Resistance to HIV Integrase Strand Transfer Inhibitors Among Clinical Specimens in the United States, 2009–2012

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Background. Data on integrase inhibitor resistance come primarily from clinical trials and in vitro studies. We examined results of all clinically indicated integrase genotypic resistance tests (GRTs) performed at a US national referral lab from 2009 through 2012.

Methods. Integrase sequences and demographic data were compiled with paired protease–reverse transcriptase (PR-RT) GRT results, when available. Analyses utilized the Stanford HIV Drug Resistance Database. "Major" integrase mutations included T66AIK, E92QV, F121Y, Y143CHR, S147G, Q148HKR, and N155H; multiple accessory mutations were also assessed.

Results. Among 3294 sequences from 3012 patients, 471 patients had viruses with ≥1 raltegravir or elvitegravir resistance mutation (15.6%). Q148 and N155 pathways were equally represented (both n = 197); 84 had Y143 mutations. Q148 rarely occurred without accessory mutations (n = 3). Among 224 patients with serial integrase GRTs, 22 with baseline wild-type acquired a major mutation, after a median 224 days between tests (interquartile range, 148–335 days). Major mutations were observed to persist up to 462 days. Most (62%) had paired PR-RT results. Patients with integrase-resistant viruses were older and more likely to have PR-RT mutations (both $P < .001$). Among those with PR-RT data, 42 patients had 4-class resistance (2.3%). Sex, geographic region, and test year were not associated with integrase resistance. High-level dolutegravir resistance was predicted in 12% of patients with raltegravir- or elvitegravir-resistant viruses (2% of all patients).

Conclusions. Approximately 1 in 6 US patients undergoing integrase GRT for clinical decision making harbors significant resistance, with Q148 and N155 pathways equally common. Dolutegravir is likely to have full or partial activity against most variants observed.

Keywords. human immunodeficiency virus; antiretroviral resistance; raltegravir; elvitegravir; dolutegravir.

As the newest class of antiretrovirals (ARVs), integrase strand transfer inhibitors (INSTIs) have assumed an important role in treating human immunodeficiency virus (HIV) infection. Raltegravir became part of a preferred initial regimen in the United States for HIV-infected adults [1] within 2 years of Food and Drug Administration (FDA) approval [2], owing to its demonstrated efficacy and favorable safety profile in treatment-experienced [3] and -naive patients [4]. The second drug in the class, elvitegravir [5, 6], is a component of an alternative INSTI-based regimen for treatment-naive patients [7], in a fixed-dose combination tablet with tenofovir disoproxil fumarate, emtricitabine, and the pharmacologic booster cobicistat [8]. Dolutegravir, a second-generation INSTI, was approved by the FDA in August 2013 [9].

Despite the potency, tolerability, and durability of first-generation INSTIs, resistance mutations are detected in up to 60% of patients with virologic failure in

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clinical trials studying highly treatment-experienced patients, and up to 8% in studies of initial therapy [10, 11]. Three principal mutation pathways reduce susceptibility to raltegravir: Y143CHR, Q148HKR, and N155H. These codons are located in close proximity to integrase's active site, and each mutation significantly reduces viral fitness [12]. Certain compensatory mutations can partially or fully restore viral replicative capacity: T97A rescues catalytic function in the presence of Y143 mutants, similar to G140ACS or E138AK for Q148 mutants [13]. In the case of N155H, its main accessory mutation, E92QV, further reduces susceptibility without restoring fitness—a fact that helps explain why N155 mutants are frequently replaced by Q148 ± G140 [14] in vivo. Interestingly, E92Q is the most common initial mutation to arise during failure of elvitegravir-based regimens, followed by N155H and Q148R [15]. Due to unique interactions between active site residues and raltegravir, substitutions at Y143 unaccompanied by additional mutations have no effect on in vitro susceptibility to dolutegravir [16] and little [17] to no [18] effect on elvitegravir. Indeed, dolutegravir retains activity against all single-mutation variants [16, 19, 20]. Patients continued on failing raltegravir-containing regimens may accumulate multiple mutations over time [21]—a scenario that can reduce susceptibilities to other INSTIs, including dolutegravir. In 2 studies of dolutegravir among patients who failed raltegravir (VIKING-2 and -3), the greatest reduction in dolutegravir susceptibility occurred when Q148 was accompanied by ≥2 other major mutations. However, a reduced but measurable antiretroviral effect was still observed in most patients [19, 20]. Two recently identified mutations, G118R and R263K, each confer low-level resistance to dolutegravir [22, 23]. Both have been reported in vivo [24, 25].

