Emergent and Detectable Proviral DNA Drug Resistance in a Community Treatment Program

Anne Derache[1], Hyoung Shik Shin[2], Elizabeth White[1], Dennis Israelski[1], Jeffrey D Klausner[3], Maya Balamane[1]

David Katzenstein[1]

[1]Stanford University, CA, USA, [2]National Medical Center, Seoul, South Korea, [3]University of California, Los Angeles, CA, USA

588

Contact Information
Anne Derache, PhD
Katzenstein's Lab
Department of Infectious Diseases
Stanford University
Email: aderache@stanford.edu

Background

. Introduction of antiretroviral therapy (ART) has changed the dynamic and outcomes of the HIV epidemic. Effective long-term suppressive ART leads to a "normal" life expectancy for HIV people who receive treatment. However, the long-term sequential use of ART is complex. HIV-1 is constantly changing in response to drug and immune pressures through evolutionary mechanisms, leading to the selection of drug resistance mutations (DRM) which may contribute to treatment outcomes and lead to transmission of drug resistance (TDR).

. HIV infection persists in viral reservoirs despite suppressive therapies, necessitating life-long ART. CD4+ T cells represent the predominant reservoir which is established at the beginning of the infection and maintained over time. DRM selected under treatment pressure during virologic failure may be archived and serve as a source of newly replicative, drug resistant virus if therapy is suboptimal.

. Rates of TDR depend on the geographical region, risk group and transmission dynamics within communities. In California, particularly in the San Francisco Bay Area, the frequency of TDR may be as high as 15%. Acquisition of drug resistance, whether through treatment failure or TDR can compromise the effectiveness of future therapies and further the dissemination of drug resistance.

. Drug resistance testing, genotyping, commonly uses viral RNA as a template, which raises challenges in settings where laboratory and transport facilities are not optimum. Furthermore, HIV RNA cannot be sequenced at very low levels, but proviral DNA contains genotypic information even among those on treatment and suppressed. Genotyping proviral DNA may be easier, cheaper and would improve HIV patient care. But, the clinical significance and impact of DRM detected in proviral DNA are not clear yet. Thus, we compared RNA and DNA genotyping in a cohort of 120 subjects receiving care, including maintenance of ART for 2 years through community public health programs in the "peninsula" extending from San Mateo County to San Francisco County public health services.

Methodology

. One hundred and twenty patients were enrolled and followed over 2 years in community-based treatment programs in San Mateo County hospital and a San Francisco Public Health clinic.

. Viremic subjects (Viral Load > 50 copies/ml) had genotypes from both viral RNA and PBMC proviral DNA, and those who suppressed (VL < 50 copies/ml) from PBMC proviral DNA only.

. Drug resistance mutations were identified using the Stanford HIV Drug Resistance Database http://HIVDB.stanford.edu

. Neighbor Joining tree had been inferred with Phylip package, with 2-Kimura parameters and 1000 bootstrap replicates, and vizualized with FigTree.

Characteristic		N = 120
Gender	Female	21 (17.5%)
	Male	98 (81.7%)
	Transgender	1 (0.8%)
Ethnicity	White	46 (8.37%)
	Afro American	33 (27.5%)
	Hispanic / Latino	30 (25%)
	Other (Asian, Natives)	11 (9.2%)
Age	Median (range)	44 (20-64)
On HAART	Yes	96 (80%)
CD4 count	<200	21 (17.5%)
	>200	99 (82.5%)
Viral Load	<50	82 (68.3%)
	>50	38 (31.7%)
Patients on HAART	Viral Load < 50	79 (82.3%)
	Viral Load >50	17 (17.7%)
	CD4 count < 200	17 (17.7%)
	CD4 count > 200	79 (82.4%)
Patients not currently on HAART	Viral Load < 50	3 (12.5%)
	Viral Load >50	2 (87.5%)
	CD4 count < 200	4 (16.7%)
	CD4 count > 200	20 (83.3%)
Ethnicity by suppression	White	34 (73.9%)
	Afro American	19 (57.6%)
	Hispanic / Latino	20 (66.7%)
	Other (Asian, Natives)	9 (81.8%)

Table 1 : Demographic, immunologic and virologic characteristics of the study population n = 120

