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MAJOR ARTICLE

Prevalence of Human Immunodeficiency Virus-1 Integrase Strand Transfer Inhibitor Resistance in British Columbia, Canada Between 2009 and 2016: A Longitudinal Analysis

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Background. Integrase strand transfer inhibitors (INSTIs) are highly efficacious and well tolerated antiretrovirals with fewer adverse side-effects relative to other classes of antiretrovirals. The use of INSTIs raltegravir, elvitegravir, and dolutegravir has increased dramatically over recent years. However, there is limited information about the evolution and prevalence of INSTI resistance mutations in clinical human immunodeficiency virus populations.

Methods. Human immunodeficiency virus-1-positive individuals ≥19 years were included if they received ≥1 dispensed prescription of antiretroviral therapy (ART) in British Columbia between 2009 and 2016 (N = 9358). Physician-ordered drug resistance tests were analyzed and protease inhibitor (PI), reverse-transcriptase inhibitor (RT), and INSTI resistance were defined as having ≥1 sample with a combined, cumulative score ≥30 by Stanford HIV Drug Resistance Algorithm version 7.0.1.

Results. Although most ART-treated individuals were tested for PI and RT resistance, INSTI resistance testing lagged behind the uptake of INSTIs among INSTI-treated individuals (11% in 2009; 34% in 2016). The prevalence of INSTI resistance was relatively low, but it increased from 1 to 7 per 1000 ART-treated individuals between 2009 and 2016 ($P < .0001$, $R^2 = 0.98$). Integrase strand transfer inhibitor resistance mutations increased at integrase codons 66, 97, 140, 148, 155, and 263.

Conclusions. The prevalence of INSTI resistance remains low compared with PI and RT resistance in ART-treated populations but is expanding with increased INSTI use.

Keywords. dolutegravir; drug resistance; elvitegravir; HIV integrase strand transfer inhibitor; raltegravir.

Integrase strand transfer inhibitors (INSTIs) are effective and well tolerated antiretrovirals (ARVs) [1, 2]. With increasing prevalence of pretreatment resistance to nonnucleoside/nonnucleotide reverse-transcriptase inhibitors (NNRTIs) [3], INSTIs are increasingly recommended in first-line and alternative first-line regimens [4–7]. Integrase strand transfer inhibitor-containing therapy also provides an alternative treatment option for individuals experiencing multidrug resistance or adverse reactions to other human immunodeficiency virus (HIV) ARVs [1, 4]. However, INSTI resistance remains a barrier to the ongoing success of HIV treatment [8]. Although ARVs effectively suppress HIV replication enabling immune restitution, selection of resistance-conferring mutations in the presence of ARVs is associated with lack of viral suppression, treatment failure, and increased likelihood of HIV transmission [8–10]. Furthermore, long-term persistence of drug resistance mutations has previously been reported and can limit regimen options [11]. Although pretreatment protease inhibitor (PI) and reverse-transcriptase (RT) inhibitor resistance testing is the current standard of care in British Columbia (BC) [12, 13], standard clinical guidelines currently do not recommend testing for INSTI resistance at initiation of therapy [14]. Instead, INSTI resistance testing is only recommended in patients who experience virologic failure while on INSTI-containing therapy [4, 13].

There have been previous reports of transmitted and treatment-emergent INSTI resistance in clinical settings [15–18]. However, to better monitor and evaluate the current modalities of INSTI resistance and assess adequacy of INSTI resistance testing, more information is required about the change in yearly prevalence of INSTI resistance and the specific integrase mutations associated with INSTI resistance selected, in large clinical HIV populations.

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In a previous study, we observed low rates of treatment-emergent INSTI resistance (approximately 1 case per 100 person-years INSTI exposure) among individuals registered in BC's Centre for Excellence in HIV/AIDS (BC-CfE) Drug Treatment Program (DTP) who initiated INSTI-containing therapies between 2012 and 2014 [19]. This longitudinal, observational study examines the prevalence of INSTI resistance and the specific INSTI resistance mutations selected in antiretroviral therapy (ART)-treated DTP individuals in each calendar year between 2009 and 2016. For context, the yearly prevalence of PI and RT (PI-RT) resistance as well as the frequency of INSTI and PI-RT resistance testing were also investigated in the same time period.

