Improved Virological Outcomes in British Columbia Concomitant with Decreasing Incidence of HIV Type 1 Drug Resistance Detection


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Background. There have been limited studies evaluating temporal changes in the incidence of detection of drug resistance among human immunodeficiency virus type 1 (HIV-1) isolates and concomitant changes in plasma HIV load for treated individuals in a population-wide setting.

Methods. Longitudinal plasma viral load and genotypic resistance data were obtained from patients receiving antiretroviral therapy from the British Columbia Drug Treatment Program from July 1996 through December 2008. A total of 24,652 resistance tests were available from 5422 individuals. The incidence of successful plasma viral load suppression and of resistance to each of 3 antiretroviral categories (nucleoside/nucleotide reverse-transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, and protease inhibitors) was calculated for the population receiving therapy.

Results. There has been a drastic decrease in the incidence of new cases of HIV-1 drug resistance in individuals followed during 1996–2008. In 1997, the incidence rate of any newly detected resistance was 1.73 cases per 100 person-months of therapy, and by 2008, the incidence rate had decreased >12-fold, to 0.13 cases per 100 person-months of therapy. This decrease in the incidence of resistance has occurred at an exponential rate, with half-times on the order of 2–3 years. Concomitantly, the proportion of individuals with plasma viral load suppression has increased linearly over time (from 64.7% with HIV RNA levels <50 copies/mL in 2000 to 87.0% in 2008; \( R^2 = 0.97; P < .001 \)).

Conclusions. Our results suggest an increasing effectiveness of highly active antiretroviral therapy at the population level. The vast majority of treated patients in British Columbia now have either suppressed plasma viral load or drug-susceptible HIV-1, according to their most recent test results.

Since the introduction of combination drug regimens to treat human immunodeficiency virus (HIV) infection, known as highly active antiretroviral therapy (HAART), the rates of HIV-related morbidity and mortality have been markedly reduced [1, 2]. Over time, there has been an increased variety and availability of antiretroviral drugs. However, suboptimal therapy response can lead to the development of antiretroviral drug resistance, which is a significant barrier to the future success of therapy. Virological failure while receiving HAART is dependent on several factors, including drug toxicity, insufficient adherence to therapy, and problems with drug pharmacokinetics.

The prevalence of drug resistance can be defined as the amount of drug resistance present in an investigated population in a given period of time. Several cross-sectional studies have investigated the prevalence of HIV drug resistance in relatively small populations [3–5], as well as in larger populations [6–8]. Some of these studies have investigated the prevalence of transmitted resistance, whereas others have addressed the prevalence of acquired drug resistance among patients receiving treatment. Although studies that determine prevalence are important for assessing the current impact of HIV drug...
resistance, a critical indicator of the future success of HAART is the incidence of resistance over time while patients receive therapy, which remains relatively poorly defined. The incidence of drug resistance is defined as the number of new cases of drug resistance that develop in a population at risk for developing drug resistance during a given period of time. Thus far, one of the few studies that has investigated HIV drug resistance incidence on the scale of a large treatment population was done in Portugal and showed that the crude incidence of both multidrug resistance and full-drug-class resistance has decreased over time [9]. We undertook this study to evaluate the incidence rate of HIV drug resistance after initiation of therapy, reflecting the entire population of individuals being treated for HIV-1 infection in British Columbia from July 1996 through December 2008.

METHODS

HIV/AIDS drug treatment program. The British Columbia Centre for Excellence in HIV/AIDS distributes antiretroviral agents at no cost to all eligible HIV-infected individuals through its drug treatment program. This program has been described in detail elsewhere [10]. The center’s HIV/AIDS drug treatment program has received ethical approval from the University of British Columbia Ethics Review Committee at its St. Paul’s Hospital site.

