

# Differential Persistence of Transmitted HIV-1 Drug Resistance Mutation Classes

Vivek Jain,<sup>1</sup> Maria C. Sucupira,<sup>4</sup> Peter Bacchetti,<sup>2</sup> Wendy Hartogensis,<sup>1</sup> Ricardo S. Diaz,<sup>4</sup> Esper G. Kallas,<sup>4,5</sup> Luiz M. Janini,<sup>4</sup> Teri Liegler,<sup>1</sup> Christopher D. Pilcher,<sup>1</sup> Robert M. Grant,<sup>3</sup> Rodrigo Cortes,<sup>4</sup> Steven G. Deeks,<sup>1,a</sup> and Frederick M. Hecht<sup>1,a</sup>

<sup>1</sup>HIV/AIDS Division, San Francisco General Hospital, <sup>2</sup>Department of Epidemiology and Biostatistics, and <sup>3</sup>Gladstone Institute for Immunology and Virology, University of California, San Francisco; <sup>4</sup>Infectious Diseases Division, Federal University of São Paulo, and <sup>5</sup>Division of Clinical Immunology and Allergy, University of São Paulo, Brazil

**Background.** Transmitted human immunodeficiency virus type 1 (HIV-1) drug resistance (TDR) mutations can become replaced over time by emerging wild-type viral variants with improved fitness. The impact of class-specific mutations on this rate of mutation replacement is uncertain.

**Methods.** We studied participants with acute and/or early HIV infection and TDR in 2 cohorts (San Francisco, California, and São Paulo, Brazil). We followed baseline mutations longitudinally and compared replacement rates between mutation classes with use of a parametric proportional hazards model.

**Results.** Among 75 individuals with 195 TDR mutations, M184V/I became undetectable markedly faster than did nonnucleoside reverse-transcriptase inhibitor (NNRTI) mutations (hazard ratio, 77.5; 95% confidence interval [CI], 14.7–408.2;  $P < .0001$ ), while protease inhibitor and NNRTI replacement rates were similar. Higher plasma HIV-1 RNA level predicted faster mutation replacement, but this was not statistically significant (hazard ratio, 1.71 log<sub>10</sub> copies/mL; 95% CI, .90–3.25 log<sub>10</sub> copies/mL;  $P = .11$ ). We found substantial person-to-person variability in mutation replacement rates not accounted for by viral load or mutation class ( $P < .0001$ ).

**Conclusions.** The rapid replacement of M184V/I mutations is consistent with known fitness costs. The long-term persistence of NNRTI and protease inhibitor mutations suggests a risk for person-to-person propagation. Host and/or viral factors not accounted for by viral load or mutation class are likely influencing mutation replacement and warrant further study.

Although the epidemiology of transmitted human immunodeficiency virus type 1 (HIV-1) drug resistance (TDR) mutations has been studied in a variety of settings [1–5], less is known about whether transmitted mutations persist without selection pressure from antiretroviral therapy (ART). Because many drug resistance mutations impair HIV fitness [6–10], replacement of TDR mutations with wild-type variants may confer

a potential survival advantage. This is particularly true when resistant and wild-type viral populations co-exist, as typically occurs when resistance develops during treatment. However, when HIV is transmitted by a single drug-resistant virion, the emergence of wild-type variants requires evolution and back-mutation rather than emergence of an existing wild-type variant.

Several studies have reported long-term persistence of most TDR mutations but have also documented replacement of certain mutations with wild-type variants [11–16]. Although valuable, these investigations did not have sufficient person-years of follow-up to quantify and compare replacement rates for each therapeutic drug class.

Improved understanding of how quickly different mutation classes are replaced by wild-type variants is important for several reasons.

First, most HIV diagnoses occur during chronic infection, rather than during acute and/or early infection. By the time genotyping is performed, TDR mutations

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<sup>a</sup>S.G.D. and F.M.H. Authors contributed equally to this research

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Reprints or correspondence: Frederick M. Hecht, MD, HIV/AIDS Division, San Francisco General Hospital, Box 0874, University of California, San Francisco, San Francisco, CA 94143 (rhecht@php.ucsf.edu).

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may have waned below the detection threshold for population genotyping, but persist as minority variants. Therefore, knowledge about mutation replacement rates for each drug class could help clinicians interpret resistance tests performed during chronic infection.

Second, the degree to which drug resistance will propagate among recently infected individuals depends on several factors including (1) the prevalence of drug resistance among chronically infected persons who are receiving partially suppressive therapy and (2) how long TDR persists in untreated persons and, thus, can propagate even without drug selection. The importance of propagation of transmitted mutations becomes amplified in resource-limited settings. Without genotyping and viral load monitoring, patients may initiate incompletely suppressive treatment regimens and sustain prolonged exposure to them. This leads to additional selection of drug resistance mutations [17, 18], further increasing the risk of resistance propagation and limiting patients' second-line treatment options.

