Subtype A1, D, and Recombinant HIV-1 Natural Polymorphisms Associated with Lenacapavir Drug Resistance in Mbarara, Uganda

Daniel Omoding[1,7]*, Nicholas Musinguzi[1], Yap Boum II[2], Conrad Muzoora[1], Simone Kigozi[1], Peter W. Hunt[3], Jeffrey N. Martin[3], David R. Bangsberg[4], Jessica E. Haberer[5,6], Mark J. Siedner[1,5,6], Suzanne McCluskey[1,5,6]**, Guinevere Q. Lee[7]**

*presenting author, **co-last-authors

[1]Mbarara University of Science and Technology, Mbarara, Uganda, [2]Institut Pasteur de Bangui, Bangui, Central African Republic, [3]University of California, San Francisco, CA, USA, [4]Oregon Health Sciences University, Portland, OR, USA, [5]Massachusetts General Hospital, Boston, USA, [6]Harvard Medical School, Boston, MA, USA, [7]Department of Medicine, Division of Infectious Diseases, Weill Cornell Medicine, New York, NY, USA.

Contact
domoding@must.ac.ug
AS-IAS-2023-02236

Background

Lenacapavir (LEN) belongs to a new class of HIV drugs called capsid inhibitors, which target viral Gag p24. LEN is effective in combination with other antiretrovirals (ART) for subtype B HIV-1 infections. However, less data is available for its activity in non-subtype-B HIV-1, which could harbor natural polymorphisms that may reduce LEN susceptibility.

Methods

The Uganda AIDS Rural Treatment Outcomes (UARTO) cohort enrolled ART-naïve adults in Mbarara, Uganda between 2002-2010 just prior to their initiation of ART. Participants were followed longitudinally until 2015. **All study participants were LEN-naïve.** Archived plasma samples collected at study visits both pre- and post-ART-initiation were subjected to HIV-1 *gag* p24 Sanger sequencing, subtyped by RIP 3.0 and aligned using MUSCLE against reference sequence HXB2. Lenacapavir-associated resistance mutations were defined according to the 2022 IAS-USA drug resistance mutations list including L56I, M66I, Q67H, K70N/S/R, N74D/S, A105T and T107N. We also examined mutations associated with lenacapavir activity reported in other studies including Q67K/N, K70H, N74H, A105S, and T107A/C (Ogbuagu 2022 and Margot 2022).

Table 2. List of Gag p24 mutations evaluated and detected in this study. Only mutations K70R and T107A were observed in the UARTO cohort.

IAS 2022 List of Resistance Mutations	Other mutations examined*	Frequency detected in this cohort (n=546)	Other non-wildtype mutations detected
L56I	None	0/546 (0%)	L56M, 258/546 (47%)
M66I	None	0/546 (0%)	None
Q67H	Q67K/N (Ogbuagu 2022)	0/546 (0%)	Q67Q/R, 1/546 (0.2%)
K70N/S/R	K70H (Margot 2022)	K70R, 1/546 (0.2%)	None
N74D/S	N74H (Ogbuagu 2022)	0/546 (0%)	N74N/K, 1/546 (0.2%)
A105T	A105S (Ogbuagu 2022)	0/546 (0%)	A105I, 1/546 (0.2%) A105V, 1/546 (0.2%) A105A/V, 2/546 (0.4%)
T107N	T107A/C (Ogbuagu 2022)	T107N, 0/546 (0%) T107A or T/A, 7/546 (1%)	T107I, 1/546 (0.2%) T107S or T/S or T/S/C, 10/546 (2%)
Any of the above		8/546 (1%)	

*These mutations have been associated with resistance in published studies but were not included into the IAS 2022 List of Drug Resistance Mutations in HIV-1.

Results

Figure 1. The UARTO cohort. (A) Sampling year. The UARTO cohort enrolled between 2002-2010. Overall, we obtained *gag* sequences from 546/609 (90%) study participants from archived plasma samples. (B) Map of Uganda showing Mbarara district.