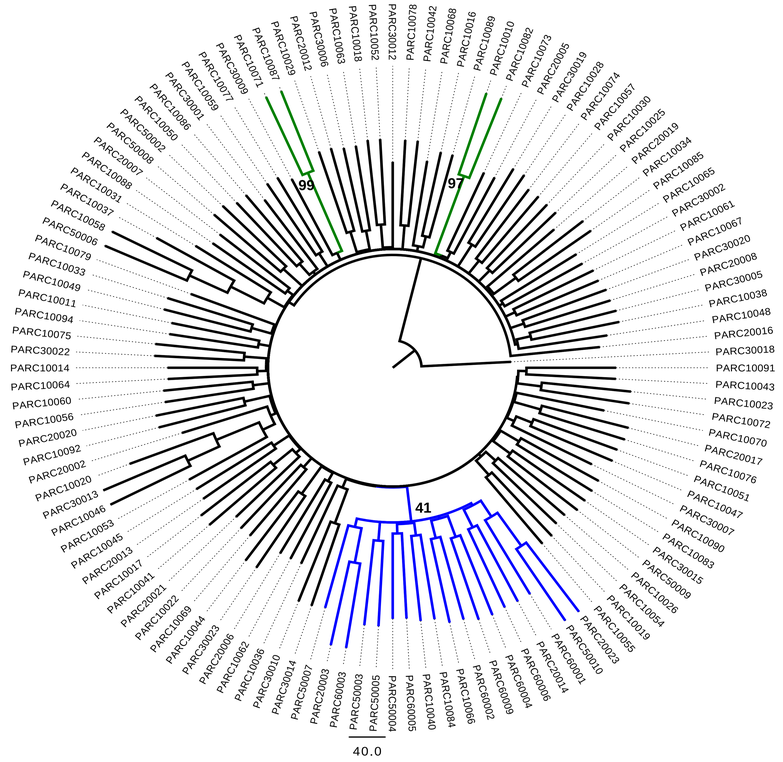


Figure 1 : Neighbor Joining tree with 2-Kimura parameters and 1000 replicates. Bolded in green 2 cluster pairs and in blue a cluster of 18 recipients. Only their bootstrap values are represented

Proviral DNA				Viral RNA				Treated at enrollment	VL at baseline
TAMs	Non TAMS	NNRTI	PI	TAMs	Non TAMS	NNRTI	PI		
		K103N			**M184MV**	K103N		yes	67
T215FIST, K70KR, D67DN, K219KQ				T215F, K70R, D67N, K219Q, **M41L, T69ADNT**	**M184V**			yes	68
T215Y, M41L	M184MV		D30, M46L	T215Y, M41L, **L210W**	M184V		D30N, M46L	yes	149
	M184V		**V82AITV**					no	156
T215ILPT			I54V, V82IT	T215IL			I54V, V82IT	yes	788
D67DG, K219KN	M184MV	K103KN, V106AV		D67G, K219N	M184V, **L74IL**	K103N, V106A		yes	969
	M184MV							no	2,770
T215F	M184MV			T215F	M184V			yes	3,320
		K103R				K103R		no	4,114
T215NSTY	M184V	K103N		T215Y	M184V	K103N		yes	7,190
		K103N				K103N		no	12,548
		K103N				K103N		no	29,268
		K103N, Y181C				K103N, Y181YC		yes	41,700
		V179D				V179D		yes	49,100

Table 2 : Drug Resistance mutations profiles of viremic patients at baseline (n = 14) with their viral load and treatment status at study entry. Bolded in blue the mutations found in a compartment and not in the other one.

Results

. Demographic, virologic and immunologic patients characteristics are summarized in Table 1. Ninety-six patients were on ART (80%) and had been treated for a median time of 7 years. The starting regimen was non-nucleoside reverse transcriptase inhibitor (NNRTI) based for 41% and Protease Inhibitor (PI) based for 59%.
Thirty eight patients were viremic: the medians of plasma HIV-1 RNA and CD4 counts were 5,685 copies/ml and 305 cells/mm^3 respectively.
Eighty-two patients were suppressed and had median CD4 count 422. cells/mm3.

. Among viremic patients (n=38), 14 harbored DRM in proviral DNA and 12 in viral RNA (profiles described in Table 2). There were concordant genotypes for 32 patients (84%) : the discordant profiles were mainly observed in patients with low-level viremia. Four patients harbored some nucleoside reverse transcriptase inhibitor (NRTI) DRM only in vRNA, and 2 only in proviral DNA as mixtures.

. Among suppressed patients (n=82), 21 had DRM in proviral DNA : 16 demonstrated mutations with potential resistance to one ARV class (mainly NRTI), 4 patient to 2 ARV classes (3NNRTI and 1 PI and NRTI), and 1 to all 3 of ARV classes.

. Of 31/38 viremic patients, 13 suppressed viremia to <50 copies/ml during follow-up. Neither presence of DRM or being on PI or NNRTI based regimen were associated with suppression.

. Among 82 suppressed patients (<300 copies/ml) 14 became viremic during the follow-up.
Five had low level viremia <1,000 copies/ml: of these 4 patients had the same genotype at baseline and failure; 3 were wild-type and 1 had 2 Thymidine analog mutations (TAM) and 1 selected a TAM mixture at 48 weeks.
Among the 9 patients who failed with > 1,000 copies/ml, 5 had a wild-type (wt) virus at baseline and 2 developed DRM associated with ART study. The other 4 harbored DRM at baseline : 2 failed with the same resistance mutations, one with multiple TAM at baseline developed more TAM and PI resistance. The last one had NNRTI mutations as mixtures at baseline in proviral DNA and failed with a wt virus (susceptible) virus from plasma RNA.

. Phylogenetic analysis (Figure 1) showed 2 transmission pairs with high bootstrap values (bolded in green). One cluster of 18 recipients is distinctly differentiated from the tree with a low bootstrap value (bolded in blue): these were older, median age 50 years, 16 were African-American, 16 were former IDU, 10 were female and 10 live in East Palo Alto.

Conclusions

. There was 84% concordance (agreement) in the DRM detected in genotypes from proviral DNA and viral RNA. Among those with virologic failure > 1,000 copies/ml, the agreement was 96%. These observations suggest that DNA genotyping might be used to guide treatment options or to assess drug resistance in resource-limited settings.

. A prevalence of DRM was observed in 26% of subjects receiving long-term of ART (median 7 years). The presence of DRM in PBMC DNA of suppressed patients poses a risk for the transmission of drug resistant virus with virologic failure, treatment interruption or adherence issues.

. The cluster of 18 recipients and their characteristics highlights the dynamic of HIV transmission in an African-American IDU community from East Palo Alto in the 90's.