Short communication

A decade of HIV-1 drug resistance in the United States: trends and characteristics in a large protease/reverse transcriptase and co-receptor tropism database from 2003 to 2012

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Background: Drug resistance testing and co-receptor tropism determination are key components of the management of antiretroviral therapy for HIV-1-infected individuals. The purpose of this study was to examine trends of HIV-1 resistance and viral evolution in the past decade by surveying a large commercial patient testing database.

Methods: Temporal trends of drug resistance, viral fitness and co-receptor usage among samples submitted for routine phenotypic and genotypic resistance testing to protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), as well as for tropism determination were investigated.

Results: Within 62,397 resistant viruses reported from 2003 to 2012, we observed a decreasing trend in the prevalence of three-class resistance (from 25% to 9%) driven by decreased resistance to PIs (43% to 21%) and NRTIs (79% to 57%), while observing a slight increase in NNRTI resistance (68% to 75%). The prevalence of CXCR4-mediated entry among tropism testing samples (*n*=52,945) declined over time from 47% in 2007 to 40% in 2012. A higher proportion of CXCR4-tropic viruses was observed within samples with three-class resistance (50%) compared with the group with no resistance (36%).

Conclusions: Decreased prevalence of three-class resistance and increased prevalence of one-class resistance was observed within samples reported between 2003 and 2012. The fraction of CXCR4-tropic viruses has decreased over time; however, CXCR4 usage was more prevalent among multi-class-resistant samples, which may be due to the more advanced disease stage of treatment-experienced patients. These trends have important implications for clinical practice and future drug discovery and development.

Introduction

Since the beginning of the HIV type-1 (HIV) epidemic, a large number of antiretroviral drugs have been developed that target several different aspects of virus replication [1–3], including seven nucleoside reverse transcriptase inhibitors (NRTIs), five non-nucleoside reverse transcriptase inhibitors (NNRTIs), nine protease inhibitors (PIs), six antiretroviral drug combinations, two entry inhibitors and three integrase strand-transfer inhibitors (INSTIs; Additional file 1). However, in the absence of complete suppression of virus replication, HIV rapidly evolves to evade these drugs [4,5], making drug resistance and co-receptor tropism testing key components of treatment management for individuals infected with HIV.

Global surveys of the epidemic are great resources that help monitor the evolution of drug resistance. As a provider of routine HIV drug susceptibility testing, Monogram Biosciences has accumulated a large set of drug resistance data over the past decade (2003–2012). This unique database is well-suited to interrogate variations, distributions, temporal trends and associations of HIV characteristics, including antiretroviral drug resistance.

Methods

We examined fully de-identified HIV samples submitted from the US to Monogram Biosciences (South San Francisco, CA, USA) for routine phenotypic and genotypic resistance testing, as well as for tropism determination between 1 January 2003 and 31 December 2012. Patients with multiple testing were not excluded

because our goal was to incorporate the contribution of longitudinal samples to the viral evolution. This study does not include INSTI trends due to the relatively short existence of the class with the first drug, raltegravir, approved in 2008 and second drug, elvitegravir, approved late 2012.

Protease and reverse transcriptase resistance

To minimize bias by sample submission trends as a result of changes in resistance testing behaviours, we restricted our investigation to samples that demonstrated substantial phenotypic resistance to at least one PI, NRTI or NNRTI as measured by a fold change in 50% inhibitory concentration (IC_{50}) greater than 2× the lower cutoff. A total of 62,397 viruses exhibiting resistance to one or more PI, NRTI and/or NNRTI were analysed. We also evaluated the resistance patterns by basic demographic characteristics such as geographic region, sex and age, as well as by HIV-1 subtype. Protease/reverse transcriptase (PR/RT)-dependent replication capacity (RC) as a measure of viral fitness was also examined.

