Background: The selection of antiretroviral (ARV) drugs for treatment of HIV-1 infection is based on several factors including potency, toxicity, resistance and ease of administration. Emtricitabine (FTC) or lamivudine (3TC), components of recommended initial ARV regimens, are structurally related and share the same resistance mutation (M184V/I). However they differ with respect to potency and incidence of M184V/I.

Methods: Resistance-associated mutation (RAM) prevalence data were obtained from genotype test results performed in a large reference laboratory from 2003–2010; subsets of data were defined by mutation pattern to resemble those following failure of non-nucleoside reverse transcriptase inhibitor (NNRTI)-based combination therapy. Mutational trend data were compared to contemporaneous ARV prescription information.

Results: In the unfiltered data set (n=107,231), the prevalence in 2010 decreased compared to 2003 for all nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) RAMs, such as M184V/I (44.0% to 17.9%), T215Y (22.7% to 4.1%), and K65R (4.3% to 2.1%). Among samples resembling those typical of first-line NNRTI-based failures, prevalence of K103N increased slightly, but prevalence of M184V/I decreased (49.8% to 36.8%), as did other NRTI RAMs. These decreases were coincident with a shift in ARV prescriptions away from zidovudine and 3TC towards tenofovir and FTC, and an increase in use of fixed-dose combinations.

Conclusions: RAM prevalence decreased substantially since 2003 among samples submitted for resistance testing in the US. The causes of this decrease are multifactorial, but our results suggest a possible role of increased use of potent ARVs that are available as fixed-dose combinations or as single-tablet regimens.

Introduction

Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs) are important components of HAART for treatment of HIV-1 infection [1]. Treatment regimens recommended for previously untreated patients include two NRTIs in combination with a non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor or integrase inhibitor; recommended NRTI combinations include lamivudine (2',3'-dideoxy-3'-thiacytidine; 3TC) or emtricitabine (2',3'-dideoxy-S-fluoro-3'-thiacytidine; FTC) [2,3]. Drug selection in general is driven by several factors, including drug potency and efficacy, toxicity and side effects, facility of resistant virus selection and consequences of resistance, pill burden, dosing frequency and availability as a coformulation with other drugs. In vitro and in vivo analyses suggest that FTC has several advantages compared to 3TC [4–9]. By contrast, one recent retrospective observational cohort study showed no difference between virological efficacy of abacavir/3TC and TDF/FTC [10]. The improved clinical efficacy observed in most studies may be related to a longer plasma and intracellular FTC triphosphate half-life compared to 3TC triphosphate, or differences between TDF and the comparators zidovudine or abacavir in these combinations [11,12].

The dominant pathway to drug resistance for both FTC and 3TC is the selection of the M184V or M184I mutation in HIV-1 reverse transcriptase [13,14]. M184V/I engenders high-level (>100-fold) resistance to 3TC and FTC, and variable levels of cross-resistance to several other NRTIs, including abacavir [15] and...
didanosine [16]. It is also associated with increased *in vitro* susceptibility to zidovudine, stavudine and TDF [17–20], and with decreased replication capacity [21,22]. These phenotypes may in part be responsible for the observation that HIV viral load does not return completely to baseline following failure of 3TC-containing, dual NRTI regimens, and that the NRTI component of HAART regimens appear to retain partial potency after loss of viral load suppression in HAART-experienced patients [23].

While both FTC and 3TC select for and are affected to a similar degree by M184V/I, the use of FTC has been associated with a lower prevalence of M184V/I than 3TC among patients with virological failure. In various clinical studies, regimens that include 3TC appear to result in the presence of M184V/I in 22–50% of virological failures compared to approximately 10–24% with FTC [7,24–31]. In a retrospective analysis of patients failing FTC/TDF- versus 3TC/TDF-containing HAART regimens in Italy, the NRTI resistance-associated mutations (RAMs) K70R, M184V and T215F were significantly more common in 3TC-treated than in FTC-treated patients [32].

