Replication-Competent HIV-1 Harboring Resistance-Associated Mutations is Present in the Viral Reservoir

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Conclusions

- This study demonstrates that RAMs reported by proviral DNA genotyping may also be found on replication-competent virus, indicating that potentially infectious, antiretroviral-resistant viruses can persist in the latent reservoir
  - Of the 70 RAMs reported by proviral DNA genotyping, 47 (67%) were also found on replication-competent virus
  - As replication-competent virus may not always be induced following stimulation in the QVOA, the actual number of RAMs on replication-competent virus may be higher[1]
- When reporting from proviral DNA genotyping was more reproducible, there was a greater chance that detected RAMs were present on replication-competent virus
  - When RAMs were detected in 100% of replicates by proviral DNA genotyping (n = 55), 42 (76%) of them were on replication-competent virus
  - When RAMs were reported 33-67% of the time by proviral DNA genotyping (n = 15), 5 (33%) of them were on replication-competent virus
- Additional RAMs (n = 17; 20%) were on replication-competent virus but were not detected by proviral DNA genotyping
  - These data reinforce guidelines that proviral DNA genotyping results should be interpreted with caution, as not all mutations may be reported

Plain Language Summary

- Genetic changes occur by chance in the human immunodeficiency virus (HIV). Some of these changes stop HIV medicines from working; these changes are called resistance mutations
- When cells are infected with HIV, the genetic code of the virus becomes part of the cell's DNA
- HIV medication prevents the cells from making virus, but if the medicine is stopped, the cells can restart making more infectious virus, helping it to spread to more cells
- It is not clear how often cells with HIV resistance mutations in their DNA can also make viruses with these mutations that are infectious and can spread to other cells
- To understand this, researchers looked at HIV resistance mutations in DNA from blood cells (called HIV DNA genotype) and whether the cells also made infectious viruses with these mutations
- Twelve blood samples from people with HIV were tested
- Most of the time, resistance mutations found in the DNA were also found on infectious viruses
- Testing for resistance mutations in DNA from blood samples can help to find mutations in the virus, and can be used by healthcare providers to choose medicines that work against viruses with these mutations

Introduction

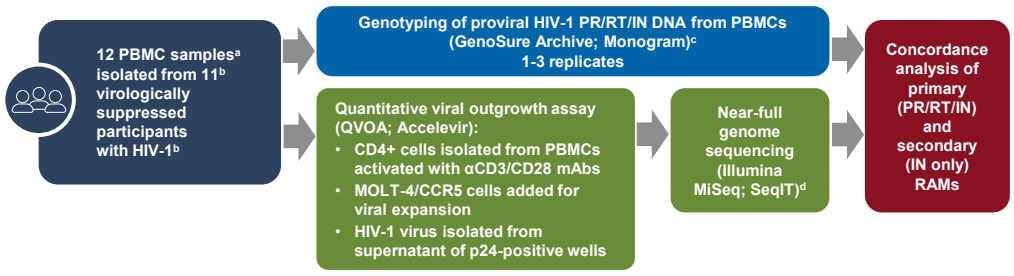
- HIV-1 variants, including those with resistance-associated mutations (RAMs), develop during periods of viremia and are archived as proviral DNA in the latent reservoir[1]
- Even during effective antiretroviral therapy (ART), when viral load is undetectable, these archived viral variants persist and may become activated if ART is interrupted[2,3]
- Genotyping to detect RAMs in proviral HIV-1 DNA can be useful for regimen selection, but results should be interpreted with caution, as not all mutations may be reported[4,5]
  - This method is insensitive due to sampling limited numbers of latently infected peripheral CD4+ T cells
- Interpretation of proviral DNA genotyping results in terms of clinical relevance of archived resistance is also confounded by not knowing whether identified RAMs are in intact, replication-competent virus
  - Most (> 97%) of the proviral DNA is defective[6]
  - 5-16% of detected sequences from PCR assays amplifying regions of polymerase are intact viruses[6]

Objective

- To examine whether RAMs identified by proviral DNA genotyping were also present on replication-competent virus

Methods

Study Design



[a]Samples were selected from the Gilead internal biobank based on availability of suitable cell numbers to carry out both assays.
[b]One participant provided samples at two different timepoints (4.8 years apart).
[c]Genotyping of PBMCs is for research use only.
[d]Mutation frequency cutoff ≥ 15%.
CCR5, C-C chemokine receptor type 5; CD, cluster of differentiation; IN, integrase; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cell; PR, protease; QVOA, quantitative viral outgrowth assay; RAM, resistance-associated mutation; RT, reverse transcriptase.

