COMPARATIVE ANALYSIS OF HIV-1 RNA AND ARCHIVED PROVIRAL HIV-1 DNA GENOTYPING

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BACKGROUND

- Baseline bulk sequencing of the HIV pol gene in blood plasma is the gold standard for HIV genotyping and detection of drug resistance mutations (DRMs).
- Next-Generation Sequencing (NGS) of archived proviral HIV DNA can detect minority DRMs that may not be found in standard bulk genotyping.[1]
- Here, we investigate if NGS of proviral DNA can detect minority DRM that will impact biological outcomes of individuals identified during acute and early HIV infection and started rapidly on antiretroviral therapy (ART).

METHODS

Population: This study included adults with acute/early HIV infection (Classified by estimated date of infection (EDI)), who were enrolled in the San Diego Primary Infection Resource Consortium (PIRC).[2]

Samples: Blood plasma and Peripheral blood mononuclear cells (PBMC) were collected before ART initiation in participants.

Sequencing

- Bulk genotyping of HIV reverse transcriptase was performed in blood plasma samples.
- NGS of HIV partial pol was performed in PBMC.

DRM Screening

- Nucleoside/Nonnucleoside Inhibitor (NRTI/NNRTI) mutations were assessed in both blood plasma and PBMC. In addition, Integrase Inhibitors (INSTI) mutations were evaluated in PBMC.[3]
- Cleaned filtered reads from NGS were used to detect DRM in HIV proviral DNA. DRM >2% prevalence were considered for further analysis.
- DRM categories were defined as Major (freq. >20%) or Minor (freq. 2-20%)

Table 1. Participant Demographics

Category	[ALL]	N
Number of Participants	**N = 190**	190
Age	31.5 [IQR:20.0,58.3]	188
Time from diagnosis to ART (days)	20 [IQR:2.7]	190
Number of Samples (Sequencing)		
PBMC	**N = 190**	
Blood Plamsa	**N = 188**	
Gender		190
Female	4 (2.11%)	
Male	184 (96.8%)	
Non-Binary	1 (0.53%)	
Transgender (Female to Male)	1 (0.53%)	

RESULTS

Among participants that started ART early, DRM identified in archived proviral DNA (>2%) was not associated with virologic failure during the follow-up period of 25.39 ± 3.13 **(Figure 4)**

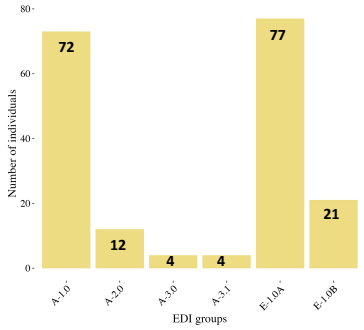
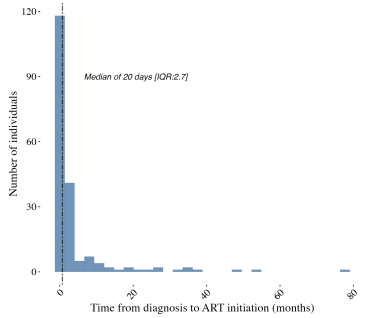


Figure 1. 78% of the cohort had an EDI status of either A-1.0 or E-1.0A

Figure 2. 62% of participants started ART within the first 3 months from their diagnosis



Cohort Characteristics

- EDI status were defined as follows: **A-1.0** (seronegative acute), **A-2.0, A-3.0, A-3.1** (Very early infection), **E-1.0A** (Antibody positive (0-6 months)), and **E-1.0B** (Antibody positive (6-12 months)) **(Figure 1)**.[4]
- Among the 190 participants, the median time from infection (days) to the first ART regimen was 81.0 days. Interquartile Range (IQR):[12.7;1064] **(Table 1,Figure 2)**.

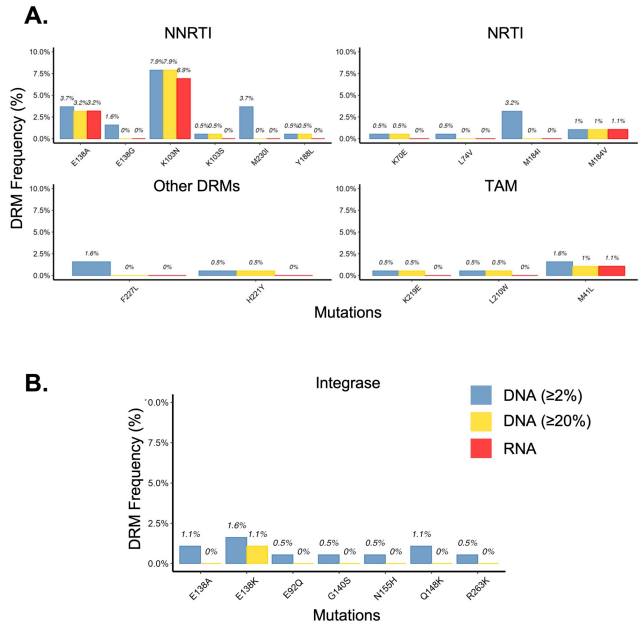
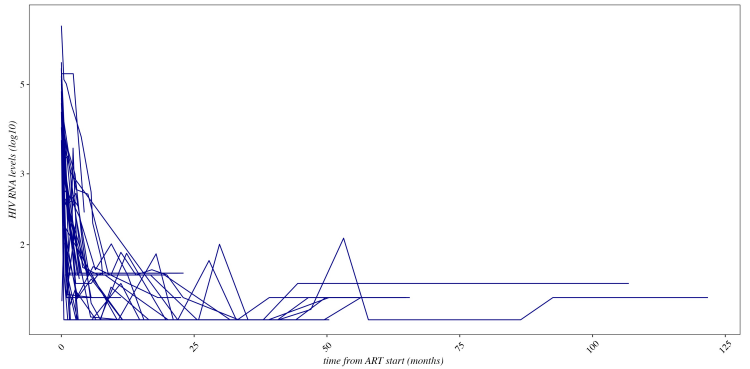


Figure 3A. While 2 major M184V mutations appeared in both PBMC and blood plasma, 6 low-frequency M184I were found only in PMBC. **B.** INSTI mutations were additionally screened in Proviral DNA

DRM Frequency

- Among the 190 participants, Major DRMs detected in blood plasma were congruent with DRM detected in PBMC aside from 2 K103N (1.1%,NNRTI) mutations found only in PBMC
- Minor DRMs detected in blood plasma were congruent with DRM detected in PBMC aside from 13 NNRTI (6.8%) and 9 NRTI (4.7%) mutations found only in PBMC.
- We also screened for INSTI mutations and detected 11 DRMs in HIV DNA.

Figure 4. Participants with DRM remained virally suppressed through the follow-up period



CONCLUSIONS

- For recently infected individuals who started ART early, detected DRM did not lead to viral rebound for participants with archived DRMs potentially limiting the diversification of quasispecies and newly resistant variants.
- NGS of HIV DNA additionally identified 22 low frequency DRMs and 2 Major K103N mutations that were not detected in blood plasma.
- Even in the absence of detectable NNRTI resistance via bulk genotyping, clinicians should be cautious regarding selecting NNRTI for ART regimens.

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ADDITIONAL KEY INFORMATION

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