In-depth Proviral Sequence Analysis Reveals Conserved Reservoir Landscape Composition Across Group M

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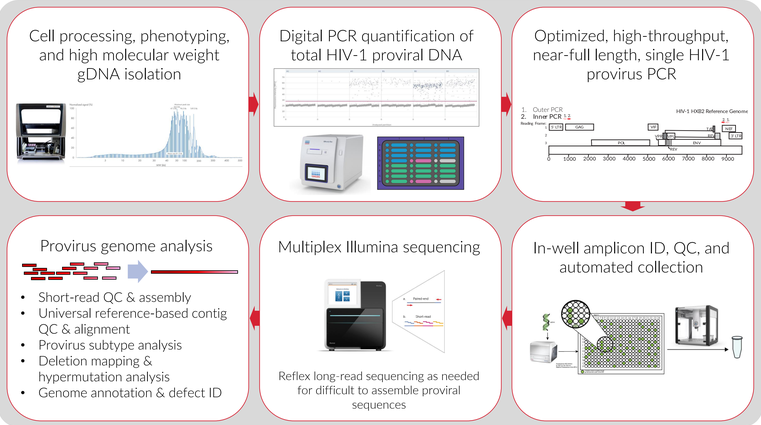
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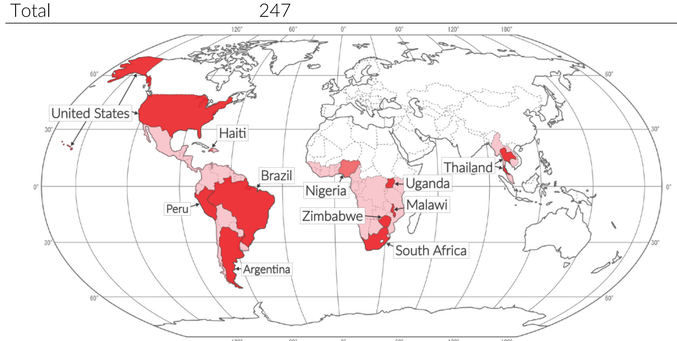
BACKGROUND

The biology of HIV-1 replication and the epidemiology of transmission led to a diverse landscape of viral subtypes heterogeneously distributed across the globe. Most people with HIV-1 (PWH) are infected with non-subtype B virus, yet the landscape of persistent HIV-1 is understudied outside of subtype B. In-depth analysis of persistent HIV-1 in PWH across subtypes is essential to globalize HIV-1 cure research and clinical trials. Such analysis is also foundational to design and validate molecular assays for latent HIV-1, such as a cross-Group M Accelevir IPDA®. We therefore interrogated the genetic landscape of persistent HIV-1 proviruses in hundreds of adult male and female PWH on ART enrolled in several independent studies. We employed an optimized high-throughput pipeline for near-full length proviral amplification, single genome sequencing, and bioinformatic analysis, allowing for robust assembly, alignment, and defect classification of thousands of HIV-1 proviral genomes across Group M.