Although much is known about the mutation pathways affecting INSTIs, all such data come from in vitro experiments or clinical trials. In this report, we focus on integrase genotypic resistance tests (GRTs) sent to a US national referral laboratory, in order to characterize the profile of INSTI resistance among specimens obtained for clinical decision making. Our principal aims were to (1) describe the prevalence of INSTI resistance and the patterns of mutations resulting from INSTI failures in a clinical population; (2) determine the association between integrase and protease–reverse transcriptase (PR-RT) mutations among patients with paired GRTs; and (3) assess the frequency of mutation patterns likely to impact dolutegravir susceptibility among patients harboring viruses with resistance to first-generation INSTIs.

METHODS

Study Population and Data Collection

Integrase and PR-RT GRTs require 2 separate amplifications, each sequencing distinct areas of the HIV genome and reporting mutations only for their respective *pol* gene segment(s). We analyzed results from all specimens sent to the referral laboratory (Laboratory Corporation of America, Research Triangle Park, North Carolina) for integrase GRT over the 4-year period beginning on the date this assay became commercially available (1 January 2009) and ending on 31 December 2012. In some cases, multiple specimens were sent during the study period for a given patient; individual results were considered separately in our analyses. In addition to an internal patient identification number and the date of specimen collection, the referral laboratory collected the patient's age and sex along with the state and postal (ZIP) code of the ordering clinic or provider. The laboratory does not obtain data on the patient's treatment status (naive or experienced) or history of prior ARV exposures. For this analysis, laboratory data managers searched for PR-RT GRT results available for each patient, and all such records accompanied the final integrase GRT results. We considered integrase and PR-RT GRTs to be paired if specimens were submitted within 30 days of one another. No specimens associated with clinical trials were included in this study.

To ensure that we did not duplicate patients or integrase GRT nucleotide sequences in the final data set, we compared sex, specimen dates, clinic location, and specimen tracking numbers. We also created a maximum likelihood phylogenetic tree and examined the same descriptive data elements to identify any potential duplicates in clusters of sequences separated by a genetic distance of ≤0.015. Details of these analyses are included in this article's Supplementary Data.

Genotyping and Analysis of Nucleotide Sequence Data

HIV-1 RNA was extracted from each submitted plasma specimen and subjected to RT-PCR to generate complementary DNA. Dideoxynucleotide sequencing was then performed using GenoSure primers spanning sections of the *pol* gene encoding amino acids 1–288, 1–99, and 1–400 of integrase, PR, and RT, respectively. Integrase and PR-RT sequences were analyzed separately using the Stanford University HIV Drug Resistance Database genotypic resistance interpretation algorithm (HIVdb Program, version 6.3.0, http://hivdb.stanford.edu). Sequence analyses were conducted on 7 June 2013.

Definitions of Resistance Mutations

After a review of relevant abstracts, published data, and the June 2013 update of the Stanford University HIV Database [13–15, 21, 26–29], we defined a "major" integrase mutation as any of the following: T66AIK, E92QV, F121Y, Y143CHR, S147G, Q148HKR, or N155H. "Accessory" mutations included H51Y, L68IV, L74IM, T97A, E138AK, G140ACS, S153F, E157Q, G163KR, and R263K. We used the 2009 World Health Organization table of surveillance drug resistance mutations (SDRMs) to define resistance to nucleoside RT inhibitors

(NRTIs), nonnucleoside RT inhibitors (NNRTIs), and protease inhibitors (PIs) [30]. Patterns of mutations affecting susceptibility to dolutegravir were determined from a separate review of results from in vitro experiments [16, 22, 23], clinical cohorts [31], and randomized trials [19, 20, 24]. We categorized these patterns according to the definitions used in 2 clinical trials of dolutegravir: VIKING-2 [19] and VIKING-3 [20]. Treatment response to dolutegravir was categorized by the presence of Q148 plus ≥2 major mutations, Q148 plus 1 major mutation, N155 alone, Y143 alone, or ≥2 major mutations. We also assessed predicted dolutegravir susceptibilities from the HIVdb Program to determine which mutational patterns in our data conferred the greatest resistance.