METHODS

Study Population

Participants within this study were HIV-1-positive individuals living in BC, Canada who received ART through the BC-CfE DTP. The BC-CfE monitors and maintains clinical patient profiles (ART, plasma HIV-1 ribonucleic acid [RNA] viral load, CD4 cell count, etc) and collects sociodemographic data of DTP participants. A patient information sheet describing the potential uses of data for health research is provided at DTP enrollment, and consent is not required for use of anonymized data. The DTP is an open cohort and members can enter and leave at any time, for any duration of time, or leave indefinitely. These programs have been described in detail elsewhere [20, 21]. The University of British Columbia Providence Health Care Research Ethics Board granted ethical approval for the DTP (H05-50123).

For each calendar year from January 1, 2009 to December 31, 2016, participants were counted in the denominator of ART-treated individuals if they were ≥19 years of age and were treated with at least 1 day of ART within that year. Treatment with ART was determined as ≥1 dispensed ART prescription in a calendar year through the BC-CfE's administrative records ART prescription database.

Drug Resistance Testing

Drug resistance tests were ordered by each individual's physician and processed at the BC-CfE research laboratories. However, if an initial HIV-1 RNA viral load level was requested by an individual's physician and the HIV-1 RNA viral load was >250 copies/mL, a drug resistance test was automatically ordered by the BC-CfE and the results were sent to the physician. Each individual in the study contributed cumulative drug resistance data from time of DTP enrollment until December 31, 2016. An individual contributed to the count of "tested for resistance" in the first year that the test was performed, and this "tested" status was automatically carried forward to each subsequent year the individual was treated with ART in the DTP. Viral nucleic acids were extracted from 500 µL plasma using the NucliSENS easyMAG from bioMérieux (Montreal, Canada). The protease, RT, and integrase genes were amplified by "nested" reverse transcription-polymerase chain reaction (PCR) using the Expand High Fidelity PCR system from Roche Diagnostics (Laval, Canada) as described elsewhere [22]. Sanger sequencing was performed bidirectionally using the BigDye Terminator version 3.1 Cycle Sequencing Kit from Applied Biosystems (ABI, Foster City, CA) on an ABI 3730 DNA Sequencer. A consensus sequence was produced, and chromatograms were analyzed by in-house custom software called RECall [23].

Prevalence of Integrase Strand Transfer Inhibitor and Protease Inhibitor and Reverse-Transcriptase Inhibitor Resistance

In each year, an individual contributed to the total count of ART-treated individuals if they were treated with ART through the DTP within that year. Because annual prevalence is calculated separately for each year, deceased and/or lost to follow-up participants are accounted for accordingly. Samples were defined as drug resistant to either INSTI or PI-RT if they contained cumulative mutations that result in intermediate- or high-level resistance to at least 1 ARV in the INSTI or PI-RT drug classes, respectively, as defined by a score ≥30 based on the Stanford University HIV Drug Resistance Database Genotypic Resistance Interpretation Algorithm version 7.0.1 [24]. To account for the potential long-term persistence of drug resistance mutations [11], each ART-treated individual tested for drug resistance was classified as having drug resistance (INSTI or PI-RT resistance) in the first year they had a sample meeting the criteria for resistance, and this resistance status was carried forward to each subsequent year the individual was treated with ART in the DTP. The annual prevalence of INSTI and PI-RT resistance per 1000 ART-treated individuals was calculated at the end of each calendar year.

Integrase Strand Transfer Inhibitor Resistance by Year of First Detection

To estimate the contribution of treatment-emergent INSTI resistance to the prevalence of INSTI resistance over time, new cases of INSTI resistance among ART-treated individuals were identified in each year and counted in the first year they were detected. It is important to note that newly identified cases of INSTI resistance do not correspond to incidence of INSTI resistance during the study period, but rather to the first year an individual who contributed to the count of prevalence had study-defined INSTI resistance during the study period. The drug associated with newly detected INSTI resistance was categorized as the last prescribed INSTI (either raltegravir, elvitegravir, or dolutegravir) before detection of resistance. If the first identified INSTI resistance occurred before the first known INSTI dispensed date in the DTP, the INSTI drug exposure was termed unclassifiable.