RAMs Analyzed

Primary HIV-1 Drug Resistance Substitutions (Based on IAS-USA List)[7]	
NRTI-R	K65R/E/N, T69 insertions, K70E, L74V/I, Y115F, Q151M, M184V/I, TAMs (M41L, D67N, K70R, L210W, T215F/Y, K219E/N/Q/R)
NNRTI-R	L100I, K101E/P, K103N/S, V106A/M, V108I, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188C/H/L, G190A/E/Q/S, H221Y, P225H, F227C, M230I/L
PI-R	D30N, V32I, M46I/L, I47A/V, G48V, I50L/V, I54M/L, Q58E, T74P, L76V, V82A/F/L/S/T, N83D, I84V, N88S, L90M
INSTI-R	T66I/A/K, E92Q/G, F121Y, Y143R/H/C, S147G, Q148H/K/R, N155H/S, R263K
Secondary HIV-1 Drug Resistance Substitutions	
INSTI-R	M50I, H51Y, L68V/I, V72A/N/T, L74M, Q95K/R, T97A, G118R, S119P/R/T, F121C, A128T, E138K/A, G140A/C/S, P145S, Q146R/I/K/L/P, V151L/A, S153A/F/Y, E157K/Q, G163K/R, E170A

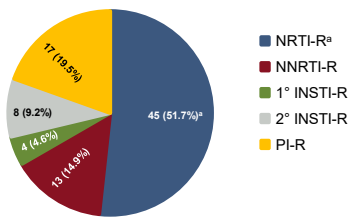
IAS-USA, International Antiviral Society-USA; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; R, resistance; TAM, thymidine analog mutation.

- The frequency of RAM reporting for proviral genotyping and quantitative viral outgrowth assay (QVOA) was compared:

$$\text{Reporting (\%)} = \frac{\text{No. of times mutation was detected}}{\text{No. of assays run (GenoSure Archive) or reactivated wells (QVOA)}}$$

Results

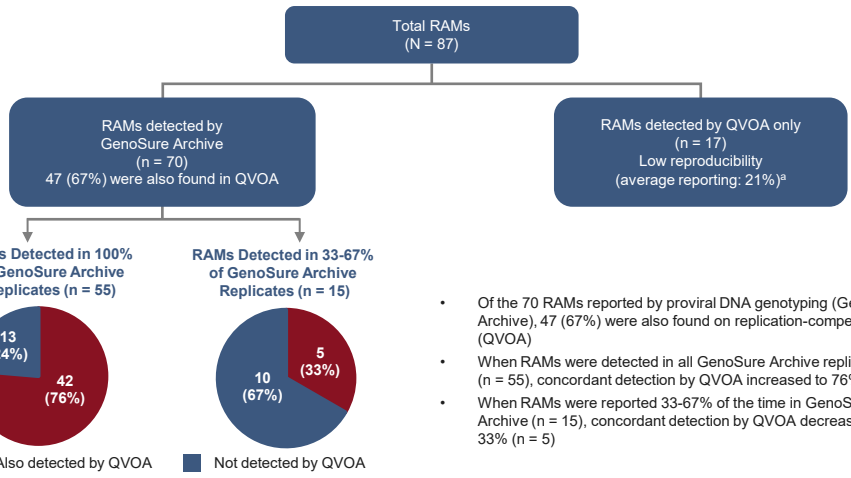
RAMs Detected by GenoSure Archive and/or QVOA



- From 12 peripheral blood mononuclear cell samples, 87 primary or secondary RAMs were identified by GenoSure Archive and/or QVOA sequencing
- RAMs were detected in the following number of samples: NRTI-R (including TAMs): 10; TAMs: 9; NNRTI-R: 4; 1° INSTI-R: 4; 2° INSTI-R: 8; PI-R: 5

[a]Including 36 TAMs.
INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; QVOA, quantitative viral outgrowth assay; R, resistance; RAM, resistance-associated mutation; TAM, thymidine analog mutation.