Ethical Approval

Because the data were fully de-identified prior to analysis, the Institutional Review Board at the University of North Carolina at Chapel Hill determined this study was exempt from review.

Statistical Analysis

Differences in demographic, clinical, and virologic characteristics were tested using Student *t* test or the Wilcoxon rank-sum test for continuous variables, and Pearson χ^2 test or Fisher exact test for categorical variables, as appropriate. We used bivariable logistic regression to determine the association between factors of interest and the presence of major integrase mutations, and calculated 95% confidence intervals (CIs) for each estimate. Statistical significance was defined as $P < .05$ for all tests. Analyses were performed using Stata/IC, version 11.2 (StataCorp, College Station, Texas).

RESULTS

Integrase GRT results were available for 3012 patients tested between January 2009 and December 2012. The average age was 42.8 years, and the majority (71%) of patients were male (Table 1). Sixty percent of patients were from the southern United States, with lesser proportions from the Northeast (17%), West (13%), and Midwest (10%). Integrase GRT utilization expanded considerably over time, rising from 80 patients tested in 2009 to 1378 in 2012. Eight different viral subtypes were observed, with subtype B predominating (98%). In all, 224 patients had >1 integrase GRT sent, yielding a total of 3294 sequences available for analysis. The median time between sequential integrase GRTs was 214 days (interquartile range [IQR], 84–317 days).

Four hundred seventy-one patients (15.6%) had ≥1 major integrase mutation detected. Compared with patients whose specimens had no detectable resistance, individuals with INSTI-resistant viruses were older (46.2 vs 42.2 years, $P < .001$), and a greater proportion had multiple integrase GRTs sent for analysis (10% vs 7%, $P = .02$). We observed no differences between the 2 groups with respect to sex or geographic location. Among patients with >1 integrase GRT, the median time between tests was similar for those with and without INSTI-resistant HIV (211 vs 215 days, $P = .29$).

Q148 and N155 pathways predominated, each observed in 197 patients (6.5% of all patients; 42% of patients with INSTI-resistant viruses), whereas Y143 was detected in only 84 patients (2.8% overall; 18% of those with ≥1 major mutation) (Supplementary Table 1). A mutation at Q148 was accompanied by G140 in 172 patients, and by E138 in 58; in only 3 patients was Q148 detected by itself. Eighty-six patients had N155H without other integrase mutations; 15 had Y143 alone (Figure 1). At key codons, certain amino acid substitutions were more common than others: Q148H (n = 144), G140S (n = 162), E138K (n = 55), and Y143R (n = 51) were most prevalent. High-level resistance to raltegravir and elvitegravir was predicted in 15% (n = 453) and 13% (n = 401) of patients (96% and 85% of those with INSTI-resistant viruses), respectively (Table 2); the difference was entirely attributable to the differential impact of Y143 mutants.

Forty-seven patients had serial integrase GRT results and at least 1 sample with a major INSTI mutation detected (Table 1). Twenty-five of these individuals (53%) had a major INSTI mutation in their first sample; the remaining 22 (47%) had wild-type virus at baseline and subsequently acquired a major mutation. Equal proportions of patients acquiring major mutations had Y143 (n = 7), Q148 (n = 7), and N155 (n = 8). No patient developed additional major INSTI mutations over time; all accumulated changes were among accessory mutations only. Fourteen patients with an initial major mutation had no change on subsequent GRTs, demonstrating stable persistence of these mutations up to 462 days (median, 140 days; IQR, 39–307 days). Thirty-seven patients had persistence of accessory mutations up to 756 days (median, 196 days; IQR, 104–276 days). We observed persistence of N155 in 5 patients, ranging between 36 and 462 days. One patient with Y143C + N155H at baseline was unchanged on a repeat GRT 28 days later. No serial specimens revealed pathways switching from one to another, although 25 patients with single integrase GRT specimens had viruses with 2 mutation pathways present (Y143 + Q148, n = 3; Y143 + N155, n = 9; Q148 + N155, n = 13).

