Pretreatment integrase strand transfer inhibitor resistance in North Carolina from 2010–2016

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Objective: We sought to define the prevalence of pretreatment integrase strand transfer inhibitor (INSTI) resistance and assess the transmission networks of those with pretreatment INSTI resistance.

Design: A retrospective cohort study of HIV-positive patients with genotypic resistance testing sent to a single referral laboratory in North Carolina between 2010 and 2016.

Methods: We linked genotype and public health data for in-care HIV-positive individuals to determine the prevalence of INSTI resistance among treatment-naive (defined as those with a first genotype $\leq$3 months after diagnosis) and treatment-experienced (defined as those with a first genotype >3 months after diagnosis) patients. We performed molecular and phylogenetic analyses to assess whether pretreatment INSTI resistance mutations represented clustered HIV transmission.

Results: Of 8825 individuals who contributed sequences for protease, reverse transcriptase, or INSTI genotypic resistance testing during the study period, 2784 (31%) contributed at least one sequence for INSTI resistance testing. Of these, 840 were treatment-naive individuals and 20 [2.4%, 95% confidence interval (CI): 1.5, 3.6%] had INSTI mutations; only two (0.2%, 95% CI: 0.02, 0.9%) had major mutations. Of 1944 treatment-experienced individuals, 9.6% (95% CI: 8.3, 11.0%) had any INSTI mutation and 7.0% (95% CI: 5.9, 8.3%) had major mutations; the prevalence of INSTI mutations among treatment-experienced patients decreased overtime ($P < 0.001$). In total 12 of 20 individuals with pretreatment INSTI mutations were part of 10 molecular transmission clusters; only one cluster shared identical minor mutations.

Conclusion: The prevalence of major pretreatment INSTI resistance is very low. Pretreatment INSTI mutations do not appear to represent clustered HIV transmission.

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Introduction

Integrase strand transfer inhibitors (INSTIs) are part of recommended first-line regimens for the treatment of HIV infection [1]. As observed with other classes of antiretroviral medications, increasing use of INSTIs and treatment failures on INSTIs may subsequently lead to an increase in pretreatment INSTI resistance [2,3]. Although there are two case reports of antiretroviral-naive individuals with major INSTI mutations in the context of multiclass antiretroviral drug resistance [4,5], to date, major INSTI mutations are rare among cohorts of treatment-naive individuals in Europe [6–10], the Middle East [11], and the United States [12].

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There are currently no data on pretreatment INSTI resistance among HIV-positive individuals in the South, the epicenter of the HIV epidemic in the United States. Currently, the United States Department of Health and Human Services recommends routine pretreatment reverse transcriptase and protease resistance testing but recommends INSTI resistance testing only if transmitted INSTI resistance is a concern (e.g. in the setting of multiclass drug resistance) [13]. In an analysis of INSTI resistance in the United States, 16.5% of HIV-positive patients in the south with INSTI resistance testing between 2009 and 2012 had a major mutation [14]. This analysis, however, did not distinguish between treatment-naïve and treatment-experienced patients. HIV-positive patients initiating antiretroviral therapy at the University of North Carolina from 1996 to 2014 whose initial regimen contained an INSTI were less likely to discontinue therapy and less likely to experience virologic failure compared to those whose initial regimen did not contain an INSTI [15]. The durability of INSTI-containing regimens likely captures their safety, tolerability, and efficacy, factors that might reduce the risk of development and subsequent transmission of resistance compared to other regimens [16].

The present study has three main objectives. First, we define the prevalence of INSTI resistance mutations among treatment-naive and treatment-experienced individuals in North Carolina from 2010 to 2016. Second, we assess the sociodemographic and clinical characteristics of patients with INSTI resistance mutations. Finally, we use HIV sequences to construct transmission clusters and phylogenetic trees to investigate transmission networks of individuals with INSTI resistance mutations.

Methods

Study population

We analyzed HIV-1 sequences derived from samples sent to the largest referral laboratory in North Carolina (Laboratory Corporation of America, Research Triangle Park, North Carolina, USA) for genotypic resistance testing from 16 November 2010 through 22 September 2016. We linked sequence data to the North Carolina State Division of Public Health's Enhanced HIV/AIDS Reporting System that included age, sex, race/ethnicity, transmission risk category, $CD4^+$ cell count and viral load at the time of genotyping, and dates of diagnosis and genotypic resistance testing. We included individuals who were at least 18 years of age at resistance testing. The final dataset included 8825 individuals with 12 159 sequences for protease, reverse transcriptase, or INSTI resistance testing during the study period; 2784 (31%) of these individuals had 3162 sequences for INSTI resistance testing. With the exception of the cluster analysis described below, the 2784 individuals with INSTI resistance testing represent the population of interest for all analyses.