The present study analysed HIV-1 NRTI and NNRTI RAM prevalence trends in the US since the commercial availability of FTC in 2003. The objective of the study was to provide additional information regarding the relative propensity of regimens containing FTC or 3TC to select for M184V/I and other mutations in clinical samples predicted to be representative of HIV patients experiencing early failure of their first antiretroviral (ARV) regimen.

**Methods**

RAM data were drawn from the database of resistance test results generated and maintained by Monogram Biosciences, Inc. (South San Francisco, CA, USA). All samples submitted for routine resistance analysis (genotyping only or phenotyping and genotyping) to Monogram Biosciences, Inc. from 2003–2010, after removal of duplicate samples from the same patient, were considered (*n* = 107,231). When duplicate samples were present, the earlier one was retained (Figure 1). Since drug treatment information is not provided at the time of test requisition, criteria based on the type of test requested and mutation pattern detected were used to define multiple groups of samples. Repeat samples were removed after applying filters based on presence or absence of RAMs such that a sample with a qualifying resistance pattern would be retained when an earlier sample with wild-type or a non-qualifying resistance pattern was in the database (Figure 1).

Statistical significance of trends in prevalence over time was assessed using a two-sided Jonckheere-Terpstra test [33,34] as implemented in the SAGx package for R.
Total US retail and mail order prescription data from 2003 to 2010 for ARV drugs were obtained from Wolters Kluwer Health Source® (Phoenix, AZ, USA) Pharmaceutical Audit Suite, which provides syndicated retail data projected from 82% of all prescriptions dispensed in the US, and mail order data projected from 60% of all prescriptions dispensed in the mail order channel in the US. Total prescriptions for each ARV were calculated by adding the prescriptions for the single ARV molecule to the prescriptions for any fixed-dose combination (FDC) that included that ARV molecule (for example, Viread, Truvada and Atripla for TDF or Epivir, Combivir, Epzicom and Trizivir for 3TC).

Results

The most commonly observed reverse transcriptase inhibitor RAMs in the unfiltered data set (n = 107,231), without regard to time of sampling, were M41L, D67N, K70R, K103N, Y181C, M184V/I, L210W and T215Y (prevalence 12–44% in 2003). The prevalence of all NRTI RAMs decreased over time between 2003 and 2010; data for several important mutations are shown in Figure 2. For example, M184V/I decreased from 44.0% in 2003 to 17.9% in 2010 (P = 0.0005), with consistent decreases every year; T215Y decreased from 22.7% to 4.1% (P = 0.0005), K103N decreased from 32.2% to 17.9% (P = 0.0005) and K65R decreased from 4.3% to 2.1% (P = 0.002). The prevalence of M41L (25.4% to 6.7%; P = 0.0005), K70R (16.7% to 3.8%; P = 0.0005), and L74V (8.4% to 1.7%; P = 0.0005) also decrease significantly.

Many factors related to the treatment histories of the patients from whom the samples were collected could be contributing to the decrease in RAM prevalence over time. One of the most important of these is the proportion of samples submitted for resistance testing that are from untreated patients, or from patients who are less than optimally adherent to their regimens, both of which might be expected to be predominantly drug-susceptible. Untreated patients are more likely to have a genotype test requested without an accompanying phenotype, whereas those failing multiple regimens are more likely to seek the combination of phenotype and genotype. To attempt to reduce the contribution from both untreated and heavily ARV-experienced patients, results from samples where only a genotype was requested, that contained any protease inhibitor (PI) RAM, Q151M or insertions near T69, or >2 thymidine analogue mutations (TAMs), were excluded. Excluding the genotype-only samples had little effect on prevalence of the most common RAMs and, therefore, this filter was not applied in subsequent groups. Excluding samples with PI RAMs, Q151M, T69 insertions, or >2 TAMs resulted in a reduction in overall RAM prevalence, as expected; the reduction in prevalence over time, however, followed a similar pattern compared to the unfiltered sample set (data not shown).

