multidrug-resistant HIV-1 in combination with other antiretrovirals, as well as for HIV-1 pre-exposure prophylaxis (PrEP) in people who may need or want PrEP[1,2]
- LEN binds at a conserved interface between two adjacent CA monomers (**Figure 1**) with high affinity and has a sub-nanomolar half-maximal effective concentration (EC_{50}) in *in vitro* HIV-1 inhibition assays[3]
- In heavily treatment-experienced PWH, LEN resistance mutations in the CA protein have been observed in the context of LEN functional monotherapy, associated either with resistance to the optimized background regimen (OBR) components or non-adherence to the OBR[4]
  - One such CA mutation, T107N, has been observed in PWH and *in vitro* where it was associated with a 4-fold decrease in LEN susceptibility[5]
- Other T107 mutations, including T107A and T107S, occur in 1–2% of circulating HIV-1 sequences and have an unknown impact on LEN susceptibility[6]

Figure 1. HIV-1 CA Protein Hexamer with Bound LEN



Structural visualization based on the protein data bank (PDB) structure 6V2F.
LEN, lenacapavir.

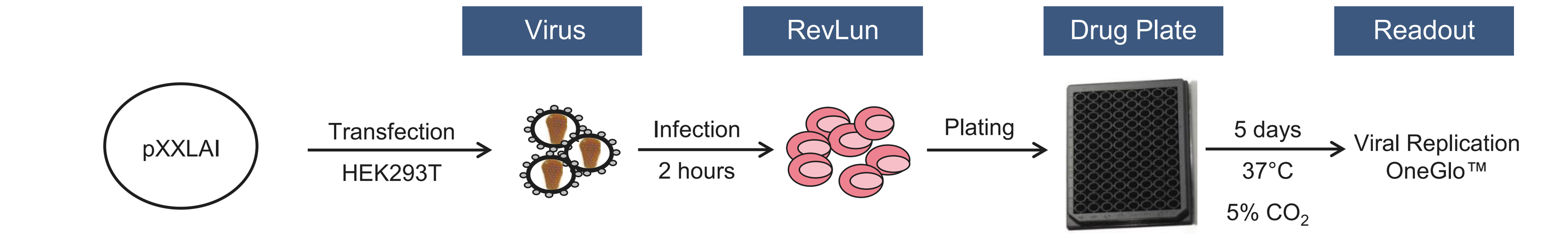
Objective

- Here, we describe the phenotypic susceptibility of viral isolates with T107 polymorphisms

Methods

- Clinical isolates with either wild-type (WT) T107T (n=5) or polymorphic residues T107A (n=5), T107S (n=7), and corresponding site-directed mutants constructed from isolates to express the known LEN minor resistance mutation T107N (n=8) were cloned into the pXXLAI infectious clone and transfected into HEK293T cells (**Figure 2**)
- Viral supernatants were used to infect Rev-CEM-GFP/Luc (RevLun) HIV reporter cells in the presence of LEN dose titration (**Figure 2**), as described previously[7]
  - Luminescence data were used to calculate the EC_{50} and fold-change (Microsoft Excel and XLFit)
  - The known N74D CA mutant was used as a resistant control for comparison
- Images of WT and mutant HIV-1 CA in complex with LEN were created using USCF ChimeraX[8] based on the Protein Data Bank (PDB) structure 6V2F
  - Rotamers and potential molecular clashes of mutant amino acids were evaluated in ChimeraX using the Dunbrack 2010 backbone-dependent rotamer library[9]

Figure 2. Multi-Cycle Phenotyping Workflow



Results

LEN Susceptibility (Table 1, Figure 3)