We examined diagnosis and sequence dates in Enhanced HIV/AIDS Reporting System to define individuals as treatment naive and treatment experienced. The Centers for Disease Control and Prevention estimate that 80% of HIV-positive people linked to care within 3 months of diagnosis in 2011 [17] and that 72% of blacks and 79% of whites with HIV infection linked to care within 1 month of diagnosis in 2014 [18]. Thus, we defined an individual as treatment naive if their first genotypic resistance test was sent within 3 months of diagnosis to capture the majority of newly diagnosed individuals linking to care for the first time. We classified individuals as treatment experienced if their first genotypic resistance test was sent more than 3 months after diagnosis.

The University of North Carolina Institutional Review Board (IRB #16–2345) approved this study.

Definition of resistance mutations

Based on the 2015 International Antiviral Society Update of the Drug Resistance Mutations in HIV-1, we defined major INSTI mutations as: T66I, E92Q, F121Y, Y143RHC, S147G, Q148HKR, and N155H [19]. Minor or accessory mutations were defined as: T66AK, L74M, E92G, T97A, E138AK, G140AS, and R263K.

Genotyping and analysis of nucleotide sequence data

Genotypic resistance testing was performed using GenoSure MG (Monogram Biosciences, South San Francisco, California, USA), GenoSure Integrase (Laboratory Corporation of America, Research Triangle Park, North Carolina, USA), and GenoSure PRIme (Monogram Biosciences). We identified INSTI, protease, and reverse transcriptase mutations using the Stanford University HIV Drug Resistance Database genotypic resistance interpretation algorithm with Sierra v1.1 [20]. We confirmed HIV subtypes using the context-based modeling for expeditious typing tool [21].

Cluster analysis

We performed a molecular cluster analysis using HIV-TRACE, available at www.hivtrace.org [22] to describe the transmission networks of treatment-naive individuals with INSTI resistance mutations. We included all 8825 individuals with genotypic resistance testing in the cluster analysis. We based the analysis on the partial polymerase (*pol*) gene (2042 with protease/reverse transcriptase, and INSTI genotypes, 6656 with only protease/reverse transcriptase sequences, and 127 with only INSTI sequences) using the first available sequence per patient in the 2010–2016 study period. We aligned sequences to HBX2 using multiple sequence comparison by log expectation (MUSCLE); and edited sequences manually for gapped positions [23]. We identified pairs of sequences whose pairwise genetic distance was 0.015 or less expected substitutions per site divergent based on the Tamura–Nei 93 substitution model implemented in

HIV-TRACE as putative linkage between individuals [24]. These linkages were constructed into clusters composed of at least two linked individuals. We counted any matching resolutions in nucleotide ambiguities as a perfect match.

Phylogenetic analysis

We then performed a phylogenetic analysis to identify clades defined by INSTI resistance mutations. For this analysis, we used the first INSTI sequence available during the 2010–2016 study periods from the 2784 individuals with INSTI resistance testing. Sequences were aligned as above using MUSCLE and a maximum-likelihood tree was constructed in FastTree v.2.1.4 with the general time reversible model of nucleotide substitution [25,26]. The purpose of this analysis was to evaluate for any INSTI resistance mutations circulating in clades at larger genetic thresholds than would be identified in the HIV-TRACE analysis. Statistical support of clades was assessed with local support values [Shimodaira–Hasegawa-like (SH-like) test] in FastTree.

Statistical analysis

We used the χ^2 test to compare the distributions of categorical variables and the Kruskal–Wallis test for continuous variables. We calculated the prevalence of INSTI resistance mutations over the study period and by year for those whose first genotype was sent 3 or less months after diagnosis and for those whose first genotype was sent more than 3 months after diagnosis. We calculated binomial exact 95% confidence interval (CI) for prevalence estimates. We performed a sensitivity analysis and calculated the prevalence of INSTI resistance mutations using alternative cutoffs of 1 month and 6 months to define the treatment-naïve population. We used logistic regression to determine trends in the prevalence of INSTI resistance mutations over the study period. Statistical significance was defined at the *P* value less than 0.05 levels. We used STATA 14.2 for all analyses (College Station, Texas, USA).

Results

Between 2010 and 2016, 2784 individuals contributed 3162 INSTI sequences [2289 (72%) by GenoSure PRIme including protease/reverse transcriptase and INSTI sequencing and 873 (28%) by GenoSure Integrase]. Of the 2784 individuals with INSTI testing, 2470 (89%) contributed one sequence and 314 (11%) contributed more than one sequence (range 2–6).

