Decay Rate of Archived HIV-1 Drug Resistance Mutations

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Background and Abstract

Antiretroviral therapy (ART) can reduce plasma HIV-1 levels below the limit of detection of clinical assays. However, incomplete suppression of virus can lead to rapid emergence of significant drug resistance mutations (DRM) that render certain drugs ineffective. The integration of drug resistant viruses into the host DNA and persistence in CD4$^+$ cells in a latent state poses a major obstacle to effective treatment strategies and challenges new universal treatment paradigms for the eradication of HIV infection. The latent cellular reservoir functions as a historical archive of viral genotypes that have previously circulated in the host including provirus harboring DRM. However, little is known about the duration, persistence, and decay of DRM in the proviral reservoir over time.

To address these questions, we identified subjects with known dates of virologic failure of Efavirenz-based regimens with emergence of drug resistant viremia (with the K103N mutation). These subjects were previously enrolled in ACTG trials but were subsequently treated with an effective second-line, boosted protease inhibitor-based regimen. CD4 count and viral load were measured and cryopreserved PBMC were collected every 6 months for up to 11 years. Following a failed first-line regimen, we hypothesize that consensus resistance mutations rapidly decay to levels below detection (<20%) in proviral DNA but persist in the latent reservoir with gradual decay over months to years.

To test this hypothesis, our proposed study will –

1) Examine the frequency of DRM in proviral DNA following virologic failure and the detection of DRM in plasma RNA
2) Define the factors that affect fixation, retention, and decay over time. PBMC proviral DNA was genotyped to determine the kinetics of retention and decay of K103N and other DRMs.

The information generated will have important implications for developing future treatment options for ART strategies to reduce clinical failure and mitigate the transmission of drug resistance.

Methods

Subjects under second-line suppression

We assessed proviral DNA for the presence of drug resistance mutations in cells obtained 6-12 months after viral suppression to <50 copies/ml in 29 subjects from ACTG364 and ACTG5095 with Efavirenz failure and documented K103N in plasma viral RNA.

Population sequencing

Genomic DNA was collected from whole peripheral blood mononuclear cells (PBMCs) or enriched CD4$^+$. Whole HIV-1 *pol* was amplified from proviral DNA and sequenced. Editing, amino acid alignment, and phylogenetic analysis were performed using Geneious 6.2. For most samples, genotypes were obtained at 3 time points (first suppression (t_1), most recent suppression (t_3), and an intermediate (t_2)).

Next-generation sequencing

Regions of the *RT* gene harboring DRMs were amplified to generate size-appropriate amplicons to barcode for multiplex 2x250bp paired-end sequencing using Illumina Miseq v2 (500 cycle). For comparison, whole HIV-1 *pol* was sequenced on PacBio *RS* II using P4-C2 chemistry.

Statistical Analyses

Repeated measures logistic regression (generalized estimating equations) was used to identify factors associated with detectable K103N in proviral DNA. All of the analyses were performed on SAS 9.2.

Results

	INTERVAL 1			INTERVAL 2		
ID	NRTI AT K103N DATE IN PLASMA	NNRTI AT K103N DATE IN PLASMA	YEARS FROM K103N TO START OF SUPPRESSION	NRTI AT MOST RECENT SUPPRESSION DATE IN PROVIRAL DNA	NNRTI AT MOST RECENT SUPPRESSION DATE IN PROVIRAL DNA	YEARS SUPPRESSED
1	M41L, T215Y	E138AE, G190GRS	3.05			10.93
2	D67N, K70R, M184V, K219Q		6.64	D67DN, K70KR, M184MV, K219KQ	K103KN	6.96
3	D67E, K70R, T215F		8.38	D67E, K70KR, T215SF	K103KN	5.18
4	M41L, D67N, T215Y		9.83	M41ML, K70KR, M184MV		4.56
5	D67N, K70R, K219Q	K101P	7.16	K219KQ		2.14
6	D67N, K70R, K219Q		12.4	D67DN, K70KR, M184MV, K219KQ		1.86
7	M41L, L210LW, T215Y	G190A	11.43	M184MI	G190R	1.2
8	M41L, D67N, L210W, T215Y, K219N		13.18	M41L, T215TA		0.78
9	M184V		0.54		K103KN	10.26
10			1.12			9.55
11	K65R		0.21			8.78
12	M184V		0.23			7.92
13			2.28		K103N	7.13
14	L74FL, Q151K, M184IV	V106AV	2.06			6.86
15	Y115F, M184V		0.9			4.92
16	T215C	Y188L	0.77	M41MI, M184MI, T215C	K103N	4.77
17	D67N, K70R, M184V, T215IT, K219E		0.59			4.67
18	M184V	V106I	1.71	M184V	V106I, Y181C	3.85
19			7.67		K103N	3.42
20			6.78		K103KN	3.38
21		V106I	1.4		K103KN, V106I	2.91
22	D67N	K101KR	8.28	D67N	K101KR, K103N	2.76
23		G190A	2.51			2.51
24			5.85		K103KN	2.46
25	T215Y	V106I, G190A	1.53	T215Y	K103N, V106I, G190A	1.86
26		Y181CY	6.16	n/a	n/a	1.05
27	M41L, L210LW, T215Y		2.96			1.01
28	M41L, L210W, T215C, K219EK	Y181CY	7.92	K219KN	K103KN	0.92
29	K219N	Y181C	1.35	M41L, M184MVI, T215C	K103N, Y181C	0.35