Study population. Longitudinal plasma viral load (pVL) and genotypic resistance data were obtained from all archived plasma samples from patients receiving antiretroviral therapy from the drug treatment program from July 1996 through December 2008 (8016 patients), regardless of when they initiated therapy and whether they started receiving mono- or dual-therapy regimens or HAART. A total of 7730 patients (96%) had at least 1 pVL measurement. In total, 24,652 resistance tests were available from 5422 (70%) of the 7730 individuals. The prevalence of successful pVL suppression and the incidence rate of detection of resistance to each of 3 antiretroviral categories—nucleoside/nucleotide reverse-transcriptase inhibitors (NRTIs), nonnucleoside reverse-transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs)—were calculated for the population receiving therapy. The incidence rates of the most common individual drug-resistance mutations in each class were also investigated. These mutations were V82A/F/T/S and L90M in the protease, associated with PI resistance; M41L, M184V/I, and T215F/Y in reverse transcriptase, associated with resistance to the NRTIs; and K103N and Y181C/I in reverse transcriptase, associated with NNRTI resistance [11]. For all incidence calculations, individuals were included in the denominator (ie, they were considered at risk for developing resistance) until the first detection of resistance of interest. From this point, subjects were considered resistant to that drug class until the end of follow-up but still at risk for developing additional mutations and resistance to other antiretroviral classes. Time at risk, in a given year, was defined in terms of total person-months of exposure to each of the antiretroviral categories investigated, with the exception of 1996, for which all available time receiving antiretrovirals before the end of 1996 was used in the incidence calculation for that year. Duration of therapy was recorded from 1992 onward; therefore, time before 1992 was not available. A total of 338 (4%) of the 8016 patients have missing data for time receiving therapy before 1992. Detection of resistance was considered only from July 1996, when samples became available for resistance testing. Therefore, the incidence rates calculated for the year 1996 include the cumulative number of resistance cases developed and the cumulative available exposure time from 1992 to the end of 1996.

Data collection. HIV-infected individuals receiving antiretroviral treatment in British Columbia are entered into an Oracle-based monitoring and evaluation system that uses standardized indicators to prospectively track their antiretroviral use and clinical health status [12]. The treatment guidelines of the British Columbia Centre for Excellence in HIV/AIDS recommend that pVLs and CD4 cell counts be monitored at baseline, at 4 weeks after initiation of antiretroviral therapy, and every 3 months thereafter. pVLs are determined with the Roche Amplicor Monitor assay (Roche Diagnostics); the standard method was used until April 1999, and the ultrasensitive adaptation was used thereafter.

Resistance testing was performed on stored plasma samples extracted manually or automatically using guanidinium-based buffer, followed by ethanol washes. Genotyping does not yield consistently successful results for samples with low pVL; therefore, samples with pVL <50 copies/mL could not be genotyped, and those with pVL ≥50 copies/mL and <250 copies/mL were rarely genotyped. Protease and reverse transcriptase genes were amplified from plasma HIV-1 RNA using nested reverse-transcriptase polymerase chain reaction (PCR) as described elsewhere [13]. PCR products were sequenced in both the 5’ and 3’ directions using an ABI automated sequencer, and a consensus sequence was generated. Results of the genotyping analysis are reported here as amino acid changes in the HIV-1 protease and reverse transcriptase, with respect to a wild-type reference sequence (HIV-1 HXB2). Samples were considered resistant if they displayed ≥1 major resistance mutation for any of the 3 categories: NRTI, NNRTI, or PI. These resistance categories are based on the key resistance mutations from the International AIDS Society–USA table [14].

Of note, a specific group of patients in our study had samples systematically genotyped more frequently than would have occurred as a result of clinical indications alone. These individuals were members of the HAART Observational Medical Evaluation and Research cohort who were antiretroviral naive before beginning a HAART regimen between 1 August 1996 and
Table 1. Annual Incidence of Drug Resistance and Corresponding Patient Exposure to Any Antiretroviral Drug and to Nucleoside Reverse-Transcriptase Inhibitors (NRTIs), Nonnucleoside Reverse-Transcriptase Inhibitors (NNRTIs), and Protease Inhibitors Individually

<table>
<thead>
<tr>
<th>Year</th>
<th>Any antiretroviral drug</th>
<th>NRTIs</th>
<th>NNRTIs</th>
<th>Protease inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of incident cases</td>
<td>Person-months of exposure</td>
<td>Cumulative person-months of exposure</td>
<td>No. of incident cases</td>
</tr>
<tr>
<td>1996</td>
<td>571</td>
<td>39,188</td>
<td>39,188</td>
<td>564</td>
</tr>
<tr>
<td>1997</td>
<td>418</td>
<td>24,224</td>
<td>63,412</td>
<td>399</td>
</tr>
<tr>
<td>1998</td>
<td>299</td>
<td>28,177</td>
<td>91,589</td>
<td>265</td>
</tr>
<tr>
<td>1999</td>
<td>366</td>
<td>29,801</td>
<td>121,390</td>
<td>322</td>
</tr>
<tr>
<td>2000</td>
<td>270</td>
<td>30,309</td>
<td>151,699</td>
<td>244</td>
</tr>
<tr>
<td>2001</td>
<td>184</td>
<td>30,446</td>
<td>182,145</td>
<td>153</td>
</tr>
<tr>
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<td>170</td>
<td>30,621</td>
<td>212,966</td>
<td>150</td>
</tr>
<tr>
<td>2003</td>
<td>123</td>
<td>31,911</td>
<td>244,877</td>
<td>109</td>
</tr>
<tr>
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<td>2007</td>
<td>82</td>
<td>47,495</td>
<td>409,149</td>
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</tr>
<tr>
<td>2008</td>
<td>71</td>
<td>52,638</td>
<td>461,787</td>
<td>51</td>
</tr>
</tbody>
</table>