Third, the interpretation of population-based surveys of TDR prevalence depends on understanding how long mutations persist after they are transmitted, because surveys will underestimate mutations that are quickly replaced by wild-type variants.

To provide more detailed information about persistence of specific mutations, we analyzed participants in 2 large early infection cohorts with sustained periods of follow-up without treatment.

## METHODS

### Study Populations and Settings

We included individuals with acute and/or early HIV infection who were enrolled in 2 prospective cohort studies: the Options Project (San Francisco General Hospital, University of California, San Francisco [UCSF]) and an acute and/or early HIV infection cohort in São Paulo, Brazil. The studies were approved by the UCSF Committee on Human Subjects Research and the Federal University of São Paulo Ethics Committee and Institutional Review Board. All participants gave informed, written consent for participation.

The Options Project is a prospective cohort study of individuals enrolled within 12 months after HIV antibody seroconversion (restricted to within 6 months after seroconversion since 2003). Participants are enrolled after screening for acute and/or early HIV infection with use of a combination of clinical history, serologic testing, and plasma HIV RNA determination, as described elsewhere [19]. Individuals enrolled during 1996–2009 were included. The São Paulo cohort enrolled patients, starting in 2002, who had experienced seroconversion within the previous 6 months and had evidence of acute and/or early HIV infection by the Serologic Testing Algorithm for Recent HIV Seroconversion [20]. Individuals enrolled during 2002–2009 were included.

We included all ART-naïve individuals in each cohort meeting 3 criteria: (1) TDR on initial genotyping, (2)  $\geq 6$  months of

observed follow-up time without ART ( $\geq 3$  months for São Paulo participants), and (3)  $\geq 1$  follow-up genotype. TDR was defined according to guidelines for epidemiologic studies [21] that include mutations known to be selected by therapy and exclude common polymorphic mutations that may or may not be selected by therapy.

For ART-naïve patients, we analyzed all available genotypes. We also included some individuals who had initiated ART. Here, we analyzed genotypes in 2 instances: (1) patient initiated ART during early HIV disease, with genotyping performed  $\leq 10$  days after initiation of ART (to minimize the possibility of an ART-induced mutation being mistaken for a TDR mutation), or (2) patient initiated but then later stopped ART, with genotyping performed  $> 6$  months after ART cessation. In the latter situation, we assumed that the virus population would be evolutionarily fixed during fully suppressive ART. If individuals started ART a second time, they were censored, and subsequent genotypes were not assessed.

### Clinical and Laboratory Evaluations

Demographic and behavioral data were collected from individuals in both cohorts with use of standardized interviews. For Options Project participants, CD4<sup>+</sup> T cell counts and plasma HIV-1 RNA levels were measured at baseline and every 3–4 months. For São Paulo cohort participants, these measurements were repeated 3 months after initial determination.

### HIV Genotype Determinations

In Options Project patients, baseline HIV-1 population sequence genotypes (TRUGENE genotyping system; Siemens Healthcare Diagnostics) were determined as described elsewhere [22, 23]. In São Paulo cohort patients, genotypes were performed as described elsewhere [24].

Follow-up genotypes were obtained using a strategy to accurately estimate the time at which baseline TDR mutations became undetectable by population sequencing while limiting the number of assays. For each individual, we genotyped the last available specimen before ART initiation or, for ART-naïve individuals, the last available specimen. If all baseline TDR mutations were present in the final sample, we did not genotype intervening specimens. If any baseline TDR mutations were not detected (indicating a loss), we bracketed backward, first genotyping the specimen closest to the midpoint of the prior 2 specimens. We continued genotyping intervening specimens to pinpoint the 2 time points bracketing the loss of the mutation.

### Time to Replacement of Drug Resistance Mutations.

When a baseline TDR mutation was not detected subsequently, it was considered to have been replaced. This contrasts to other approaches that focus on the first appearance of wild-type variants.

For ART-exposed patients, because we assumed that mutation loss or gain would not occur during effective ART, the

duration of observation was defined as the total number of days elapsed since HIV infection minus the number of days of ART.

We estimated a date for each mutation replacement event with use of midpoint imputation between the date of the last specimen on which the mutation was detected and the first specimen in which it was absent. Our primary statistical analyses, however, did not rely on these imputed dates.

### Data Analysis

Drug resistance mutations were grouped into 6 categories: (1) lamivudine/emtricitabine-associated mutations M184V/I; (2) thymidine analog-associated (TAM) mutations M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E; (3) T215 partial revertant mutations T215C, T215D, T215E, T215I, T215S, and T215V; (4) other nucleoside reverse-transcriptase inhibitor (NRTI) mutations; (5) nonnucleoside reverse-transcriptase inhibitor (NNRTI) mutations; and (6) protease inhibitor (PI) mutations. We analyzed the T215 partial revertant mutations as a separate group, because T215Y/F mutations first progress to T215 partial revertants in one step, then progress to wild-type in a second step [25]. In each mutation group, a Kaplan-Meier plot was made of the cumulative probability of mutation replacement versus the number of ART-free days since the estimated date of infection, using imputed dates of replacement.