Prevalence of Integrase Strand Transfer Inhibitor Resistance Mutations

Each ART-treated individual with newly detected study-defined INSTI resistance was counted once per mutation position in each calender year they received ART in the DTP. Samples were defined as drug resistant to INSTIs if they contained cumulative mutations that result in intermediate- or high-level resistance to at least 1 ARV in the INSTI drug class, as defined by a score ≥30 based on the Stanford University HIV Drug Resistance Database Genotypic Resistance Interpretation Algorithm version 7.0.1 [24]. An individual's INSTI mutation contributed to the mutation count in the first year it was detected and automatically carried forward to all subsequent years the individual received ART in the DTP.

Statistical Analysis

Generalized additive models were used to model the nonlinear trend of the prevalence of PI-RT and INSTI drug resistance [25, 26]. Statistical software R version 3.2.2 was used with the mgcv package [25], assuming a Beta distribution and a logit link. The year or percentage of patients tested for INSTI resistance were the explanatory variables being smoothed. Restricted maximum likelihood was used to estimate the parameters. A cubic regression spline was used to smooth the yearly trend in each of the outcomes.

RESULTS

Characteristics and Yearly Number of Participants

Between January 1, 2009 and December 31, 2016, 9358 unique individuals received ART through the DTP in BC. Patients were predominantly male (83%) infected with HIV-1 subtype B (81%). The median age of participants at the year of first inclusion in the study was 46 years old (25th–75th percentile [Q1–Q3] 38–53) (Table 1). In total, 3645 unique individuals (39%) received INSTI-containing therapy in the DTP during the study period. A patient may have been prescribed more than 1 INSTI during the study period. During this time period, there were 1546 individuals ever treated with raltegravir, 830 individuals ever treated with elvitegravir, and 1856 individuals ever treated with dolutegravir.

Between 2009 and 2016, the number of individuals enrolled and receiving treatment through the DTP each year increased from 5587 to 7772 individuals (39% increase), and the proportion receiving an INSTI increased from 10% to 40% (542 to 3117 individuals) (Supplementary Table 1).

Table 1. Characteristics of Individuals Within the BC Drug Treatment Program Between 2009 and 2016

Variable	N = 9358 n (%)
ART-treated individuals[a]	9358 (100)
INSTI-treated individuals[b]	3645 (39)
INSTI prescribed in the DTP[c]	
Raltegravir	1546 (17)
Elvitegravir	830 (9)
Dolutegravir	1856 (20)
Age, years, median (Q1–Q3)[d]	46 (38–53)
Sex	
Male	7768 (83)
Female	1590 (17)
HIV subtype	
B subtype	7542 (81)
Non-B subtype	586 (6)
Unknown	1230 (13)
Number of years individuals contributed resistance data, median (Q1–Q3)	6 (2–8)
Number of years individuals contributed any type of data, median (Q1–Q3)	7 (4–8)
Number of ART-treated individuals ever tested for PI-RT resistance	7883 (84)
Number of physician-ordered resistance tests done per person, median (Q1–Q3)	2 (1–5)
Number of individuals with a single resistance test only, at some point in time	2647 (28)
Number of individuals with a single resistance test performed at baseline	2109 (23)
Number of INSTI-treated individuals ever tested for INSTI resistance	1244 (13)
Number of physician-ordered resistance tests done per person, median (Q1–Q3)	1 (1–2)
Number of individuals with a single resistance test only, at some point in time	752 (8)
Number of individuals with a single resistance test performed at baseline	482 (5)

Abbreviations: ART, antiretroviral therapy; BC, British Columbia; DTP, Drug Treatment Program; HIV, human immunodeficiency virus; INSTI, integrase strand transfer inhibitor; Q1–Q3, 25th–75th percentile range.

[a]An individual contributed to the count of ART-treated if they were ever dispensed ART in the DTP during the study period.

[b]An individual contributed to the count of INSTI-treated if they were ever dispensed INSTIs in the DTP during the study period.

[c]Median age of individuals at first year of inclusion in the study.

[d]An individual could be prescribed more than 1 INSTI during the study period.