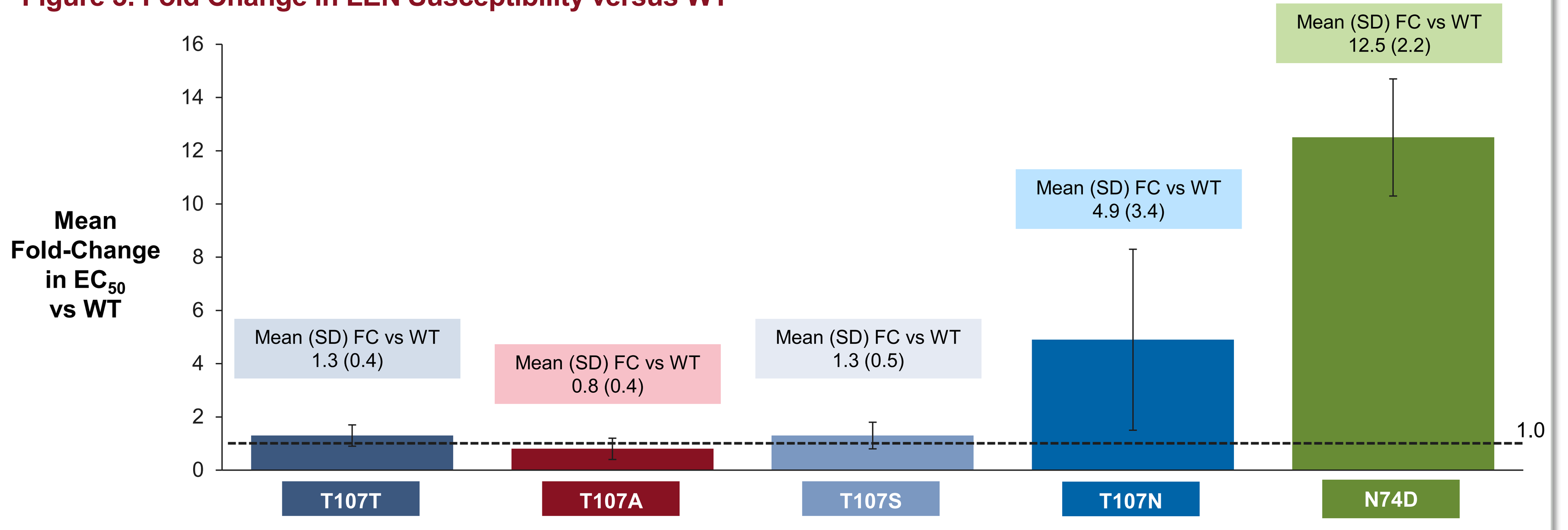
- The XXLAI WT reference showed an average LEN EC_{50} of 78 pM
  - The resistant control N74D had a mean LEN EC_{50} fold-change of 12.5 from the WT control
- In comparison to the WT reference, clinical isolates with the WT (T107T) residue, T107A residue, and T107S residue had a mean (SD) LEN EC_{50} fold-change of 1.3 (0.4), 0.8 (0.4), and 1.3 (0.5), respectively
- WT and T107A/S clinical isolates engineered to express T107N instead of T107T, T107A, or T107S showed low-level phenotypic LEN resistance, with a mean (SD) LEN EC_{50} fold-change of 4.9 (3.4) compared to WT
  - When comparing the T107N site-directed mutant to the clinical isolates from which they had been constructed, we observed a mean (SD) LEN EC_{50} fold-change of 3.1 (1.8) (**Table 2**)

Table 1. LEN Susceptibility Data

Construct	Sample #	Subtype	Mean LEN EC_{50} (nM)	%CV	FC vs WT
WT	XXLAI		0.078	7.7	1
T107T	1	AE	0.058	72.4	0.7
	2	BF	0.127	1.6	1.6
	3	B	0.145	88.3	1.8
	4	B	0.126	31.0	1.6
	5	B	0.072	33.3	0.9
				Mean (SD) FC vs WT	**1.3 (0.4)**
T107A	13	C	0.166	75.9	2.1
	14	C	0.042	21.4	0.5
	15	C	0.031	19.4	0.4
	16	B	0.045	28.9	0.6
	17	C	0.116	53.4	1.5
				Mean (SD) FC vs WT	**0.8 (0.4)**
T107S	6	AE	0.081	53.1	1.0
	7	AE	0.189	63.0	2.4
	8	B	0.136	37.5	1.7
	9	B	0.086	65.1	1.1
	10	B	0.061	31.1	0.8
	11	B	0.067	19.4	0.9
	12	B	0.174	29.9	2.2
				Mean (SD) FC vs WT	**1.3 (0.5)**
T107N	18	AE	0.200	4.5	2.6
	19	AE	0.266	24.1	3.4
	20	B	0.354	64.7	4.5
	21	C	0.472	19.9	6.0
	22	B	0.369	44.2	4.7
	23	AE	0.152	14.5	1.9
	24	BF	0.216	45.8	2.8
	25	B	1.029	67.9	13.1
				Mean (SD) FC vs WT	**4.9 (3.4)**
N74D	26	B	0.975	17.9	12.5

%CV, percent coefficient of variation; EC_{50}, half maximal concentration; FC; fold-change; LEN, lenacapavir; SD, standard deviation; WT, wild-type

Figure 3. Fold Change in LEN Susceptibility versus WT



EC_{50}, half maximal concentration; FC, fold-change; LEN, lenacapavir; SD, standard deviation; WT, wild-type