We sought to address several features of these data that make optimal statistical analysis challenging. First, transmitted mutations that become undetectable before a person's initial genotype cannot be identified or included in this study. The time of individuals' baseline genotypes, therefore, are late-entry times (also known as left-truncation times). Second, as mentioned, the exact time of mutation replacement lies between the last genotype with the mutation and the first genotype without it. Such interval-censored data require specialized calculations. Third, an important a priori predictor that we sought to analyze—HIV plasma RNA level—changed throughout follow-up (ie, it is a time-varying covariate), and those changes could impact the chance of mutation replacement. Fourth, many persons had multiple baseline mutations, requiring assessment of possible within-person clustering or dependence.

Methods have been published for fitting parametric survival models to interval-censored data with clustering but without time-varying covariates [26] and for interval-censored data with time-varying covariates but no clustering [27]. We combined features of these models [28] and added calculations to account for late-entry times. Details of the calculations and example SAS code (SAS Institute) are provided in online supplements A and B.

This produced a parametric proportional hazards model that we used to analyze the association between mutation group and the hazard of mutation replacement. To control for plasma RNA levels as a time-varying covariate, for each interval of time modeled, we used the last RNA level measured at or before the start of that interval to ensure that RNA levels were predicting

mutation loss rather than reflecting it. Using the NNRTI mutation group as the referent, we computed hazard ratios for mutation loss over time for the other 5 mutation groups. Our method allows individual mutations to be assessed singly but accounts for within-subject correlation in participants with multiple baseline mutations.

Using this model, we computed the percentage of transmitted mutations that would be expected to become undetectable by 6 months, 1 year, 2 years, and 3 years after HIV infection, assuming a constant HIV plasma RNA level of 40,000 copies/mm<sup>3</sup> and assuming that mutations were present at 3 months after infection (because there were no observations during very early times due to the aforementioned late-entry phenomenon).

## RESULTS

### Clinical Characteristics of Participants

Of 697 participants who entered either the Options Project or the São Paulo cohort during 1996–2009, 676 had baseline genotyping performed (21 individuals had plasma RNA levels that were too low for genotyping or were lost to follow-up). Of the 676 participants with baseline genotypes, 20 additional persons were excluded because they began ART >10 days before genotyping ( $n = 7$ ), had evidence of superinfection with a second HIV variant ( $n = 6$ ), or had missing data ( $n = 7$ ). Of the 656 eligible individuals, 136 (21%) had TDR. Of these, a total of 75 persons had a follow-up genotype that could be assessed (the other 61 individuals either began ART and achieved virologic suppression [ $n = 57$ ] or were lost to follow-up or had missing data [ $n = 4$ ]). The 75 patients with TDR and follow-up genotypes formed the basis for this analysis ( $n = 59$  from Options cohort and  $n = 16$  from the São Paulo cohort).

Overall, individuals in the 2 cohorts were similar; our sample was predominantly men whose main risk for HIV acquisition was sex with other men and who enrolled in the cohorts at a median of 2.7–3.0 months after HIV infection (Table 1). HIV-1 plasma RNA levels were slightly higher among São Paulo patients, but this difference was not statistically significant (Wilcoxon rank sum test,  $P = .37$ ).

### Mutation Replacement Over Time

The 75 participants had a total of 195 baseline TDR mutations (Table 2). Participants' genotypes are listed in online supplement C and have been uploaded to GenBank (accession HQ528536-HQ529037; HQ585024-HQ585055). Over a median follow-up period of 25 months (interquartile range, 8–41 months), 75% of individuals with transmitted M184V/I mutations exhibited loss of this mutation; the proportion with mutation replacement was 40% for those with T215 revertants, 28% for those with TAMs, 11% for those with other NRTI mutations (including K65R), 25% for those with NNRTI mutations, and 20% for those with PI mutations (Table 2).

**Table 1. Baseline Characteristics of 75 Participants in the Options Project and São Paulo Acute and/or Early HIV Infection Cohorts.**

Variable	Options Project cohort	São Paulo cohort
No. of participants	59	16
Age at cohort enrollment, median (IQR), years	36 (31–42)	30 (24–32)
Male sex, %	95	81
MSM status, %	93	81
Recent injection drug use, %	7	N/A
Estimated duration of HIV infection <sup>a</sup> (IQR), months	2.7 (2.4–3.5)	3.0 (2.8–4.8)
Baseline HIV-1 plasma RNA level, median (IQR), copies/mm <sup>3</sup>	12,710 (2549–79,060)	34,050 (7725–53,350)
Baseline CD4 cell count, median (IQR), cells/mm <sup>3</sup>	560 (465–693)	469 (372–567)

**NOTE.** IQR, interquartile range; MSM, men who have sex with men; N/A, not available.

<sup>a</sup> Duration from estimated date of HIV infection to date of first genotype determination.

