Impact of HIV-1 Capsid Polymorphisms on Viral Fitness and Susceptibility to Lenacapavir

Poster #00304

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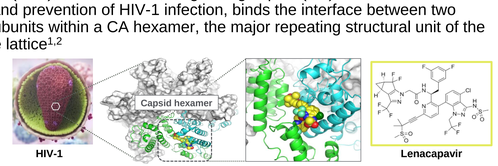
Conclusions

- We identified 55 capsid polymorphisms across 29 binding site residues within 5 angstroms of LEN with a >0.5% prevalence in at least one of the HIV-1 subtypes analyzed, half of which impaired virus infectivity at least 2-fold.
- With few exceptions, this mutant HIV panel comprising rare naturally occurring LEN binding site variants in CA remained fully susceptible to LEN. LEN-resistant variants L56V and N57H displayed profoundly impaired replication capacity in primary human CD4$^+$ T-cells relative to the WT virus.
- These data confirm a high degree of HIV-1 capsid sequence conservation and suggest that the existing natural viral diversity should minimally impact LEN efficacy in the clinic.

Introduction

- The capsid core, which encapsidates and protects the viral RNA genome and its replicative enzymes, plays multiple essential roles throughout HIV-1 replication
- Lenacapavir (LEN), a first-in-class, long-acting capsid (CA) inhibitor for the treatment and prevention of HIV-1 infection, binds the interface between two adjacent subunits within a CA hexamer, the major repeating structural unit of the capsid core lattice[1,2]



- LEN binding to CA interferes with the late and early stages of HIV-1 replication[1-3]
- LEN maintains full antiviral potency against a small multiclade HIV-1 panel (mean EC_{50} of 50 pM) and has properties that make it well suited as a long-acting agent[1,4]
- LEN has shown high clinical efficacy as a twice-yearly injectable agent and was approved in 2022 as Sunlenca®, an add on therapy for the treatment of adults with multidrug resistant HIV-1 infection[5,6]
- LEN is also under Ph2 clinical investigation as a twice-yearly injectable add on therapy for people with treatment-naïve HIV-1 infection and is in Ph3 development as a twice-yearly injectable medicine for HIV-1 prevention in people who could benefit from pre-exposure prophylaxis (PrEP)[7,8]
- In cell culture, LEN can select for HIV-1 with substitutions in one or more CA binding site codons (L56, N57, M66, Q67, K70, N74, A105 and T107) that either alone or in combination contribute to LEN resistance in vitro and in the clinic[1,9]
- Presently, it remains unclear how viral diversity encountered in the clinic may impact LEN efficacy
- Here we analyzed HIV-1 CA sequence diversity to identify natural polymorphisms within the LEN binding site and assessed each for their impact on viral fitness and susceptibility to LEN

Methods

- CA binding site (bs) residues were identified in Pymol using x-ray co-crystal structure PDB 6V2F.
- Unique curated HIV-1 CA sequences from public and Gilead trial datasets were analyzed for naturally occurring bs polymorphisms across subtypes and aligned to the HXB2 reference sequence.
- Publicly available HIV-1 group M *gag* sequences were obtained (www.lanl.gov) and deduplicated by keeping only the first sequence among those with the same patient ID.
- For Gilead clinical trial samples, viral RNA was isolated from patient-derived plasma samples and the *gag* region was amplified and deep sequenced using the Illumina-MiSeq NGS platform. CA amino acid substitutions observed in the viral population above a 15% prevalence were reported.
- A panel of 55 SDMs encompassing CA polymorphisms with a > 0.5% prevalence were expressed in HEK293T cells as VSV(G)-pseudotyped NL4.3 reporter viruses encoding firefly luciferase.
- Reporter virus infectivity was assessed 3 dpi in MT-4 cells and expressed relative to wildtype (WT).
- EC_{50}s for LEN and BIC (control) against WT and mutant reporter viruses was determined in MT-4 cells using a 3-day antiviral luciferase-based assay. EC_{50} fold-change values for each CA mutant relative to the WT virus was determined from 3 independent experiments assayed in triplicate.
- CA mutations conferring >3-fold resistance to LEN were evaluated for their impact on LEN binding in Bioluminate[10] and expressed in HEK293T cells as replication-competent NL4.3 reporter viruses encoding NanoLuc luciferase. Viruses were infected onto primary human CD4+ T-cells activated with PHA/IL-2 and their outgrowth assessed over a 2-wk period and expressed relative to the WT.