Concordance Between GenoSure Archive and QVOA



- Of the 70 RAMs reported by proviral DNA genotyping (GenoSure Archive), 47 (67%) were also found on replication-competent virus (QVOA)
- When RAMs were detected in all GenoSure Archive replicates (n = 55), concordant detection by QVOA increased to 76% (n = 42)
- When RAMs were reported 33-67% of the time in GenoSure Archive (n = 15), concordant detection by QVOA decreased to 33% (n = 5)

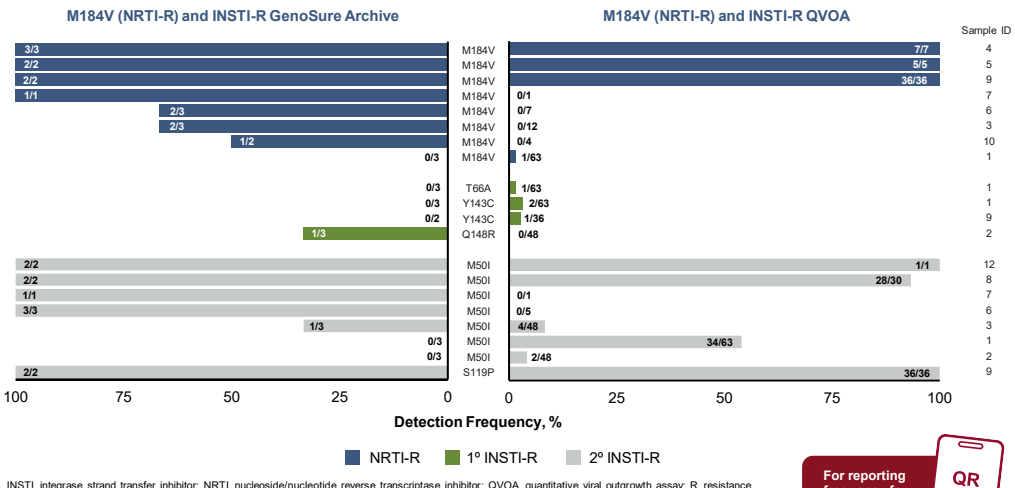
[a]Average reporting calculated from the % reporting for each RAM detected by QVOA only.
QVOA, quantitative viral outgrowth assay; RAM, resistance-associated mutation.

Class	RAM	100% Reproducible GenoSure Archive Detection: QVOA Detection for Each Observation, %	33-67% Reproducible GenoSure Archive Detection: QVOA Detection for Each Observation, %	0% Reproducible GenoSure Archive Detection: QVOA Detection for Each Observation, %
NRTI	L74V	100		
	M184V	100 100 100 0	0 0 0	2
TAM	M41L	100 100 100 75 75 0	50	6
	D67N	100 100 73 67	0	6
	K70R	100	0	8
	L210W	100 100 75 75	50	6
	T215Y/F	100 100 100 78 75 0	50	6
	K219N/Q/R/E	100 100 0	0 0	
NNRTI	K101E	0		50
	K103N	100		
	V106M	0		
	V108I	100 86		
	E138A	0		
	Y181C	100 80		50
	G190A	100 0		
	H221Y	0		
PI	D30N		50	
	V32I	100		
	M46I/L	100 100	0	50 8
	G48V	100		
	I50V	100		
	I54V	0		
	Q58E			50
	V82A/S	100 100 0	0	
	I84V	100		
	L90M			50
INSTI	T66A			2
	Y143C			3 3
	Q148R		0	
INSTI SEC	M50I	100 93 0 0	8	54 4
	S119P	100		

INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; QVOA, quantitative viral outgrowth assay; RAM, resistance-associated mutation; SEC, secondary; TAM, thymidine analog mutation.

- Higher reproducibility of RAM reporting from GenoSure Archive testing is linked to higher reproducibility of RAM reporting by QVOA and overall better concordance

M184V (NRTI-R) and INSTI-R Reporting Frequency



INSTI, integrase strand transfer inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; QVOA, quantitative viral outgrowth assay; R, resistance.

- For M184V (n = 8), when detection by GenoSure Archive was reproducible (100%) versus variable (33-67%), detection by QVOA was 3/4 versus 0/3; n = 1 was detected by QVOA only
- Of the four primary INSTI RAMs detected, only one was detected by GenoSure Archive but not by QVOA

For reporting frequency for all RAMs, please scan the QR code

References: **1.** Brooks K, et al. *PLOS Pathog*. 2020;3;16:e1008378. **2.** Cohn LB, et al. *Cell Host Microbe*. 2020;27:519-30. **3.** McMyn NF, et al. *J Clin Invest*. 2023;133:e171554. **4.** EACS. https://www.eacsociety.org/media/guidelines-12.0.pdf (accessed Aug. 2, 2024). **5.** DHHS. https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/adult-adolescent-arv/guidelines-adult-adolescent-arv.pdf (accessed Aug. 2, 2024). **6.** Bruner KM, et al. *Nature*. 2019:566:120-5. **7.** Wensing AM, et al. *Top Antiv Med*. 2022;30:559-74.

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Disclosures: **MLD'A, SC, LAV**, and **CC** are employees of, and own stocks/shares in, Gilead Sciences, Inc.

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