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Evaluation of HIV-1 capsid genetic variability and lenacapavir (GS-6207) drug resistance-associated mutations according to viral clades among drug-naive individuals

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Objectives: We evaluated the HIV-1 capsid genetic variability and lenacapavir drug resistance-associated mutations (DRMs) among drug-naive individuals across HIV-1 clades.

Methods: A total of 2031 HIV-1 sequences from drug-naive patients were analysed for capsid amino acid modification and the prevalence of lenacapavir DRMs. Amino acid positions with <5% variability were considered as conserved and variability was analysed by HIV-1 clades.

Results: Overall, 63% (148/232) of amino acid positions were conserved in the capsid protein. Of note, conservation was consistent in specific binding residues of cellular factors involved in viral replication [CypA (G89, P90), CPSF6 (Q4, N57, N74, A77, K182) and TRIM-NUP153 (R143)], while N183 (12.31%) was the only non-conserved lenacapavir binding residue. The overall prevalence (95% CI) of lenacapavir DRMs was 0.14% (0.05–0.44) (3/2031), with M66I (0.05%) and Q67H (0.05%) observed in subtype C, and T107N (0.05%) observed in CRF01_AE. Moreover, polymorphic mutations M66C ($n=85$; 4.18%), Q67K ($n=78$; 3.84%), K70R ($n=7$; 0.34%), N74R ($n=57$; 2.81%) and T107L ($n=82$; 4.03%) were observed at lenacapavir resistance-associated positions.

Conclusions: The low level of lenacapavir DRMs (<1%) supports its predicted effectiveness for treatment and prevention, regardless of HIV-1 clades. The established conserved regions hence serve as a hallmark for the surveillance of novel mutations potentially relevant for lenacapavir resistance.

Introduction

HIV-1 Gag proteins are necessary for virion assembly, virion maturation and early stages of virus replication.[1] One of the important Gag proteins is the capsid (CA), which is generated by the cleavage of the Gag polyprotein by the protease enzyme.[2] In the final phase of maturation, Gag is cleaved and CA is released, allowing the assembly of CA into a fullerene cone, known as the capsid core.[2,3] The C-terminal domain (CTD) third of CA (152–231) appears to function primarily as an assembly domain and is required for Gag oligomerization,[4] CA dimerization[5] and viral assembly.[6] In contrast, substitutions and deletions in the N-terminal domain (NTD) of the HIV-1 CA (1–151) generally result in virions that assemble, but are non-infectious.[7] The correlation between proper core assembly and viral infectivity suggests that the structure of the CA core plays an essential role in the early stages of viral replication.[6] HIV-1 replication also requires packaging of the cellular peptidyl prolyl isomerase, cyclophilin A

(CypA).[8] CypA binds directly to the CA domain of Gag (at residues G89, P90, H87, A92 and G94), with substantial CA–CypA stoichiometry estimates of 2000:200 per virion.[6] Formation of the CA/CypA complex is inhibited by a series of mutations in the N-terminal domain of CA.[8] Reagents and mutations that inhibit the CA–CypA interaction *in vitro* also block CypA packaging and HIV-1 replication in culture, demonstrating that this interaction is essential for viral infectivity.[8]

Further investigations on the role of CA in the HIV-1 life cycle are concordant on the therapeutic potentials as an HIV-1 inhibitor.[2] Of note, lenacapavir (GS-6207) is the first-in-class HIV CA inhibitor with a unique multistep mechanism of action, including inhibition of HIV assembly, formation of appropriate viral and nuclear entry of viral DNA.[9] GS-6207 contains a difluorobenzyl ring that occupies the CPSF6/Nup153 binding pocket, overlapping with the benzyl group of F321 for CPSF6 and F1417 for Nup153.[3] Crystal structures of GS-6207 bound to CA hexamers reveal that six GS-6207 molecules bind to each hexamer, establishing extensive hydrophobic interactions, two cation-π interactions and seven hydrogen bonds, which contact ∼2000 Å^2 of buried protein surface area.[3] Strong binding of GS-6207 therefore competitively interrupts CA interactions with CPSF6 and Nup153, and GS-6207 binding sites are N57, K70, N74 and N183.[3] The picomolar potency of GS-6207 and its unique physicochemical properties make it a good candidate for a long-acting antiretroviral agent.[9] Recent findings on GS-6207 *in vitro* dose escalation and viral breakthrough selections in T cell lines and PBMCs, infected with clonal and clinical HIV-1 isolates, identified the following CA variants associated with HIV drug resistance to GS-6207: L56I, M66I, Q67H, K70N, N74D, N74S and T107N.[9–11]

