Longitudinal Analysis of Preexisting Resistance-Associated Mutations Prior to B/F/TAF Switch

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695

GS-US-380-4449, GS-US-380-4030, and GS-US-380-4580 studies

Conclusions

- This retrospective subanalysis of longitudinal genotyping data demonstrated:
  - The majority of resistance-associated mutations (RAMs) in the two timepoint analysis were detected 100% of the time or were newly detected at the second timepoint
  - Almost half of RAMs in the multiple timepoint analysis were characterized as having variable detection, demonstrating that reporting of a mutation as negative at a given timepoint does not indicate that the mutation will not be detected at future timepoints
- These data most likely reflect the dynamic nature of the HIV latent reservoir where decay and proliferation are occurring,[1] the detection sensitivities of assays and the presence of drug pressure from various ARV regimens[a]
- Since substitutions are not always consistently detected, the data from the present analysis reinforce the need to consider an individual's treatment history and all past genotyping results for optimal treatment management

[a]As ARV treatment history was not analyzed as part of this study, no conclusions can be drawn as to whether the persistence of RAMs was impacted by drug-related selection pressure.

Plain Language Summary

- Genetic changes occur in the human immunodeficiency virus (HIV) by chance. Some of these changes stop HIV medicines from working; these changes are called resistance mutations
- To understand the length of time that resistance mutations stay in the body, researchers looked at the genetic patterns of HIV (called HIV genotype) over time in people who took part in three clinical studies
- Genotype data were collected at the beginning of the clinical trials, and for some people, earlier genotyping reports were also available. Researchers looked at these reports to find out whether resistance mutations kept being found or if they were found in some, but not all of the reports
- No consistent patterns were seen, but most resistance mutations continued to be present on all reports, appeared on a later report, or were not always consistently reported
- Given that reporting of most resistance mutations does not disappear with time, healthcare providers must take into account all drug treatments used in the past, and prior genotype reports, and not suppose that resistance mutations are no longer present because they are not reported in the latest genotype report, since they may reappear in future reports

Introduction

- Resistance-associated mutations (RAMs) that develop during a period of viremia may persist in the latent reservoir, even when viral load is undetectable[2]
- The latent reservoir has dynamic properties, expanding and contracting over time[3]
  - The pool of memory CD4+ T cells latently infected with HIV variants is modulated in response to stimuli, including cytokines and antigenic exposure[3]
  - Detection of these variants is dependent on clone size being above the assay detection threshold
- Given these fluctuations, the persistence of the RAMs in the reservoir has not been well defined
- Since preexisting resistance may affect antiretroviral (ARV) efficacy,[4-6] it is important to understand the persistence of RAMs over time
- To understand the effect of preexisting resistance on bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) efficacy in switch studies, historical genotype reports previously generated by commercial or local assays were collected at enrollment, and retrospective analyses of baseline whole blood samples were performed
- Here we compare all available genotype data for the same participants to investigate variation in longitudinal RAM detection

Objective

- A retrospective subanalysis of a cohort of 242 people with HIV (PWH) from B/F/TAF switch studies, to investigate the persistence of RAMs by comparing longitudinal genotyping data prior to B/F/TAF initiation for participants with historical and/or baseline visit data

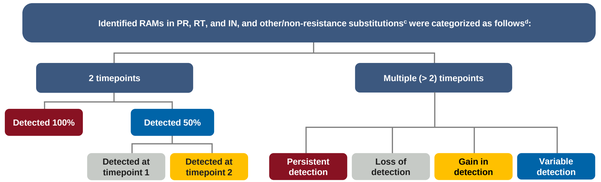
Methods

Study Design

Adults with HIV-1 (N = 242) from the B/F/TAF switch studies GS-US-380-4449 (n = 35), GS-US-380-4030 (n = 94), and GS-US-380-4580 (n = 113) with:

- Virologic suppression ≥ 3-6 months
- No prior virologic failure on INSTI-containing regimens (4030, 4580) or > 400 copies/mL (4449)
- Genotyping history that includes HIV-1 RNA or proviral HIV-1 DNA genotyping[a] from ≥ 2 preswitch timepoints

Genotypic data were available at 2 timepoints for 223 participants and > 2 timepoints for 26 participants[b]



[a]Genotyping data were obtained from local laboratories or commercial sites (including Monogram Biosciences and Quest Diagnostics); analyses from RNA (plasma) and DNA (whole blood/cells) were reported on a population sequencing level.
[b]Seven participants were included in both the 2 timepoint and multiple timepoint analyses.
[c]Other/non-resistance mutations were all non-primary resistance mutations including accessory, secondary mutations, and polymorphisms; non-resistance substitutions were analyzed where pairs of Monogram reports were available.
[d]Non-parametric statistics were used for data analysis; only mutations detected at ≥ 1 timepoint were included in the analyses.
B/F/TAF, bictegravir/emtricitabine/tenofovir alafenamide; IN, integrase; INSTI, integrase strand transfer inhibitor; PR, protease; RAM, resistance-associated mutation; RT, reverse transcriptase.