Finally, a requirement for ≥1 NNRTI RAM was added to the above filter, to attempt to focus on viruses from patients failing an NNRTI-based regimen typical of most initial HAART combinations (Figure 1). In this group of 21,453 samples, RAM prevalence decreased in most cases; for example, M184V/I in 2003 was 49.8%, decreasing to 36.8% in 2010 (P = 0.002), T215Y was present in 17.5% of the NNRTI-resistant samples in 2003, and 5.6% in 2010 (P = 0.0008) and K65R, initially in 8.8% of these samples, decreased to 6.5% in 2009 then returned to 7.4% in 2010 (P = 0.033; Figure 3). K103N prevalence in this group was high (between 70% and 72%) and increased slightly over time (P = 0.006). Other common NNRTI RAMs, such as Y181C and G190A, were present at lower levels, which decreased over time (20.6% to 13.7% for Y181C, P = 0.004; and 17.8% to 10.2% for G190A, P = 0.001). By contrast, prevalence of L100I (5.1% to 6.1%; P = 0.02) and P225H (8.9% to 12.8%; P = 0.006) increased between 2003 and 2010 (Figure 3A).

Resistance mutation patterns are largely influenced by the ARVs prescribed to the patient. ARV use at the population level can be inferred from prescription data, and analysed over time to look for correlations with the mutation information described above. Using a large commercial database representing approximately 82%...
of retail and 60% of mail order prescriptions in the US, the numbers of prescriptions written annually for seven NRTIs, efavirenz and nevirapine between 2003 and 2010 are shown in Figure 4A. There was a shift over this time period from zidovudine and 3TC being the two most often-prescribed NRTIs in 2003 to mostly TDF and FTC since 2007. The use of efavirenz increased modestly, while that of abacavir remained stable. Didanosine and stavudine prescriptions were relatively low in number in 2003 and decreased further through 2010.

Another factor that can influence the prevalence of RAMs is adherence to the regimen, which in turn can be affected by the use of FDCs of ARVs. The proportion of FTC, 3TC, TDF, zidovudine or efavirenz prescribed in an FDC is shown in Figure 4B. The preference for FDC for all of these drugs clearly increased from 2003 to 2010, illustrated notably by the near exclusive use of FTC as an FDC since its approval as a single drug in 2003 and in combination with TDF in 2004. Since 2007, over 50% of any NRTI available in an FDC was prescribed as an FDC. For efavirenz, use in the complete single tablet regimen of Atripla has similarly reached nearly 80% of total efavirenz usage in 2010.

**Discussion**

The data presented in this report demonstrate a striking decrease in the percentage of HIV-1 samples from infected individuals that contain mutations associated with resistance to reverse transcriptase inhibitors, among samples submitted for resistance testing to a large centralized reference laboratory in the US between 2003 and 2010. Decreases were consistently observed year after year, and over the 8-year period, several decreased by a factor of two- to five-fold. However, interpretation of these observations is complicated by uncertainty regarding the ARV drug use by these patients. To mimic patients treated with two NRTIs and an NNRTI, a recommended first-line ARV regimen, we focused on a subset of the samples that demonstrated evidence of moderate NRTI/NNRTI use. In this group, NRTI mutation prevalence still decreased over time, albeit less dramatically and consistently. M184V/I, the most frequently detected NRTI RAM, was 1.4-fold less prevalent in 2010 compared to 2003 in NNRTI-resistant samples; T215Y and other TAMs were 2–3-fold less prevalent. There was evidence for different trends among NNRTI RAMs, as K103N remained relatively constant, Y181C and G190A decreased, and P225H increased. The decrease in Y181C and increase in P225H prevalence is most likely explained by the reduction in prescription of nevirapine and increase in efavirenz use over time.