However, there is a scarcity of evidence on GS-6207 resistance-associated mutations in clinical isolates, especially on a large set of individuals infected with HIV-1 non-B subtypes.[9,12] In this frame, we sought to evaluate HIV-1 CA variability and GS-6207 drug resistance-associated mutations across HIV-1 viral subtypes among ART-naive individuals.

Methods

Sequence analysis

A study was conducted on HIV-1 Gag sequences retrieved from the Los Alamos HIV Database (https://www.hiv.lanl.gov/content/index). One sequence per patient was selected from plasma samples of ART-naive individuals. Multiple sequence alignment of the Gag gene was performed by using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and manually edited with the BioEdit program, version 7.0.5.

Analysis of the CA amino acid (aa) conservation

Within the 232 aas of the HIV-1 CA protein, analysis of conserved regions was based on consensus B as reference strain, with <5% variability defined as conserved positions. CA long conserved regions were defined as a minimum of five consecutive positions with <5% variability. The CA variability was classified as follows: highly conserved aas (<1% variability); weakly conserved aas (1%–4.9% variability); non-conserved aas (5%–19.9% variability) and highly polymorphic positions (≥ 20% variability).

Analysis of CA variants at GS-6207 resistance-associated positions

For all CA sequences, the presence and the frequency of mutations at the six CA positions found to be associated with GS-6207 resistance were investigated,[3,13] and these included L56, M66, Q67, K70, N74 and T107. For all these positions, the frequency of CA variants was then evaluated according to HIV-1 viral subtypes.

Results

Sequence characteristics and genetic diversity

Overall, 2031 HIV-1 group M full CA sequences obtained between 1985 and 2020 were analysed. Twelve HIV-1 clades were found, including HIV-1 pure subtypes (A, B, C, D and F1) representing 63.1% (1281/2031), and recombinant forms (07_BC, 01_AE, 02_AG, 49.cpx, 65.cpx, 76.01B and 89_BF1) representing 36.9% (750/2031). The most frequent clades were subtype C ($n=834$; 41.0%), CRF01_AE ($n=267$; 22.0%), CRF07_ BC ($n=267$; 13.1%) and subtype B ($n=205$; 10.1%).

Conservation analysis of the CA protein

Overall, a total of 148/232 (63.0%) CA aa positions in the entire sequences were conserved (<5% variability), among which only 4.7% ($n=11/232$) were highly conserved (<1% variability). The conserved residues were distributed throughout the entire CA protein, either singly ($n=16$), in pairs ($n=19$), in triplets ($n=9$), quadruplets ($n=1$) or in long invariant regions (five consecutively conserved aa positions <5%; $n=8$), as shown in Figure 1. The long invariant regions were as follows: I (P17 to K25; aa=9); II (G60 to Q67; aa=8); III (P99 to G106; aa=8); IV (K140 to Y145; aa=6); V (Q155 to F168; aa=14); VI (L172 to Q176; aa=5); VII (N195 to K199; aa=5); and VIII (A217 to P224; aa=8). The aa positions with a variability of 5.0%–19.9% and ≥20% variability had proportions of 53/232 (22.8%) and 31/232 (13.4%), respectively. The CypA binding residues G89 and P90 were conserved (<5% variability) while H87, A92 and G94 were not conserved. The CPSF6 binding residues (Q4, N57, N74, A77, K182) were conserved, as well as position R143, which is known to be associated with resistance to TRIM-NUP153, was also conserved. Regarding lenacapavir binding sites (N57, K70, N74, N183), only N183 was not conserved ($n=250$; 12.31%). Regarding the CA domains, NTD (1–151) and CTD (152–231) were conserved, respectively, in 70.4% (57/81) and 60.3% of aa positions.