Sixty-two percent of patients had paired integrase and PR-RT GRTs sent within 30 days of each other (n = 1866), of whom 239 had ≥1 integrase major mutation (13%; Table 3). SDRMs (certain key nonpolymorphic resistance mutations in PR and RT [30]) were detected in just over half of all patients (n = 954), and individuals with INSTI-resistant viruses were significantly more likely to have an SDRM detected in any class (all $P < 0.001$). Of the 110 patients whose viruses had SDRMs present in all 3 PR-RT classes, 42 (38%) also harbored ≥1

Table 1. Demographic and Viral Characteristics for Patients With Integrase Genotypic Resistance Tests, 2009–2012

Characteristic	All Patients (N = 3012)	Integrase GRT Results[a]		*P* Value[b]
		Major Mutation(s) Present (n = 471)	No Major Mutations (n = 2541)	
Age, y, mean (SD)[c]	42.8 (11.2)	46.2 (9.4)	42.2 (11.4)	<.001
Sex[d]				
Female	851 (28.6)	122 (26.5)	729 (29.0)	.27
Male	2123 (71.4)	339 (73.5)	1784 (71.0)	
Region[e]				
Northeast	508 (16.9)	65 (13.8)	443 (17.4)	.2
South	1818 (60.4)	301 (64.0)	1517 (59.7)	
Midwest	300 (10.0)	43 (9.2)	257 (10.1)	
West	384 (12.8)	61 (13.0)	323 (12.7)	
Year of test[f]				
2009	80 (2.7)	13 (2.8)	67 (2.6)	<.001
2010	479 (15.9)	96 (20.4)	383 (15.1)	
2011	1075 (35.7)	223 (47.4)	852 (33.5)	
2012	1378 (45.8)	139 (29.5)	1239 (48.8)	
HIV-1 subtype[g]				
A	4 (0.13)	1 (0.2)	3 (0.1)	. . .
B	2964 (98.4)	464 (98.5)	2500 (98.4)	
C	17 (0.6)	2 (0.4)	15 (0.6)	
CRF01_AE	7 (0.2)	1 (0.2)	6 (0.2)	
CRF02_AG	11 (0.4)	2 (0.4)	9 (0.4)	
D	3 (0.1)	1 (0.2)	2 (0.1)	
F	3 (0.1)	0 (0)	3 (0.1)	
G	3 (0.1)	0 (0)	3 (0.1)	
>1 integrase GRT sent	224 (7.4)	47 (10.0)	177 (7.0)	.02
Days between serial integrase GRTs, median (IQR)[h]	214 (84–317)	211 (84–317)	215 (111–389)	.29
Pattern of mutations among patients with >1 integrase GRT sent				
Major mutations				. . .
None	177/224 (79.0)	0/47 (0)	177 (100)	
Acquired	22 (9.8)	22 (46.8)	0 (0)	
Accumulated	0 (0)	0 (0)	0 (0)	
Lost	11 (4.9)	11 (23.4)	0 (0)	
No change over time	14 (6.3)	14 (29.8)	0 (0)	
Accessory mutations				
None	154 (68.8)	13/47 (27.7)	141/177 (79.7)	
Acquired	18 (8.0)	13 (27.7)	5 (2.8)	
Accumulated	4 (1.8)	4 (8.5)	0 (0)	
Lost	11 (4.9)	6 (12.8)	5 (2.8)	
No change over time	37 (16.5)	11 (23.4)	26 (14.7)	

Data are presented as No. (%) of patients unless otherwise indicated.

Abbreviations: GRT, genotypic resistance test; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; SD, standard deviation.

[a] "Major" mutations included T66AIK, E92QV, F121Y, Y143CHR, S147G, Q148HKR, N155H. "Accessory" mutations included H51Y, L68IV, L74M, T97A, E138AK, G140ACS, V151AL, S153F, G163KR, R263K.

[b] Pearson χ^2 and Fisher exact test compared categorical variables; continuous variables were assessed with Student *t* test.

[c] n = 2978 (461 with integrase strand transfer inhibitor [INSTI] resistance, 2517 without).

[d] n = 2974 (461 with INSTI resistance, 2513 without).

[e] n = 3010 (470 with INSTI resistance, 2540 without); Northeast included Connecticut, Massachusetts, New Jersey, New York, Pennsylvania, Rhode Island; South included Alabama, Arkansas, Delaware, District of Columbia, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, South Carolina, Tennessee, Texas, Virginia, West Virginia; Midwest included Iowa, Illinois, Indiana, Kansas, Michigan, Minnesota, Missouri, Ohio, Wisconsin; West included Alaska, Arizona, California, Colorado, Idaho, New Mexico, Nevada, Utah, Washington.