Drug Resistance Testing

During the study period, the number of ART-treated individuals tested for PI-RT resistance increased from 4520 to 6614 individuals (81% to 85%) (Figure 1), and the number of all ART-treated individuals tested for INSTI resistance increased from 188 to 1440 individuals (3% to 19%) ($P < .0001$, $R^2 = 0.99$) (Supplementary Table 1). Among those treated with INSTIs each year, the number of individuals tested for INSTI resistance increased from 60 to 1059 individuals (11% to 34%) ($P < .0001$, $R^2 = 0.97$) (Figure 1).

Prevalence of Resistance in All Antiretroviral Therapy-Treated Individuals

Figure 2 shows the prevalence of PI-RT and INSTI resistance in all ART-treated individuals treated with ART in BC in each calendar year from 2009 to 2016. The prevalence of study-defined PI-RT resistance in all ART-treated individuals declined significantly from 337 per 1000 ART-treated individuals in 2009 to 285 per 1000 ART-treated individuals in 2016 ($P < .0001$, $R^2 = 0.98$) (Figure 2). In contrast, the prevalence of study-defined INSTI resistance was lower than that of PI-RT resistance, but it increased from 1 per 1000 ART-treated individuals in 2009 to 7 per 1000 ART-treated individuals in 2016 ($P < .0001$, $R^2 = 0.98$) (Figure 2).

Integrase Strand Transfer Inhibitor Resistance by Year of First Detection

From 2009 to 2016, 64 unique individuals (69% male) were newly identified as having study-defined INSTI resistance (Table 2). Median age was 47 years old ([Q1–Q3] 40–53), and the majority were infected with HIV-1 subtype B (91%). Among these individuals, 88% (56 of 64) have also had mutations conferring PI-RT resistance. The observed number of newly identified cases of INSTI resistance ranged from 4 to 15 new cases per year during the study period, and it remained relatively stable at 6–9 cases per year after peaking in 2011 (Table 2). Patient characteristics of a subset of these 64 individuals have been previously described in detail elsewhere [19]. During the study period, there was an apparent shift from raltegravir-containing regimens to elvitegravir- and dolutegravir-containing regimens (Supplementary Figure 1) and until 2014, most new cases of INSTI resistance were associated with the use of raltegravir (Table 2). In 2015 and 2016, the first 2 full years the 3 INSTIs were widely prescribed, 80% of new INSTI resistance cases (12 of 15) were attributed to elvitegravir and dolutegravir use compared with 2013 and 2014, where 88% of INSTI resistance cases (15 of 17) were associated with raltegravir use (Table 2). With decreasing use of raltegravir, most new cases of INSTI resistance have been associated with the use of elvitegravir. In 4 individuals, INSTI drug resistance mutations were documented in a sample drawn before treatment with an INSTI-containing regimen in the DTP. It is unknown whether these unclassifiable individuals had received prior INSTI treatment elsewhere (Table 2).

Prevalence of Integrase Strand Transfer Inhibitor Resistance Mutations

The prevalence of specific INSTI resistance mutations for individuals with newly identified study-defined INSTI resistance from 2009 to 2016 is depicted in Figure 3. Nucleotide substitutions at codons 51, 66, 74, 92, 95, 97, 118, 121, 138, 140, 143,

