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Lack of resistance to integrase inhibitors among antiretroviral-naive subjects with primary HIV-1 infection, 2007–2013

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Abstract

Background—U.S. guidelines recommend genotyping for persons newly diagnosed with HIV infection to identify transmitted drug resistance mutations associated with decreased susceptibility to NRTIs, NNRTIs, and PIs. To date, testing for integrase strand transfer inhibitor (INSTI) mutations has not been routinely recommended. We aimed to evaluate the prevalence of transmitted INSTI mutations among persons with primary HIV-1 infection in Seattle, WA.

Methods—Persons with primary HIV-1 infection have enrolled in an observational cohort at the University of Washington Primary Infection Clinic since 1992. We performed a retrospective analysis of plasma specimens collected prospectively from the 82 antiretroviral-naive subjects who were enrolled from 2007–13, after FDA-approval of the first INSTI. Resistance testing was performed by consensus sequencing.

Results—Specimens for analysis had been obtained a median of 24 (IQR 18–41, range 8–108) days after the estimated date of HIV-1 infection. All subjects were infected with HIV-1 subtype B except for one subject infected with subtype C. Consensus sequencing identified no subjects with major INSTI mutations (T66I, E92Q, G140S, Y143C/H/R, S147G, Q148H/K/R, N155H). Using exact binomial confidence intervals, the upper bound of the 95% CI was 4.4%.

Conclusions—Although our sample size was small, this study does not support the need at this time to evaluate integrase mutations as part of routine consensus sequencing among persons newly diagnosed with HIV-1 infection. However, it is likely that the prevalence of transmitted INSTI mutations may increase with the recent commercial introduction of additional INSTIs and presumably greater INSTI use among persons living with HIV-1.

Introduction

In the United States, guidelines recommend genotypic resistance testing for persons newly diagnosed with HIV-1 infection to identify transmitted drug resistance (TDR) mutations associated with decreased susceptibility to nucleoside and non-nucleoside reverse transcriptase (RT) inhibitors and protease inhibitors [1]. This recommendation followed findings from public health surveillance that TDR can be identified in greater than 10% of newly infected persons in the United States [2–4] and may impact the effectiveness of antiretroviral (ARV) therapy [3].

Raltegravir became the first FDA-approved integrase strand transfer inhibitor (INSTI) in 2007. Fortunately, while cases of transmitted INSTI resistance have been reported [5,6], major INSTI mutations have been rarely identified in cross-sectional surveys of ARV-naïve patients in Europe [7–10]. U.S. guidelines therefore only recommend testing for INSTI resistance when clinically suspected [1]. One challenge is that the Centers for Disease Control and Prevention Variant, Atypical, and Resistant HIV Surveillance System never included monitoring for INSTI mutations and active surveillance of TDR nationally was discontinued in 2012. A new program called Molecular HIV Surveillance that provides passive surveillance of viral resistance reports done clinically and can collect INSTI mutations is underway but not yet fully operational. At the Harborview Madison Clinic (the largest provider of HIV care in Washington) and University of Washington (UW) Virology Clinic, clinical testing for INSTI resistance remains uncommon despite inclusion of an INSTI in nearly one-third of ARV regimens in 2013 [Dr. Mari Kitahata, personal communication].

Transmitted drug resistance mutations are more likely to be detected in persons with recent compared to established infection [11] for a variety of reasons. We estimated the prevalence of transmitted INSTI mutations among ARV-naïve persons with primary HIV-1 infection in Seattle, Washington in order to assist decision-making regarding resistance testing for persons newly diagnosed with HIV-1 infection.

Methods

Patient population

Persons with primary HIV-1 infection have enrolled in an observational cohort at the UW Primary Infection Clinic since 1992 [12–14]. All subjects in the cohort were HIV antibody-negative with detectable HIV RNA (acute infection) or HIV antibody-positive with a negative or indeterminate Western Blot, negative "detuned" antibody test, or negative HIV test within one year of screening (early infection). Subjects for this analysis were enrolled within 30 days of HIV infection, estimated to be the date of onset of seroconversion symptoms [15] or, for asymptomatic subjects, the midpoint between the last negative and first positive HIV tests. We performed a retrospective analysis of plasma specimens collected prospectively from the 82 ARV-naïve subjects enrolled from 2007–2013. The UW Institutional Review Board approved these studies, and subjects gave written consent for study participation.

