Journal of Antimicrobial Chemotherapy Advance Access published July 17, 2015

J Antimicrob Chemother
doi:10.1093/jac/dkv202

Journal of Antimicrobial Chemotherapy

Primary resistance to integrase strand-transfer inhibitors in Europe

M. Casadellà[1]*, P. M. van Ham[2], M. Noguera-Julian[1,3], A. van Kessel[2], C. Pou[1], L. M. Hofstra[2,4], J. R. Santos[5], F. Garcia[6], D. Struck[4], I. Alexiev[7], A. M. Bakken Kran[8], A. I. Hoepelman[2], L. G. Kostrikis[9], S. Somogyi[10], K. Liitsola[11], M. Linka[12], C. Nielsen[13], D. Otelea[14], D. Paraskevis[15], M. Poljak[16], E. Puchhammer-Stöckl[17], D. Staneková[18], M. Stanojevic[19], K. Van Laethem[20], S. Zidovec Lepej[21], B. Clotet[1,3,4], C. A. B. Boucher[22], R. Paredes[1,3,4] and A. M. J. Wensing[2] on behalf of the SPREAD programme†

[1]IrsiCaixa AIDS Research Institute, Universitat Autònoma de Barcelona, Badalona, Catalonia, Spain; [2]Department of Virology, Medical Microbiology, Utrecht Medical Centre, Utrecht, The Netherlands; [3]Universitat de Vic-Universitat Central de Catalunya, Vic, Spain; [4]Laboratory of Retrovirology, Luxembourg Institute of Health, Luxembourg; [5]HIV Unit, Hospital Universitari Germans Trias I Pujol, Universitat Autònoma de Barcelona, Badalona, Catalonia, Spain; [6]Complejo Hospitalario Univeristario de Granada, Instituto de Investigación IBS, Granada, Cohorte de Adultos de la Red de Investigación en SIDA (CoRIS) Spain; [7]National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria; [8]Institute of Clinical Medicine, Oslo, Norway; [9]University of Cyprus, Nicosia, Cyprus; [10]Robert Koch-Institute, Berlin, Germany; [11]National Institute of Health and Welfare, Helsinki, Finland; [12]National Reference Laboratory for HIV/AIDS, National Institute of Public Health, Prague, Czech Republic; [13]Statens Serum Institut, Copenhagen, Denmark; [14]National Institute for Infectious Diseases 'Prof. Dr. Matei Bals', Bucharest, Romania; [15]National Retrovirus Reference Center, University of Athens, Athens, Greece; [16]Slovenian HIV/AIDS Reference Centre, University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia; [17]University of Vienna, Vienna, Austria; [18]Slovak Medical University, Bratislava, Slovakia; [19]Faculty of Medicine, University of Belgrade, Belgrade, Serbia; [20]Clinical and Epidemiological Virology, Rega Institute for Medical Research, Department of Microbiology and Immunology, KU Leuven, Belgium; [21]University Hospital for Infectious Diseases 'Dr. Fran Mihaljevic', Zagreb, Croatia; [22]Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands

*Corresponding author. Tel: +34-93-465-6374, ext. 169; Fax: +34-93-465-3968; E-mail: mcasadella@irsicaixa.es
†SPREAD programme investigators are listed in the Acknowledgements section.

Received 2 March 2015; returned 12 April 2015; revised 8 June 2015; accepted 16 June 2015

Objectives: The objective of this study was to define the natural genotypic variation of the HIV-1 integrase gene across Europe for epidemiological surveillance of integrase strand-transfer inhibitor (InSTI) resistance.

Methods: This was a multicentre, cross-sectional study within the European SPREAD HIV resistance surveillance programme. A representative set of 300 samples was selected from 1950 naive HIV-positive subjects newly diagnosed in 2006–07. The prevalence of InSTI resistance was evaluated using quality-controlled baseline population sequencing of integrase. Signature raltegravir, elvitegravir and dolutegravir resistance mutations were defined according to the IAS-USA 2014 list. In addition, all integrase substitutions relative to HXB2 were identified, including those with a Stanford HIVdb score ≥10 to at least one InSTI. To rule out circulation of minority InSTI-resistant HIV, 65 samples were selected for 454 integrase sequencing.

Results: For the population sequencing analysis, 278 samples were retrieved and successfully analysed. No signature resistance mutations to any of the InSTIs were detected. Eleven (4%) subjects had mutations at resistance-associated positions with an HIVdb score ≥10. Of the 56 samples successfully analysed with 454 sequencing, no InSTI signature mutations were detected, whereas integrase substitutions with an HIVdb score ≥10 were found in 8 (14.3%) individuals.

Conclusions: No signature InSTI-resistant variants were circulating in Europe before the introduction of InSTIs. However, polymorphisms contributing to InSTI resistance were not rare. As InSTI use becomes more widespread, continuous surveillance of primary InSTI resistance is warranted. These data will be key to modelling the kinetics of InSTI resistance transmission in Europe in the coming years.