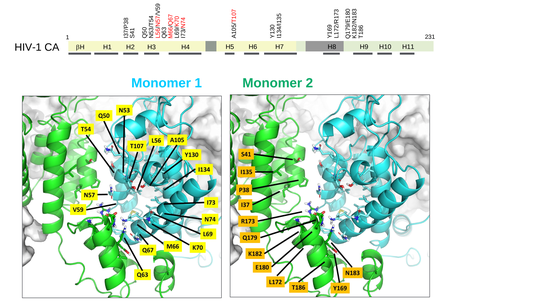
Results

Prevalence of CA binding site variants across HIV-1 subtypes

Capsid Amino Acid	Known resistant variants	New variants identified in this study	Capsid amino acid substitutions within HIV-1 group M subtypes (N)								
			B - Public (4,970)	B – Clinical (719)	A1 (489)	C (1,558)	D (430)	F1 (89)	G (128)	CRF01_AE (1,328)	CRF02_AG (346)
I37		V,Y			$V^{0.6}$				$Y^{0.8}$		
P38		None									
S41		A,I,M,Q,T,V	$T^{22}A^{4.5}$	$T^{33}A^{5.9}$	$T^{6.8}A^{0.6}$	$T^{96}A^{0.5}$	$T^{39}A^{1.6}I^{1.2}M^{0.7}$	$T^{5.6}A^{2.3}V^{1.1}$	$T^{47}Q^{1.6}V^{0.8}$	$T^{3.0}$	T^{43}
Q50		A,E,G,H,P,S,T,Y	$S^{1.1}G/H^{0.7}$	$S^{3.1}A^{1.7}T^{1.3}G/H^{1.1}$	$G^{4.4}H^{2.5}S^{1.5}T^{1.3}Y^{1.2}$	$S^{4.6}T^{2.4}G^{1.5}A/H^{1.2}P^{0.6}$**$E^{0.4*}$**	$G^{6.1}S^{4.9}A^{2.8}T^{1.9}H^{1.6}$	$H/T^{1.1}$	$G/S/T^{0.8}$		$T^{1.2}H^{0.9}G^{0.6}$
N53		K							$K^{0.8}$		
T54		A,I,L,M,S,V	$L^{0.7}$	$S^{0.7}L^{0.6}$	$M^{85}V^{3.9}L^{0.8}$	$S^{5.0}A^{0.6}$	$S^{4.0}M^{3.3}L^{1.6}V^{1.4}$	$S^{2.3}I^{1.2}$	$S^{2.4}L^{1.6}I^{0.8}V^{0.8}$	$M^{97}V^{0.7}$	$M^{92}V^{3.5}L^{1.2}A^{0.9}$
L56	I	F,M,V			$M^{0.8}$	$F^{0.6}$					**$V^{0.3*}$**
N57	S	H					**$H^{0.47*}$**				
V59		I	$I^{1.1}$	$I^{2.2}$	$I^{2.3}$	$I^{2.8}$	$I^{3.8}$		$I^{1.6}$		$I^{1.2}$
Q63		None									
M66	I	None									
Q67	H/Y	None									
L69		None									
K70	N/R	None									
I73		F							$F^{0.8}$		
N74	D/S	None									
A105	S/T	None									
T107	N	A,S,V	$S^{1.4}$	$S^{2.0}$	$A^{1.8}S^{0.6}$	A/$S^{1.2}$	$S^{0.7}$	$S^{2.3}A^{1.1}$	$A^{3.9}S^{2.3}$	$S^{2.6}$	$S^{0.9}V^{0.6}$
Y130		None									
I134		V		$V^{0.7}$							
I135		H,V	$V^{2.1}$	$V^{2.6}$		$V^{3.5}$	$V^{6.8}$	$H^{1.1}V^{1.1}$	$V^{2.4}$	$V^{1.1}$	V^{56}
Y169		F			F^{100}	F^{99}	$F^{2.8}$	F^{99}	F^{100}	$F^{1.6}$	F^{99}
L172		V						$V^{1.1}$			
R173		K							$K^{0.8}$		
Q179		A,P,T			$A^{0.8}P^{0.6}$				$T^{0.8}$		
E180		A,D,P	D^{37}	D^{44}	$D^{34}A^{0.6}$	D^{65}	D^{83}	D^{14}	D^{43}	$D^{2.9}$	$D^{20}P^{0.9}$
K182		R						$R^{1.1}$			
N183		A,D,G,H,Q,R,S,T,V	$T^{1.0}G/H^{0.6}$	$G^{2.9}S^{2.2}T^{1.8}H^{0.9}A^{0.7}$	$G^{53}S^{4.7}H^{2.3}D/R^{1.0}T/A^{0.6}$	$G^{2.7}H^{1.2}S^{0.6}$	$T^{2.8}G^{2.6}Q^{0.9}S^{0.7}$	$G^{65}S^{2.3}A/H^{1.1}$	$G^{40}S^{7.9}H^{2.4}D^{1.6}V/A^{0.8}$	$H^{1.7}$	$G^{8.4}S^{1.7}H^{0.9}A^{0.6}$
T186		None									