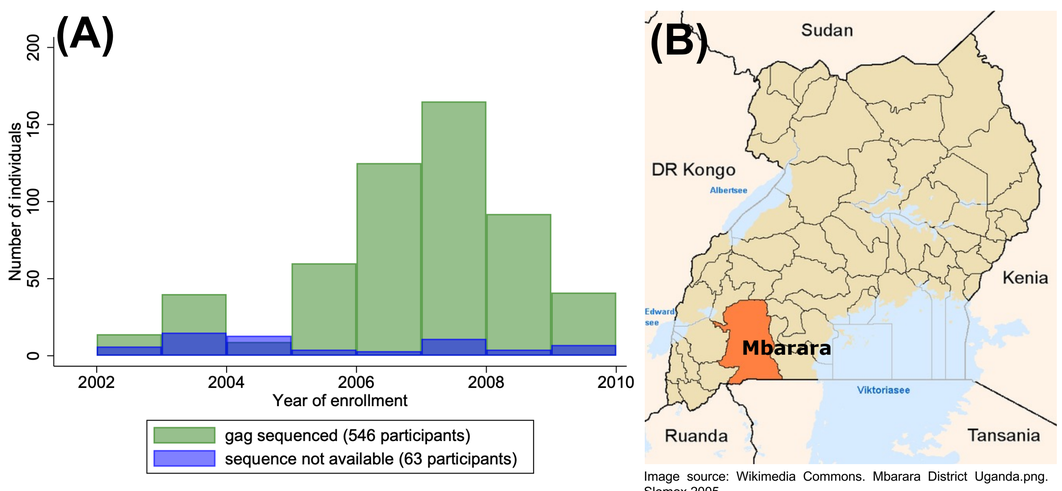
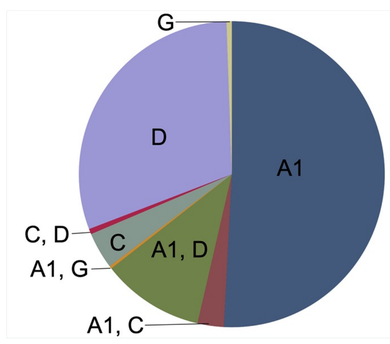


Image source: Wikimedia Commons. Mbarara District Uganda.png. Slomox 2005.

Figure 3. UARTO cohort HIV-1 subtype distribution. The primary subtyping method used in this study was RIP 3.0 (Los Alamos HIV Sequence Database). Results were compared to subtyping by Hamming distances against subtype references and REGA.



HIV-1 Subtype by RIP 3.0	Counts	Percentage
A1	278	50.9%
A1, C	15	2.7%
A1, D	59	10.8%
A1, G	2	0.4%
C	20	3.7%
C, D	3	0.5%
D	166	30.4%
G	3	0.5%
Total	546	100.0%

Subtyping Tools	Count Concordance	Total	Percentage
Hamming-RIP	630	741	85%
Hamming-REGA	604	741	82%
RIP-REGA	694	741	94%
Hamming-RIP-REGA	604	741	82%

Figure 4. Overall prevalence. Lenacapavir-associated resistance mutations were detected in 1% of UARTO participants (95% confidence level 0.6-2.9%).

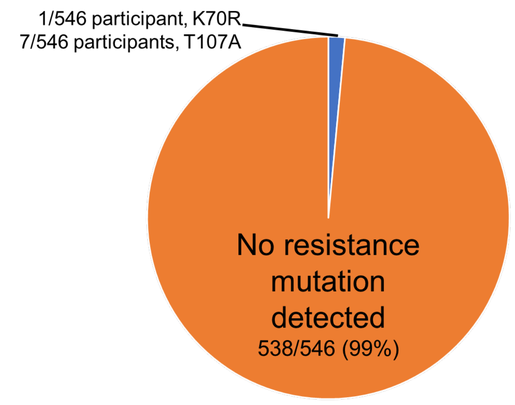
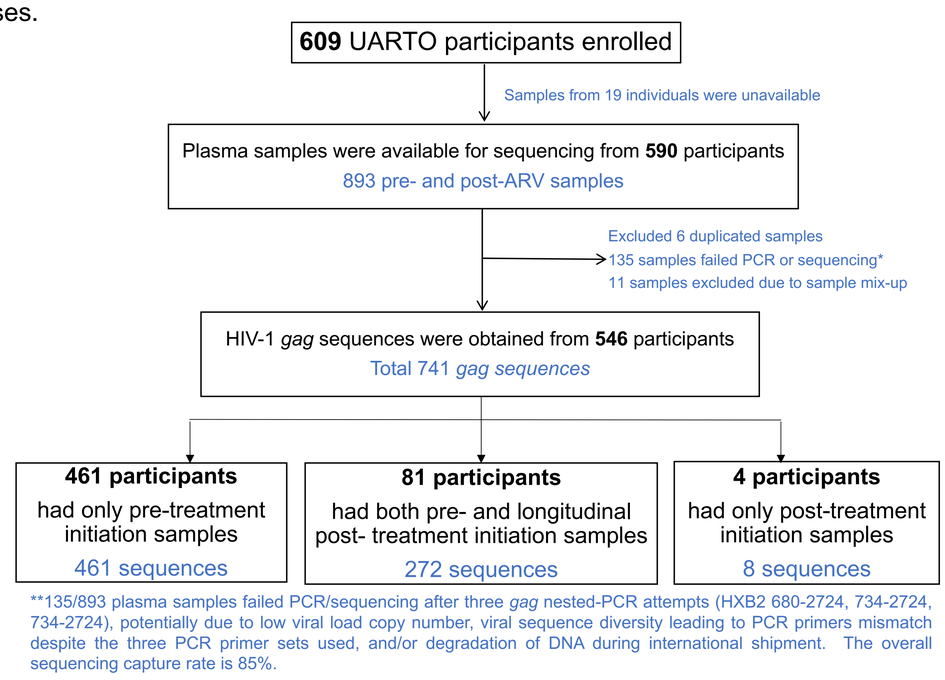