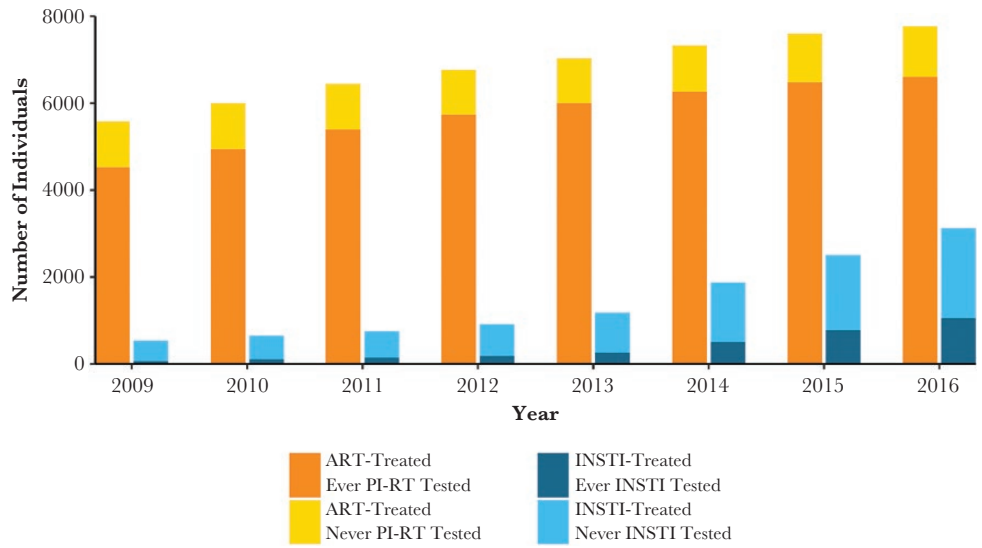

Figure 1. Antiretroviral therapy (ART) and drug resistance testing. All active ART-treated individuals tested and not tested for protease inhibitor and reverse-transcriptase inhibitor (PI-RT) resistance and active integrase strand transfer inhibitor (INSTI)-treated individuals tested and not tested for INSTI resistance as of December 31st of each year from 2009 to 2016 are indicated. Individuals contributed to the count of PI-RT or INSTI tested in the first year that a PI-RT or INSTI resistance test was performed, and this tested status was automatically carried forward to each subsequent year the individual was treated with ART in the Drug Treatment Program. ART-treated ever PI-RT tested, ART-treated individuals ever tested for PI-RT resistance; ART-treated never PI-RT tested, ART-treated individuals never tested for PI-RT resistance; ART-treated ever INSTI tested, ART-treated individuals ever tested for INSTI resistance; ART-treated never INSTI tested, ART-treated individuals never tested for INSTI resistance.

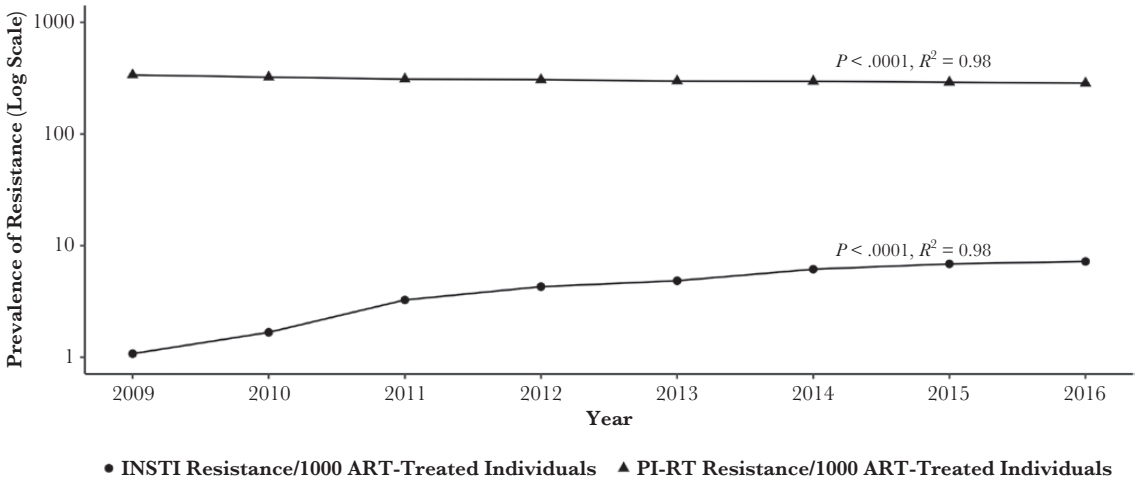

Figure 2. Prevalence of protease inhibitor and reverse-transcriptase inhibitor (PI-RT) and integrase strand transfer inhibitor (INSTI) drug resistance. The annual prevalence of PI-RT and INSTI drug resistance per 1000 antiretroviral therapy (ART)-treated individuals between 2009 and 2016 within the Drug Treatment Program is shown. The trend shows a decrease in the prevalence of PI-RT resistance from 337 per 1000 ART-treated individuals in 2009 to 285 per 1000 ART-treated individuals in 2016 ($P < .0001$, $R^2 = 0.98$); the trend shows an increase in the prevalence of INSTI resistance from 1 per 1000 ART-treated individuals in 2009 to 7 per 1000 ART-treated individuals in 2016 ($P < .0001$, $R^2 = 0.98$).