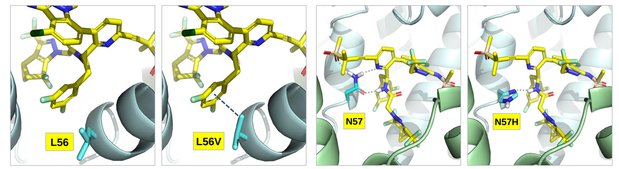
* Three polymorphs (Q50E, L56V, N57H) highlighted in **blue** were below the final 0.5% prevalence cutoff but were included here as there were identified and characterized during an interim sequence analysis

HIV-1 CA amino acid side-chains within a 5-angstroms of LEN



Structural impact of key CA substitutions on LEN binding

- L56V introduces steric clashing with di-flouro Phe group
- N57H eliminates 2 hydrogen bonds with LEN



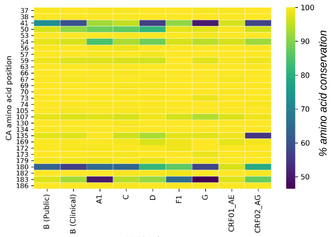
- Not shown: Q50E may alter the organization of water molecules adjacent to LEN's sulfonyl group

Summary of curated unique HIV-1 capsid (CA) sequences

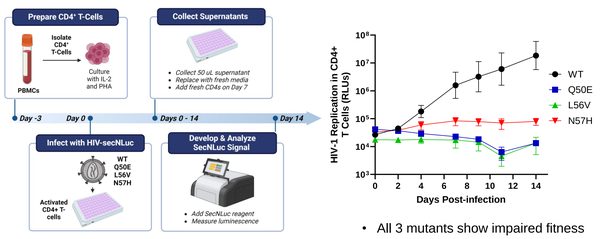
HIV-1 Subtype	Public[a] (n = 9,232)	Clinical[b] (n = 825)	Total Sequences (n=10,057)
B	4,970	719	5,689
A1	485	4	489
C	1,543	15	1,558
D	376	54	430
F1	88	1	89
G	125	3	128
CRF01_AE	1,308	20	1,328
CRF02_AG	337	9	346

[a] Curated *gag* sequences from Los Alamos HIV database (www.lanl.gov) as of June 2023
[b] Population and NGS data from plasma derived from PWH enrolled in Gilead clinical trials

Cross-clade CA sequence conservation within the LEN binding site

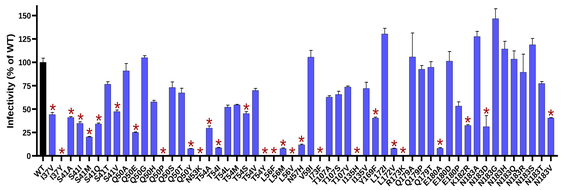


Impact of key CA polymorphisms on HIV-1 fitness



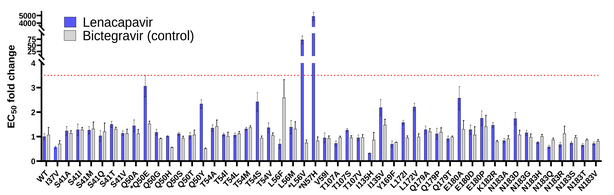
- All 3 mutants show impaired fitness

Infectivity of 55 site-directed HIV-1 CA mutants in MT-4 cells



- (*) Approximately half (28/55) of these mutants showed impaired infectivity (<50%, range 0.006 – 47%) relative to the WT
- The infectivity of 6 mutants (I37Y, Q50P, N53K, T54Y, I73F, R173K) was so severely impaired that it prevented LEN resistance profiling

Impact of CA polymorphisms on LEN potency in MT-4 cells



- With few exceptions (L56V, N57H), a mutant HIV panel composed of rare naturally occurring LEN binding site variants in CA remained fully susceptible to LEN

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