[f] Data were available from 1 January 2009 through 31 December 2012.

[g] Subtype predicted by the Stanford HIV Database, based on integrase sequence data.

[h] There were 282 calculated day values overall (62 for patients with INSTI resistance, 220 without).

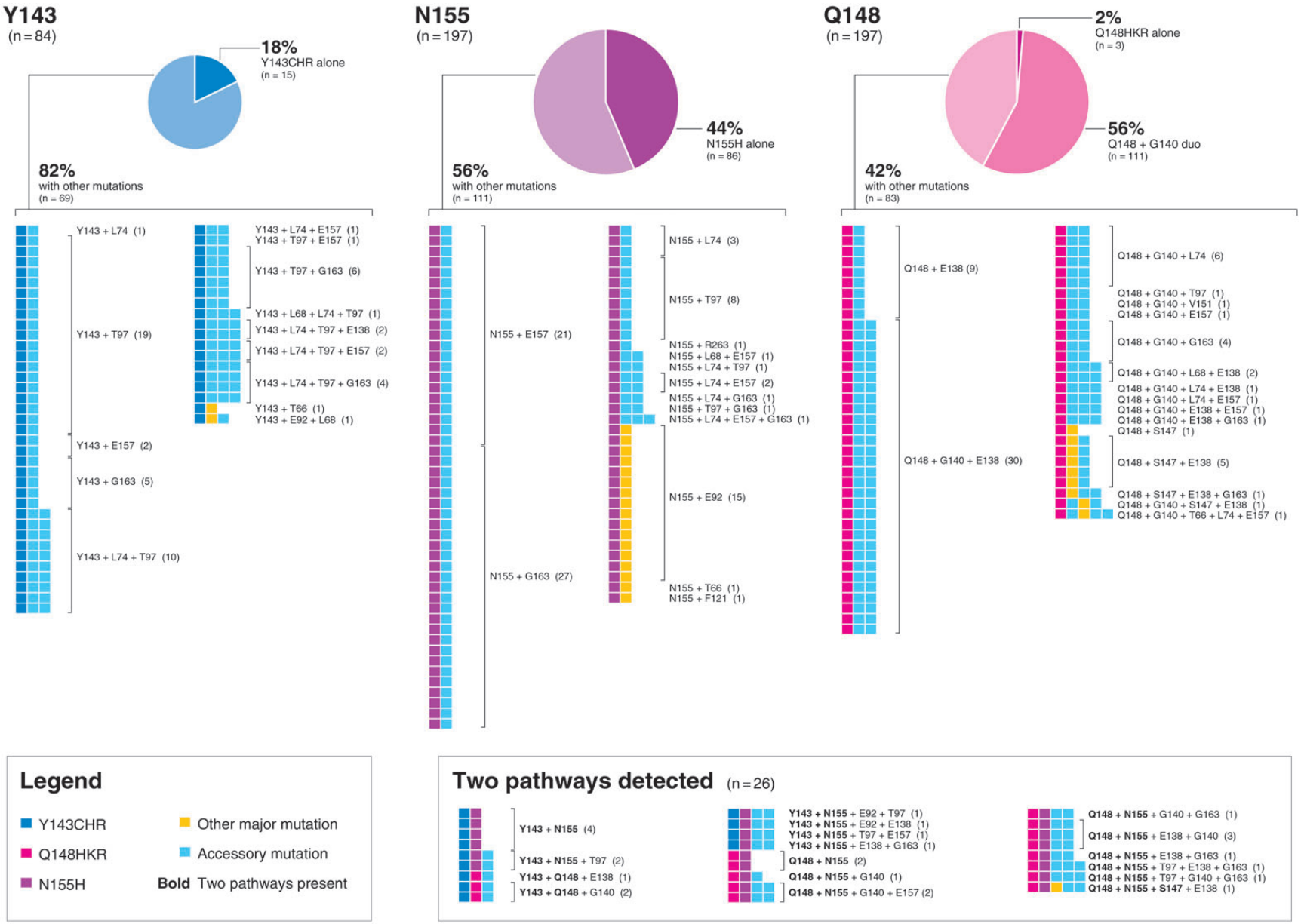

Figure 1. Integrase mutation patterns among 3012 patients in the United States, 2009–2012. The frequency of Y143, N155, and Q148 pathways and their associated accessory mutations is shown. Isolates in which 2 major pathways were identified are depicted separately in the lower box.