145, 146, 147, 148, 151, 153, 155, 157, 163, 230, and 263 of the integrase gene were identified among individuals with study-defined INSTI resistance. Overall, there was an increase in the prevalence of INSTI resistance mutations counted at codons 66, 97, 140, 148, 155, and 263 of the integrase gene (Figure 3). By the end of the study, mutation 155H/S/T was detected in 18 individuals and had the highest prevalence of all detected mutations. The second most prevalent mutation, 263K, was identified in 14 individuals by the end of 2016, after increasing from 1 to 11 individuals between 2010 and 2011.

DISCUSSION

Drug-resistant strains of HIV have emerged for all ARVs used in clinical settings, including those of newer classes such as INSTIs [3]. In this study, we conducted a retrospective study of over 9000 HIV-1-positive, ART-treated individuals who participated in the BC DTP from 2009 to 2016. During this study period, there was a dramatic increase in the number of individuals receiving INSTIs raltegravir, elvitegravir, and dolutegravir, and a small but significant increase in the prevalence of intermediate- to high-level INSTI resistance in our ART-treated population.

Similar to findings in the United States [27], we observed an increase in the proportion of ART-treated individuals tested for INSTI resistance. This increase may be due to fewer restrictions on INSTI resistance testing in BC in recent years as well as the partially automated INSTI resistance testing implemented in 2014 for individuals treated with ART in the DTP. This automated INSTI resistance testing system involves the addition of INSTI resistance tests to the first drug resistance test ever done in BC, and, to any standard, physician-ordered PI-RT resistance

Table 2. Newly Identified Cases of INSTI Resistance Within the BC Drug Treatment Program Between 2009 and 2016

	Year								
INSTI Drug Exposure[a]	2009	2010	2011	2012	2013	2014	2015	2016	Total Resistance Cases per INSTI
Raltegravir	6	3	12	7	8	7	2	1	46
Elvitegravir	0	0	0	0	0	2	4	5	11
Dolutegravir	0	0	0	0	0	0	3	0	3
Unclassifiable[b]	0	1	3	0	0	0	0	0	4
Total per Year	6	4	15	7	8	9	9	6	64

Abbreviations: ART, antiretroviral therapy; BC, British Columbia; DTP, Drug Treatment Program; INSTI, integrase strand transfer inhibitor.

[a]The INSTI a person was last exposed to before the first detection of INSTI resistance.

[b]If a person had mutations conferring INSTI resistance before the first known INSTI dispensing date in the DTP, the INSTI drug exposure was termed unclassifiable.

Newly identified ART-treated individuals with mutations conferring INSTI resistance with a total score ≥30 by the Stanford University HIV Drug Resistance Database Genotypic Resistance Interpretation Algorithm version 7.0.1 to at least 1 INSTI is displayed. Note that in December 2009, as shown in Supplementary Figure 1, 475 individuals were prescribed raltegravir, 2 individuals were prescribed elvitegravir, and 0 individuals were prescribed dolutegravir, compared with December 2016, where 751 individuals were prescribed raltegravir, 579 individuals were prescribed elvitegravir, and 1467 individuals were prescribed dolutegravir.