Figure 2. HIV-1 *gag* PCR and sequencing workflow. We obtained 546 pre- and 195 post-therapy initiation *gag* sequences. The p24 region in *gag* was extracted for downstream analyses.



**135/893 plasma samples failed PCR/sequencing after three *gag* nested-PCR attempts (HXB2 680-2724, 734-2724, 734-2724), potentially due to low viral load copy number, viral sequence diversity leading to PCR primers mismatch despite the three PCR primer sets used, and/or degradation of DNA during international shipment. The overall sequencing capture rate is 85%.

Figure 5. Details of the eight individuals who had lenacapavir-associated resistance mutations detected. Longitudinal samples were available from participants MBA1123 and MBA1057. Only pre-therapy initiation samples were available from the remaining six study participants.

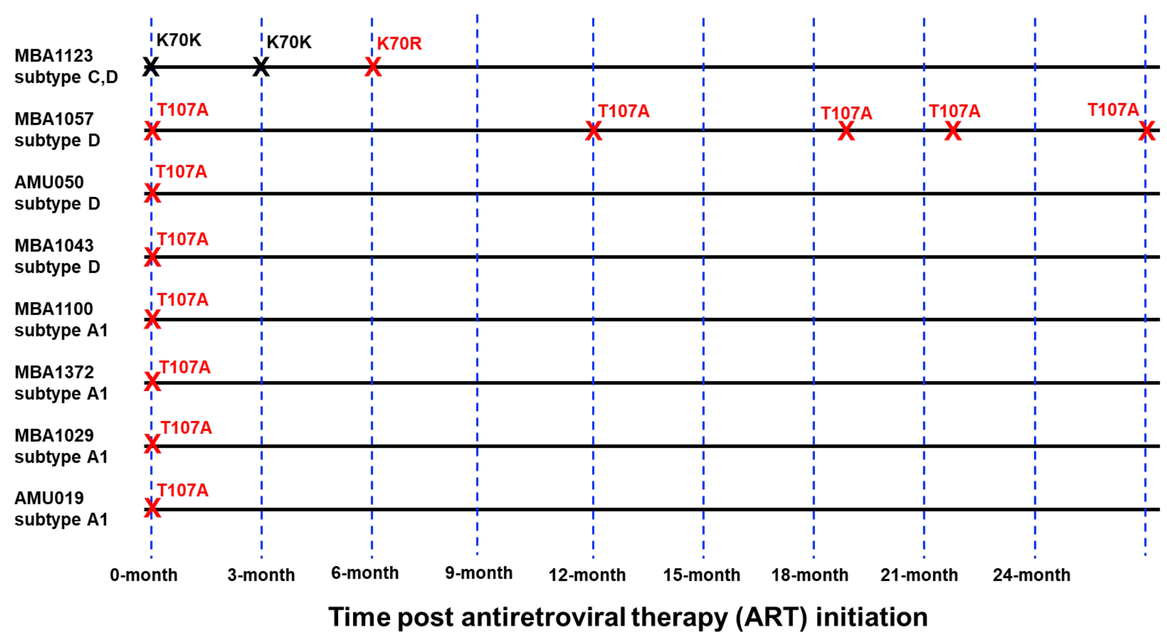
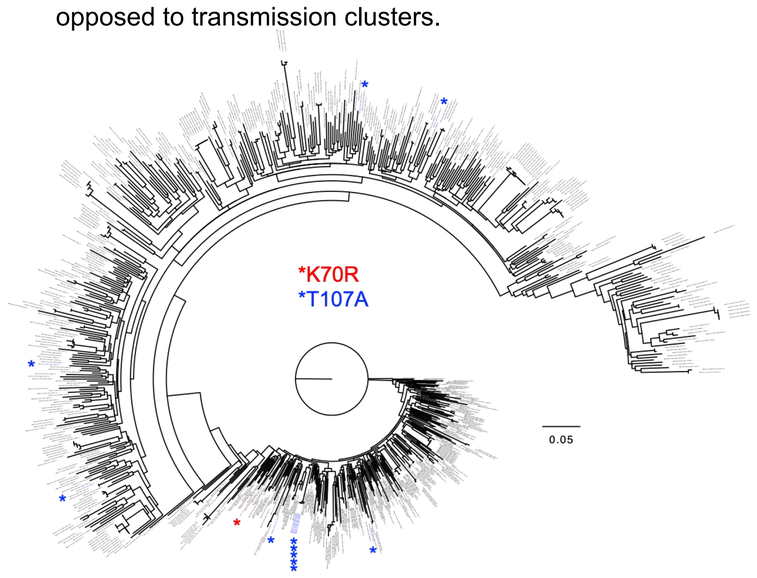


