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Prevalence of Primary Drug Resistance Against HIV-1 Integrase Inhibitors in Canada

To the Editors:

An estimated 36.9 million people are living with HIV-1 worldwide,¹ of which there are approximately 75,500 infected people in Canada.² Although varied prevention strategies have been successful in reducing the HIV-1 incidence,³ over 2000 new infections occur each year in Canada.² Typical antiretroviral therapy (ART) regimens primarily include HIV-1 protease inhibitors (PIs) and reverse transcriptase inhibitors (RTIs). However, drug resistance (DR)-associated mutations (DRMs) have been identified against all existing PIs and RTIs; thus, new drugs are continually required to ensure the efficacy of ART regimens. Over the past decade, considerable effort has been directed toward the development of compounds targeting HIV-1 integrase, an essential HIV-1 enzyme that interrupts HIV-1 replication by preventing proviral DNA from integrating into the host genome. The first HIV-1 integrase inhibitor (INI) approved in Canada was raltegravir (RAL) in 2007. Since then, 2 other INIs have been approved: elvitegravir (EVG) in 2012 followed by dolutegravir (DTG) in 2013. These drugs are currently used in ART individually as: Isentress (Merck), Tivicay (ViiV Healthcare), and Vitekta (Gilead), or in combination with other ART drugs: Stribild (Gilead)

or Triumeq (ViiV Healthcare).^{4–8} With excellent antiviral potency and safety profile, INIs have now been recommended as first-line ART options for both treatment-experienced patients failing conventional ART, and ART-naive patients in both high-income and low-income countries.^{9–11}

Although INIs are generally well tolerated and effective against HIV-1 variants resistant to PIs and RTIs,^{12–14} DRMs have been detected against INIs.^{15,16} Significantly, many INI DRMs may lead to cross-resistance against 2 or more INIs targeting similar integrase pathways.^{16,17} The Stanford HIV Drug Resistance Database (HIVdb) (<http://hivdb.stanford.edu>) classifies INI DRMs into 3 groups (major, accessory, and other mutations) based on their associations with decreased drug susceptibility. Major mutations are nonpolymorphic mutations that by themselves contribute to reduced susceptibility to 1 or more INIs. Accessory mutations are nonpolymorphic or polymorphic mutations that reduce susceptibility in combination with major DRMs. Other mutations are nonpolymorphic or polymorphic mutations shown to be selected for under INI therapy, but may or may not effectively reduce susceptibility. In Canada, transmitted HIV DR (TDR) is monitored at national level through the Canadian HIV Strain and Drug Resistance Surveillance Program (CHSDRSP), which examines PI and RTI resistance in residual diagnostic specimens from participating public health laboratories across Canada.¹⁸ From 1999 to 2008, the CHSDRSP found an overall 9.8% TDR prevalence against PIs and RTIs among participating provinces.¹⁹ However, prevalence of INI TDR in Canada has never been assessed. Using 587 CHSDRSP specimens collected from ART-naive patients with HIV during 2007–2013, we examined the INI TDR prevalence in 4 Western Canadian provinces, including British Columbia, Alberta, Saskatchewan, and Manitoba. To determine the baseline HIV-1 integrase gene polymorphism before INI introduction, 435 pre-INI specimens collected from 2002 to 2006 were also examined. All samples were randomly selected representing

approximately 20% of CHSDRSP specimens from each sampling year from all 4 provinces combined.