Tropism

The Trofile® [6] assay was specifically developed to determine the co-receptor tropism (CCR5, CXCR4 or dual-mixed) of patient viruses as a means to select patients who are likely to respond to treatment regimens that include a co-receptor antagonist (for example, maraviroc). For this study, CXCR4 and dual tropic viruses were grouped together as CXCR4-using. Test results from 52,945 samples sent to Monogram Biosciences for tropism determination between 2007 and 2012 were analysed. We studied the prevalence of co-receptor usage over time, and by envelope subtype as assessed by the HyPhy SCUEAL [7] package. For the 9,643 specimens with phenotypic PR/RT resistance profiles as well as tropism results, we also evaluated prevalence of CXCR4-using viruses and distribution of RC by the PR/RT resistance status.

Statistical analysis

Jonckheere–Terpstra test was performed to evaluate the significance of the temporal trends. Wilcoxon test was used to examine the differences in the resistance levels and the RC distributions within various subtype and tropism groups. Differences in CCR5 usage compared with subtype B were assessed using Fisher's exact test.

Results

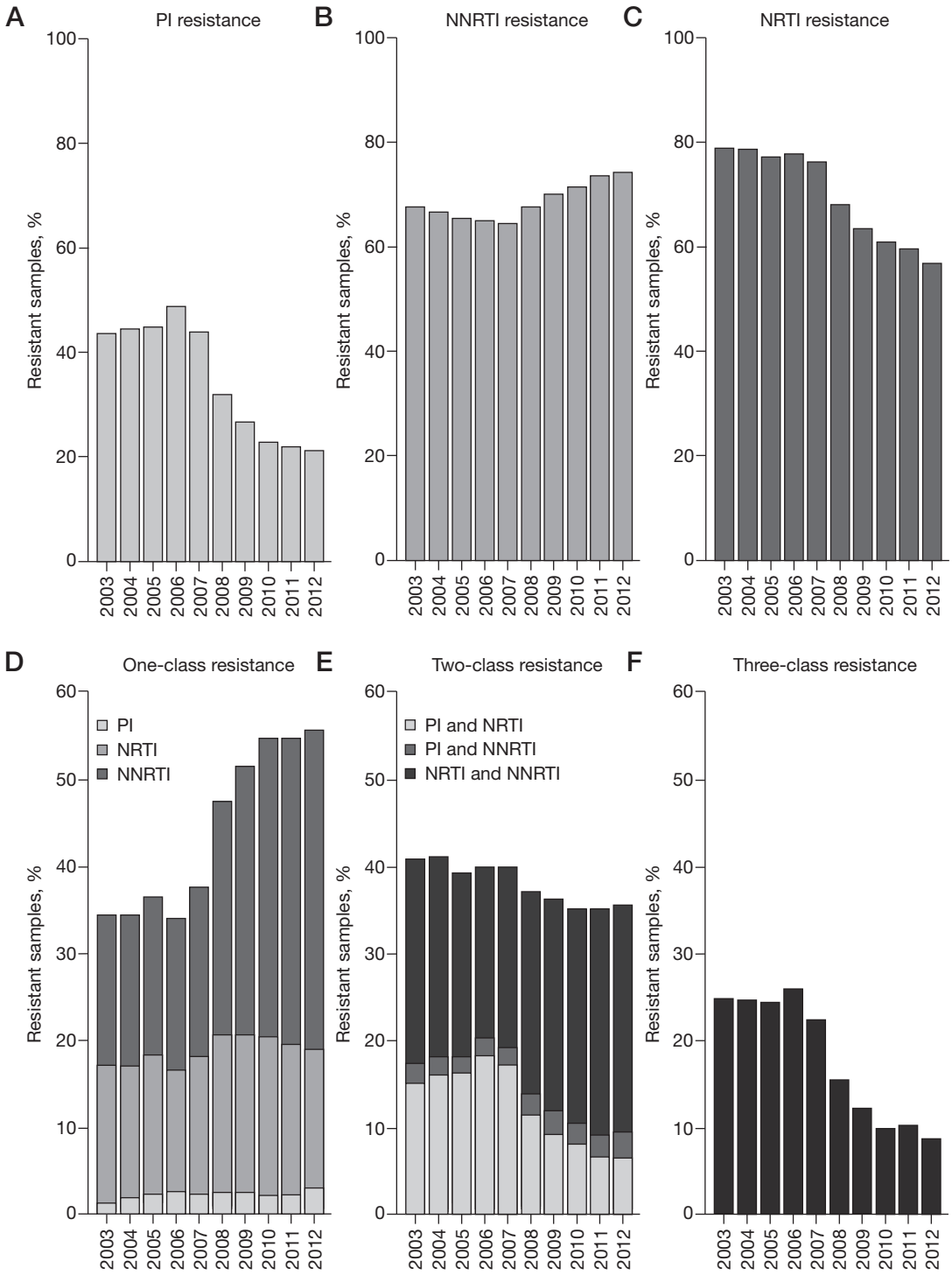
Trends in PI/RT inhibitor resistance

Over the past decade a marked decrease in the fraction of samples with measurable reductions in susceptibility to PIs and NRTIs was observed in our database. PI resistance decreased from 43.4% in 2003 to 21.2% in 2012 (P=0.006; Figure 1A) and NRTI resistance decreased from 79.3% to 57.4% (P<0.001, Figure 1C). By contrast, NNRTI resistance increased from 67.8% to 74.6% over the same time period (P=0.02; Figure 1B). To rule out population density as a possible confounder, we evaluated resistance patterns within four geographic regions of the US grouped based on the submitting physician's address. As shown in Additional file 1, regional trends in NRTI, NNRTI and PI resistance were consistent with the trends in the entire country.