Prevalence of integrase strand transfer inhibitor resistance mutations among those with integrase strand transfer inhibitor resistance testing within 3 months of diagnosis

Both patients who had first resistance testing 3 or less months and more than 3 months after diagnosis experienced an increase in INSTI resistance testing overtime (*P* for trend < 0.001 for both groups; Table 1). Compared to patients who had first resistance testing more than 3 months after diagnosis, patients with testing 3 or less months after diagnosis were less likely to have an INSTI sequence from 2010 to 2013; were younger; and, more likely to be men, white or Hispanic, and identify as men who have sex with men. Those with first resistance testing 3 or less months after diagnosis had a greater viral load and $CD4^+$ cell count, and a shorter duration to first genotype.

About 840 of 2784 (30%) individuals provided their first sample for INSTI resistance testing 3 or less months after diagnosis. In total 20 (2.4%, 95% CI: 1.5, 3.6%) of these individuals had INSTI mutations, 18 (2.1%, 95% CI: 1.3, 3.4%) with only a minor mutation and two (0.2%, 95% CI: 0.02, 0.9%) with a major mutation (Table 2). Both individuals with major INSTI mutations had concomitant reverse transcriptase resistance mutations.

The median age of the 20 individuals with INSTI mutations was 27.5 (interquartile range; IQR 24–41). In total 80% were men, 80% identified as black, and the most common transmission risk was sex with another men. All had subtype B virus. Median HIV viral load was 29 800 copies/ml (range: 600–6 856 570 copies/ml) and median $CD4^+$ cell count was 666 (range: 5–1148 cells/μl). Median time from HIV diagnosis to first genotype was 32 days (range: 4–74 days). In total 11 (55%) had a T97A/T mutation, 6 (30%) had a L74M mutation, and 1 (5%) had an E138K mutation. Two patients had major mutations: one with an S147G major mutation and a T66A minor mutation (Patient 3) and one with an N155H major mutation without minor mutations (Patient 20). The most common reverse transcriptase mutations were K103N (25%) followed by G190A (5%), M184V (5%), and D67N (5%). There were no major protease mutations. Patient 20 was identified through the acute infection program of the North Carolina Division of Public Health.

Sensitivity analysis of the definition of treatment-naive individuals

Using a definition of treatment-naive individuals as those with a genotype 1 month or less after HIV diagnosis, the prevalence of any INSTI mutation was 10/520 or 1.9% (95% CI: 0.9, 3.5%). One patient, patient 20 who was identified with acute infection, had a major mutation (0.2%, 95% CI: 0.005, 1.1%). Using a cutoff of 6 months or less, the prevalence of any INSTI mutation was 22/908, or 2.4% (95% CI: 1.5, 3.6%). Three had a major mutation (0.3%, 95% CI: 0.07, 1.0%). The additional patient captured by increasing the cutoff to 6 months had an N155H mutation with seven reverse transcriptase mutations: M41L, D67N, V75M, M184V, L210W, T215Y, K103N, and E138Q.

Table 1. Sociodemographic and clinical characteristics of HIV-positive individuals by timing of first integrase strand transfer inhibitor resistance testing after HIV diagnosis, North Carolina, 2010–2016.

	Individuals with first genotype 3 or less months after diagnosis ($n = 840$)	Individuals with first genotype more than 3 months after diagnosis ($n = 1944$)	*P* value
Sociodemographic characteristics			
Year			<0.001
2010	0	6 (<1)	
2011	3 (<1)	50 (3)	
2012	7 (<1)	76 (4)	
2013	13 (1)	80 (4)	
2014	135 (16)	340 (17)	
2015	355 (42)	672 (34)	
2016	327 (39)	720 (37)	
Age, median (IQR)	30 (24–44)	43 (32–50)	<0.001
Sex			<0.001
Male	685 (82)	1378 (71)	
Female	155 (18)	566 (29)	
Race/ethnicity			<0.001
Black	537 (64)	1452 (74)	
White	190 (23)	337 (17)	
Hispanic/Latino	85 (10)	87 (4)	
Native American	8 (<1)	2 (<1)	
Asian, Pacific Islander	6 (<1)	8 (<1)	
Multiracial	14 (2)	57 (3)	
Transmission risk			
MSM (includes MSM/IDU)	522 (62)	910 (47)	<0.001
IDU	16 (2)	129 (6)	
Heterosexual	104 (12)	305 (16)	
Other	198 (23)	600 (31)	
Clinical characteristics			
Subtype B	820 (98)	1908 (98)	0.361
HIV viral load (copies/ml), median (IQR)	43081 (14970–115538) [$n = 574$]	21981 (3000–79700) [$n = 1278$]	<0.001
$CD4^+$ cell count (cells/μl), median (IQR)	392 (216–575) [$n = 574$]	283 (113–488) [$n = 1084$]	<0.001
Time to first sequence, days, median (IQR)	24 (12–41)	3639 (1885–5819)	<0.001

Data are presented as *n* (%) unless otherwise specified. Numbers may not add to total because of missing data. HET, heterosexual; IDU, intravenous drug use; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; MSM, men who have sex with men.