Conventional genotypic DR testing was performed on the HIV-1 integrase genes of all examined specimens. In brief, total nucleic acid was first extracted from patient plasma using NucliSENS easyMag system (BioMerieux, Canada) following manufacturer's instructions. Full length HIV-1 integrase gene was then reverse transcribed and amplified using Superscript III one-step system (ThermoFisher, Canada). The RT-polymerase chain reaction (PCR) primers included Out-F: 5'-CACAYAARG-GRATTGGAGGAAATG-3' and Out-R: 5'-TARTGGRATGTGTACTTCTGAAC-3'. The resulting amplicon was then amplified with nested PCR using primers Nest-F: 5'-AACARGTAGATAAAT-TAGTHAGT-3' and Nest-R: 5'-ATACATATGRTGYTTTACTARACT-3'. The derived 944bp amplicons were then purified, quantified, and subject to sequencing PCR using ABI BigDye system (ThermoFisher, Canada). The sequencing PCR primers included both nested PCR primers and Seq-F1 (5'-TACAATCCCCAAAGTCARG-GAG-3') and Seq-R1 (5'-AY-TATTCTTTCCCCTGCACTGT-3'). The HIV-1 integrase sequences were then bidirectionally resolved using Genetic Analyzer 3730 (ABI Foster City, CA). The derived sequences were assembled using RECall 2.0 and the Stanford HIV-1 Genotypic Resistance Interpretation Algorithm (<http://hivdb.stanford.edu>) was used to identify HIV-1 INI DRMs and subtypes.

Among all 1022 specimens, the HIV-1 subtypes included B(848, 83.0%), C(116, 11.4%), CRF01_AE (26, 2.5%), A(17, 1.7%), CRF02_AG (9, 0.9%), F2(3, 0.3%), D(2, 0.2%), and G(1, 0.1%). No significant difference was observed between pre-INI (2002–2007) and the INI (2007 and after) eras concerning HIV-1 subtype distributions. Eighty-two (8.0%) samples contained INI DRMs, of which only 1 contained >1 DRMs (L68V and L74M). The only major DRM identified was S147G from 1 subtype C specimen collected in 2008. S147G is a nonpolymorphic mutation

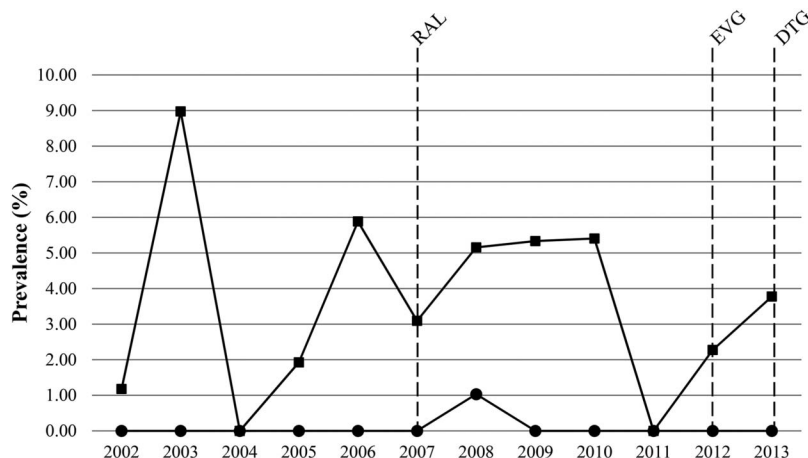
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FIGURE 1. HIV-1 INI DRM prevalence in ART-naive patients from 2002 to 2013. The prevalence of major (circle) and accessory (square) INI DRMs is represented for each year sampled. Graph is overlaid with Canadian approval dates of each INI. DTG, dolutegravir; EVG, elvitegravir; RAL, raltegravir.



resulting in reduced EVG susceptibility. Notably, EVG was not approved in Canada until 2012 and the patient self-declared as ART-naive, suggestive of possible acquisition of this DRM from patient receiving EVG in a clinical trial.

Accessory mutations were found at a prevalence of 0.1%–1.2%, including L74M(9, 0.9%), T97A(12, 1.2%), E138K(8, 0.8%), V151L(1, 0.1%), S153Y/F(3, 0.3%), and R263K(5, 0.5%). Similarly, other mutations were identified at a prevalence of 0.2%–1.3%, including V54I(8, 0.8%), L68V(6, 0.6%), Q95K(3, 0.3%), A128T(2, 0.2%), V151I(13, 1.3%), and E157Q(12, 1.2%). The overall prevalence of all major and accessory INI DRMs during 2002–2013 is shown in Figure 1. No temporal trend was observed for any of these mutations over time. Despite the minor discrepancies between the INI DRM lists from Stanford HIVdb and IAS-USA, the overall DRM prevalence remains the same when IAS-USA list was applied in this specific study because none of the discrepant DRMs were present in this cohort.