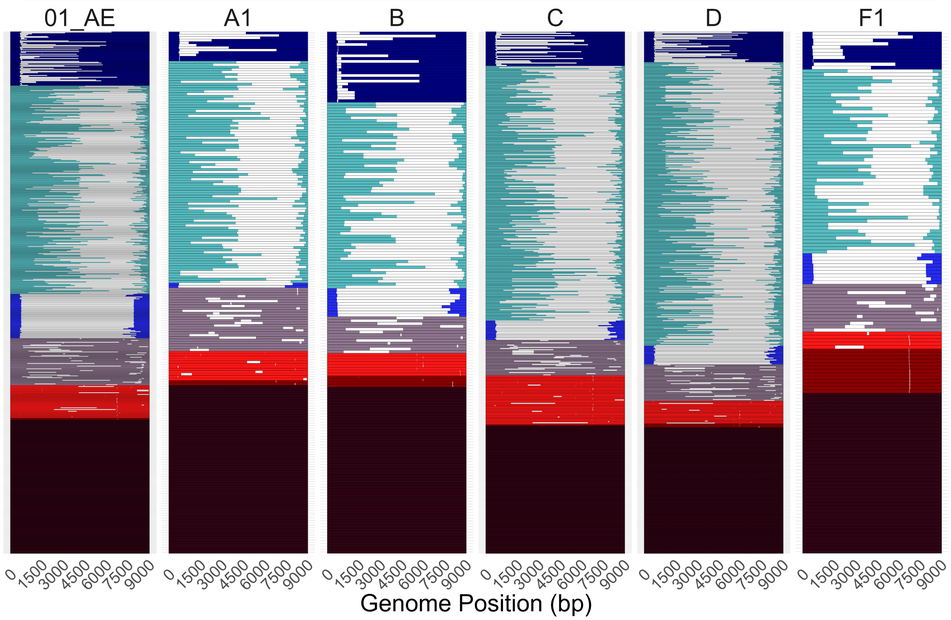
METHODS



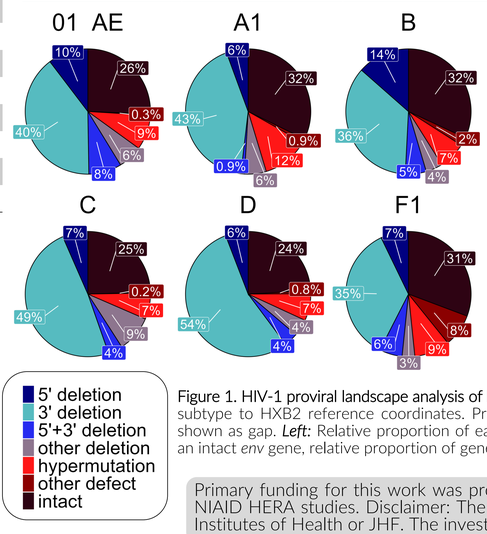
Study	Sample *N*	Date	Subtypes
ACTG A5175	31	2008-2010	C
ACTG A5345	2	2019	01_AE, B, C
ACTG A5354	16	2018-2020	01_AE, C
IMPAACT 1077HS	66	2011-2016	01_AE, B, C, F1 recombinants
NIAID HERA	47	2018-2021	A, D
US MHRP RV254/304	23	2010-2020	CRF01_AE
Other studies	62		A, B, D
Total	247		



The landscape of persistent proviruses in PWH on ART is conserved across HIV-1 Group M. This new insight has important implications for the design of assays and therapeutics targeting the HIV-1 latent reservoir.



N sequences= 716	N sequences= 214	N sequences= 378	N sequences= 457	N sequences= 530	N sequences= 153
N participants = 64	N participants = 25	N participants = 52	N participants = 50	N participants = 46	N participants = 13



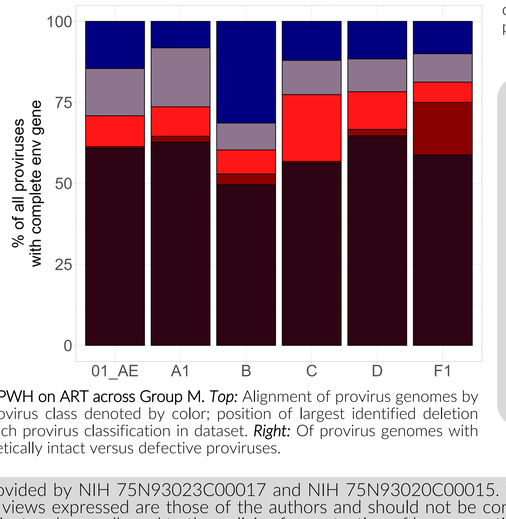


Figure 1. HIV-1 proviral landscape analysis of PWH on ART across Group M. *Top:* Alignment of provirus genomes by subtype to HXB2 reference coordinates. Provirus class denoted by color; position of largest identified deletion shown as gap. *Left:* Relative proportion of each provirus classification in dataset. *Right:* Of provirus genomes with an intact *env* gene, relative proportion of genetically intact versus defective proviruses.