Introduction

HIV integrase is a key enzyme for retroviral replication and one of the main targets of modern HIV therapy.[1,2] Integrase strand-transfer inhibitors (InSTIs) reached clinical practice in Europe in 2007–08, after proving their efficacy in antiretroviral treatment-naive and -experienced subjects.[3–6] Virological failure (VF) to the first-generation InSTIs raltegravir and elvitegravir is associated with

development of resistance through three mutually exclusive pathways characterized by one signature resistance mutation in the catalytic domain of the enzyme, i.e. Y143R/C, N155H or Q148K/R/H, alongside accessory mutations that improve viral fitness or further reduce InSTI susceptibility.[7–10] The resistance profile of the second-generation InSTI dolutegravir is being defined, as few subjects developed VF in clinical trials and data from routine care are still scarce. Viruses with Q148K/R/H plus at least one additional mutation, however, may also affect susceptibility to dolutegravir.[11,12] Based on the low genetic barrier of first-generation InSTIs and with the increasing use of them in clinical practice, surveillance of transmitted InSTI-resistant HIV will be a key to optimizing InSTI efficacy. Primary InSTI resistance is still rare. However, it has started to be reported[13,14] and will likely increase in the coming years. In addition, up to 34% of published sequences, 56% of those obtained from recent HIV infection,[15] contain polymorphisms in the integrase,[16] which modulate InSTI resistance in particular to raltegravir and elvitegravir and are frequently observed in InSTI VF. In this study, we performed a systematic, representative description of the natural sequence variation of the integrase gene across Europe, before InSTI drugs were commercially available. We also aimed to clarify the chances that spontaneously generated InSTI-resistant mutants could be circulating as minority species and be missed by routine population sequencing approaches.

Methods

A sample of 300 subjects was randomly selected from 1950 individuals enrolled in the European SPREAD programme in 2006–07, before InSTIs were introduced into routine clinical care in Europe. SPREAD is a prospective HIV-1 resistance surveillance programme that collects representative data on the spread of HIV-1 resistance among newly diagnosed patients from all risk groups in Europe. Ethics requirements were fulfilled according to the procedure described in the ethics committee contract. Additionally, written informed consent was obtained for each patient. Population sequencing of plasma HIV-1 was performed (ViroSeq® HIV-1 Genotyping System, Abbott; Trugene® HIV-1 Genotyping Kit, Siemens; or in-house methods) in laboratories that successfully participated in the SPREAD quality control (QC) programme for population sequencing. Samples from laboratories that did not participate in the integrase sequencing QC programme or did not successfully meet the QC criteria were tested by one of the qualified laboratories that passed the QC programme within the SPREAD network using the above-mentioned commercial or in-house Sanger sequencing methods. HIV subtypes were determined using Rega Subtyping Tool v2 based on *pol* sequence data.[17] To screen for circulating low-frequency InSTI-resistant mutants, ultradeep integrase sequencing was additionally attempted in 65 subjects randomly selected from those included in the population sequencing analyses, using a 454 FLX Genome Sequencer with Titanium chemistry and a 1% threshold for mutant detection. Sequences were analysed using the Roches's proprietary Amplicon Variant Analyser software (v2.7). Sample contamination was ruled out by similarity analysis both against a pNL4.3 reference sequence and by per-amplicon phylogenetic analysis of all sequences >1% within an ultradeep sequencing run. Given the lack of a widely accepted list of integrase mutations for surveillance, we first listed all substitutions relative to the reference sequence HXB2 (GenBank accession number K03455) according to their frequency in the patient population. We then evaluated which substitutions achieved an HIVdb score ≥10 to at least one InSTI, representing substitutions with potential impact on InSTI susceptibility. We also listed integrase mutations included in the IAS-USA list (July 2014 update). 'Signature mutations' were: Y143R/C/H, N155H and Q148K/R/H for raltegravir; T66I, E92Q, F121Y, S147G, Q148R and N155H for elvitegravir; and G140S and Q148H for dolutegravir.

Results

Samples were retrieved and successfully analysed by population sequencing in 278 out of the 300 (92.7%) subjects selected. The prevalence of transmitted drug resistance mutations found by Sanger sequencing for PIs, NRTIs and NNRTIs was 2.5%, 9.71% and 7.91%, respectively. No signature InSTI mutations were detected. By contrast, we observed integrase-associated mutations with an HIVdb score ≥10 in 11 (4.0%) patients (Table 1 and Table S1, available as Supplementary data at *JAC* Online). Samples unsuccessfully processed had a median (IQR) number of copies/mL of 57 000 (13 212–374 154). The HIV subtype from