Prevalence of integrase strand transfer inhibitor mutations among those with integrase strand transfer inhibitor resistance testing 3 or more months after diagnosis

Of the 1944 individuals who provided their first sample for INSTI genotypic resistance testing more than 3 months after diagnosis, 187 (9.6%, 95% CI: 8.3, 11.0%) had INSTI mutations. Of these 187 individuals, 50 (27%) had only minor mutations, 128 (68%) had one major mutation (with or without minor mutations), and 9 (5%) had two major mutations (with or without minor mutations). Overall, the prevalence of any major mutation was 137/1944 or 7.0% (95% CI: 5.9, 8.3%). The prevalence of any and major INSTI resistance decreased overtime. In 2010, 2/6 (33%, 95% CI: 4.3, 78%) had any resistance mutation, whereas in 2016 54/720 had any resistance mutation (7.5%, 95% CI: 5.7, 9.7%; *P* for trend <0.001; Fig. 1a). One of six (17%, 95% CI: 0.4, 64%) patients in 2010 and 36/720 (5.0%, 95% CI: 3.5, 6.8%) in 2016 had major mutations (*P* for trend <0.001; Fig. 1b).

Table 3 describes the sociodemographic and clinical characteristics of those with no, minor, and major mutations detected more than 3 months after diagnosis. Few patients had two major mutations. Of the 10 patients with two major INSTI mutations, three had S147G, Q148R, and E138K mutations; two had S147 and N155H mutations; and one each had E138EK, S147GS, Q148QR; G140GS, Y143CHRY, Q148HQ; G140S, Q148H, N115H; L74M, T97A, Y143C, S147G; and, T97AT, G149GS, Q148QE, N115HN mutations.

Cluster analysis

Of the 8825 individuals who contributed sequences between 2010 and 2016, 2899 (33%) comprised 774 clusters. In total 12 of the 20 individuals with INSTI mutations in sequences collected within 3 or less months of diagnosis were members of 10 distinct clusters with median size three (IQR 2–8) and median node degree of one (IQR 1–2.5; Fig. 2). In total 41% (55/93) of cluster members had INSTI resistance testing. We observed two clusters involving at least two INSTI mutations (clusters 270 and 222). In cluster 270, the pretreatment patient had a T97A mutation and the treatment-experienced cluster member had an R263KR mutation. In cluster 222, all three members had L74M and K103N mutations. We identified only one patient with pretreatment major INSTI resistance in a cluster. Patient 3 with an S147G major mutation was part of a cluster 1319; he and the

Table 2. Sociodemographic, clinical, and transmission cluster characteristics of individuals with integrase strand transfer inhibitor mutations captured within 3 months of HIV diagnosis, North Carolina, 2010–2016.

Subject	Year	Age	Sex	Race	Risk	HIV VL, (copies/ml)	$CD4^+$ (cells/μl)	Days to genotype	INSTI mutations	Reverse transcriptase/ protease mutations	Cluster characteristics	
											ID, size	Degree
1	2012	24	Male	Black	MSM	ND	ND	34	L74M	None		
2	2014	22	Male	Black	MSM	600	387	24	T97A	None	270, 4	1
3	2015	45	Male	Black	HET	141 910	666	42	T66A, S147G	G190A	1319, 2	1
4	2015	40	Male	Black	HET	6450	486	30	T97AT	K103N	366, 14	4
5	2015	22	Male	Black	MSM	54 635	702	12	T97AT	None	1358, 2	1
6	2015	42	Male	White	NIR	6 856 570	5	35	E138K	None		
7	2015	20	Male	Black	NIR	5410	805	23	L74M	None	1299, 2	1
8	2015	32	Male	Black	MSM	288 872	383	34	T97A	None	151, 8	1
9	2015	37	Male	Hispanic	MSM	11 676	775	54	T97A	None		
10	2015	26	Male	Black	MSM	46 326	155	23	T97AT	None	1452, 2	1
11	2015	24	Male	Hispanic	MSM	847	760	74	T97AT	None		
12	2015	24	Male	Black	MSM	324 720	656	53	T97A	K103N	211, 8	3
13	2015	19	Male	Black	MSM	15 200	836	13	T97A	None	22, 48	11
14	2015	52	Female	Black	HET	23 400	964	39	L74M	K103N	222, 3	2
15	2016	32	Female	Black	NRR	46 630	572	15	L74M	K103N	222, 3	1
16	2016	29	Male	Black	MSM	29 800	538	68	L74LM	None		
17	2016	24	Female	White	HET	1450	1148	14	T97A	None		
18	2016	65	Male	Black	NRR	224 380	13	59	T97A	None		
19	2016	42	Female	Black	NRR	8828	732	4	L74M	K103N	222, 3	1
20	2016	25	Male	Black	MSM	30 554	760	20	N155H	D67N, M184V		