In a Kaplan-Meier analysis of time to mutation replacement (Figure 1), the M184V/I group displayed more rapid replacement compared with all other mutation groups, and the other mutation groups were similar to one another (Figure 1).

Table 2 shows results of our parametric proportional hazards model (which assumed an exponential parametric form). Because of the steady state plasma RNA level of 40,000 copies/mm<sup>3</sup>, the model predicts that, after 6 months of HIV infection, 68% of transmitted M184V/I mutations that were present at 3 months would wane below the detection limit, whereas ≤6% of other mutation groups would wane to this level (Table 2). After 3 years of HIV infection, the model predicts that all mutation groups except other NRTIs would show >15% replacement and M184V/I mutations would show 100% replacement.

#### Predictors of Mutation Replacement

Compared with the NNRTI mutation group, the M184V/I mutation group had a markedly higher hazard for mutation

replacement (hazard ratio, 77.5; 95% CI, 14.7–408.2;  $P < .001$ ) (Table 2). TAMs and T215 partial revertants exhibited a trend toward elevated hazard of replacement, although neither was statistically significant. Other NRTI mutations had a lower hazard, but the CIs around this estimate were very wide. The hazard for PI mutation replacement was not substantially different from that of NNRTI mutations.

Higher plasma HIV RNA levels were associated with a trend toward increased mutation loss, but this was not statistically significant (hazard ratio for replacement per log<sub>10</sub> increase in plasma HIV RNA, 1.77; 95% CI, .90–3.25;  $P = .11$ ).

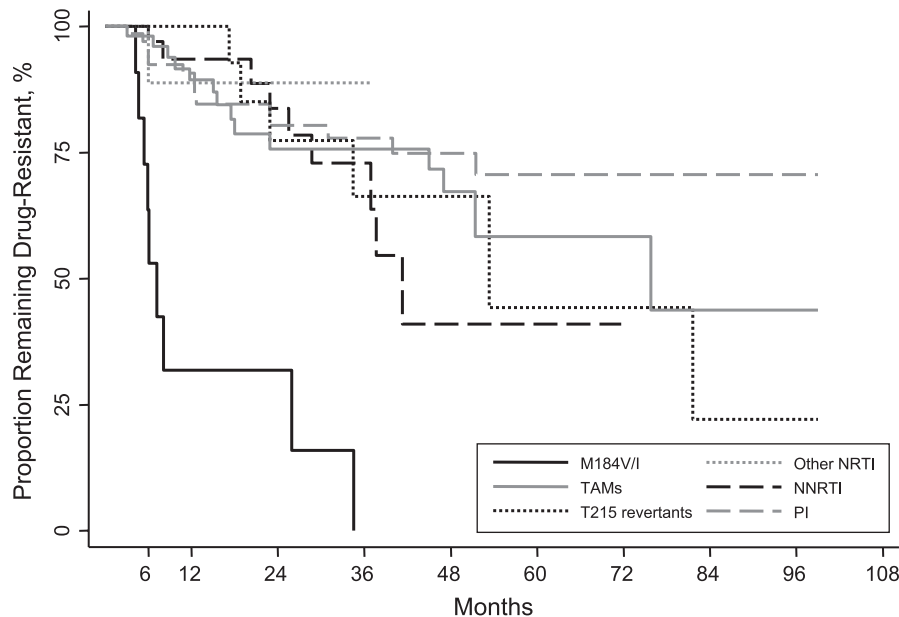
In addition, we found strong evidence for substantial within-person correlation in the risk of mutation loss that was not accounted for by plasma HIV RNA levels ( $P < .001$ ) or by drug class.

We examined possible departures from the proportional hazards assumption, including possible change in the effect of HIV plasma RNA level in the first 6 months after infection

**Table 2. Frequency of Transmitted Drug Resistance Mutations, by Drug Class, and Proportions Receding to Undetectable Status on Population Sequencing among 75 Participants Followed Up from Early HIV Infection.**