After grouping samples into one-, two- or three-class resistance categories, a steady increase in reduced susceptibility to a single drug class from 34.3% to 55.6% was observed (P<0.001; Figure 1D). This increase is mostly driven by an increased percentage of samples with reduced susceptibility to NNRTIs in the absence of PI or NRTI resistance (17.1–36.6%). Over the same period, the percentage of samples with resistance to all three drug classes decreased almost threefold, from 24.8% in 2003 to 8.7% in 2012 (P<0.001; Figure 1F). The fraction of samples with two-class resistance decreased slightly from 40.9% to 35.6% (P=0.002; Figure 1E). The decrease in the percentage of patients with reduced susceptibility to both PI and NRTI classes appears to account for the overall decrease in multidrug class resistance. We also evaluated resistance levels within different subtypes; only non-B subtypes with 50 or more samples were included: A, AG, C and D, which made up 1.2% of all resistant samples. We observed lower levels of resistance within non-B samples compared with subtype B samples. Prevalence of one-, two- and three-class resistance within subtype B samples was 45%, 38% and 17%, respectively, compared with 54%, 37% and 9% within non-B samples (P<0.001). This may indicate that the subtype B population, the most common subtype in North America, is more treatment-experienced than the non-B group. There could be other, less well-understood subtype-specific factors that play a role as well.

When examining resistance patterns based on gender, we observed significantly lower prevalence in three-class resistance in women versus men (P<0.001), which seems to be driven by the lower prevalence in PI and NRTI resistance in women (29% and 65%, respectively, versus 41% and 76%). Prevalence of NNRTI resistance is the same in men and women (68%). This finding is consistent with a study performed by Patel *et al.* [8] evaluating antiretroviral efficacy in men versus women. We also examined the level of resistance in the following age groups: 0–12, >12–17, >17–25, >25–40, >40–55, >55–65 and 65+, with age calculated at the time of sample collection. As shown in the Additional file 1, age groups >17–25 and >25–40 exhibited lowest prevalence of three-class resistance (both at 13%) and highest level of one-class resistance (55% and 49%,

Figure 1. Phenotypic trends of PR/RT class resistance and of one-, two- and three-class resistance