Prevalence of CA variants at GS-6207 known resistance-associated positions

The overall prevalence (95% CI) of GS-6207 resistance-associated mutations from our analysis was 0.14% (3/2031) (0.05–0.44) in the entire sequence dataset. The lenacapavir-associated resistance mutations observed in our study were: M66I (1/2031), Q67H (1/2031) and T107N (1/2031), while L56I, K70N and N74S/D were completely absent. Moreover, no resistance mutation associated with TRIM-NUP153 was found. Distribution of mutations by viral clades showed M66I and T107N present in subtype C, and Q67H in circulating recombinant form CRF01_AE. Other aa substitutions found at GS-6207 resistance positions were: L56F ($n=3$/2031; 0.14%); M66C (85/2031; 4.18%); Q67K (78/2031; 3.8%);

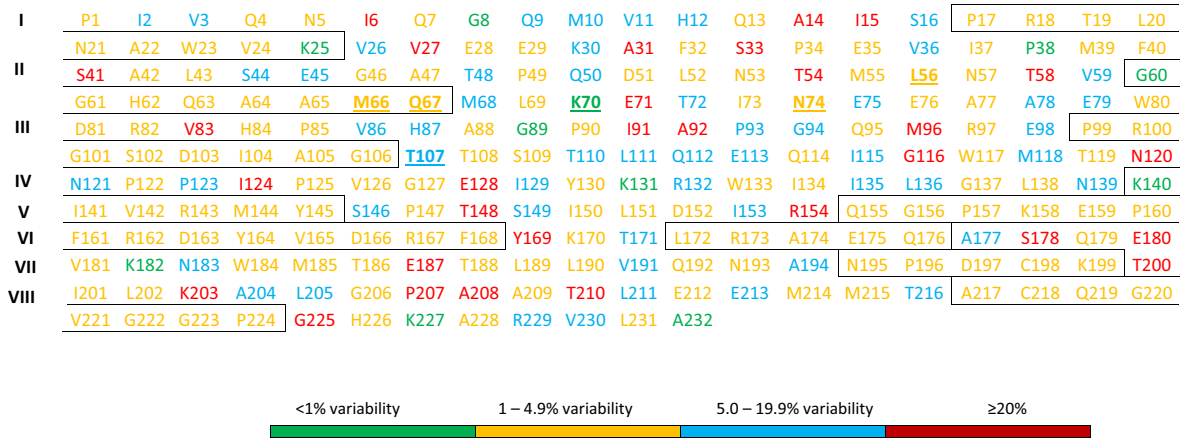

Figure 1. Sequences containing the 232 amino acids of HIV-1 capsid were aligned and compared to the reference strain B (HXB2) for the entire study sequences. Colour at each amino acid position was attributed according to the frequency rate of variation such that blue corresponds to highly conserved amino acids (<1% variability), green corresponds to weakly conserved amino acids (1%–4.9% variability), orange corresponds to non-conserved amino acids or polymorphisms (5%–19.9% variability) and red represents highly polymorphic positions (≥20% variability). The amino acids in the box represent the long invariants regions. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Table 1. CA polymorphisms at lenacapavir resistance-associated positions