Table 1. Summary of significant NRTI- and NNRTI-associated DRM. Identified mutations (in context of K103N) present in plasma HIV-1 at time of initial treatment failure. Subject 26 has withdrawn from the study.
K103N date – date of K103N detection following Efavirenz failure
NRTI/NNRTI mutations at K103N date – DRMs identified in plasma in the context of K103N
NRTI/NNRTI mutations at most recent suppression date – total persisting DRM in PBMC
Interval 1 – # Years from K103N to start suppression
Interval 2 – # Years suppressed

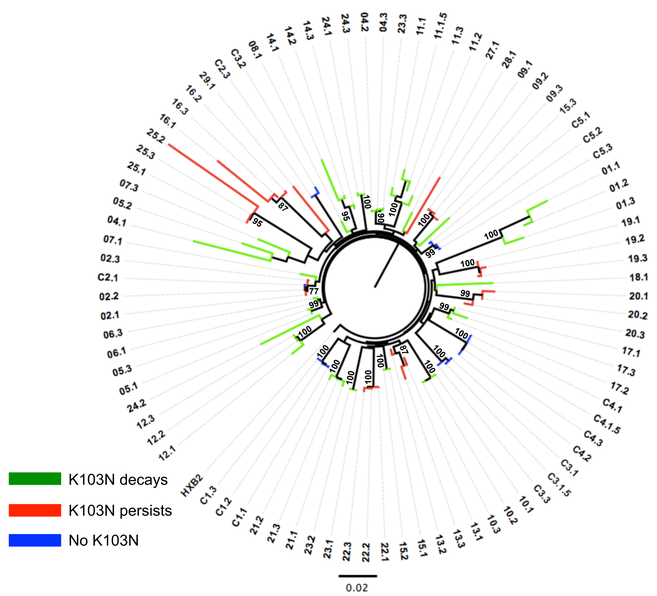

Figure 1. Phylogenetic analysis of HIV-1 from each subject. A neighbor-joining phylogenetic tree of *prot-RT* sequences derived from each subject time point. For each sample, it is shown whether the K103N mutation decays (**green**) or persists (**red**) over time. Control subjects that failed first-line therapy without K103N are shown in **blue**. The tree is rooted on Clade B consensus and evaluated with 100 bootstrap replicates.

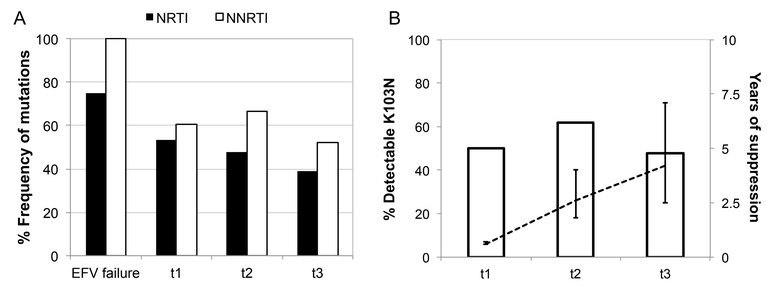

Figure 2. Frequency of NRTI and NNRTI mutations from each time point. A) Percentage of subjects with detectable NRTI versus NNRTI mutations at Efavirenz (EFV) failure, first suppression (t_1), most recent suppression (t_3), and an intermediate (t_2). Detection of NRTI mutations in DNA decreased with time on suppression (P<0.01). In adjusted model with years since suppression, $\log_{10}$ plasma HIV RNA level at EFV failure was associated with increased likelihood of observing proviral K103N (P=0.04); odds of detectable proviral K103N was 2.6 times higher (95% CI: 1.0-6.4) per $\log_{10}$ HIV RNA at EFV failure. B) Percentage of subjects having detectable proviral K103N (□) with median years of suppression (---) (bars indicate Q1-Q3 values). Years since start of suppression was not associated with the likelihood of detectable K103N (P=0.53).

Sample	Mutation	Time point	Sanger	PacBio RSII	Illumina MiSeq
Control	K103N	plasmid	Y	50.4	56.0
Subject 24	K103N	1	N	0	0
		2	N	0	0
		3	Y	15.3	19.1
	M41I	1	N	4.34	0
		2	N	0	0
		3	N	0	0
	G190R	1	N	5.66	10.8
		2	N	0	0
		3	N	0	0

Table 2. Quantification of minority DRM in Subject 24 using next-generation sequencing (NGS). Control K103N plasmid was mixed in equal proportion with control WT plasmid and sequenced in parallel with Subject 24 across three sequencing platforms. Standard population sequencing was used to detect presence or absence of the indicated mutations. PacBio RSII and Illumina MiSeq NGS technologies were used to quantify % variant with respect to HXB2 reference sequence. Using Sanger, a K103KN mixture was observed in Subject 24, which is accurately reflected by the levels detected on both NGS platforms. Values >2% were reported and statistically significant (P<0.05).

Conclusions

1. K103N in plasma was subsequently identified in the proviral DNA of ~50% of subjects for up to 11 years during PI-based suppression
2. Results imply seeding of the HIV reservoir during viral failure
   - Confirms the stability of drug resistance in HIV reservoirs during long-term suppressive ART
3. Persistent drug resistance at lower levels may be clinically relevant in those with undetected proviral resistance by population sequencing

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