Table 1. Participants Pre-therapy Initiation Baseline Characteristics (UARTO cohort, n=609). There was no statistically significant differences in age, viral load and sex between participants with and without *gag* sequence data. Participants who did not have *gag* sequence data had significantly lower median CD4 count/μL and more frequently received 3TC/d4T/NVP as their initial ART regimen.

	Participants with *gag* sequence data (n=546)	Participants without *gag* sequence data (n=63)	p-value
Age median (IQR)	34 (29 - 39)	36 (31 - 40)	0.150
CD4 count/μL median (IQR)	127 (65 - 196)*	73 (9 - 204)**	0.005
Viral load copies/mL median (IQR)	5.16 (4.67 - 5.65)	5.12 (4.14 - 5.64)	0.098
Sex n, female (%)	377 (69)	42 (67)	0.699
Initial ART n (%)			
3TC/AZT/NVP	314 (58)	16 (25)	<0.001
3TC/d4T/NVP	170 (31)	42 (67)	
3TC/AZT/EFV	50 (9)	1 (2)	
Others	8 (1)	0 (0)	
Missing	4 (1)	4 (6)	

All p-values <0.05 are highlighted in red. Antiretroviral therapy (ART), Lamivudine (3TC), zidovudine (AZT), nevirapine (NVP), stavudine (d4T), efavirenz (EFZ). *In the group with *gag* sequence data (n=546), CD4 count information was missing from two individuals (MBA1007, MBA1476) and were excluded from the statistical analysis. **In the group without *gag* sequence data (n=63), CD4 count information were missing from four individuals (MBA1200, MBA1254, MBA1424, MBA1032) and were excluded from the statistical analysis.

Figure 6. Phylogenetic analysis of *gag* p24 by PhyML. Phylogenetic analysis of all 741 *gag* p24 sequences in this study shows the lack of clustering of T107A (blue asterisks), suggesting independent mutation events as opposed to transmission clusters.



Notes about mutations. **K70R**, when occurred alone, was associated with 1.2-fold reduced LEN susceptibility (Margot 2022, SDM), and when occurred in conjunction with Q67H was associated with 15-20-fold reduced LEN susceptibility (Margot 2022, CALIBRATE and CAPELLA). **T107A**, when occurred with M66I, was associated with 240-fold reduced LEN susceptibility (Margot 2022, CAPELLA). Both K70 and T107 are in the HIV-1 CA N-terminal domain and are involved in forming both intra- and inter-CA-hexameric interactions.

Conclusions

Among individuals in western Uganda living with subtype A1, D, and intersubtype recombinant HIV-1, we observed a 1% prevalence of natural viral polymorphisms associated with lenacapavir resistance.

Our findings provide preliminary evidence that LEN is likely to be active against circulating HIV-1 viruses in this region.

Presented at IAS 2023, the 12th IAS Conference on HIV Science