WT aas and their positions	Resistant variants[a]	HIV-1 group M subtypes							
		A ($n=157$)	B ($n=205$)	C ($n=834$)	D ($n=71$)	F1 ($n=14$)	CRF07_BC ($n=267$)	CRF01_AE ($n=446$)	ORFs ($n=37$)
L56	I	—	—	F (3), V (2), M (1)	—	—	—	K (1)	—
M66	I	C (1)	C (5)	C (7), H (2), **I (1)**	C (71)	—	V (1)	A (1), C (1)	—
Q67	H	K (1)	K (5)	K (4), R (3), A (2)	K (67)	—	—	N (1), K (1), **H (1)**	—
K70	N	—	—	R (1)	R (5)	—	—	R (1), I (1)	—
N74	D, S	M (1)	M (5)	M (7), Q (2)	R (57)	—	—	M (1)	—
T107	N	L (1)	A (1), V (2), L (4), P (1), S (1)	A (1), S (2), L (7), **N (1)**	L (69), P (3)	—	—	S (9), L (2), Q (1)	—

Other recombinant forms (ORFs) refers to CRF89_BF1 ($n=18$), CRF02_AG ($n=11$), CRF_49.cpx ($n=3$), CRF76_01B ($n=3$) and CRF65_cpx ($n=2$). Numbers in brackets are the number of sequences harbouring the variant. Reference to HXB2. In bold they are amino acid substitution associated to Lenacapavir drug resistance.
[a]Resistant variants were defined according to previous studies.[9,13,17]

K70R (7/2031; 0.34%); N74R (57/2031; 2.80%); and T107L (83/2031; 4.08%). Of note, the GS-6207 resistance-associated position with the highest variability was T107 (5.22%).

Discussion

In the present study dedicated to the natural variability of the HIV-1 CA protein according to HIV-1 viral clades, with a focus on the lenacapavir positions associated with resistance among ART-naive individuals, the molecular epidemiology showed a wide range of HIV-1 clades, including pure and recombinant strains. As previously reported, our analysis revealed that HIV-1 CA is a well-conserved region (about 63% of the entire aa positions), which includes the cellular factors CypA, CPSF6 and NUP153, known to play an important role in HIV viral replication.[14] Our data also show that most CA residues interacting with these factors are conserved. For instance, G89 and P90 were conserved for CypA. Q4, N57, N74, A77 and K182 were also conserved for CPSF6, and 153 and R143 were the conserved residues for NUP. Interestingly, we have identified up to eight invariant regions, of which the V region (Q155 to F168; $n=14$ aa) was the longest and is located in the major homology region (MHR). This represents a set of residues (L151 to Q179) well conserved across HIV-1 clades and which is known to play an essential role for viral assembly, maturation and infectivity.[15] This high conservation of HIV-1 CA underscores the suitability of this target for new antiretrovirals such as GS-6207.[13] This is further reassuring as, apart from N183, all the binding sites of lenacapavir[16] are conserved.

Concerning resistance, we have found that the prevalence of lenacapavir-resistance mutations is low (0.14%) among drug-naive individuals, thus ensuring its efficacy across a wide range of HIV-1 subtypes.[16] As compared with the literature, where GS-6207 resistance mutations have been well documented,[17] GS-6207 resistance mutations were rare in our study. However, we identified some polymorphisms at GS-6207

resistance positions (Table 1) that deserve further monitoring when transitioning to this new drug in clinical practice, including an in-depth analysis. Giving that lenacapavir is still under investigation, clinical trials are needed to ensure timely translational applications.

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Transparency declarations

The authors declare no competing interests.

Author contributions

Conceived the study: A.D.N., Y.B., G.T., J.F., M.M.S., D.K.T., F.C.S., E.N.J.S., S.M.S., A.N. Collected and analysed the data: A.D.N., Y.B., G.T., L.C., D.A., L.V., A.M.K.N., W.P. Initiated the manuscript: A.D.N., Y.B., G.T., J.F., M.M.S., F.C.S., L.F., D.A., E.N.J.S., D.T. Revised the manuscript: all authors read and approved the final manuscript.

Availability of data and materials

See https://www.hiv.lanl.gov/content/index.

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