Resistance Mutations in Intact vs Defective HIV Proviruses: Implications for Proviral DNA Testing

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BACKGROUND

- HIV-1 RNA resistance testing guides antiretroviral therapy (ART) when viral load is detectable.
- For suppressed individuals, DNA resistance testing is limited in clinical utility due to poor predictive values for treatment outcomes.
- Intact proviruses, which can drive viral rebound, are rare in the proviral reservoir and archival patterns of resistance mutations may differ across donors.
- **We hypothesize that drug resistance mutations (DRMs) will be differentially archived in intact versus defective proviruses, informing whether proviral DRM testing should distinguish between these compartments.**

Participant 1

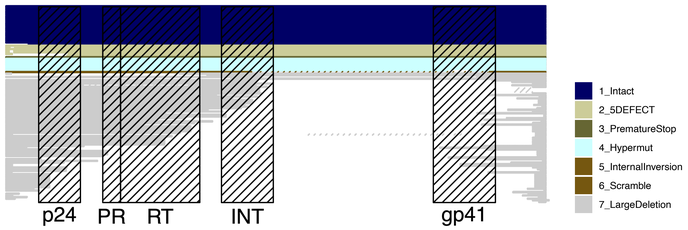


Figure 1. Proviral landscape sampled from a single time point from a representative donor. Each bar represents a single HIV-1 genome.

METHODS

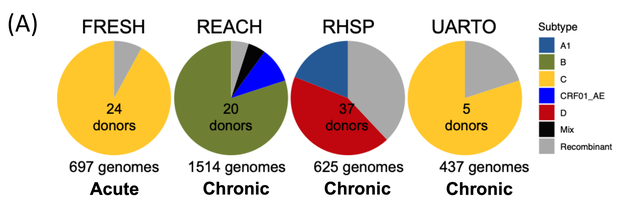


Figure 2. (A) Study cohorts, subtype distribution and (B) experimental workflow.

Interpretation of proviral drug resistance genotyping may be impacted by differential archival of resistance mutations in intact vs defective proviral compartments, even after hypermutated genomes were excluded.

RESULTS

Table 1. (A) Fraction of participants with any DRMs associated with each drug class by cohort. Subtype-polymorphic DRMs were included. (B) Fisher's Exact tests (2-tailed) were performed on 11 individuals who had at least 5 intact and 5 defective proviral genomes captured. Two individuals had statistically significant differential archival of DRMs across intact versus defective proviral compartments.

(A)

Donors with Mutations by Class

(Each cell = number of donors with ≥1 mutation / total donors with percent)

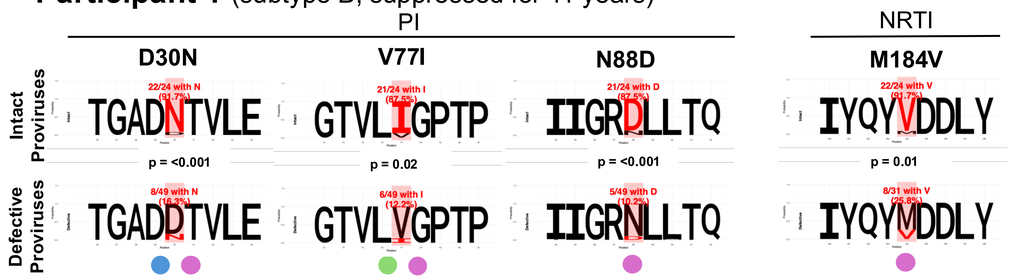
	FRESH	REACH	Rakai	UARTO
CAI	1/24 (4.2%)	0/20 (0%)	1/37 (2.7%)	0/5 (0%)
Entry	0/24 (0%)	0/20 (0%)	0/37 (0%)	0/5 (0%)
INSTI	6/24 (25%)	4/20 (20%)	7/37 (18.9%)	2/5 (40%)
NNRTI	2/24 (8.3%)	6/20 (30%)	4/37 (10.8%)	4/5 (80%)
NRTI	0/24 (0%)	5/20 (25%)	1/37 (2.7%)	3/5 (60%)
PI	24/24 (100%)	20/20 (100%)	34/37 (91.9%)	5/5 (100%)

(B)

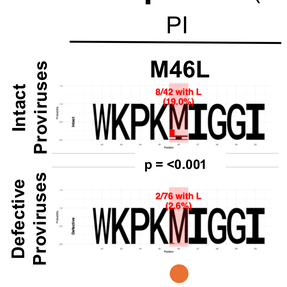
	FRESH	REACH	RHSP	UARTO
Participants with at least 5 intact and 5 defective sequences per timepoint	3 participants	6 participants	1 participant	1 participant
Participants with any instance of differential archival in intact versus defective	0	2 participants	0	0
Details of DRMs differentially archived	0	**Participant 1** M184V (NRTI), N88D (PI), D30N (PI), V77I (PI) **Participant 2** M46L (PI)	0	0

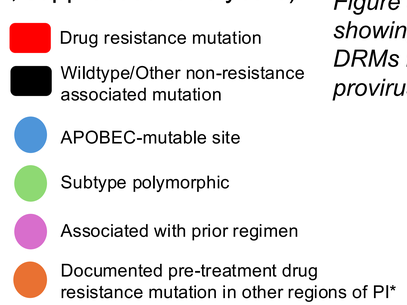
When differentially archived, DRMs were detected more frequently in intact, as opposed to defective proviral genomes.

Participant 1 (subtype B, suppressed for 11 years)



Participant 2 (subtype B, suppressed for 8 years)





*No pretreatment drug resistance information was available for Participant 1

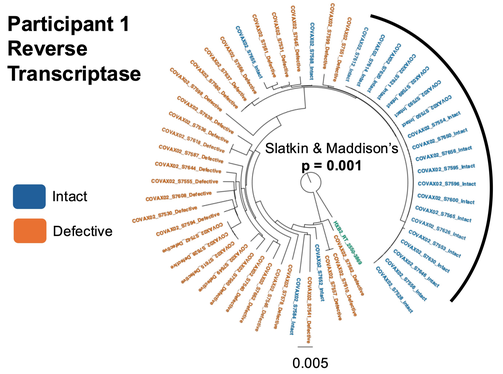
Figure 3. Sequence logo plots showing distinct frequencies of DRMs in intact versus defective proviruses.

RESULTS (CONT.)

Clonal expansion propagated drug resistance mutations in the intact compartment.

- Intact and defective genomes were significantly compartmentalized in both Participant 1 and 2.
- Phylogenetic analysis of the full-length genome showed 100% nucleotide identity in 18/24 intact genomes carrying linked M184V (NRTI), N88D, D30N, and V77I (PI) in Participant 1 and 6/42 intact genomes carrying M46L (PI) in Participant 2.

Participant 1 Reverse Transcriptase



Clonal cluster of intact proviruses containing DRM

Figure 4. Phylogenetic tree for Participant 1.

CONCLUSIONS

While most participants showed no significant compartmentalization of drug resistance mutations, select individuals had significant enrichment in the intact compartment.

This supports further evaluation on whether a focus on intact genomes would improve proviral resistance testing predictability of treatment responses.

FUNDING SUPPORT

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