30 September 1999. More-detailed descriptions of this study population have been published elsewhere [10, 15]. For this particular subset of patients, HIV drug resistance genotyping was systematically attempted for all plasma samples with pVL \( \geq 1000 \) copies/mL that were collected in the first 30 months of treatment.

We conducted 2 sensitivity analyses, the first of which addressed the impact of the time of therapy initiation on the incidence rate. For this sensitivity analysis, we repeated the original analysis, stratifying patients by the date on which they began antiretroviral therapy into the following groups: 1987–1995, 1996–1999, 2000–2004, and 2005–2008. We also conducted a sensitivity analysis to assess the impact of patients with no baseline (pretherapy) genotypes, and the potential transmitted resistance they may harbor, on our calculation of the incidence rate. For this sensitivity analysis, we repeated the original analysis using only those individuals who had an available baseline genotype.

Outcome measures. The primary outcome in this analysis was emergence of drug resistance to any of the 3 categories described above (yes vs. no) and the investigated variable while receiving therapy was pVL (log10 transformed). In 1996, the lower limit of the viral load assay was changed from 500 to 400 copies/mL. In April 1999, the lower limit of the viral load assay changed again from 400 to 50 copies/mL, with the introduction of the ultrasensitive adaptation. Because pVL was measured over time (starting in 1996), our earliest pVLs were obtained on the basis of the standard pVL assay, and our most recent measurements were obtained on the basis of the ultrasensitive pVL assay. Thus, our lower and upper limits of pVL ranged over time, from 500 and \( 1 \times 10^4 \) copies/mL, to 400 and \( 7.5 \times 10^3 \) copies/mL, and then to 50 and \( 1 \times 10^3 \) copies/mL, respectively.

Statistical analyses. Calculations of the incidence rate of resistance detection in each antiretroviral category were conducted using the number of new cases of resistance detected in each year (ie, excluding previously identified resistance) divided by the number of person-months of exposure to antiretroviral drugs in each category. Incidence rates were natural-log transformed and were plotted against calendar year. The proportion of individuals achieving a pVL <50 copies/mL within a given year was calculated from 2000 to 2008. Trend analyses were conducted using linear or log-linear regressions and were reported in order to estimate the slope of any change in the incidence rate. \( R^2 \) values were generated for the regressions.

RESULTS

The numbers of patients with newly detected resistance suggest that there have been significant decreases in the occurrence of new HIV-1 drug resistance during the period 1996–2008 (Table 1). The number of incident cases of new resistance to any category of antiretrovirals was 571 in 1996, considerably more than the 71 cases detected in 2008, despite increased exposure to antiretroviral therapy in 2008 and a cumulative exposure of 461,787 person-months of therapy (Table 1). These results were broadly similar across all the drug classes after the year 2000.
Note that resistance to both the PI and NNRTI classes showed an initial increase in the absolute number of cases soon after their introduction in 1996 (Table 1), but the number has subsequently decreased. As an illustration, only 14 new cases of PI resistance were detected in the entire province of British Columbia in 2008, despite >30,000 person-months of PI therapy in 2008 and a cumulative total of >269,000 person-months of PI therapy by the end of 2008.

When expressed as the incidence rate per 100 person-months of therapy for resistance to any drug category, there is an exponential decrease in resistance incidence since 1996, with a half-time (the time it takes for the incidence rate to decrease to half its original value) of ~3.2 years ($R^2 = 0.98; P < .001$) (Figure 1A). Overall, the incidence rate decreased ~12-fold, from 1.73 cases per 100 person-months in 1997 (the first year with complete exposure time and incidence data available) to 0.13 cases per 100 person-months in 2008. This trend toward exponential decrease was consistent across the drug classes (Figure 1B). The incidence rate of resistance to the NRTIs decreased exponentially, with a half-time of 2.9 years ($R^2 = 0.98$), whereas the half-time for the PI class was 2.0 years ($R^2 = 0.98$) (Figure 1B). The incidence of NNRTI resistance per 100 person-months of therapy has been consistently higher than that for the other drug classes and has been decreasing with a half-time of 2.5 years ($R^2 = 0.94$) (Figure 1B). The incidence of PI resistance is now the lowest of the 3 classes (Figure 1B).