HET, heterosexual; ID, identification; INSTI, integrase strand transfer inhibitor; MSM, men who have sex with men; ND, not done; NIR, adult with no identifiable risk; NRR, adult with no reported risk; PR, protease inhibitor; RT, reverse transcriptase; VL, viral load.

treatment-experienced cluster member shared a G190A mutation, but the cluster member did not have INSTI genotypic resistance testing. Patient 20, the patient with acute infection and an N155H major mutation, was not a member of a molecular cluster.

Phylogenetic analysis

We identified nine clades with at least two sequences with identical INSTI resistance mutations. Three clades included pretreatment patients with INSTI mutations in clusters 211, 222, and 270. Cluster 270 had a patient with a pretreatment T97A mutation and a treatment-experienced patient with an R263K mutation; phylogenetic analysis included an additional treatment-experienced patient with T97A and F121Y mutations (SH-like test = 0.96). In cluster 211, a cluster member whose first sequence in the study period did not contain an INSTI region, had a subsequent, but initial INSTI sequence with a T97A mutation on phylogenetic analysis (SH-like test = 1.0); this cluster also included three sequences without INSTI mutations. Cluster 222 included no additional sequences or INSTI mutations (SH-like test = 0.96). The six additional clades included treatment-experienced individuals interspersed with

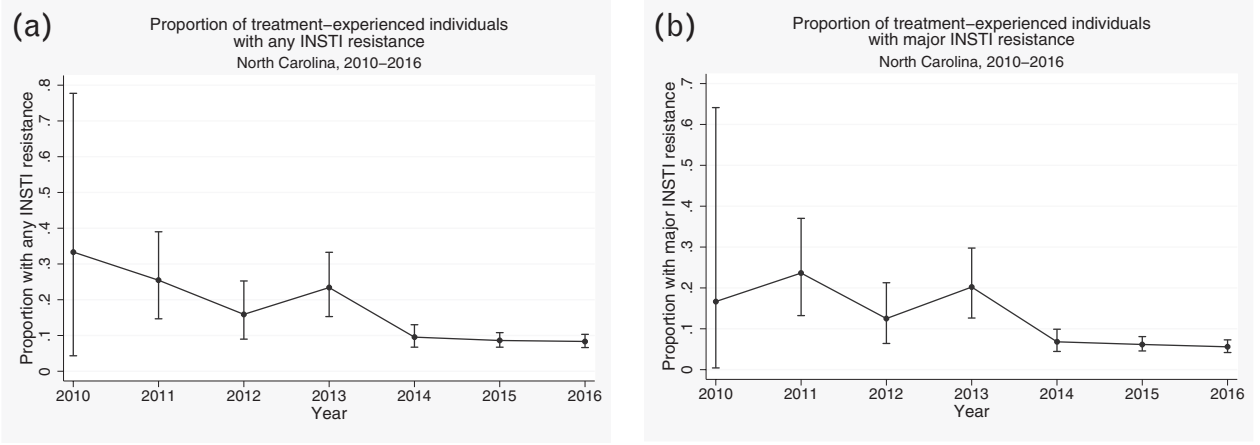


Fig. 1. (a) Prevalence of any INSTI resistance among treatment-experienced patients in North Carolina, 2010–2016. (b) Prevalence of major INSTI resistance among treatment-experienced patients in North Carolina, 2010–2016. Error bars indicate 95% CIs for each prevalence estimate. CI, confidence interval; INSTI, integrase strand transfer inhibitor.

Table 3. Sociodemographic, clinical, and transmission cluster characteristics of HIV-positive individuals with and without integrase strand transfer inhibitor mutations on genotype testing more than 3 months after HIV diagnosis, North Carolina, 2010–2016.