Observed Data	Mutation Group					
	NNRTI	M184V	TAMs	T215 Revertants	Other NRTI	PI
No. of baseline mutations(no. of patients)	36 (33)	12 (12)	54 (29)	15 (15)	9 (5)	69 (31)
No. replaced by wild-type(% of total mutations)	9 (25)	9 (75)	15 (28)	6 (40)	1 (11)	14 (20)
<b>Model projections</b>						
Hazard ratio for replacement (95% CI)	Reference	77.5 (14.7–408.2)	2.54 (.65–9.92)	4.24 (.76–23.51)	0.39 (.024–6.33)	1.12 (.31–3.95)
<i>P</i>	–	<.001	.19	.10	.51	.87
Percentage replacement (95% CI)						
6 months	1 (0–5)	68 (27–98)	4 (1–9)	6 (2–21)	1 (0–9)	2 (1–4)
1 year	4 (1–15)	97 (61–100)	11 (4–26)	17 (5–50)	2 (0–24)	5 (2–13)
2 years	10 (3–32)	100 (89–100)	23 (9–50)	35 (11–80)	4 (0–47)	11 (4–28)
3 years	15 (4–45)	100 (97–100)	34 (14–67)	50 (17–92)	6 (0–63)	17 (6–40)
4 years	20 (6–56)	100 (99–100)	43 (19–78)	61 (22–97)	8 (1–74)	22 (8–50)

**NOTE.** Hazard ratios for replacement by wild-type over time are displayed for each mutation group, derived from a parametric proportional hazards model accounting for time-varying plasma HIV RNA levels and within-subject clustering. The proportion of baseline mutations expected to be replaced at specified times after HIV infection are also displayed. CI, confidence interval; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; TAMs, thymidine analog-associated mutations.



**Figure 1.** Kaplan-Meier plot of cumulative probability of mutation replacement over time in different classes of transmitted drug resistance mutations among 75 patients followed up from early infection. Probabilities of mutation replacement for each of 6 mutation groups are plotted against the number of antiretroviral treatment-free days since the estimated date of HIV infection, as follows: Lamivudine/emtricitabine-associated mutations M184V/I (solid black line), TAMs M41I/D67N/K70R/L210W/T215Y/F/K219Q/E (solid gray line), T215 revertant mutations T215C/D/E/I/S/V (black dotted line), other nucleoside reverse-transcriptase inhibitor (NRTI) mutations (gray dotted line), nonnucleoside reverse-transcriptase inhibitor (NNRTI) mutations (black dashed line), and protease inhibitor (PI) mutations (gray dashed line). TAM, thymidine analog-associated mutations.

versus after 6 months, and did not find strong evidence for nonproportionality; therefore, we used the simplest model, assuming proportional hazards for all covariates. Generalizing from an exponential model to a Weibull model did not improve fit substantially.

## DISCUSSION

By analyzing a large number of patients with TDR, we were able to show that the M184V/I mutations become undetectable in population sequencing substantially faster than do other mutation groups, which are generally similar to one another. Although rates of replacement for mutations other than M184V/I were relatively low, there was still appreciable replacement. Although NNRTI mutations are assumed to have minimal effects on fitness, replacement of these mutations occurred over time. In addition, our results suggest that higher viral load may promote mutation loss, but this does not account for the very substantial person-to-person variation that we observed in the rate of mutation loss.

Prior studies of TDR mutations have reported that replacement with wild-type variants occurs but that most mutations are maintained for at least 1–2 years after transmission [11–16]. The largest and most definitive study grouped all mutations together and estimated a median time-to-loss of detectable drug resistance (using population-based assays) ranging from 4.1 years, using a conservative estimate, to longer

than the lifetime of the individual, using a less conservative estimate [12]. Although our results might appear to differ from this estimate, the difference is primarily attributable to our ability, with larger numbers, to assess groups of mutations separately. The high rate of replacement that we observed among M184V/I mutations is consistent with prior reports [11–13, 29] and almost certainly reflects this mutation’s association with reduced fitness in the absence of therapy [6, 9, 30]. The relative stability of thymidine analog-associated, NNRTI, and PI mutations over time is also consistent with other reports [12–14].

We had hypothesized that PI mutations would be replaced more rapidly than would NNRTI mutations. However, we found that the PI group had a rate of replacement similar to that of the NNRTI group, although CIs were wide (HR for replacement of PI vs NNRTI, 1.12; 95% CI, 0.3–4.0;  $P = .9$ ). The slow replacement of PI mutations is notable, because these mutations, similar to M184V/I, are known to affect viral fitness [31, 32]. Our group previously performed a partial treatment interruption study in which patients receiving a stable partially suppressive regimen discontinued one drug class while maintaining the other drug classes. Continuation of at least some ART prevented rapid rebound of archived variants, forcing HIV to back-mutate in the same way that occurs after the acquisition of a TDR variant. In the study, selective removal of NRTIs was associated with loss of the M184V/I mutation, and removing PIs was associated with limited mutation replacement, even after several years [33]. The mechanism for the persistence

of PI-resistant variants despite fitness costs was investigated by van Maarseveen et al [34], who argued that viral variants with both PI mutations and compensatory mutations must travel through a “fitness valley” to lose both types of mutations— first reducing fitness in order to ultimately maximize it. Our findings are consistent with the theoretical framework advanced by these 2 studies and with prior reports of infrequent replacement of PI mutations [11, 12, 16].