Of all 82 DRMs, 61 (74%) were polymorphic mutations (V54I, L68V, L74M, T97A, V151I, and E157Q) and 21 (26%) were nonpolymorphic mutations or very rare polymorphisms (Q95K, A128T, E138K, V151L, S153Y/F, R263K, and S147G) with subtype B representing >80% of samples in each group. Notably, V151L is an extremely rare nonpolymorphic mutation selected *in vitro* by early investigational INIs. Despite some inconsistency in its DRM status definition in HIVdb overtime and its absence

in IAS-USA list, V151L is currently listed in current HIVdb (last updated June 16, 2017) as accessory DRM and, therefore, it is categorized accordingly here. Except for L74M, no association could be inferred between specific HIV-1 subtypes and INI DRM, as there were too few non-B subtypes to determine whether subtype B selects certain integrase mutations. By contrast, L74M trends to be more prevalent in non-B subtypes ($P = 0.051$).

Of the 0.5% of specimens harboring R263K mutations, all were infected by HIV-1 subtype B. Although classified as an accessory DRM, R263K has been shown to confer low-level resistance against DTG.²⁰ If found together with E157Q, this mutation may increase DTG resistance because it may be a compensatory mutation to partially restore enzymatic activity and infectivity lost with the R263K mutation.²¹ E157Q was found in 12 (1.2%) samples; however, none of them contained R263K. DTG was approved in Canada in 2013; therefore, the observed R263K is unlikely a result from DTG application but rather reflects naturally occurring HIV-1 polymorphisms. In particular, R263K has been reported at a prevalence rate of 4% in HIV-1 CRF-02-infected patients in a small sub-Saharan cohort.²² R263K was not detected in any non-B specimens in our study. A recent study of treatment-experienced patients in British Columbia observed emergence of INI DRMs, including both major and accessory DRMs such as R263K, after the introduction of each INI, suggesting that acquired INI resistance may be an emerging phenomenon.²³

Nonetheless, our results suggest that close monitoring of R263K mutation is necessary given the current treatment regimens.

Further examination on the coexistence of INI DRMs with those RTI and PI DRMs revealed that the only S147G positive subject was also had 2 RTI mutations (M184V and G190A). Among all subjects with accessory INI DRMs, 1 E138K positive subject also had K103N against RTI and 1 L74M positive specimen also had M46I against PI and T215I against RTI, with too few specimens having DRMs in multiple HIV-1 genes. No potential linkage was detected among the identified DRMs in these genes.

INIs are becoming an integral part of ART, which warrants a baseline INI TDR survey in ART-naive subjects.^{24,25} Fortunately, major INI DRM transmission remained rare in Canada during 2007–2013 with only S147G identified in a single specimen from 2008. This finding is similar to those found in studies from the United States²⁶ and Europe²⁷ where INI DRMs were rarely, if ever, identified in ART-naive patients. As this study was based only on approximately 20% of the total samples collected from 4 Western Canada provinces, it is possible that overall INI TDR here are not fully representative of the greater HIV population in Canada. In addition, conventional Sanger sequencing was performed on these samples, which is able to reliably detect nucleotide variants present at >20% of the viral population, therefore, we cannot comment on transmitted INI DRMs at lower abundance in these samples.

In conclusion, the prevalence of transmitted HIV-1 INI resistance during 2007–2013 remained low, and major INI DRMs were rare in Western Canada. No identifiable trend was observed for any recognized mutations. However, INI TDR prevalence may increase in the coming years with increased availability and clinical usage of INIs, particularly DTG. Although interpretation of HIV-1 resistance profile is critical to guide ART regimen selection for optimal patient care, continued INI TDR surveillance is essential to generate data on potentially evolving patterns of resistance against INI in Canada.

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