	No INSTI mutations ($n=1757$)	Only minor INSTI mutations ($n=50$)	Any major INSTI mutation ($n=137$)	*P* value
Sociodemographic characteristics				
Year				<0.001
2010	4 (<1)	1 (2)	1 (<1)	
2011	38 (2)	1 (2)	11 (8)	
2012	66 (4)	2 (4)	8 (6)	
2013	61 (3)	2 (4)	17 (12)	
2014	307 (17)	10 (20)	23 (17)	
2015	615 (35)	16 (32)	41 (30)	
2016	666 (38)	18 (36)	36 (26)	
Age, median (IQR)	42 (32–51)	38 (29–48)	46 (37–50)	0.015
Sex				0.431
Female	512 (29)	18 (36)	36 (26)	
Male	1245 (71)	32 (64)	101 (74)	
Race/ethnicity				0.904
Black	1311 (75)	38 (76)	104 (76)	
White	306 (17)	11 (22)	20 (15)	
Hispanic/Latino	80 (4)	0	7 (5)	
Native American	2 (<1)	0	0	
Asian, Pacific Islander	7 (<1)	0	1 (<1)	
Multiracial	51 (3)	1 (2)	5 (4)	
Transmission risk				0.722
MSM (includes MSM/IDU)	820 (47)	26 (52)	64 (47)	
IDU	118 (7)	2 (4)	9 (7)	
Heterosexual	271 (15)	11 (22)	23 (17)	
Other	548 (31)	11 (22)	41 (30)	
Clinical characteristics				
Subtype B	1727 (98)	47 (94)	134 (98)	0.081
HIV viral load (copies/ml), median (IQR)	24060 (3055–80520) [$n=1176$]	31420 (2930–92350) [$n=37$]	6000 (2266–34589) [$n=65$]	0.051
$CD4^+$ cell count (cells/μl), median (IQR)	285 (113–490) [$n=1011$]	276 (95–430) [$n=25$]	273 (102–470) [$n=48$]	0.590
Years to first sequence, median (IQR)	9 (5–15)	9.5 (3–15)	12 (7–18)	<0.001
NRTI resistance mutations	262 (15)	15 (30)	66 (48)	<0.001
NNRTI resistance mutations	381 (22)	15 (30)	38 (28)	0.109
PI resistance mutations	66 (4)	2 (4)	11 (8)	0.051
Extent of resistance (excludes INSTI resistance)				<0.001
Single class	355 (20)	17 (34)	30 (22)	
Dual class	141 (8)	6 (12)	35 (25)	
Triple class	24 (1)	1 (2)	5 (4)	
INSTI minor mutations				NA
T66AK		2 (4)	0	
T97A		32 (64)	19 (14)	
L74M		8 (16)	10 (7)	
E92G		4 (8)	4 (3)	
E138AK		2 (4)	13 (9)	
G140AS		1 (2)	32 (23)	
R263K		6 (12)	0	
INSTI major mutations				
T66I			0	NA

Table 3 (*continued*)

	No INSTI mutations ($n = 1757$)	Only minor INSTI mutations ($n = 50$)	Any major INSTI mutation ($n = 137$)	*P* value
E92Q			34 (25)	
F121Y			1 (<1)	
Y143RHC			20 (15)	
S147G			10 (7)	
Q148HKR			51 (37)	
N155H			67 (49)	

Data are presented as *n* (%) unless otherwise specified. Numbers may not add to total because of missing data. HET, heterosexual; IDU, intravenous drug use; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; MSM, men who have sex with men; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