The clinical implication of our finding that PI mutations are replaced at a similar rate to NNRTI mutations is that one potential advantage of including PIs in first-line ART regimens in resource-limited settings—namely, faster replacement of transmitted mutations by wild-type and lower risk of spread of resistance—does not appear to be present. The risk of drug resistance propagation is further amplified by the substantial fraction of all HIV transmissions that occur from source patients who have acute and/or early HIV infection [35, 36], implying that, in many patients with TDR, the available time for mutation replacement before retransmission may be quite limited.

A second clinical implication of our findings relates to the rapid replacement of M184V that we observed. Because of the frequent use of drugs that select for this mutation, should a patient who has received a new diagnosis of HIV who has other mutations be assumed to have lamivudine/emtricitabine-resistant virus even if this is not present on genotyping? The rapid mutation loss that we observed suggests that the lack of M184V in this context may be attributable to replacement, which is supported by a prior study showing that very early replacement of M184V is common: 11% of persons with early HIV infection had M184V variants detected using a minor variant assay but not in population sequencing [37]. Other studies, however, have shown that genotyping with population sequencing is usually a reliable guide to choosing therapy for patients with TDR [38, 39]. One way to reconcile these studies is that M184V variants may rapidly decrease to levels that are not clinically significant, leaving a relatively short window during which clinically important frequencies are missed. Precise quantitation of the prevalence of M184V variants after the loss of detection on population sequencing, along with correlation with treatment outcomes, could help better define the threshold for clinically significant levels of M184V.

In analyzing predictors of mutation replacement, we made 2 observations that together raise interesting questions about host-virus interactions. First, we hypothesized that higher viral load would predict faster mutation loss but found only a modest trend. Second, our study included many persons with multiple baseline mutations, allowing us to assess whether there are person-level factors influencing mutation replacement. We found that, after accounting for viral load and mutation drug class, there was marked person-to-person variability in the likelihood of mutation replacement. This suggests that there are additional patient-level factors driving mutation loss. Our

observations about mutation replacement are consistent with the concept that viral evolution, rather than being driven exclusively by selective (deterministic) events based only on the fitness costs of mutations, may also be driven by stochastic forces. This idea that evolution is influenced by a combination of factors and that the effective viral population size is not simply reflected by the plasma HIV RNA level has been supported by seminal studies [40–42] and helps contextualize our results.

Our study has several important limitations. First, we used population sequence genotypes rather than more sensitive methods capable of detecting minor variants. This limits our ability to assess mutations below the detection threshold of conventional sequencing, which may persist in the viral quasi-species in sufficient quantities to remain clinically significant. This phenomenon has been illustrated by studies of the K103N mutation among women who received single-dose nevirapine to prevent vertical HIV transmission [43]. Our inability to detect M184V minor variants in particular suggests that our data may underestimate the rate at which replacement with wild-type virus occurs. Second, we included 11 individuals who received and subsequently interrupted ART. Because viral evolution is minimal or nonexistent during effective therapy [44–46], we do not believe that this affected our central observations. Third, because of the challenges of identifying patients with very early HIV infection, we could not determine whether any of our participants acquired mutations and lost them in the earliest weeks of infection. Fourth, we did not assess all factors that might influence replacement rates, such as viral tropism. Although the effective viral population size discussed earlier is highly pertinent to analyzing rates of mutation replacement, estimating this factor is complex and requires measurement of multiple clonal populations that we did not perform. Finally, caution should be exercised in generalizing our results from sexual transmission cases to injection drug use transmission. Because intravenous infections have been shown to be caused by a multiplicity of viral variants in most cases [47], whereas the majority of sexual transmissions are caused by single virions [48], there may be more rapid replacement of TDR in transmissions related to injection drug use.

Overall, our data indicate that transmitted M184V/I mutations are unique in their high propensity to wane below the detection threshold of population genotyping over time. Clinicians assessing new patients should consider the possibility that mutations not present in baseline genotyping may have been present previously and may have been replaced with wild-type viral variants, particularly in the case of M184V/I. Further investigation is warranted on both host and viral factors that are influencing mutation replacement.

