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Results of Repeat HIV-1 DNA Resistance Tests Are Highly Concordant

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I. Background

- HIV-1 proviral DNA sequencing can identify drug resistance mutations (DRMs) contained in the host's viral reservoir[1]
- Host cells that harbor HIV-1 proviral DNA are subject to proliferation and turnover,[2-4] which may affect the detection of HIV-1 DNA DRMs
- The Department of Health and Human Services HIV-1 treatment guidelines recommend caution when interpreting HIV-1 DNA resistance testing because all previously identified DRMs may not be captured[5]
- Comparison of multiple HIV-1 DNA resistance tests from the same patient was performed to assess the ability of HIV-1 DNA testing to consistently identify wild-type and drug resistance variants

II. Methods

- Patients with 3 HIV-1 DNA resistance tests (GenoSure Archive®, Monogram Biosciences, South San Francisco, CA) and corresponding HIV-1 viral load (VL) measurements within ~3 months of each resistance test were identified in a commercial database
- All drug resistance sequencing data were reanalyzed using the most current bioinformatics pipeline to account for any pipeline updates that occurred over the testing interval
- Amino acids evaluated for presence or absence of drug resistance mutations were:
  - M41, K65, K66, D67, T69, K70, L74, V75, F77, Y115, F116, Q151, M184, L210, T215, K219 for nucleos(t)ide reverse transcriptase inhibitors (NRTIs)
  - A98, L100, K101, K103, V106, V108, E138, V179, Y181, Y188, G190, H221, P225, F227, L234, K238 for non-nucleoside reverse transcriptase inhibitors (NNRTIs)
  - L23, D30, V32, L33, K43, M46, I47, G48, I50, F53, I54, Q58, G73, T74, L76, V82, N83, I84, N88, L90 for protease inhibitors (PIs)
  - T66, E92, F121, E138, G140, Y143, S147, Q148, N155, S230 for integrase inhibitors (INIs)
- Result concordance across the 3 HIV-1 DNA tests (trios) and DRM redetection rates were assessed per patient
- The effects of viral load at time of testing and interval between tests on result concordance were evaluated using Spearman correlation and Mann-Whitney U tests

III. Results

Table 1. Patient and virus characteristics

Characteristic	n (%)
Male	46 (83.6%)
Female	8 (14.5%)
Undisclosed	1 (1.8%)
Mean age at last resistance test, years	51
Range	26 - 75
HIV-1 subtype	
B	51 (92.7%)
A1	2 (3.6%)
C	1 (1.8%)
D	1 (1.8%)
Median VL at resistance testing, c/mL	125
Range	TND - 3,780,000
Suppressed (<200 c/mL) on all 3 tests	25 (45.5%)
Not suppressed (>200 c/mL) on all 3 tests	16 (29.1%)
Average number of DRMs per test	3.36
Range	0 - 18
Patients without DRMs	11 (20.0%)
Patients with DRMs	44 (80.0%)
NRTI	29 (52.7%)
NNRTI	24 (43.6%)
PI	27 (49.1%)
INI	7 (12.7%)
Average trio testing interval, weeks	100
Range	17 - 228

