Sequence Note

Analysis of *pol* Integrase Sequences in Diverse HIV Type 1 Strains Using a Prototype Genotyping Assay

Susan H. Eshleman,[1] Sarah E. Hudelson,[1] Peter Smith,[2] John Hackett, Jr.,[3] Vera Holzmayer,[3] Priscilla Swanson,[3] Sushil G. Devare,[3] and Natalia Marlowe[2]

Abstract

A prototype assay was used to genotype integrase (IN) from 120 HIV-1- infected IN inhibitor-naive adults from Argentina, Brazil, Cameroon, South Africa, Thailand, and Uganda. Subtype designations based on analysis of *pol* IN sequences were A (14), B (15), C (12), D (11), F (12), G (7), H (1), CRF01_AE (9), CRF02_AG (34), CRF22_01A1 (4), and CRF37_cpx (1). Ten (8.3%) of 120 samples had mutations associated with reduced susceptibility to the IN inhibitors, raltegravir and elvitegravir. Two samples had E92Q (both subtype B) and eight had E157Q (2A, 1C, 1D, 1F, 3 CRF02_AG). Some samples had other mutations selected by these drugs including T97A, and some had amino acid polymorphisms at positions associated with raltegravir and elvitegravir resistance. Mutations associated with other investigational HIV IN inhibitors were also identified. This suggests that HIV strains may vary in their natural susceptibility to HIV IN inhibitors.

HIV-1 INTEGRASE (IN) is a 288 amino acid protein that functions to insert HIV-1 cDNA into the chromosomes of infected cells. In 2007, the FDA approved the first HIV-1 IN inhibitor for treatment of HIV-1 infection, raltegravir (RAL, Isentress, MK-0518, Merck & Co., Inc, Whitehouse Station, NJ). Another HIV-1 IN inhibitor, elvitegravir (EVG, JTK-303, GS-9137, Gilead Sciences, Foster City, CA) is currently in clinical trials. These inhibitors block HIV-1 replication by inhibiting DNA strand transfer.[1] Mutations in the IN region of HIV-1 *pol* have been associated with RAL and EVG resistance.[2] Naturally occurring polymorphisms in HIV-1 IN have been observed in some subtype B and non-subtype B HIV-1 strains from IN inhibitor-naive individuals.[3,4] In this study, we used a prototype HIV-1 IN genotyping assay developed by Celera (Alameda, CA) to analyze genetically and geographically diverse HIV-1 strains.

Plasma samples were collected from 125 asymptomatic blood donors in Cameroon ($N = 61$), Brazil ($N = 20$), Uganda ($N = 17$), South Africa ($N = 15$), Thailand ($N = 9$), and Argentina ($N = 3$) who were subsequently found to be HIV-1 infected. Because antiretroviral therapy was not widely available in these countries at the time of sample collection (1993–2001), these individuals were assumed to be antiretroviral drug naive; furthermore, the lack of availability of IN inhibitor therapy during this time period precluded exposure to this class of compounds. Viral loads were measured using the LCx HIV RNA Quantitative assay (LCx HIV; Abbott Molecular Inc, Des Plaines, IL; not available in the United States). Viral loads ranged from 2.92 to greater than 6 $\log_{10}$ RNA copies/ml, the upper limit of quantification (ULQ) of the LCx HIV assay. The mean viral load was 4.24 $\log_{10}$ RNA copies/ml (excluding one sample with a viral load value of >ULQ).

HIV-1 RNA was isolated from 0.5 ml plasma using the ViroSeq HIV-1 Genotyping System Sample Preparation Module (Celera) and was stored at −70°C. Ten microliters of each HIV-1 RNA extract was used per single-step reverse transcription polymerase chain reaction (RT-PCR), which yielded a 1056 bp DNA fragment encompassing the entire IN gene. The prototype IN genotyping reagents incorporate a heat-labile UNG/dUTP system for contamination control. PCR products were purified using ExoSap-IT (USB Corporation, Cleveland, OH). Cycle sequencing was performed using

[1]School of Medicine, Johns Hopkins University, Baltimore, Maryland 21205.
[2]Celera, Alameda, California 94502.
[3]Abbott Diagnostics, Abbott Park, Illinois 60064.

TABLE 1. MUTATIONS IDENTIFIED IN THE SAMPLE SET THAT HAVE BEEN ASSOCIATED WITH REDUCED *IN VITRO* SUSCEPTIBILITY
OR HAVE BEEN SELECTED *IN VIVO* OR *IN VITRO* BY RALTEGRAVIR AND ELVITEGRAVIR[a]

	HIV-1 subtype/CRF[b]											
Mutation	*A* (N=14)	*B* (N=15)	*C* (N=12)	*D* (N=11)	*F* (N=12)	*G* (N=7)	*H* (N=1)	*CRF01* (N=9)	*CRF02* (N=34)	*CRF22* (N=4)	*CRF37* (N=1)	*Total* (N=120)
L74M			2 (16.7)			1 (14.3)		1 (11.1)	5 (14.7)			9 (7.5)
E92Q		2 (13.3)										2 (1.7)
T97A	1 (7.1)			1 (9.1)	1 (8.3)				2 (5.9)			5 (4.2)
V151I		4 (26.7)			1 (8.3)							5 (4.2)
E157Q	2 (14.3)		1 (8.3)	1 (9.1)	1 (8.3)				3 (8.8)			8 (6.7)
I203M			1 (8.3)					1 (11.1)				2 (1.7)
S230N				1 (9.1)								1 (0.8)