Trends for **(A)** protease inhibitor (PI)-, **(B)** non-nucleoside reverse transcriptase inhibitor (NNRTI)- and **(C)** nucleoside reverse transcriptase inhibitor (NRTI) resistance are shown. Each bar represents the percentage of samples that exhibited reduced phenotypic susceptibility to either one, two or three drug classes (NRTI, NNRTI and PI) compared with the sum total of samples that exhibited reduced susceptibility to any drug class ($P \leq 0.002$). Trends for **(D)** one-, **(E)** two- and **(F)** three-class resistance are shown. Each bar represents the percentage of samples that exhibited reduced phenotypic susceptibility to either one, two or three drug classes (NRTI, NNRTI and PI) compared with the sum total of samples that exhibited reduced susceptibility to any drug class (NRTI, NNRTI and PI). The decreasing trends in NRTI and PI resistance, and increasing trend in NNRTI resistance over time within all samples with evidence of phenotypic resistance to at least one protease/reverse transcriptase (PR/RT) were significant ($P<0.03$).

respectively), whereas age groups >55–65 and >12–17 showed highest level of three-class resistance with 24% and 23%, respectively.

Trends in PI/RT inhibitor resistance associated mutations

Next, we characterized the temporal trends of major resistance-associated mutations (RAMs) in this cohort. In order to distinguish temporal changes of the class-specific RAMs from overall resistance trends, we restricted these examinations to samples that exhibited a measurable reduction in susceptibility to one or more drugs of that class. Comma delimited mutations denote substitutions involving more than one amino acid: for instance, M184I,V means M184I or M184V.

Trends in the frequencies of PI RAMs are shown in Figure 2A. PI RAMs with statistically significant upward trends include V32I, I47A,V, I50V, I54L,M, Q58E, and T74P. The PI mutation with the most significant decline in frequency is L90M, which decreased from 61% in 2003 to 48% in 2012 ($P<0.001$). Selection of the L90M mutation is strongly associated with the use of first-generation PIs (some of which are still being prescribed, such as atazanavir and fosamprenavir), but not with newer PIs such as darunavir or tipranavir [9].

Significant downward trends in the prevalence of all NRTI RAMs were observed between 2003 to 2012, with the exception of M184I,V (from 75% to 90%, $P<0.001$) and K65R (from 5.9% to 9.8%, $P=0.003$; Figure 2B). The selection of M184I,V variants is associated with the use of emtricitabine and lamivudine while the selection of K65R variants is associated with tenofovir use [9,10]. The increasing trends noted in M184V and K65R mutations may be attributed to the increased utilization of emtricitabine and tenofovir among current ARV regimens. Although emtricitabine+tenofovir combination is a leading candidate for pre-exposure prophylaxis and treatment as prevention strategies, it is unlikely that the increased prevalence in M184V and K65R reported here were significantly impacted by these emerging prevention strategies. However, if such trends continue, these increases may become problematic as emtricitabine+tenofovir for pre-exposure prophylaxis is more broadly implemented.

Only two NNRTI mutations exhibited statistically significant upward trends in frequency: L100I (from 7% to 8%, $P=0.025$) and P225H (from 7% to 11%, $P=0.001$). The emergence of L100I variants is associated with the use of all NNRTIs and was recently reported to confer reductions in rilpivirine susceptibility when present in combination with K103N,R,S mutations [11]. The frequency of the K103N mutation associated with efavirenz use was relatively stable over time (65% in 2003 and 63% in 2012, $P=0.8$; Figure 2C).

Tropism and replication capacity

We evaluated trends in co-receptor usage within all samples submitted for co-receptor tropism testing within the US between 2007 and 2012 ($n=52,945$). Prevalence of CXCR4-using viruses (X4+DM) declined from 47% in 2007 to 40% in 2012 (data not shown). Among samples with corresponding PI/RT inhibitor resistance data ($n=9,643$), we observed a significant increase in the fraction of CXCR4-tropic viruses as the number of resistant drug classes increased: 36.4%, 41.3%, 46.8% and 50.2% for 0-, 1-, 2- and 3-class resistance, respectively ($P=0.02$; Additional file 1).