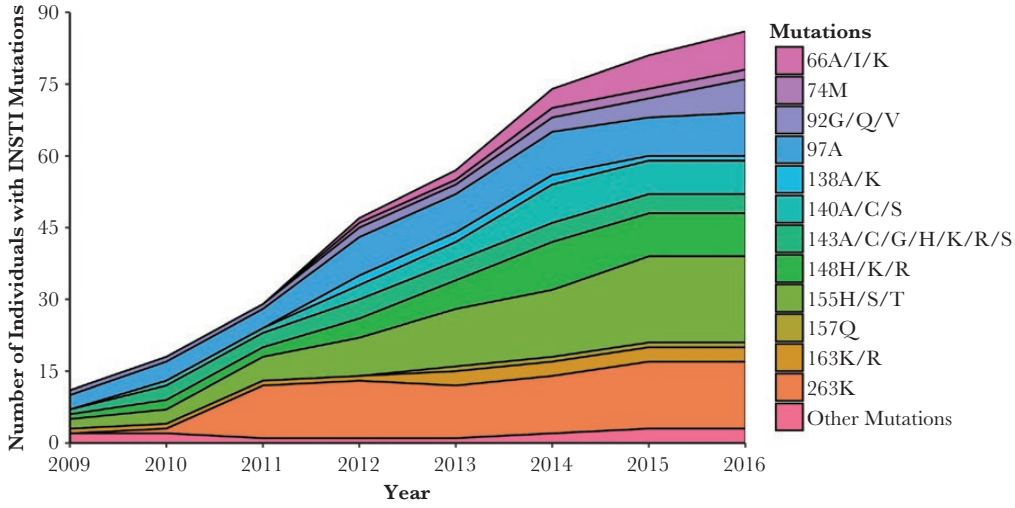

Figure 3. Prevalence of mutations conferring integrase strand transfer inhibitor (INSTI) resistance within newly identified INSTI-resistant individuals. The prevalence of INSTI resistance mutations in INSTI-resistant individuals within the Drug Treatment Program (DTP) between 2009 and 2016 is shown. An individual's INSTI mutation contributed to the mutation count in the first year it was detected and automatically carried forward to all subsequent years the individual received antiretroviral therapy in the DTP. A person with mutations in 3 separate codons (eg, 92, 95, and 97) in a year will contribute 3 counts for that year, whereas a person with mutations in multiple positions within a codon only contributes a count of 1 for that codon (eg, mutations in positons 155H and 155S will only be counted once under 155H/S/T). The "Other Mutations" category represents the sum of mutation counts from 118R, 121Y, 145S, 146P, 147G, 151A/L, 153F/Y, 230R, 51Y, and 95K. NOTE: Certain specific positions associated with INSTI resistance increased in prevalence over the time period.

test if the individual tested was currently or previously treated with INSTIs in BC. Nevertheless, although the number and proportion of INSTI-treated individuals tested for INSTI resistance increased each year during our study period, the proportion of INSTI resistance testing among INSTI-treated individuals has lagged behind the uptake of INSTI-containing therapy (11% in 2009; 34% in 2016) and lagged behind PI-RT resistance testing. This may be a result of the current physician-accessible ART guidelines, which do not support INSTI resistance testing for individuals unless there is evidence of virological failure and suspected INSTI resistance [4, 13].

Over the study period, we observed a decrease in the prevalence of PI-RT resistance in our ART-treated population. A decreasing trend in PI-RT resistance has recently been reported in other clinical HIV-positive populations [28] and may be attributed to the introduction of newer ARVs with greater genetic barriers to resistance. For example, second-generation NNRTIs rilpivirine and etravirine are not significantly impacted by single NNRTI resistance mutations [29] and have shown similar viral suppression efficacy to efavirenz [30]. In addition, increased production of novel fixed-dose combination products, which reduce pill burden and simplify regimens, may lead to better adherence and lower likelihood of ARV resistance [31, 32].

The prevalence of INSTI resistance reported in our study is similar to a recent study observing the prevalence of transmitted INSTI resistance in a Swiss HIV cohort [33] but is lower than those reported in other North American and European cohorts [15, 16, 27, 28]. This difference may in part be due to our method of defining drug resistance, which was restricted to individuals who had intermediate- to high-level INSTI resistance as defined by the Stanford University HIV Drug Resistance Database Genotypic Resistance Interpretation Algorithm version 7.0.1. Other studies have defined INSTI resistance as individual [15, 16, 27, 33] and/or cumulative [16, 27] presence of mutations. Nevertheless, there is an increase in the prevalence of intermediate- to high-level INSTI resistance within our population from 2009 to 2016 (1 to 7 per 1000 ART-treated individuals), likely due to the increase in the number of individuals receiving INSTI-containing therapies. In previous studies, we have identified <80% ARV adherence and CD4 cell count <200 cells/μL as strong risk factors of emergent INSTI resistance in DTP participants treated with INSTI-containing regimens [19], and these factors may contribute to the increase in the prevalence of INSTI resistance over time.