[a]The number and percentage (in parentheses) of each mutation are shown for each subtype/CRF. Other amino acid substitutions were detected at positions of mutations that are either associated with reduced susceptibility to RAL or EVG, or selected by these drugs. These include L74I (2 A, 9 CRF02_AG), Q95S (1 A), Q95P (1 CRF02_AG), E138D (1 F, 1 H), G163E (1 D, 2 CRF02_AG), G163N (1 F), G163Q (1 B, 1 C), G163S (4 F), and G163V (1 D, 1 G). Two of the 120 sequences did not include data for codon 263 (1 A, 1 B).
[b]HIV-1 subtype/CRF assignment is based on IN.

BigDye Terminator v1.1 chemistry (Applied Biosystems, Foster City, CA), using two forward primers (A and B) and two reverse primers (C and D). Sequencing products were analyzed on a 3130xl Genetic Analyzer; for comparison, sequencing products were also analyzed on a 3100 Genetic Analyzer (Applied Biosystems). SeqScape v2.5 software (Applied Biosystems) was used to assemble and align the resulting sequences.

RT- PCR amplification was successful for 120 (96%) of the 125 samples (Table 1). Five samples failed to amplify sufficient product for sequence analysis. Based on analysis of *gag* p24, *pol* IN, and *env* gp41 immunodominant regions,[5] these included two South African subtype C samples ($\log_{10}$ viral load: 3.10 and 3.22), two Cameroonian CRF02_AG samples ($\log_{10}$ viral load: 3.07 and 3.41), and one Brazilian sample with a unique recombinant form (F/B/F; $\log_{10}$ viral load: 4.10). All 120 of the amplified samples were successfully sequenced. Bidirectional sequences were obtained for 116 (96.7%) of the 120 amplified samples. The forward sequencing primer A failed for one subtype D sample from South Africa; the reverse sequencing primer D failed for three CRF02_AG samples from Cameroon. Overall, sequences obtained on the 3130xl instrument were longer than those obtained on the 3100 instrument; 114 (95%) of the 120 sequences obtained with the 3130xl instrument contained the full-length 864 nucleotide IN sequence, compared to only 102 (85%) of 120 sequences ob-

tained with the 3100 instrument. In addition, the 3130xl instrument appeared to be more sensitive for detecting mixed nucleotide positions.

The 120 IN sequences were aligned with HIV-1 group M references and phylogenetic analysis was performed to determine the subtype of this gene.[5,6] Results of this analysis revealed that successfully genotyped IN genes included 14 subtype A (11 A1, 1 A2, 2 A3), 15 B, 12 C, 11 D, 12 F (8 F1, 4 F2), 7 G, 1 H, 9 CFR01_AE, 34 CRF02_AG, 4 CRF22_01A1, and 1 CRF37_cpx.

HIV-1 IN sequences from the sample set were aligned and compared to the reference sequence HXB2 (GenBank accession number K03455) to identify amino acid differences relative to the reference strain. The Stanford University HIV Drug Resistance Database provides a summary of mutations associated with reduced susceptibility to HIV IN inhibitors, as well as mutations selected *in vivo* or *in vitro* by these drugs (updated June 10, 2008).

In this study, the sequences were analyzed for the presence of mutations associated with reduced *in vitro* susceptibility to RAL (E92Q, F121Y, E138AK, G140AS, Y143CHR, S147G, Q148HKR, N155HS, E157Q) or EVG (T66I, E92Q, F121Y, E138AK, G140AS, S147G, Q148HKR, S153Y, N155HS, E157Q, R263K).[2] Mutations associated with reduced *in vitro* susceptibility to RAL and/or EVG were identified in 10 (8.3%) of 120 samples (Table 1). Two subtype B samples from Brazil

TABLE 2. MUTATIONS IDENTIFIED IN THE SAMPLE SET THAT HAVE BEEN SELECTED *IN VIVO* OR *IN VITRO* BY OTHER
INVESTIGATIONAL HIV INTEGRASE INHIBITORS[a]

	HIV-1 subtype/CRF[b]											
Mutation	*A* (N=14)	*B* (N=15)	*C* (N=12)	*D* (N=11)	*F* (N=12)	*G* (N=7)	*H* (N=1)	*CRF01* (N=9)	*CRF02* (N=34)	*CRF22* (N=4)	*CRF37* (N=1)	*Total* (N=120)
V72I	2 (14.3)	9 (0.6)	7 (5.8)	4 (36.4)	6 (50)	7 (100)		1 (11.1)	24 (70.1)			60 (50)
M154I		2 (2.2)	1 (4.4)									3 (2.5)
V165I		4 (26.7)		1 (9.1)	2 (16.7)	1 (14.3)	1 (100)	3 (33.3)	1 (4.2)			13 (10.8)
V201I	13 (92.9)	6 (40)	12 (100)	11 (100)	12 (100)	7 (100)	1 (100)	9 (100)	34 (100)	4 (100)	1 (100)	110 (91.7)