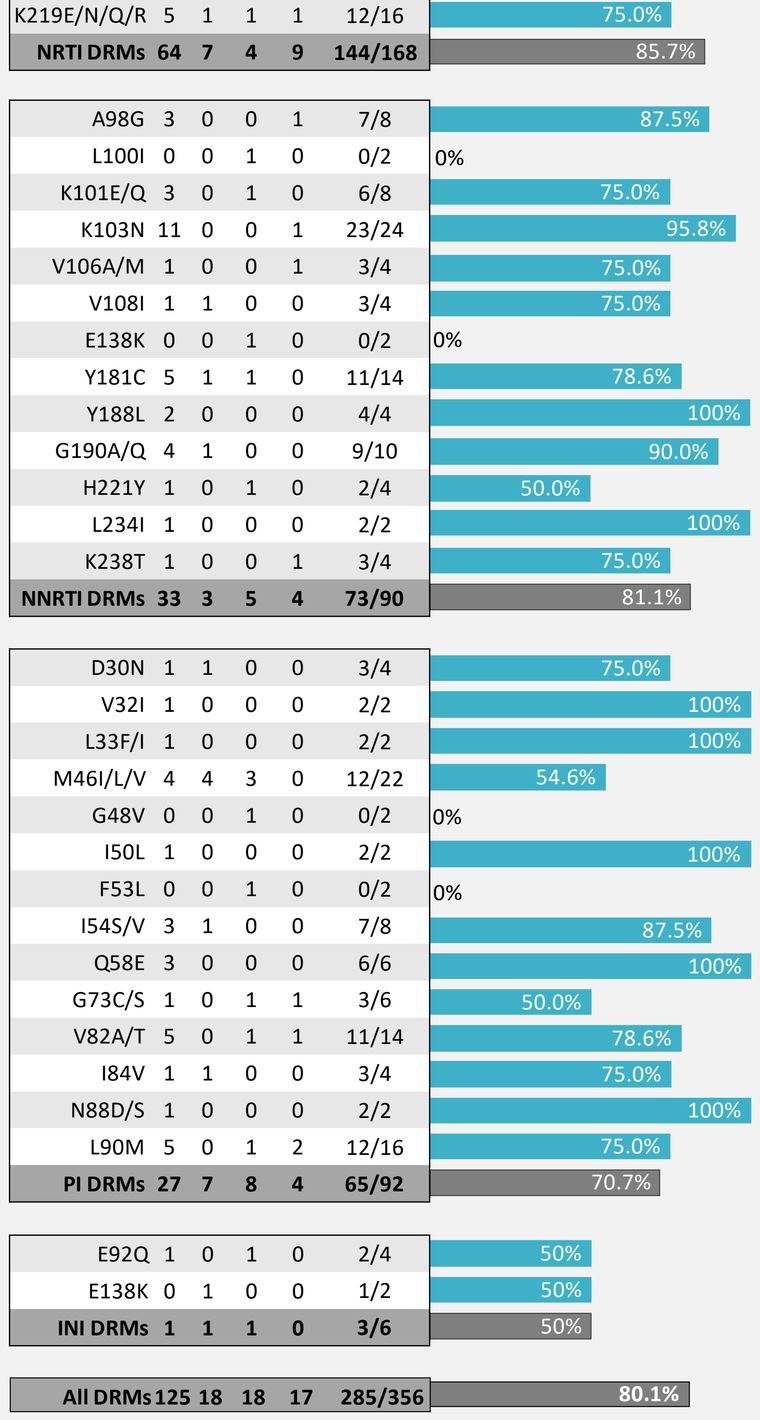
c/mL, copies per milliliter; DRM, drug resistance mutation; INI, integrase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TND, target not detected; VL, viral load.