When examining viral RC, the overall RC of viruses lacking drug resistance was higher than the RC of phenotypically resistant samples: median RC was 100% for viruses with no drug resistance versus 83%, 49% and 34% for viruses exhibiting one-, two- and three-class resistance, respectively. This trend was most striking among the pure X4-tropic viruses where a median RC of 117% was observed for the group lacking drug resistance compared a median RC of 45% for the resistant group ($P<0.001$; Additional file 1).

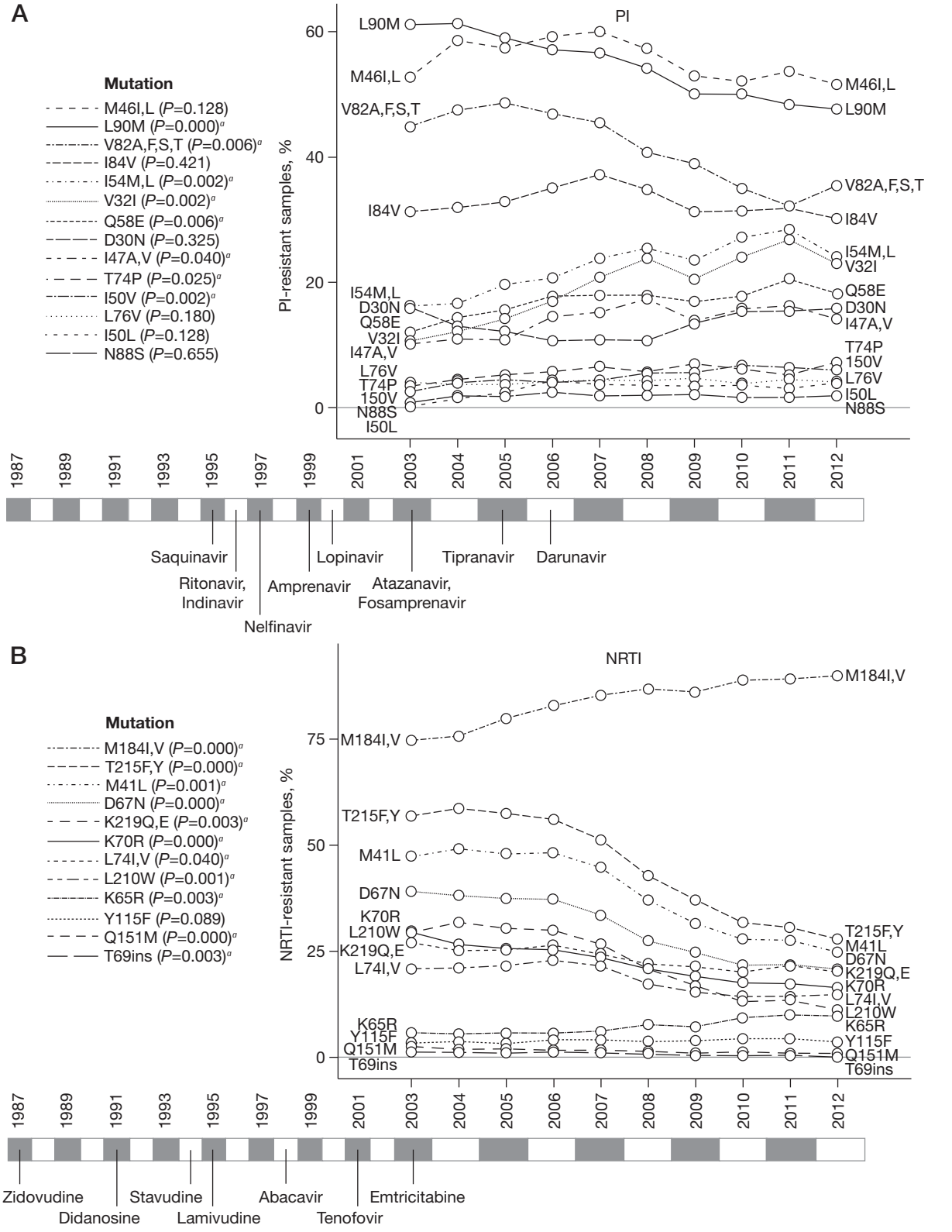
This cohort was predominantly composed of subtype B samples (96.8%), with other subtypes present at less than 1% each. The prevalence of CXCR4-using variants was 41.9% among subtype B samples. Notably, we observed a significantly lower prevalence of CXCR4-tropic viruses within subtypes C, A/A1 and G as compared with subtype B (Table 1).

