HIV-1 DNA GENOTYPING IS OFTEN VARIABLE IN REPEAT TESTING FROM SINGLE BLOOD DRAWS

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Introduction

- DHHS guidelines state that HIV-1 proviral DNA genotyping, which aims to detect archived DRMs in individuals with low or undetectable plasma HIV-1 RNA levels, can be considered but also caution the interpretation of results as reports might miss some or all prior drug resistance.

- Assays detecting archived mutations are insensitive due to:
  - Only assessing cells which are circulating in the periphery and above the assay detection threshold (Figure)
  - Sampling of small blood volumes
    - Clones which are present at low frequencies may potentially be detected but may also be missed (Table – Test #2)

- **Objective:** To characterize the variability of DNA genotyping by quantifying the reproducibility of mutation reporting from multiple assays from a single blood draw.

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1. [http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGI.pdf](http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGI.pdf) Accessed Jan 22 2021 C-14 and Table 5; 2. Figure adapted from Cohn LB. Cell Host Microbe 2020;27:519-30;
Sample Population and Calculations

♦ **Sample Population**: Baseline samples for 70 suppressed participants (67 with documented primary resistance and 3 without) from Studies 4449\(^1\), 4030\(^2\) and 4580\(^3\) were analyzed; a subset of participants had a baseline and additional time-point sample (n=20)
  - For 2–4 replicates from 90 blood draws, 257 genotype reports were analyzed

♦ **Genotyping Analysis**: DNA genotyping of PR, RT, and IN used GenoSure Archive\(^*\) from whole blood

♦ **Substitutions analyzed:**
  - Primary PI-R, NRTI-R, NNRTI-R, INSTI-R (based on IAS-USA\(^4\))
  - Other/non-R mutations were all nonprimary resistance mutations reported by the assay which include secondary mutations and polymorphisms

♦ **Reproducibility of individual substitutions were calculated as:**

\[
\text{Reproducibility (\%) } = \frac{\text{No. of times the mutation was detected}}{\text{No. of assays run}}
\]

\*Monogram Biosciences, CA, USA. IN, integrase; INSTI, IN strand transfer inhibitor; NRTI, nucleos(t)ide reverse transcriptase (RT) inhibitor; NNRTI, non-NRTI; PI, protease (PR) inhibitor; -R, resistance; SD, standard deviation.

Reproducibility of Substitutions in PR/RT/IN*

Analysis population

- Clinical and demographic characteristics: 96% HIV-1 RNA <50 copies/mL (n=3 with >50 copies/mL: 136, 105 and 107,000 copies/mL); 79% male; median age 57 years; median CD4 count 707 cells/μL; 86% subtype B

- Primary DRMs and non-R substitutions have high reproducibility, but certain replicates of reports are variable
  - M184V had a reproducibility of 82 ± 25%, 14% of cases were detected in 1/4 or 1/3 reports, 23% in 1/2, 2/4, or 2/3 reports, and 63% were detected in all reports

- Lower reproducibility may reflect more rare quasispecies

- Reproducibility was similar between PI, NRTI and NNRTI drug classes

- Reproducibility of non-R RT substitutions was higher than for primary NRTI and NNRTI DRMs
  - Polymorphisms can be more stable than primary mutations due to minimal impact on viral fitness and potential role as immune escape variants

*INSTI-R occurrence was too low for further analyses.
Means ± SD are reported. Comparisons used Wilcoxon Rank Sum tests
Reproducibility of Primary DRMs According to Historic Data

By modelling, if a participant had a PI, NRTI, or NNRTI DRM, the probability of it being reported by the proviral genotyping assay was 76–80%
- The probability of a primary DRM being detected increased to 89% if historically detected and decreased to 59% if not detected by a previous report

Reproducibility of DRMs was 10–17% higher when detected historically compared to being undocumented or not having historic data
- Of the DRMs detected, 77% were previously undocumented

*For NRTI-, NNRTI-, and PI-R, only primary mutations with n >10 are graphed. Means ± SD are reported. Comparisons used Wilcoxon Rank Sum tests
Conclusion

- The utility of HIV-1 proviral DNA genotyping for identification of archived DRMs is clinically relevant, especially in individuals with limited available clinical history; it can be beneficial in guiding regimen selection but can be insensitive as resistance substitutions are not always consistently detected
  - Proviral DNA genotypic susceptibility score can be a useful tool to predict virologic outcome in virologically suppressed people\(^1\)
  - Primary DRM reproducibility of \(~80\%\) with standard deviations of \(~25\%\) indicate that detection of mutations is variable; low reproducibility still confirms the presence of the mutation
  - The reporting of a mutation as negative does not imply that the mutation is absent
  - A limitation of DNA genotyping is that current assays cannot determine whether DRM is on an intact, infectious HIV genome
- DNA genotyping may aid clinicians when switching ARV regimens, but these data reinforce the need to interpret tests with caution, as not all mutations may be reported; the individual’s treatment history, including ART, virologic failures, and all past resistance test results should be evaluated and considered

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