Table 2. Average concordance among HIV-1 DNA resistance test trios

NRTI	NNRTI	PI	INSTI	All
96.2%	98.1%	96.9%	99.2%	**97.4%**

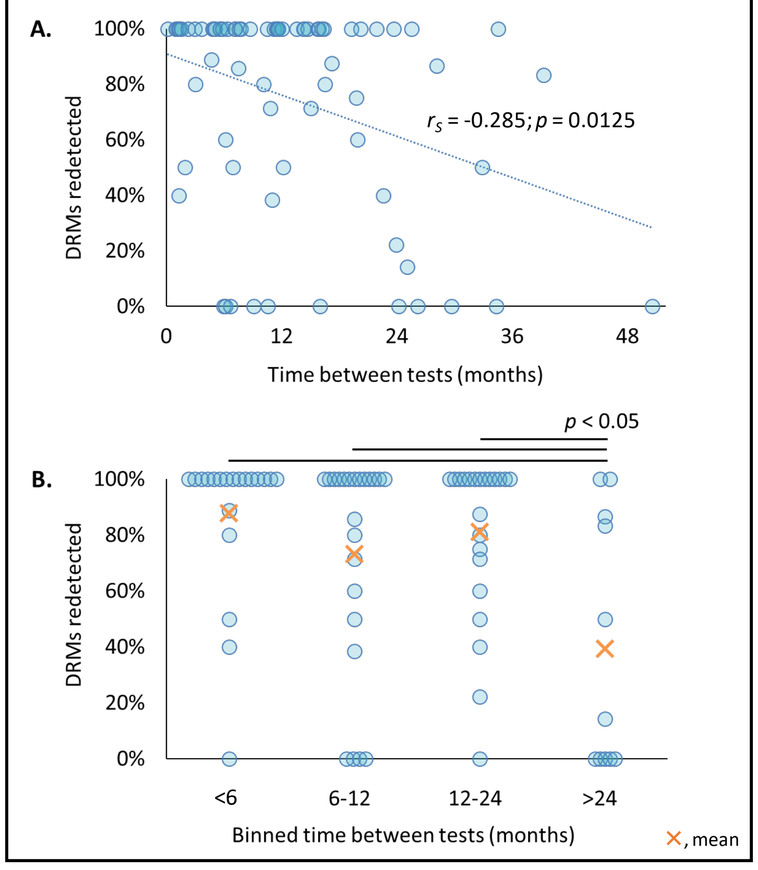
- The average concordance among 55 test trios in identifying wild-type or drug resistance variants across all drug classes was 97.4%

Figure 1. Redetection of drug resistance mutations in HIV-1 DNA



- Across the entire cohort, 178 DRMs were identified on time point 1 HIV-1 DNA drug resistance tests
- DRMs identified on the initial tests were redetected on 285/356 (80.1%) subsequent tests
- M184I/V was redetected on 30/34 (88.2%) tests
- 125/178 (70%) DRMs were redetected on both subsequent tests
- 35/178 (19.7%) DRMs were redetected only once
- 18/178 (10.1%) DRMs were not redetected

Figure 2. Percentage of DRMs redetected as a function of time between tests



- Significant negative correlation was found between percentage of DRMs redetected and time elapsed between tests (r_S = -0.285; p = 0.0125) (Figure 2A)
- Percentage of DRMs redetected were significantly lower if more than 24 months had elapsed between tests (p < 0.05); no other significant differences were found in pairwise comparisons of test interval bins (Figure 2B)
- Redetection rate did not correlate with the number of DRMs detected on the initial test (r_S = -0.0537, p = 0.644) or the viral load at time of subsequent testing (r_S = 0.144, p = 0.216)
- The average trio concordance was not different between patients who were suppressed (<200 c/mL) on all 3 resistance tests vs those with VL > 200 c/mL on all 3 resistance tests (95.9% vs 97.9%, p = 0.772)

IV. Summary and Conclusion

- The average result concordance across 55 test trios was 97.4% (Table 2)
- The overall redetection rate of DRMs identified on an initial test was 80.1% (Figure 1)
- The average DRM redetection rate was lower if more than 24 months had elapsed between tests (Figure 2B)
- The redetection pattern suggests that both sampling error and reservoir dynamics may affect test results
  - Some proviral HIV-1 genomes may not be sampled in a blood draw
  - HIV-1 DRMs may be lost over time due to host cell turnover

V. References

1. Ellis et al. "Clinical Outcomes Following the Use of Archived Proviral HIV-1 DNA Genotype to Guide Antiretroviral Therapy Adjustment." *Open Forum Infectious Diseases*. Vol. 7. No. 1. US: Oxford University Press, 2020. **2.** Chomont, Nicolas, et al. "HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation." *Nature Medicine.* 15.8 (2009): 893-900. **3.** Hosmane et al. "Proliferation of latently infected CD4+ T cells carrying replication-competent HIV-1: Potential role in latent reservoir dynamics." *Journal of Experimental Medicine* 214.4 (2017): 959-972. **4.** Wang et al. "Expanded cellular clones carrying replication-competent HIV-1 persist, wax, and wane." *Proceedings of the National Academy of Sciences.* 115.11 (2018): E2575-E2584. **5.** Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV. Department of Health and Human Services. Available at https://clinicalinfo.hiv.gov/sites/default/files/inline-files/AdultandAdolescentGL.pdf. Accessed 09/29/20.

VI. Acknowledgements

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