Table 2. Predicted Raltegravir, Elvitegravir, and Dolutegravir Resistance Among Patients With Integrase Genotypic Resistance Tests, 2009–2012[a]

Predicted Resistance	No. (%) Among All Patients (n = 3012)	No. (%) Among Patients With Integrase Major Mutation[b] (n = 471)
Predicted raltegravir resistance		
None (susceptible)	2321 (77.1)	0 (0)
Potential low-level	183 (6.1)	2 (0.4)
Low-level	33 (1.1)	2 (0.4)
Intermediate	22 (0.7)	14 (3.0)
High-level	453 (15.0)	453 (96.2)
Predicted elvitegravir resistance		
None (susceptible)	2332 (77.4)	12 (2.6)
Potential low-level	196 (6.5)	18 (3.8)
Low-level	63 (2.1)	28 (5.9)
Intermediate	20 (0.7)	12 (2.6)
High-level	401 (13.3)	401 (85.1)
Predicted dolutegravir resistance		
None (susceptible)	2566 (85.2)	57 (12.1)
Potential low-level	199 (6.6)	169 (35.9)
Low-level	53 (1.8)	51 (10.8)
Intermediate	136 (4.5)	136 (28.9)
High-level	58 (1.9)	58 (12.3)

[a] Based on Stanford HIV Database interpretation, using 5 June 2013 update. All sequences analyzed on 7 June 2013.

[b] "Major" integrase mutations included: T66AIK, E92QV, F121Y, Y143CHR, S147G, Q148HKR, N155H.

integrase major mutation, constituting resistance to at least 1 ARV in all classes except entry inhibitors.

Individuals with viruses having integrase major mutations appeared to be more highly treatment experienced, and options for active companion ARVs among these 239 patients were limited: 14% had no fully susceptible NRTIs, 27% had no fully susceptible NNRTIs, and 5% had no fully susceptible PIs available (Table 3). However, all but 6 of these patients had at least 1 fully susceptible non-INSTI medication from which to choose. Frequencies of each individual SDRM are listed in Supplementary Table 2.

Using bivariate logistic regression, we compared patients with INSTI-resistant viruses containing 1 of the main pathways (Y143, Q148, N155) to patients with viruses having either or both of the other 2 pathways. Individuals with Y143 had 8.6 times the odds of having an SDRM present, compared with those having Q148 and/or N155 (95% CI, 1.2–64). However, at the level of individual SDRM classes and the key RT mutations M184V and K103N, we found no significant associations with the presence of Y143 or either of the other pathways. No patient or virologic characteristics were predictive of acquiring

Table 3. Characteristics of Viruses From Patients With Paired Data for Both Integrase and Protease–Reverse Transcriptase Genotypic Resistance Tests, 2009–2012[a]

Characteristic	All Patients (N = 1866)	Integrase GRT Results[b]: Major Mutation(s) Present (n = 239)	Integrase GRT Results[b]: No Mutations (n = 1627)	*P*[c] Value
Any SDRM present	954 (51.1)	212 (88.7)	742 (45.6)	<.001
SDRMs present in all classes	110 (5.9)	42 (17.6)	68 (4.2)	<.001
Any NRTI SDRM present	662 (35.5)	192 (80.3)	470 (28.9)	<.001
NRTI resistance[d]				
None (fully susceptible)	1118 (59.9)	44 (18.4)	1071 (66.0)	<.001[e]
At least 1 low-level or less	665 (35.6)	162 (67.8)	503 (30.9)	
All intermediate or higher	83 (4.5)	33 (13.8)	50 (3.1)	
Any NNRTI SDRM present	632 (33.9)	122 (51.5)	510 (31.4)	<.001
NNRTI resistance[d]				
None (fully susceptible)	1108 (59.4)	96 (40.2)	1012 (62.2)	<.001[e]
At least 1 low-level or less	504 (27.0)	79 (33.1)	425 (26.1)	
All intermediate or higher	254 (13.6)	64 (26.8)	190 (11.7)	
Any PI SDRM present	208 (11.2)	65 (27.2)	143 (8.8)	<.001
PI resistance[d]				
None (fully susceptible)	1569 (84.1)	160 (67.0)	1409 (86.6)	<.001[e]
At least 1 low-level or less	271 (14.5)	67 (28.0)	204 (12.5)	
All intermediate or higher	26 (1.4)	12 (5.0)	14 (0.9)	

Data are presented as No. (%) of patients, unless otherwise indicated.