Since 2009, usage of elvitegravir and dolutegravir have risen considerably in the DTP since their introduction in BC in 2013 and 2014, respectively, whereas there has been a decline in the use of raltegravir, the first INSTI [34]. The observed increase in usage of dolutegravir may be due to its association with fewer adverse drug effects and its higher selective barrier for multidrug resistance mutations compared with elvitegravir and raltegravir [35–37]. This shift in prescribed drug therapies coincides with the shift in INSTIs associated with newly detected cases of INSTI resistance. Over time in our ART-treated population, the observed number of new raltegravir-associated resistance cases decreased as use of raltegravir declined (6 cases in 2009;

1 case in 2016). Elvitegravir-associated resistance accounted for the majority of new cases in 2015 and 2016, whereas only 3 new cases were associated with dolutegravir use, despite a marked increase in dolutegravir prescribing. Overall, the number of newly identified cases of individuals with study-defined INSTI resistance increased and then subsequently decreased during the study period.

Individuals with newly identified study-defined INSTI resistance appear to have mutations selected at codons 66, 97, 140, 148, 155, and 263. Mutation 66I is expected with increased elvitegravir usage [38]. Mutation 97A, in combination with other INSTI mutations, confers reduced susceptibility to raltegravir and dolutegravir [39, 40]. Mutations 140A/S are associated with dolutegravir and raltegravir resistance, and, in combination with mutation 148H, it is associated with reduced virological suppression [41]. Mutations 148H/R and 155H are mutations associated with 2 distinct mutational pathways that reduce susceptibility to all current INSTIs [42, 43]. Mutation 263K is associated with decreased HIV-deoxyribonucleic acid integration, viral replication capability, and integrase enzyme capacity but may also confer low- to intermediate-level susceptibility to dolutegravir and elvitegravir [44, 45]. The 263K mutation has previously been noted as an emergent INSTI mutation in treatment-experienced, INSTI-naive DTP individuals initiating raltegravir, elvitegravir, or dolutegravir for the first time [19] as well as in other treatment-experienced HIV-1-positive populations [35].

There are several limitations within our study. First, the incomplete coverage of INSTI resistance testing limits our ability to adequately characterize INSTI resistance mutations and may also underestimate the prevalence of INSTI resistance. We speculate that this poor coverage could be due to the relatively new nature of INSTIs and their significance might be overlooked. This study also observed cumulative mutations that confer intermediate- to high-level resistance to INSTIs, and this threshold does not include individual mutations that may confer low-level resistance and may be polymorphic or treatment-emergent. Most INSTI resistance tests are requested in response to virological failure rather than before initiation of ART therapy, and, therefore, individuals with intermediate- to high-level resistance to INSTIs were included in our study because they were more likely to be identified during routine clinical care. Without comprehensive baseline INSTI resistance testing, the prevalence of individual low-level resistance mutations cannot be accurately estimated. As a result, we cannot determine the true prevalence of INSTI mutations present in our ART-treated population and our INSTI prevalence may be underestimated. Our prevalence of INSTI resistance may also be underestimated do to Sanger sequencing's inability to detect rare but clinically significant drug resistance mutations present at low viral populations [46]. In addition, our assessment of nucleotide substitutions within the integrase gene can limit key identification of mutations outside of the integrase gene, which may confer high-level resistance to raltegravir, elvitegravir, and dolutegravir [47].

CONCLUSIONS

In summary, our results indicate that the prevalence of INSTI resistance in the DTP is low, but it is gradually increasing over time as INSTI prescribing increases. The ability to precisely characterize and determine the frequency of INSTI resistance is hampered by the limited usage of INSTI resistance testing. Although previous research suggests dolutegravir's genetic barrier to resistance is high, the genetic barrier of other currently prescribed INSTIs, raltegravir and elvitegravir, is comparable to other ARVs [37]. Integrase strand transfer inhibitor-treated individuals with suboptimal adherence may require more extensive monitoring to permit early detection of emergent INSTI resistance mutations. Further drug resistance monitoring is required to detect any changes in the prevalence of INSTI resistance with the introduction of newer INSTIs, such as bictegravir.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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