Discussion

Strong trends of decreasing prevalence of three-class antiretroviral drug resistance, and increasing prevalence of one-class resistance was identified within resistant samples collected in the US between 2003 and 2012. This decreasing trend of multi-class resistance is consistent with observations reported by Buchacz *et al.* [12], in the HIV outpatient study. Our analysis suggests that the downward trend in three-class resistance since 2007 is driven by decreases in the fractions of resistant viruses with reduced susceptibility to PI and NRTI classes. Temporal trends in resistance mutation profiles are consistent with the availability and use of emerging antiretroviral agents.

Drug-sensitive 'wild type' viruses exhibited higher RC compared with resistant samples, which is consistent with the loss of RC that can occur with the development of resistance [4,5]. Additionally, higher RC of antiretroviral sensitive CXCR4-using variants and lower replication of drug resistant CXCR4-using variants was observed. We also identified an association between multi-class resistance and X4 tropism, which can offer a possible explanation for part of the decline

Figure 2. Genotypic trends



Temporal trends of class-specific resistance associated mutations (RAMs) and approval dates of antiretrovirals in each drug class are shown: **(A)** protease inhibitor (PI), **(B)** nucleoside reverse transcriptase inhibitor (NRTI) and **(C)** non-nucleoside reverse transcriptase inhibitor (NNRTI). PI RAMs include D30N, V32I, M46I,L, I47A,V, G48V, I50L,V, I54L,M, Q58E, T74P, L76V, V82A,F,S,T, I84V, N88S and L90M; NRTI RAMs include M41L, K65R, D67N, T69insertion, K70R, L74I,V, Q151M, M184I,V, L210W, T215F,Y and K219E,Q; and NNRTI RAMs include L100I, K101E,P, K103N, V106A,M, Y115F, Y181C,I,V, Y188C,H,L, G190A,S and P225H. The Jonckheere-Terpstra test was performed to evaluate the significance of the trend of each mutation over 2003–2012. [a]Statistically significant trends (P-value <0.05).