Abbreviations: GRT, genotypic resistance test; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor; PI, protease inhibitor; SDRM, surveillance drug resistance mutation.

[a] GRT sequence data were considered paired if specimens were sent within 30 days of one another.

[b] "Major" integrase mutations included: T66AIK, E92QV, F121Y, E138AK, Y143CHR, G140ACS, S147G, Q148HKR, N155H.

[c] Pearson χ^2 and Fisher exact test compared categorical variables; continuous variables were assessed with the Wilcoxon rank-sum test.

[d] Categorization was based on "penalty" scores from the Stanford HIV Database. "Fully susceptible" meant every drug in class had a score of ≤9; "at least 1 low-level or less" meant there was at least 1 drug in the class with a score <29; "all intermediate or higher" meant that all drugs in the class had scores of ≥30.

[e] *P* value reflects comparison of subjects with all drugs having at least intermediate resistance vs the combination of patients with no resistance and those with at least 1 fully active drug.

Table 4. Frequencies of Integrase Strand Transfer Inhibitor Mutations and Susceptibility to Dolutegravir Among Patients With Integrase Genotypic Resistance Tests, 2009–2012

Mutation Pathway	No. of Patients	% Among All Patients (N = 3012)	% Among Patients With Integrase Major(s) (n = 471)	Predicted HIVdb Penalty Score, Mean (SD)[a]	Estimated Fold-change in DTG Susceptibility (From VIKING-3 Study)[b]	
					Range	Median (IQR)
Q148 plus ≥2 majors	57	1.9	12.1	74.7 (12.9)	2.6–37.0	10.0 (4.5–13)
Q148 plus 1 major	137	4.6	29.1	50.4 (4.6)	0.5–12.0	4.6 (3.4–6.3)
N155	157	5.2	33.3	10.6 (1.8)	0.8–3.9	1.5 (1.3–1.8)
Y143	67	2.2	14.2	4.6 (3.8)	0.8–2.0	1.1 (0.9–1.2)
≥2 major mutations	54	1.8	11.5	36.6 (25.7)	1.5–27.0	4.6 (1.7–20.0)
No major mutation	2541	84.4	0	0.3 (1.4)	0.5–4.0	0.9 (0.8–1.0)

Abbreviations: DTG, dolutegravir; HIVdb, Stanford HIV Database HIVdb Program; IQR, interquartile range.

[a] Predicted "penalty" scores from the HIVdb interpretation algorithm were used to determine the mean score for each pathway. Ranges for scoring: 0–9, fully susceptible; 10–14, potential low-level resistance; 15–29, low-level resistance; 30–59, intermediate resistance; ≥60, high-level resistance.

[b] Data from reference [16].

or accumulating integrase mutations among patients with serial GRTs.

Finally, we estimated the prevalence of patients harboring dolutegravir-resistant viruses, based on categorizations of mutation patterns and estimates of associated fold-changes in susceptibility from the VIKING-2 and VIKING-3 studies (Table 4). The VIKING patterns conferring the greatest losses in dolutegravir activity (≥2 major mutations, Q148 plus 1 major mutation, and Q148 plus ≥2 major mutations) were observed in 54, 137, and 57 patients, respectively. Results from the HIVdb Program predicted high-level dolutegravir resistance in 58 patients (2% overall; 12% of patients with ≥1 integrase major mutation), the majority of whom (n = 39 [67%]) had viruses containing Q148 + G140 + E138 with or without additional major or accessory mutations. The virus with the greatest predicted resistance contained Q148K, G140A, E138K, and N155H. Of the 42 patients with 4-class ARV resistance, 55% were predicted to have intermediate or high-level resistance to dolutegravir (n = 25). The R263K mutation was detected in sequences from 5 patients, only 1 of whom had virus with an additional integrase mutation (N155H). No patient had G118 mutations.

DISCUSSION

In this, the first study to characterize INSTI resistance among integrase GRT specimens obtained for clinical indications in the United States, we found that 15.6% of patients had viruses with ≥1 integrase major mutation—the most frequent of which, N155H and Q148HKR, were equally represented. The prevalences of predicted high-level resistance to raltegravir, elvitegravir, and dolutegravir were 15%, 13%, and 2%, respectively. Although treatment histories for the patients included in this analysis were unavailable, it seems reasonable to assume that most (if not all) were on raltegravir; clinical trial participants were excluded and elvitegravir became commercially available only 4 months before the end of the study period.