RESULTS

To date, this effort includes 2,447 proviral genomes from 247 adult PWH on suppressive ART. Our analysis reveals consistent proviral landscapes across Group M subtypes CRF01_AE, A1, B, C, D, and F1 recombinants. Most persistent proviruses harbor fatal deletions, which on average span half the genome and most frequently impact the *env* gene. Interestingly, the majority of proviruses with a complete *env* gene are genetically intact. A subset of proviruses in all subtypes are extensively hypermutated at APOBEC3G target sites. Frequencies of proviruses vary between participants but are similar across all subtypes analyzed. Optimization of NFL-PCR substantially increased provirus recovery efficiency, enabling a more accurate assessment of the HIV-1 proviral landscape across diverse cohorts of PWH on ART. Assessment of deletion position and frequency suggests comparable accuracy of proviral discrimination across subtypes by Accelevir IPDA® *psi* and *env* targets.

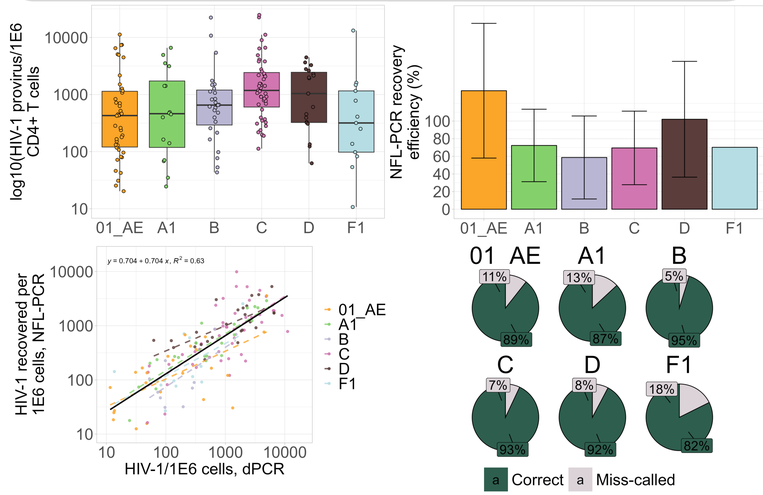


Figure 2. Group M HIV-1 reservoir size. *Top left:* Total HIV-1 proviral DNA per million CD4+ T cells quantified by dPCR; median=565.1 provirus/1E6 CD4+ T cells [CI: -205.9 – 1336.0] *Top right:* Recovery efficiency of HIV-1 provirus NFL-PCR based on dPCR-calculated proviral input (mean ± 95% confidence interval). *Bottom left:* Linear correlation of HIV-1 proviral DNA frequency quantified by dPCR vs. NFL-PCR, color-coded by subtype with black line indicating overall correlation. *Bottom right:* Predicted accuracy of proviral discrimination by Accelevir IPDA® *psi* and *env* targets.

CONCLUSIONS

This study is among the first efforts to comprehensively assess the HIV-1 reservoir in PWH on ART across Group M. The overall consistency in HIV-1 provirus deletion frequency and position between subtypes suggests that conserved mechanisms drive the formation of defective proviruses. The dominance of *env* deletions has important implications for antibody-mediated reservoir immune selection and for global deployment of env-targeting therapeutics. Our improvements to long-range proviral PCR reduce the bias against intact proviruses, resulting in a more accurate analysis of proviral landscape composition in PWH on ART, and these improvements will enable comparison of short- and long-range PCR-based reservoir quantification assays. Importantly, our results support the feasibility of a unified Accelevir IPDA® version 2 design with robust performance across Group M for global use.

ACKNOWLEDGEMENTS

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