The observed changes in RAM prevalence occurred against the backdrop of an evolution in ARV prescription preferences. A dramatic shift away from individual prescription of zidovudine, stavudine, didanosine and 3TC-based ARV combination regimens, and towards

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**Figure 3. Reverse transcriptase RAM prevalence trends among samples with low- to moderate-level NRTI resistance and ≥1 NNRTI RAM**

![Graph A](image1)

![Graph B](image2)

Sample total n=21,453. (A) M184V/I and non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance-associated mutations (RAMs): K103N, M184V/I, Y181C, G190A, P225H, L100I. (B) Nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) RAMs: M41L, T215Y, K70R, L74V, L210W, K65R. Changes in prevalence over time were all statistically significant (P≤0.05).
use of TDF and FTC, often in an FDC pill or a single-tablet regimen, was reflected by the prescription data reported here.

Decreasing prevalence of RAMs is likely due to multiple factors, including fewer patients with a history of suboptimal therapy (for example, monotherapy, dual NRTI therapy and use of relatively less potent ARVs), shorter periods of virological failure on treatment due to improved access to diagnostic monitoring, increased use of FTC relative to 3TC, and increased use of FDC-containing regimens. The increased use of FTC relative to 3TC and the observed decreases in M184V/I prevalence are consistent with multiple studies showing less development of M184V/I in FTC- versus 3TC-containing regimens [7,24–29].

Results similar to those described here have been reported by others, for example in Spain between 2000 and 2010 [35]. If confirmed in other studies, the observed reduced incidence of drug-resistant virus has important implications regarding continued use of suboptimal combination regimens in some settings. From a public health perspective, a significant predicted outcome of increased use of TDF/FTC versus zidovudine or stavudine and 3TC [36] is that the transmission of drug-resistant HIV should decrease as a result of lower prevalence of resistant virus in the population. A reduction in the prevalence of K65R was observed in one study following a shift away from non-recommended NRTI combinations to TDF plus FTC or 3TC [37].

Our study has several potential drawbacks, the most important of which is that the treatment histories of the patients from whom the viral sequences are derived are unknown. In addition, treatment guidelines were updated to include a recommendation to obtain resistance information for previously untreated patients being considered for initiation of HAART during the time period covered in this study. It is therefore expected that an increase in resistance testing requests from untreated patients could result in ‘dilution’ of resistant virus prevalence. However by examining only samples with mutations that are NNRTI-selected, without PI RAMs or extensive accumulation of TAMs, we have enriched the dataset for samples from patients failing NNRTI/ NRTI-based regimens for a relatively short period of time. Although samples could be included in this filter that were obtained from patients infected with a drug resistant virus (transmitted drug resistance), the observation that NNRTI RAMs and M184V prevalence still decreased when samples submitted for genotyping only were excluded suggests that the effect of treatment-naive samples on the mutational trends observed is minimal.

Another limitation of this study is that only indirect comparisons are possible between mutation prevalence and prescription trends. Nonetheless, the apparent correlation between increased use of FTC compared to 3TC and increased use of FDC and single tablet regimens with the decrease in prevalence of M184V/I is notable and deserves further examination in controlled studies.

Figure 4. Reverse transcriptase inhibitor prescription trends

(A) Individual nucleoside/nucleotide reverse transcriptase inhibitor prescriptions for tenofovir disoproxil fumarate (TDF), didanosine (ddI), abacavir (ABC), zidovudine (ZDV), emtricitabine (FTC), lamivudine (3TC), stavudine (d4T), nevirapine (NVP) and efavirenz (EFV). (B) Proportions of FTC, 3TC, TDF, ABC and EFV prescribed in a fixed-dose combination.
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Disclosure statement

MDM, CS, CG, DJM and BG are employees and stockholders of Gilead Sciences, Inc., which manufactures TDF and FTC. MH is an employee and stockholder of Monogram Biosciences, Inc., which performed the resistance testing.

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