When the commonly occurring major NRTI resistance mutations were investigated individually, each showed broadly similar decreasing trends over time (Figure 2A). M41L and T215F/Y are the most commonly occurring thymidine analog-associated mutations, whereas M184V/I mutations are associated with resistance to lamivudine/emtricitabine and are the most common NRTI mutations. The incidence of M41L and T215F/Y reverse-transcriptase mutations decreased with a half-time of 2.0 years and 1.8 years, respectively. The incidence of M184V/I mutations decreased more slowly in comparison (half-time, 2.8 years; $R^2 = 0.96$). The incidence of the NNRTI mutations K103N and Y181C/I decreased with a half-time of 2.9 years and 2.4 years, respectively ($R^2 = 0.96$ for both mutations), whereas the incidence of the PI resistance mutations V82A/F/
Figure 2. Annual incidence rate of drug resistance detected for the most commonly occurring mutations in human immunodeficiency virus type 1 (HIV-1) protease and reverse transcriptase, as a function of calendar year. A, Incidence rate for the major nucleoside reverse-transcriptase inhibitor (NRTI) resistance mutations M41L, M184V/I, and T215F/Y. B, Incidence rate for the major nonnucleoside reverse-transcriptase inhibitor (NNRTI) resistance mutations K103N and Y181C/I. C, Incidence rate for the major protease inhibitor (PI) resistance mutations V82A/F/T/S and L90M. Incidence rates are natural-log (ln) transformed. Dashed and solid lines represent corresponding linear regressions. The number of people exposed to each therapy, by calendar year, is indicated below the graphs.

T/S and L90M decreased with a half-time of 1.9 years ($R^2 = 0.86$) and 1.7 years ($R^2 = 0.96$), respectively. Note that, although the incidence of the most common mutations is decreasing, it is unlikely that rates of all individual mutations are decreasing. Some may even be increasing, as different therapies are introduced. For example, the incidence of the reverse-transcriptase mutation K65R has not shown a clear trend toward decreasing incidence during the period 1996–2008 (data not shown).

The decreases in the incidence of resistance can be partially explained by higher rates of virological suppression over time. The lowest (Figure 3A) and highest (Figure 3B) recorded median pVLs for each patient receiving therapy in British Columbia have decreased dramatically since 1996. In 1996, the median lowest pVL recorded was $3.76 \log_{10}$ copies/mL, and by 2007, it was below the lower limits of detection for the assay (<50 copies/mL). The range of values recorded for the lowest pVL has also decreased over time, as indicated by the shrinking interquartile range (Figure 3A). There has been an approximately linear increase in the proportion of individuals with pVL suppression over time to below the limit of detection of the viral load assay (from 64.7% with pVL <50 copies/mL in 2000 to 87.0% in 2007; $R^2 = 0.97; P < 0.001$) (Figure 3C).

When stratified by time of therapy initiation, the trend of an exponentially decreasing incidence rate is confirmed (Figure 4). This is consistent across all 4 periods of therapy initiation and all drug classes investigated. There is a temporal trend toward a faster decrease in incidence rate by the period of therapy initiation (see slopes of regression lines for 2000–2004 and 2005–2008 vs. those for 1987–1995 and 1996–1999 in Figure 4). Also, there have been smaller trends toward improvement even within the first year of therapy; the first 2 incidence rates for initiation of therapy in 2000–2004 and 2005–2008 are lower than the first 2 rates for initiation in 1987–1995 and 1996–1999. Finally, we also performed a sensitivity analysis, restricting the analysis to individuals with available pretherapy genotypes (2571 patients), and we confirmed the decreasing incidence to any category of resistance over time (95% confidence interval).