These results shed light on the development of resistance while failing INSTI-containing ARV regimens, offering support for some commonly held assumptions while calling others into question. N155-containing viruses were observed to persist for up to 10 months, challenging the idea that their fitness disadvantage favors early fading of mutants with N155H and emergence of Y143 or Q148 mutants [14]. Among patients with serial tests whose viruses evolved from wild type to INSTI resistant, we noted equal distribution of the 3 pathways, rather than a tendency for the N155 pathway to predominate. Other data suggested evolution away from the N155 pathway. In patients with a single integrase GRT and virus containing a mixture of 2 pathways, N155 was present in 22 of 25 (88%). However, without sequential GRT results for these patients, neither the pace of change nor timing relative to INSTI treatment initiation can be determined.

As indicated by data from patients with multiple integrase GRTs in this study, failing INSTI therapy may lead to the accumulation of INSTI resistance—but not in all cases. A quarter of serially tested patients had the same mutations detected in every specimen, with some major mutations persisting for as many as 15 months. Only about 1 in 10 patients had viruses accumulate additional integrase resistance following the appearance of an initial major mutation, and in every case these were accessory mutations, not major ones. This is by no means reassuring, as the accumulation of accessory mutations often raised the overall level of resistance. For example, 1 patient accumulated

mutations conferring high-level resistance to dolutegravir, with the addition of an E138A mutation in a specimen obtained 210 days after Q148H + G140S first appeared.

A majority of patients had PR-RT GRTs sent within 30 days of the integrase GRT, suggesting that testing was ordered to confirm virologic failure due to resistance. However, in nearly half of these cases no resistance mutations were detected in any ARV class, implying that failure was due to nonadherence rather than evolution of INSTI mutations. A similarly high frequency of wild-type sequences on resistance tests has been documented recently in the United Kingdom [32], although mutations may be present as minority variants or could have developed and been archived as proviruses if patients had stopped therapy prior to the resistance testing. In our study, nearly 1 in 5 patients with initial INSTI resistance had no mutations detected on subsequent GRTs, suggesting that selection pressure had been removed.

Finally, our data show that NRTI resistance does not always accompany the development of INSTI resistance. Among patients with paired integrase and PR-RT GRTs, isolated INSTI resistance was detected in 17 with the Q148 pathway, 18 with the N155 pathway, and only 1 with the Y143 pathway. This finding of INSTI-only resistance differed from findings in 2 clinical trials of raltegravir, STARTMRK and QDMRK. In the former, 3 of the 4 patients who developed raltegravir resistance had emtricitabine resistance conferred by M184V [11], while in QDMRK, all 11 patients with raltegravir-resistant viruses also had M184V detected [33]. Interestingly, we found that Y143 mutants had 8 times the odds of having an SDRM in any class, compared with Q148 and N155 mutants—but in analyses considering only NRTI SDRMs or M184V, the association did not hold.

This study has limitations, the most significant being the absence of treatment history data. Without knowing when patients initiated INSTI-containing ARV regimens, it is impossible to determine when INSTI resistance emerged. Additionally, it is not possible to know whether integrase GRTs were ordered along with PR-RT GRTs as an assessment of "baseline" or pretreatment resistance. Although transmitted INSTI resistance has been described [34, 35], and its prevalence is likely to increase over time [36], there is currently no organized surveillance for integrase mutations taking place in the United States. Despite careful efforts to eliminate duplicate patient observations, the absence of identifying information for all participants means it is possible that some individuals may be represented more than once.

The expanding use of integrase inhibitors in initial and salvage ARV regimens makes it increasingly important for HIV care providers to understand INSTI resistance and its consequences. In this study of integrase GRTs obtained for clinical decision making, approximately 1 in 6 tests identified viruses with ≥1 major integrase mutation present. For patients on incompletely suppressive raltegravir or elvitegravir-containing regimens, emergent mutations conferring intermediate to high-level dolutegravir resistance could be present in up to 40% of those whose viruses have major and accessory integrase mutations. Thoughtful application of integrase GRTs is essential for the optimal management of patients being treated for their HIV with INSTI-containing ARV regimens.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed

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