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## References

- Chaix M-L, Descamps D, Wirlden M, et al. Stable frequency of HIV-1 transmitted drug resistance in patients at the time of primary infection over 1996–2006 in France. *AIDS* **2009**; *23*:717–24.
- Hurt CB, McCoy SI, Kuruc J, et al. Transmitted antiretroviral drug resistance among acute and recent HIV infections in North Carolina from 1998 to 2007. *Antivir Ther* **2009**; *14*:673–8.
- Vercauteren J, Wensing AM, van de Vijver DA, et al. Transmission of drug-resistant HIV-1 is stabilizing in Europe. *J Infect Dis* **2009**; *200*:1503–8.
- Weinstock HS, Zaidi I, Heneine W, et al. The epidemiology of antiretroviral drug resistance among drug-naïve HIV-1-infected persons in 10 US cities. *J Infect Dis* **2004**; *189*:2174–80.
- Yerly S, von Wyl V, Ledergerber B, et al. Transmission of HIV-1 drug resistance in Switzerland: a 10-year molecular epidemiology survey. *AIDS* **2007**; *21*:2223–9.
- Campbell TB, Shulman NS, Johnson SC, et al. Antiviral activity of lamivudine in salvage therapy for multidrug-resistant HIV-1 infection. *Clin Infect Dis* **2005**; *41*:236–42.
- Cong ME, Heneine W, Garcia-Lerma JG. The fitness cost of mutations associated with human immunodeficiency virus type 1 drug resistance is modulated by mutational interactions. *J Virol* **2007**; *81*:3037–41.
- Gonzalez LM, Brindeiro RM, Aguiar RS, et al. Impact of nelfinavir resistance mutations on in vitro phenotype, fitness, and replication capacity of human immunodeficiency virus type 1 with subtype B and C proteases. *Antimicrob Agents Chemother* **2004**; *48*:3552–5.
- Paredes R, Sagar M, Marconi VC, et al. In vivo fitness cost of the M184V mutation in multidrug-resistant human immunodeficiency virus type 1 in the absence of lamivudine. *J Virol* **2009**; *83*:2038–43.
- Zimmer JM, Roman F, Lambert C, et al. Impact on replicative fitness of the G48E substitution in the protease of HIV-1: an in vitro and in silico evaluation. *J Acquir Immune Defic Syndr* **2008**; *48*:255–62.
- Barbour JD, Hecht FM, Wrin T, et al. Persistence of primary drug resistance among recently HIV-1 infected adults. *AIDS* **2004**; *18*:1683–9.
- Little SJ, Frost SD, Wong JK, et al. Persistence of transmitted drug resistance among subjects with primary human immunodeficiency virus infection. *J Virol* **2008**; *82*:5510–8.
- Brenner BG, Routy JP, Petrella M, et al. Persistence and fitness of multidrug-resistant human immunodeficiency virus type 1 acquired in primary infection. *J Virol* **2002**; *76*:1753–61.
- Pao D, Andrady U, Clarke J, et al. Long-term persistence of primary genotypic resistance after HIV-1 seroconversion. *J Acquir Immune Defic Syndr* **2004**; *37*:1570–3.
- Smith DM, Wong JK, Shao H, et al. Long-term persistence of transmitted HIV drug resistance in male genital tract secretions: implications for secondary transmission. *J Infect Dis* **2007**; *196*:356–60.
- Brenner B, Routy J-P, Quan Y, et al. Persistence of multidrug-resistant HIV-1 in primary infection leading to superinfection. *AIDS* **2004**; *18*:1653–60.
- Sungkanuparph S, Manosuthi W, Kiertiburanakul S, Piyavong B, Chumpathat N, Chantratita W. Options for a second-line antiretroviral regimen for HIV type 1-infected patients whose initial regimen of a fixed-dose combination of stavudine, lamivudine, and nevirapine fails. *Clin Infect Dis* **2007**; *44*:447–52.
- Gupta RK, Hill A, Sawyer AW, et al. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. *Lancet Infect Dis* **2009**; *9*:409–17.
- Hecht FM, Busch MP, Rawal B, et al. Use of laboratory tests and clinical symptoms for identification of primary HIV infection. *AIDS* **2002**; *16*:1119–29.
- Kallas EG, Bassichetto KC, Oliveira SM, et al. Establishment of the serologic testing algorithm for recent human immunodeficiency virus (HIV) seroconversion (STARHS) strategy in the city of Sao Paulo, Brazil. *Braz J Infect Dis* **2004**; *8*:399–406.
- Shafer RW, Rhee SY, Pillay D, et al. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. *AIDS* **2007**; *21*:215–23.
- Grant RM, Kuritzkes DR, Johnson VA, et al. Accuracy of the TRUGENE HIV-1 genotyping kit. *J Clin Microbiol* **2003**; *41*:1586–93.
- Kuritzkes DR, Grant RM, Feorino P, et al. Performance characteristics of the TRUGENE HIV-1 genotyping kit and the open gene DNA sequencing system. *J Clin Microbiol* **2003**; *41*:1594–9.
- Sucupira MC, Caseiro MM, Alves K, et al. High levels of primary antiretroviral resistance genotypic mutations and B/F recombinants in Santos, Brazil. *AIDS Patient Care STDS* **2007**; *21*:116–28.
- de Ronde A, van Dooren M, van Der Hoek L, et al. Establishment of new transmissible and drug-sensitive human immunodeficiency virus type 1 wild types due to transmission of nucleoside analogue-resistant virus. *J Virol* **2001**; *75*:595–602.
- Bellamy SL, Li Y, Ryan LM, Lipsitz S, Canner MJ, Wright R. Analysis of clustered and interval censored data from a community-based study in asthma. *Stat Med* **2004**; *23*:3607–21.
- Sparling YH, Younes N, Lachin JM, Bautista OM. Parametric survival models for interval-censored data with time-dependent covariates. *Biostatistics* **2006**; *7*:599–614.
- Kalbfleisch JD, Prentice RL. *The statistical analysis of failure time data*. New York: Wiley, 1980.
- Chan KC, Galli RA, Montaner JS, Harrigan PR. Prolonged retention of drug resistance mutations and rapid disease progression in the absence of therapy after primary HIV infection. *AIDS* **2003**; *17*:1256–8.
- Castagna A, Danise A, Menzo S, et al. Lamivudine monotherapy in HIV-1-infected patients harbouring a lamivudine-resistant virus: a randomized pilot study (E-184V study). *AIDS* **2006**; *20*:795–803.
- Croteau G, Doyon L, Thibeault D, McKercher G, Pilote L, Lamarre D. Impaired fitness of human immunodeficiency virus type 1 variants with high-level resistance to protease inhibitors. *J Virol* **1997**; *71*:1089–96.
- Martinez-Picado J, Savara AV, Sutton L, D'Aquila RT. Replicative fitness of protease inhibitor-resistant mutants of human immunodeficiency virus type 1. *J Virol* **1999**; *73*:3744–52.
- Deeks SG, Hoh R, Neilands TB, et al. Interruption of treatment with individual therapeutic drug classes in adults with multidrug-resistant HIV-1 infection. *J Infect Dis* **2005**; *192*:1537–44.
- van Maarseveen NM, Wensing AM, de Jong D, et al. Persistence of HIV-1 variants with multiple protease inhibitor (PI)-resistance mutations in the absence of PI therapy can be explained by compensatory fixation. *J Infect Dis* **2007**; *195*:399–409.
- Brenner BG, Roger M, Routy JP, et al. High rates of forward transmission events after acute/early HIV-1 infection. *J Infect Dis* **2007**; *195*:951–9.
- Brenner BG, Roger M, Moisi DD, et al. Transmission networks of drug resistance acquired in primary/early stage HIV infection. *AIDS* **2008**; *22*:2509–15.
- Toni TA, Asahchop EL, Moisi D, et al. Detection of human immunodeficiency virus (HIV) type 1 M184V and K103N minority variants