DISCUSSION

Based on a provincewide cohort of individuals followed longitudinally, our results demonstrate that there has been a drastic decrease in the incidence of new cases of HIV-1 drug resistance, despite increases in annual (and, especially, cumulative) exposure to antiretrovirals. This has occurred alongside a steady increase in the proportion of treated patients achieving virological suppression. Remarkably, the incidence of resistance per person-month of therapy appears to decrease with increasing duration of therapy (Figure 4). This is consistent across all drug classes and years of initiation. Improvements over time in HAART, including the periodic introduction of new therapeutic agents and the continual assessment of and improvement in how
those agents are prescribed, have most likely contributed to decreases in the incidence rate of detection of HIV-1 drug resistance. Before 1996, drugs were prescribed as monotherapy or dual-therapy combinations, which resulted in the rapid selection of drug resistance. Initial increases in the incident cases of drug resistance to NNRTIs and PIs are most likely linked to patients previously exposed to mono- or dual therapy who had developed NRTI resistance and were therefore more likely to develop additional resistance because of compromised HAART regimens (Table 1). The relative “fragility” of the NNRTI class likely accounts for both the higher incidence of NNRTI resistance per person-month of therapy and the slower decrease in the rate of selection of NNRTI-resistant virus. However, even for this drug class, the decrease in resistance incidence has been remarkable, with a >40-fold decrease in NNRTI resistance per 100 person-months of NNRTI exposure from 1996 to 2008.

Our results complement those found by Vercauteren et al [8], which is most likely a reflection of both studies being performed using large populations with free access to HAART. However, because we had detailed information about each patient’s antiretroviral regimen and duration of therapy, we were able to analyze the incidence in terms of rate per person-months of drug exposure. Particular strengths of this analysis are its size and the ability to monitor all HIV-infected patients receiving treatment in an entire population. The centralized system allows monitoring of both the amounts of therapy dispensed and the laboratory measures of viral load and resistance. In addition, resistance testing is widely used in British Columbia, with almost 25,000 resistance tests performed for 5422 individuals.

Although the data shown are encouraging, there are limitations. This analysis is observational and, therefore, cannot definitively establish a causal relationship between reductions in the incidence of resistance detection and concomitant decreases in pVL. Another limitation is that not every person receiving therapy is monitored clinically in exactly the same way (eg, some will be missing baseline resistance data and some will be monitored more heavily than others). The frequency of genotyping because of clinical indications varies between individual patients in the population and depends on several factors, including the date of therapy initiation, differences between physicians in the number of tests requested, and whether a particular patient sample has a pVL that is high enough to genotype. These factors could contribute to producing a detection bias. Approximately 50% of patients with at least 1 viral load measurement ≥250 copies/mL have been tested for resistance each year since 1996 (Figure 1). However, practices in genotyping have changed over time, such that genotyping is now performed before the initiation of antiretroviral therapy. This could lead to a conservative bias when the incidence of resistance is measured over time, because patients who initiated

Figure 3. Distribution of the lowest (A) and highest (B) plasma viral loads (pVLs) for human immunodeficiency virus type 1 (HIV-1) and the percentage of individuals achieving a plasma HIV-1 RNA level below the limit of detection of the RNA assay (C), for all patients in British Columbia. The box plot includes the median (solid horizontal bar), interquartile range (box), and the lower of 1.5 times the interquartile range or the most extreme value (dashed line). The number of patients who received therapy within a given year and had an available pVL test is indicated above the bars. These data include patients who recently started antiretrovirals. Note that the reporting of pVL values is capped at the limits of the pVL assay. The lower limit of the viral load assay was changed in 1999. Therefore, the newer assay would report lower values for the lower quartile and 1.5 times the interquartile range for pVL values in 1996–1998. Except for 1998, median values could not be affected, but interquartile range values could be affected. For the percentage of individuals achieving a plasma HIV-1 RNA level below the limit of detection, data are shown from the year 2000 onward because of the decrease of the lower limit of the viral load assay from 500 to 50 copies/mL in April 1999. Annual percentages were based upon the lowest available pVL from individuals, regardless of whether they were receiving therapy at the time of testing.
therapy in later calendar years would be more likely to have transmitted resistance detected. It should be noted that this analysis shows trends in the detection of resistance, and the results are therefore dependent on the frequency of genotyping and cannot tell us about the development of resistance below the pVL limits of genotype testing. However, such low viral loads suggest that any resistance that is evolving may have little clinical significance. Although there are missing exposure data for 338 individuals (4%) who were receiving therapy before 1992, these missing data only affect the incidence rates calculated for 1996, resulting in a probable slight overestimate of the cumulative rates reported for that year.

These results provide a benchmark for monitoring HIV treatment programs. Efforts to improve accessibility to HAART have the potential to greatly reduce HIV-1 levels in populations without increasing the risk of drug resistance. If current trends persist,
the continued improvement of HAART and the increased availability of new drugs could potentially make the development of new HIV drug resistance a rare event.

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