Examples of Categorization Results

- Example results and their categorization as detected 100% or 50%, or as having persistent detection, loss of detection, gain in detection, or variable detection, are shown in the tables below:

Two Timepoint Analysis

Gene	Mutation	Timepoint 1	Timepoint 2	Quality Assessment	
Protease	I15V	+	+	Detected 100%	
Reverse transcriptase	M184V	+	+	Detected 100%	
	K103N	-	+	Detected 50%	Timepoint 2
Integrase	M50I	+	-	Detected 50%	Timepoint 1

Multiple Timepoint Analysis

Gene	Mutation	Timepoint 1	Timepoint 2	Timepoint 3	Quality Assessment
Protease	D30N	-	+	+	Gain in detection
Reverse transcriptase	D67N	+	-	-	Loss of detection
	M184V	+	+	+	Persistent detection
	K70R	+	-	+	Variable detection
	K103N	-	+	-	Variable detection

RAMs Analyzed: 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/I/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

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

Results

Participant Baseline Clinical and Demographic Characteristics

	All Participants N = 242
Age, years, median (IQR)	52 (39-61)
Male at birth, n (%)	185 (76)
Black race, n (%)	130 (54)
HIV-1 RNA < 50 copies/mL, n (%)	239 (99)
HIV-1 RNA ≥ 50 copies/mL, n (%)	3 (1)
CD4 count, cells/µL, median (IQR)	644 (468-871)
HIV subtype B, n (%)	219 (90)

Timing Distribution Between Tests

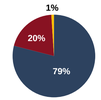
	Two Timepoint Analysis		Multiple Timepoint Analysis[a]
	IN n = 70	PR/RT n = 215	IN/PR/RT n = 26
0-0.99 years, n (%)	6 (8.6)	10 (4.7)	0 (0.0)
1-2.99 years, n (%)	48 (68.6)	62 (28.8)	2 (7.7)
3-4.99 years, n (%)	10 (14.3)	39 (18.1)	1 (3.8)
5+ years, n (%)	6 (8.6)	104 (48.4)	23 (88.5)

[a]Time between first and last test.
IN, integrase; PR, protease; RT, reverse transcriptase.

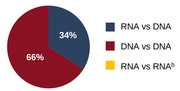
- The majority of integrase reports were 1 to 3 years apart (68.6%)
- About half of protease and reverse transcriptase reports were 5+ years apart (48.4%)
- Almost all reports in the multiple timepoint analysis had 5+ years between the first and last tests (88.5%)
  - Individuals in the multiple timepoint analysis had a median of three reports, with a median of 10.5 years between the first and last tests (IQR: 6.5-14.3)

Analysis Method Comparison (Two Timepoint Analysis[a]): Timepoint 1 vs Timepoint 2

Reverse Transcriptase/Protease (n = 215)



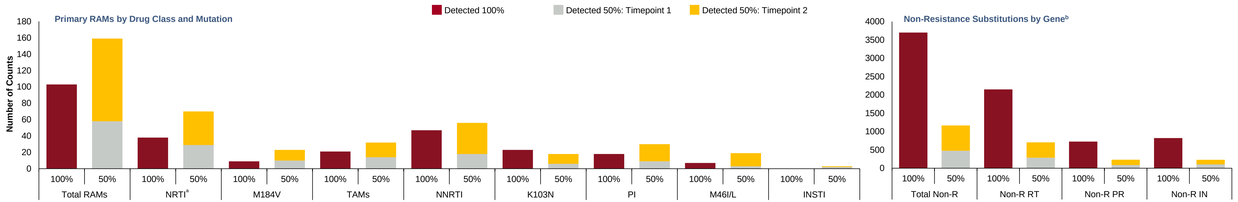
Integrase (n = 70)



- The majority of comparisons were RNA versus DNA for reverse transcriptase/protease genotypes and DNA versus DNA for integrase genotypes[c]

[a]For the multiple timepoint analysis, reports were primarily RNA (69.4%), and 21 participants had at least one DNA genotyping report analyzed.
[b]Data from two historical reports.
[c]Reasons for historical genotyping are not available.