Plasma RNA extraction and reverse transcription

RNA was extracted from 0.5 or 1 mL of plasma as previously described [16], and nucleic acids were reverse transcribed (BluePrint 1st strand cDNA Synthesis Kit, Clontech Laboratories, Inc.; Mountain View, CA) and random hexamer primers. The *pol* region encoding *integrase* (IN) was PCR-amplified (MyTaq DNA polymerase, Bioline USA Inc.; Taunton, MA, in nested reactions: 1st-round PCR primers: polF_4053 (5' AGTAAACATAGTAACAGACTCACARTATG-3') and polR_5087 (5'-CCTAGTGGGATGTGTACTTCTGAACTTA-3'), and 2nd-round PCR: polF_4195 (5'-GGRATTGGAGGAAATGAACAAGTAGA-3') and polR_5193 (5'-GTTTTACTAACTTTTCCATGTTCTMTCTCAT-3'). Amplicons were visualized in a 1% agarose gel, and, if a sample did not amplify or double bands were visualized, alternate primers were used: polF_4310 (5'-CACAGTAATTGGAGAGCAATGGCTAGTGATTTTAA-3') and polR_5194 (5'-CATTCTATGGAGACYCCMTGACCCA-3') in 1st-round; polF_4357 (5'-GTAGCAAAGAAATAGTAGCCAGCTGTGATAAAT-3') and polR_5282 (5'-CCTARTGGGATGTGTACTTCTGAACT-3') in 2nd round (Complete protocol available, on request, Lfrenkel@uw.edu).

Consensus sequencing

Second round PCR products were directly sequenced as previously described [17], spanning IN codons 1–288 or 36–288. Sequences were assembled with Sequencher (version 4.9; Gene Codes Corp, Ann Arbor, MI) and submitted to the Stanford University HIV Drug Resistance Database (version 6.3.0; <http://hivdb.stanford.edu> on August 21, 2013) to identify "major" INSTI mutations (T66I, E92Q, G140S, Y143C/H/R, S147G, Q148H/K/R, and N155H).

Statistical analyses

Results are presented primarily as descriptive analyses. The upper bound of the prevalence of transmitted INSTI resistance mutations was estimated using exact binomial confidence intervals (CIs).

Results

Demographic and baseline characteristics of subjects are shown [Table]. Plasma specimens for analysis had been obtained a median of 24 (IQR 18–41, range 8–108) days after the estimated date of HIV-1 infection. Mean HIV-1 RNA level at the time of evaluation was 4.9 (s.d. 1.2) log₁₀ copies/mL.

All subjects but one were infected with HIV-1 subtype B; one subject had subtype C. Consensus sequencing identified no subjects with major INSTI mutations. Using exact binomial confidence intervals, the upper bound of the 95% CI was 4.4%. One subject had L74M, which confers potential low-level resistance to raltegravir and elvitegravir [18] and has been found in association with major integrase resistance and as a natural polymorphism [19,20]. Viral polymorphisms in integrase were identified in 24 (28%) subjects, including V54I (n=1), L74I (n=7), V151I (n=3) M154I (n=2), M154L (n=2), G163E (n=1), I203M (n=2), and S230N (n=6).

In contrast to the lack of major INSTI mutations, 17 subjects had RT mutations and one subject had both RT mutations and major protease mutations [Table].

Discussion

Like cross-sectional surveys from Europe, this survey did not identify major INSTI mutations among antiretroviral-naïve persons who acquired HIV-1 infection after FDA-approval of the first INSTI in 2007. This contrasted with the presence of RT or protease mutations in 22% of our cohort. Although our sample size was small, these data do not currently support the need to evaluate integrase mutations as part of routine consensus sequencing among persons with newly acquired or diagnosed HIV-1 infection.

It is possible that we might have detected major mutations in this population had we used a more sensitive assay to look for low-level mutations that have been identified in ARV-naïve persons with primary [14,21] and established [22,23] HIV-1 infection. However, the impact of minority variants on HIV-1 disease progression and ARV response appears to vary by mutation and has not yet been evaluated for INSTI resistance mutations. TDR associated with high level drug resistance (not including INSTI) was more common in men who have sex with men (MSM) compared to non-MSM newly diagnosed with HIV-1 during the same time period in King County, WA, [Susan Buskin, PhD, personal communication]. As our cohort was entirely MSM, it is unlikely that INSTI mutations would be less prevalent in our study population than in all persons acquiring HIV-1 in Seattle.

We should be prepared for the likelihood that transmission of INSTI mutations may increase with the recent commercial introduction of additional INSTIs and greater INSTI use among HIV-infected persons. One recent national survey found a significant number of INSTI resistance mutations in clinical specimens submitted for testing [24], presumably from INSTI-exposed persons. It is therefore important for public health surveillance to actively monitor INSTI resistance among ARV-naïve persons and not rely on clinical suspicion and passive laboratory reporting to determine when the threshold level of INSTI TDR has been reached.

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Table

Demographic and other baseline characteristics of 82 subjects

	Number	(% or IQR)
Gender		
Male	81	99
Transgender male	1	1
Median Age (Years)	31	25–41
Race/ethnicity		
Alaska Native/American Indian	1	1
Black/African-American	3	4
Native Hawaiian	1	1
White/Caucasian	68	83
Other/Unknown	9	11
Hispanic	13	16
HIV risk		
MSM	78	95
MSM-IDU	4	5
Year of HIV infection		
2007	19	23
2008	14	17
2009	10	12
2010	11	13
2011	14	17
2012	12	15
2013	2	2
Other major mutations detected		Number
Reverse transcriptase	M41L+T215C	1*
	K103N	7
	Y181C	8
	M184V	1
	K103+M184V	1
Protease	D30N+N88D	1*

IQR: Intraquartile range; MSM: men who have sex with men; IDU: injection drug use

* The same subject had these mutations.