Table 2. Change in LEN Susceptibility

	Sample #	Residue at position 107	EC_{50} (nM)	%CV	FC vs Isolate
T107T→N	1	T107T	0.058	72.4	2.6
	23	T107N	0.152	14.5	
	2	T107T	0.127	1.6	1.7
	24	T107N	0.216	45.8	
	3	T107T	0.145	88.3	7.1
	25	T107N	1.029	67.9	
T107A→N	17	T107A	0.116	53.4	4.1
	21	T107N	0.472	19.9	
T107S→N	12	T107S	0.174	29.9	2.0
	20	T107N	0.354	64.7	
	6	T107S	0.081	53.1	2.5
	18	T107N	0.200	4.5	
	7	T107S	0.189	63.0	1.4
	19	T107N	0.266	24.1	
T107N				**Mean (SD) FC vs Isolate**	**3.1 (1.8)**

Although no phenotypic resistance cut-off have been established for LEN, changes above 2.5-fold of wild-type control are typically considered relevant.
%CV, percent coefficient of variation; EC_{50}, half maximal concentration; FC; fold-change; LEN, lenacapavir; SD, standard deviation; WT, wild-type

Structural Analysis

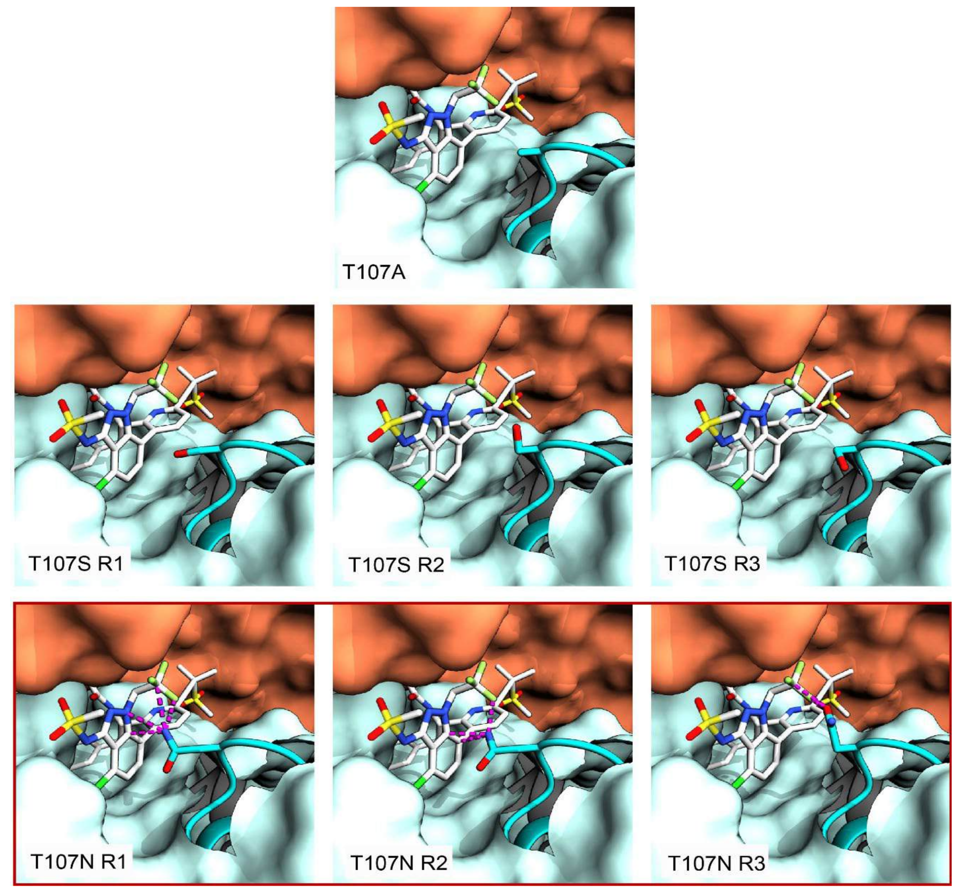
- Structural simulations of T107 polymorphisms show multiple rotamers for T107S and T107N (**Table 3, Figure 4**)
  - Neither T107A nor T107S rotamers showed steric clashes with LEN
  - Modeling of the T107N mutant, however, shows steric clash with a bound LEN molecule

Table 3: Position 107 Rotamers and LEN Steric Clash

Residue at Position 107	Number or Rotamers with prevalence >1%	# of Steric Clashes with LEN
T107T	3	0
T107A	1	0
T107S	3	0
T107N	13	14

LEN, lenacapavir

Figure 4. T107A/N/S Rotamer Models with Bound LEN



Structural visualization based on the protein data bank (PDB) structure 6V2F. T107S and T107N are illustrated with three rotamers, R1–3.
LEN, lenacapavir; R, rotamer