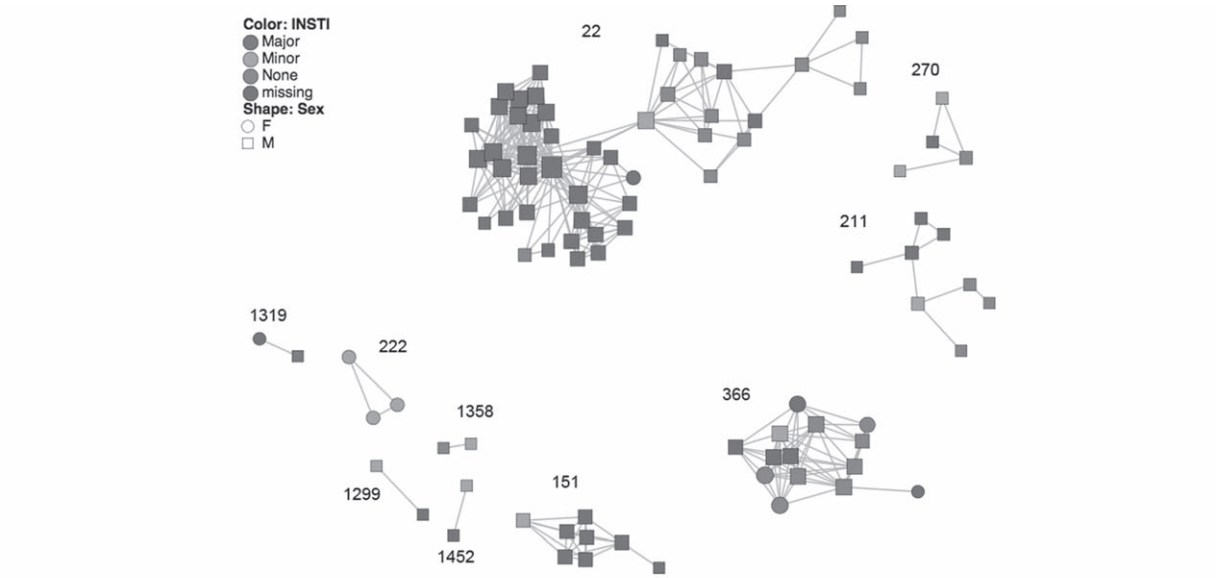
individuals without INSTI mutations. The full phylogenetic tree is available as Supplementary Figure 1, http://links.lww.com/QAD/B142.

Discussion

We found a low prevalence (0.2%) of major INSTI resistance among patients with genotypes collected within 3 months of an HIV diagnosis. To our knowledge, this is the largest North American sample of individuals with INSTI resistance testing to date. This low prevalence of transmitted major INSTI mutations is consistent with prior reports from Europe [6–10] and small sample of patients with primary infection in Seattle, Washington, USA [12]. A sensitivity analysis of alternate cutoffs to define the treatment-naive population yielded similarly low prevalence estimates.

Two patients had pretreatment major INSTI mutations. One individual had an S147G major mutation, a nonpolymorphic mutation associated with elvitegravir resistance, as well as a T66A minor mutation that has also been associated with elvitegravir resistance in combination with other INSTI mutations [27]. Neither of these mutations appear to affect susceptibility to dolutegravir or raltegravir. A second patient had an N155H mutation and was diagnosed during acute infection. Viruses with an N155H mutation show high-level resistance to raltegravir and elvitegravir and low-level resistance to dolutegravir [28,29]. This patient also had an M184V mutation as well as D67N, a thymidine analogue mutation. This particular thymidine analogue mutation has been described in treatment-naive patients as viruses with D67N retain replicative efficiency and can, thus, be transmitted [30,31]. Transmitted M184V mutations appear to occur in settings where the viral load of the population of patients who are failing treatment is high because of the lower fitness of viruses with this mutation [3]. Only the patient with the S147G major mutation was part of a transmission cluster; this patient and the cluster member shared a G190A mutation. The cluster member did not have INSTI genotypic resistance testing and we cannot know if they also shared INSTI mutations.

Minor INSTI mutations in the treatment-naive population concentrated among young, black men who have sex with men. The most common minor mutations among treatment-naive patients were T97A and L74M, natural polymorphisms that have been found in individuals without prior INSTI exposure and prior to the widespread use of INSTIs [32–34]. We also observed clustering of three women with both pretreatment L74M and K103N mutations (cluster 222). Although prior studies have documented clustering of individuals with K103N mutations [3,35,36], likely because of preserved or increased replicative fitness of viruses with this



Cluster	Size	Median degree (IQR)	Sex	Race	Transmission Risk	Index INSTI mutation	Index RT/PR mutations	Cluster member INSTI mutations	Cluster member RT/PR mutations
22	48	4 (2-6.5)	Male (47), Female (1)	Black (41), white (4), Hispanic (3)	MSM (35), HET (1), NRR (12)	T97A	None	None (12); not done (35)	E138A (1), Y188L (1), K103N (1)
151	8	1 (1-2)	Male (8)	Black (5), white (2), multiracial (1)	MSM (8)	T97A	None	Not done (7)	None (7)
211	8	1.5 (1, 2.5)	Male (8)	Black (5), white (2), multiracial (1)	MSM (8)	T97A	K103N	None (3); not done (4)	K103N (6), P225H (3)
222	3	1 (1-2)	Female (3)	Black (3)	HET (1), NRR (2)	L74M (3)	K103N (3)	NA	NA
270	4	2 (1.5, 2.5)	Male (4)	Black (4)	MSM (4)	T97A	None	R263KR (1); none (1); not done (1)	None
366	14	3.5 (2, 5)	Male (9), Female (5)	Black (10), white (3), Hispanic (1)	MSM (3), HET (4), NRR (7)	T97AT	K103N	None (8), not done (5)	K103N (8)
1299	2	1	Male (2)	Black (1), white (1)	MSM (1), NIR (1)	L74M	None	Not done	None
1319	2	1	Male (1), Female (1)	Black (2)	HET (2)	T66A, S147G	G190A	Not done	G190A, M184V, V106M
1358	2	1	Male (2)	Black (2)	MSM (2)	T97AT	None	None	None
1452	2	1	Male (2)	Black (1), white (1)	MSM (2)	T97AT	None	Not done	None