[a]The number and percentage (in parentheses) of each mutation are shown for each subtype/CRF.
[b]HIV-1 subtype/CRF assignment is based on IN.

had E92Q; in both cases, the mutation was detected as a mixture. This mutation (E92Q) is associated with a 5- to 10-fold reduction in RAL and EVG susceptibility.[2] Eight samples had E157Q (2A, 1C, 1D, 1F, 3 CRF02_AG); for the subtypes D and F, the E157Q mutation was detected as a mixture. In addition, mutations selected *in vivo* or *in vitro* by RAL[2] were detected including L74M, T97A, V151I, I203M, and S230N (Table 1); Other mutations selected *in vivo* or *in vitro* by RAL (G163R, H183P, Y226DFH, S230R, D232N) or EVG (H51Y, Q95K, Q146P) [2] were not observed. Finally, the sequences were examined for the presence of mutations that have been selected by other investigational HIV IN inhibitors.[2,3] The following polymorphic mutations were detected: V72I, M154I, V165I, and V201I; some of these were present in over 30% of the 120 samples (e.g., V72I and V201I, Table 2). In a previous study of 1304 IN sequences from drug-naive blood donors, V72I and V201I were observed as naturally occurring polymorphisms in a high proportion of samples (53% and 88.9%, respectively).[4] The nonpolymorphic mutations, T125K, A128T, Q146K, N155S, and K160D, were not detected.

Population sequencing of viruses selected with IN strand-transfer inhibitors identified three pathways for the development of resistance: N155H + E92Q, Q148K/R/H + G140A/S, and Y143C/R + T97A.[7,8] In this study we detected E92Q in two (13.3%) subtype B samples, and T97A in five (4.2%) samples from individuals who were presumed to be anti-retroviral drug naive. Whether preexisting resistance mutations direct development of resistance via a specific pathway under drug pressure is yet to be determined.

This study expands available data on the genetic heterogeneity of the HIV-1 IN domain in non-B HIV-1 strains. Knowledge of IN amino acid polymorphisms in diverse HIV-1 strains may be helpful when considering use of an IN inhibitor for HIV-1 treatment, and for elucidating potential subtype-specific effects on resistance pathways. This information is expected to become increasingly important as RAL and other IN inhibitors become more widely used in patient populations infected with non-B strains.

Sequence Data

The GenBank Accession Numbers for the 120 integrase sequences are FJ480225–FJ480344.

Disclosure Statement

Natalia Marlowe and Peter Smith are employees and stockholders of Celera, manufacturer of the HIV-1 integrase assay.

References

1. Pommier Y, Johnson AA, and Marchand C: Integrase inhibitors to treat HIV/AIDS. Nat Rev Drug Discov 2005;4:236–248.
2. Stanford University HIV Drug Resistance Database: Integrase Inhibitor Resistance Notes. http://hivdb.stanford.edu/cgi-bin/IIResiNote.cgi. Accessed 9/7/08.
3. Lataillade M, Chiarella J, and Kozal MJ: Natural polymorphism of the HIV-1 integrase gene and mutations associated with integrase inhibitor resistance. Antiviral Ther 2007;12:563–570.
4. Hackett J, Jr, Harris B, Holzmayer V, *et al.*: Naturally occurring polymorphisms in HIV-1 group M, N, and O integrase: Implications for integrase inhibitors. 15th Conference on Retroviruses and Opportunistic Infections. Boston, MA, February 3–6, 2008 (Abstract 872).
5. Swanson P, Devare SG, and Hackett J Jr: Molecular characterization of 39 HIV isolates representing group M (subtypes A-G) and group O: Sequence analysis of gag p24, pol integrase, and env gp41. AIDS Res Hum Retroviruses 2003;19:625–629.
6. Leitner T, Korber B, Daniels M, Calef C, and Foley B: HIV-1 subtype and circulating recombinant form (CRF) reference sequences. In: *HIV Sequence Compendium 2005* (Leitner T, Foley B, Hahn B, *et al.*, eds.). Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, 2005, pp. 41–48, LA-UR 06-0680.
7. Fransen S, Gupta S, Danovich R, *et al.*: Loss of raltegravir susceptibility in treated patients is conferred by multiple non-overlapping genetic pathways. Antiviral Ther 2008;13(Suppl. 3):A9.
8. Hatano H, Lampiris H, Huang W, *et al.*: Virological and immunological outcomes in a cohort of patients failing integrase inhibitors. Antiviral Ther 2008;13(Suppl. 3):A12.

Address reprint requests to:
Susan Eshleman
Department of Pathology
The Johns Hopkins Medical Institutions
Ross Bldg. 646
720 Rutland Ave.
Baltimore, Maryland 21205

E-mail: seshlem@jhmi.edu