- in patients with primary HIV infection. *Antimicrob Agents Chemother* **2009**; 53:1670–2.
38. Oette M, Kaiser R, Däumer M, et al. Primary HIV drug resistance and efficacy of first-line antiretroviral therapy guided by resistance testing. *AIDS* **2006**; 41:573–81.
  39. Chaix ML, Desquilbet L, Descamps D, et al. Response to HAART in French patients with resistant HIV-1 treated at primary infection: AaNRS Resistance Network. *Antivir Ther* **2007**; 12:1305–10.
  40. Brown AJ. Analysis of HIV-1 env gene sequences reveals evidence for a low effective number in the viral population. *Proc Natl Acad Sci U S A* **1997**; 94:1862–5.
  41. Rouzine IM, Coffin JM. Linkage disequilibrium test implies a large effective population number for HIV in vivo. *Proc Natl Acad Sci U S A* **1999**; 96:10758–63.
  42. Frost SD, Nijhuis M, Schuurman R, Boucher CA, Brown AJ. Evolution of lamivudine resistance in human immunodeficiency virus type 1-infected individuals: the relative roles of drift and selection. *J Virol* **2000**; 74:6262–8.
  43. Coovadia A, Hunt G, Abrams EJ, et al. Persistent minority K103N mutations among women exposed to single-dose nevirapine and virologic response to nonnucleoside reverse-transcriptase inhibitor-based therapy. *Clin Infect Dis* **2009**; 48:462–72.
  44. Hermankova M, Ray SC, Ruff C, et al. HIV-1 drug resistance profiles in children and adults with viral load of <50 copies/ml receiving combination therapy. *JAMA* **2001**; 286:196–207.
  45. Kieffer TL, Finucane MM, Nettles RE, et al. Genotypic analysis of HIV-1 drug resistance at the limit of detection: virus production without evolution in treated adults with undetectable HIV loads. *J Infect Dis* **2004**; 189:1452–65.
  46. Persaud D, Siberry GK, Ahonkhai A, et al. Continued production of drug-sensitive human immunodeficiency virus type 1 in children on combination antiretroviral therapy who have undetectable viral loads. *J Virol* **2004**; 78:968–79.
  47. Bar KJ, Li H, Chamberland A, et al. Wide variation in the multiplicity of HIV-1 infection among injection drug users. *J Virol* **2010**; 84:6241–7.
  48. Keele BF, Giorgi EE, Salazar-Gonzalez JF, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci U S A* **2008**; **105**:7552–7.