Numbers in parentheses indicate number of cluster members with each attribute
HET, heterosexual; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; MSM, men who have sex with men; NIR, adult with no identifiable risk; NRR, adult with no reported risk; RT, reverse transcriptase; PR, protease inhibitor

Fig. 2. Molecular transmission clusters including individuals with pretreatment INSTI resistance mutations in North Carolina, 2010–2016. INSTI, integrase strand transfer inhibitor.

mutation, none have documented similar clustering of individuals with L74M mutations. As sexual transmission of HIV between women is rare [37], it is likely that additional (men) members of the transmission cluster are missing in our data (i.e. genotyping was performed before 2010 or performed elsewhere; the individual is not linked to care; or the individual remains undiagnosed).

Phylogenetic analysis revealed three clades that included five patients with pretreatment INSTI resistance who were also part of clusters identified by molecular cluster analysis. Only cluster 222 represented potentially clustered transmission of minor INSTI mutations (L74M). Other clades and clusters with sequences with identical INSTI mutations were interspersed with sequences with no or other INSTI mutations or with sequences that frequently contained the same reverse transcriptase mutations. For example, in cluster 211, most individuals (75%) had K103N mutations, suggesting clustered transmission of K103N. Phylogenetic analysis showed that this clade included only two individuals with T97A mutations; a pretreatment patient and a cluster member who only had an initial protease/reverse transcriptase sequence in the molecular cluster analysis and a subsequent, initial INSTI sequence more than 3 months after diagnosis.

The prevalence of any and major INSTI mutation among those with resistance testing more than 3 months after diagnosis was 9.6 and 7.0%, respectively. The latter estimate is lower than the 15.6% prevalence of major INSTI mutations in the United States between 2009 and 2012 [14], but we also observed a decrease in the prevalence of any and major mutations overtime. There are a few explanations for this decrease. First, there has been an increase in the collection of INSTI genotypic resistance testing overtime. If the actual number of patients with INSTI mutations remained the same overtime (the numerator) but the number of individuals on whom tests were sent increased overtime (the

denominator), the prevalence would be lower. Second, if the denominator became enriched with treatment-naive individuals or treatment-experienced patients without INSTI exposure overtime, the prevalence of INSTI resistance would also appear lower. The use of GenoSure PRIme resistance testing for protease/reverse transcriptase and INSTI mutations, particularly in pretreatment patients, may contribute to this explanation. Finally, the decrease may reflect the effectiveness of HIV treatment in patients treated with a regimen with an INSTI backbone, particularly regimens containing dolutegravir [38,39], which have increased over the same time period [40].

The study has several limitations. First, without information on treatment history, we have likely misclassified some treatment-naive individuals as treatment experienced, particularly newly-diagnosed individuals who present to care or start treatment more than 3 months after diagnosis. Our sensitivity analysis, however, yielded similar prevalences to the primary analysis. Second, we restricted our cluster and phylogenetic analyses to individuals who provided at least 1 sequence for genotypic resistance testing between 2010 and 2016 from a single laboratory group. Thus, we are missing clusters containing individuals with sequences sent elsewhere for genotypic resistance testing, individuals with genotypes collected outside the study period, and individuals without genotype testing because of lack of care engagement. Finally, there was significant missingness in viral load and $CD4^+$ data which may limit the validity of comparisons based on these data.

In a large sample of HIV-positive patients in North Carolina, we found the prevalence of transmitted major INSTI resistance to be very low. Additionally, pretreatment INSTI resistance is largely because of minor mutations that are natural polymorphisms that are unlikely to impact treatment outcome. These polymorphisms do not appear to indicate clustered HIV transmission in this population. Nonetheless, INSTI mutation surveillance remains important in the setting of increasing use of INSTIs in the United States and worldwide.

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A.M.D. and J.J.E. conceived the study. T.W.M. and A.M.D. designed the study. R.B. and E.S. procured, prepared, and matched public health and sequence data. T.W.M. performed the analyses and drafted the manuscript. All authors reviewed the manuscript and made significant intellectual contributions to the final product.

Conflicts of interest

There are no conflicts of interest.

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