Persistence of Primary RAMs and Non-Resistance Substitutions (Two Timepoint Analysis)



[a]Total NRTI including TAMs.
[b]Most frequent non-R substitutions were: Q102K (n = 123), R277K (n = 80), and A272P (n = 78) for RT; L63P (n = 72), V77I (n = 57), and I62V (n = 47) for PR; and V113I (n = 63), V234L (n = 59), and V72I (n = 51) for IN.
IN, integrase; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PR, protease; R, resistance; RAM, resistance-associated mutation; RT, reverse transcriptase; TAM, thymidine analog mutation.

Analysis of Primary RAMs over Multiple Timepoints[a]

Type	Substitution	Persistent detection	Loss of detection	Gain in detection	Variable detection	Total
PR	D30N			3		3
PR	M46I		1		1	2
PR	I50V				1	1
PR	Q58E	1				1
RT	K65R				1	1
RT	D67N		1	1	1	3
RT	K70R		1	1	2	4
RT	L74V		1		2	3
RT	I100I				1	1
RT	K101E	1				1
RT	K103N	2		1	6	9
RT	V108I			3		3
RT	Y115F		1			1
RT	E138K	1		1	2	4
RT	M184V	2	1	4	6	13
RT	M184I	1		1		2
RT	Y188L	1			1	2
RT	Y188H			1		1
RT	G190A	2			2	4
RT	G190E				1	1
RT	L210W				1	1
RT	K219Q	1				1
RT	P225H				1	1
IN	Q148R			1		1
Total		12	6	17	29	64

[a]Number of RAMs categorized as having persistent detection, loss of detection, gain in detection, or variable detection.
IN, integrase; PR, protease; RAM, resistance-associated mutation; RT, reverse transcriptase.

Two Timepoint Analysis:

- For 223 PWH with genotypic data at two timepoints, 262 RAMs and 4866 non-resistance substitutions were analyzed
- 103 of the 262 RAMs (39.3%) had 100% detection; 159 (60.7%) had 50% detection, with 101 (63.5%) of these detected at timepoint 2 and 58 (36.5%) detected at timepoint 1
  - These data suggest that RAM frequency does not predominantly decay over the timeframe of this study
- Non-resistance substitutions were predominantly persistently detected (76.0% overall)
- RAM detection did not persist as frequently over time as non-resistance substitutions (overall and by gene) ($P < 0.0001$ for all comparisons; Fisher exact test)
  - Non-resistance substitutions may be more stable than RAMs due to their minimal impact on viral fitness and potential role as immune escape variants
  - Substitutions only detected 50% of the time may reflect rarer variants
- The time between tests was not different between RAMs with 100% and 50% detection: median 7.7 years (IQR: 3.2-11.1) versus 5.6 years (IQR: 3.1-9.4), respectively ($P = 0.109$; Mann-Whitney U test). However, the time between tests was significantly longer for RAMs that were detected at timepoint 2 versus RAMs that were detected at timepoint 1 (timepoint 2: 6.3 years [IQR: 3.4-11.0]; timepoint 1: 5.0 years [IQR: 2.6-8.2]; $P = 0.015$; Mann-Whitney U test)

Multiple Timepoint Analysis:

- For 26 PWH with genotypic data at multiple timepoints (> 2), 64 RAMs were analyzed
- The 64 RAMs were categorized as having variable detection (45.3%); gain in detection (26.6%); persistent detection (18.8%); or, least commonly, loss of detection (9.4%)
- The M184V/I RAM was not detected at timepoint 2 in 31.3% (10/32) of cases in the two timepoint analysis,[a] but had loss of detection in only 6.7% (1/15) of cases in the multiple timepoint analysis and had variable detection in 40% (6/15) of cases
- The K103N RAM was not detected at timepoint 2 in 14.6% (6/41) of cases in the two timepoint analysis, but had no loss of detection (0/9) in the multiple timepoint analysis, and had variable detection in 66.7% (6/9) of cases
- In general, time between tests were similar between RAMs that had persistent detection, loss of detection, gain in detection, or variable detection, with the exception of the gain in detection versus variable detection comparison – medians: 10.3 years (IQR: 2.7-13.0); 14.6 years (IQR: 7.4-15.8); 8.5 years (IQR: 5.4-9.3); 13.9 years (IQR: 10.6-16.1), respectively ($P = 0.0059$; Kruskal-Wallis test). For gain in detection versus variable detection, $P = 0.0152$ (Dunn's multiple comparison test)

[a]Number of RAMs categorized as having persistent detection, loss of detection, gain in detection, or variable detection.

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