Figure 2. Continued

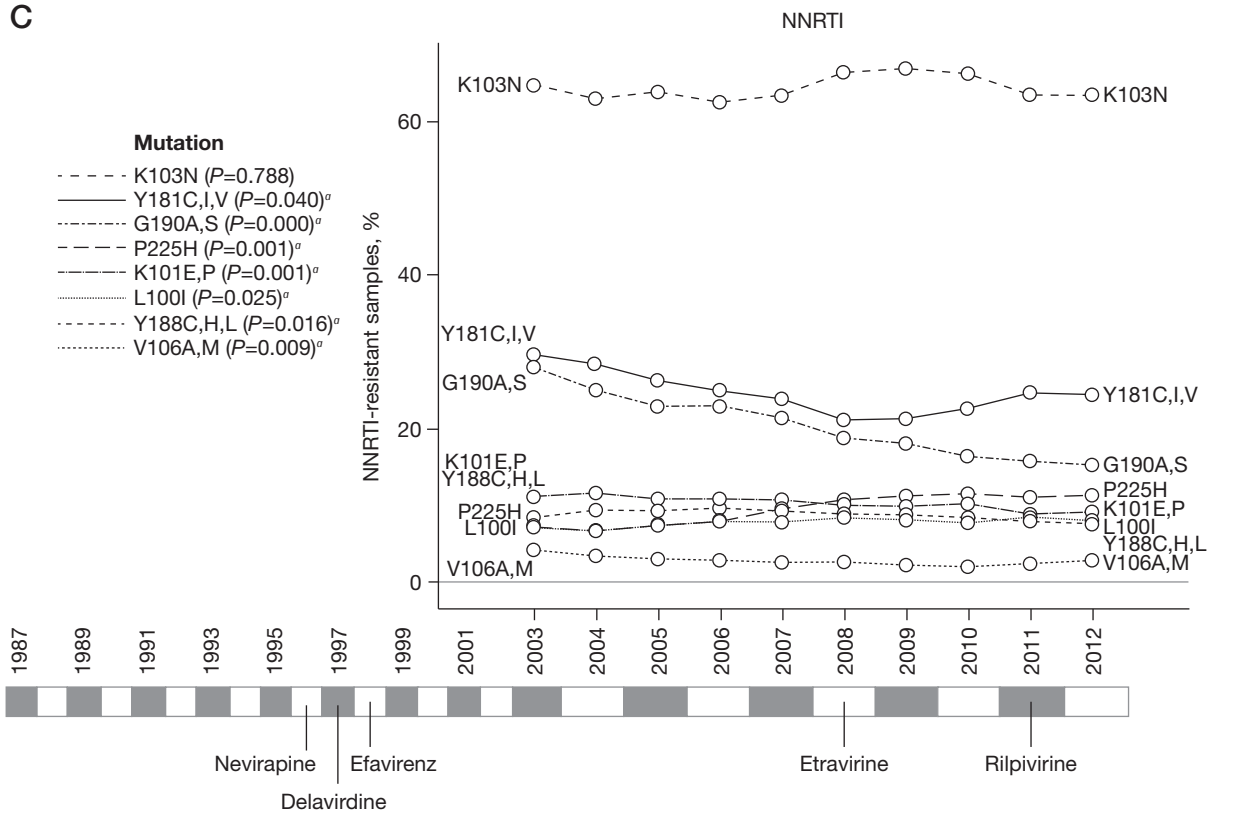


Table 1. Tropism prevalence by subtype

Subtype	R5, %	Non-R5, %	*n*	*P*-value	Odds ratio
B	58.06	41.94	46,410	–	1
B,D	53.39	46.61	251	0.14	1.21
C	83.26	16.74	233	**<0.001**	0.28
A,B	64.16	35.84	173	0.11	0.77
A/A1	72.27	27.73	119	**<0.001**	0.53
A,G	57	43	100	0.84	1.04
B,C	67.21	32.79	61	0.16	0.68
AE	67.24	32.76	58	0.18	0.67
G	78.95	21.05	57	**<0.001**	0.37
Other	61.34	38.66	494	0.16	0.87

Comparisons were made with subtype B and examined by Fisher's exact test. The *P*-value is highlighted in bold for subtypes where prevalence of CXCR4-using viruses is significantly lower than subtype B (A/A1, C and G).

in multi-class resistance since CXCR4-using viruses are generally thought to be less transmissible.

The trends described in this paper highlight a decrease in the number of HIV-infected patients with multidrug-resistant virus: a patient group for whom limited treatment options were available in earlier years of the epidemic. Availability of antiretrovirals directed at novel anti-HIV targets, including INSTIs and maraviroc, decreased dependence on the three major classes of antiretroviral drugs for HIV management. Additionally, newer agents with favourable cross-resistance profiles such as darunavir and etravirine became available after 2006. These advancements in drug treatment [13–17] likely explain the declining trends of resistance to

the PR/RT drug classes after 2007. Overall, improved efficacy of antiretroviral drugs, convenient drug co-formulations and evolving prescription patterns likely account for the decreasing prevalence of multidrug resistance observed in this clinical database.

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Disclosure statement

All authors are employees of Monogram Biosciences, a wholly owned subsidiary of Laboratory Corporation of America, provider of the PhenoSense®, PhenoSense® GT and Trofile® assays.

Additional file

Additional file 1: An additional file showing antiretroviral approval history, resistance by age groups, PR/RT inhibitor class resistance patterns in different US regions, and co-receptor usage by PR/RT drug-class resistance can be found at http://www.intmedpress.com/uploads/documents/3167_Paquet_Additional_file_1.pdf

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