Table 1. Subject characteristics and summary of sequencing results

All subjects, *n* (%)	278 (100)
Male, *n* (%)	231 (83.0)
Continent of origin, *n* (%)	
western Europe	180 (64.7)
eastern Europe	48 (17.3)
sub-Saharan Africa	20 (7.2)
Latin America	16 (5.8)
others	14 (5.0)
CDC class, *n* (%)	
A	230 (82.7)
B	21 (7.5)
C	22 (8.0)
unknown	5 (1.8)
CD4+ T count (cells/μL), median	411
Route of transmission, *n* (%)	
MSM/bisexual	180 (64.8)
heterosexual	61 (21.9)
IVDU	5 (1.8)
other	32 (11.5)
Viral subtype, *n* (%)	
B	186 (67.0)
C	15 (5.4)
A	11 (4.0)
F	12 (4.3)
G	6 (2.1)
D	1 (0.3)
unknown	47 (16.9)
Summary of Sanger sequencing, *n* (%)	
IAS-USA integrase mutations	5 (1.8) [74M (2), 97A (2) and 138A]
HIVdb score ≥10	11 (4.0)
Summary of 454 sequencing (*n*=56 subjects), *n* (%)	
IAS-USA integrase mutations	0
HIVdb score ≥10	8 (14.3)

these samples was mainly subtype B ($n=12$; 54.55%) and subtype G ($n=5$; 22.72%).

454 data were obtained from 56/65 (86.1%) subjects. Most of them (85.2%) were infected with subtype B HIV-1. Fifty of them (89.3%) had WT protease, reverse transcriptase and integrase by Sanger sequencing, whereas 6 (10.7%) had transmitted resistance to at least two antiretroviral drug classes. The median (IQR) coverage was 4593 (3066–6598) reads per substitution found. Again, no InSTI signature mutations were detected. However, 8/56 subjects (14.3%) had integrase substitutions with an HIVdb score ≥10 (Table S2). Of these, mutation E157Q was found in five (8.9%) individuals, in two of them as a low-frequency variant. The following mutations were found in one subject each: H51Y, G163R, both as low-frequency variants (1.9; 2.6% in the virus population each); G163K as major variant (100% in the virus population); E157Q was detected at a 22.1% frequency in a subject with transmitted Q58E mutations in the protease and D67N and K219Q mutations in the reverse transcriptase. No integrase substitutions with an HIVdb score ≥10 were detected in the remaining five subjects with transmitted dual-class resistance.

Discussion

No signature InSTI resistance mutations were circulating in Europe before InSTI introduction, although potentially relevant polymorphisms could be observed. This study also indicated a limited utility of ultrasensitive genotyping for surveillance of InSTI-resistant minority variants at present, which might change if the burden of transmitted InSTI resistance increases. Elvitegravir and raltegravir have a low genetic barrier to resistance and extensive overlap in their drug resistance profiles.[18] Raltegravir is often prescribed as salvage therapy to subjects with MDR HIV who could select for InSTI resistance, which could be transmitted to newly infected subjects. Dolutegravir has a higher genetic barrier to resistance than elvitegravir and raltegravir, but its long-term potency might be reduced in the presence of Q148R/H/K plus one or two additional mutations. As dolutegravir is also often prescribed as salvage ART, dolutegravir resistance might also evolve in European populations in the coming years. Continued surveillance of InSTI resistance in Europe is thus warranted, including periodic re-evaluations of the usefulness of ultrasensitive genotyping technologies, which nowadays allow faster monitoring of transmitted resistance, particularly with large sample sets.

Substitutions detected with at least some presumed impact on ART susceptibility (i.e. having an HIVdb score ≥10) were E157Q, G163R/K, L74M, T97A, E138A, S153F and R263K. E157Q is a polymorphic accessory mutation weakly selected in patients receiving raltegravir and selected *in vitro* by elvitegravir. G163R/K are non-polymorphic mutations in all subtypes except F, often selected in patients receiving raltegravir. However, their effect on InSTIs has not yet been well studied. L74M is a polymorphic accessory mutation selected in patients receiving raltegravir, elvitegravir and dolutegravir, which does not reduce InSTI susceptibility unless it is found in combination with other InSTI resistance mutations. T97A is a polymorphic accessory mutation selected by raltegravir and elvitegravir that occurs in 1%–5% of viruses from untreated persons. Combined with Y143C/R, it markedly reduces raltegravir susceptibility, although it has minimal effect alone. E138A is a non-polymorphic accessory resistance mutation usually occurring in combination with Q148 mutations, selected in patients receiving raltegravir, elvitegravir and dolutegravir. It is associated with >100-fold reduction in raltegravir and elvitegravir susceptibility and up to 10-fold reduced dolutegravir susceptibility in combination with Q148. S153F is selected *in vitro* by dolutegravir and is a rare non-polymorphic mutation, reducing raltegravir and dolutegravir susceptibility by 2-fold and elvitegravir susceptibility by 4-fold. R263K is a non-polymorphic mutation selected in patients receiving raltegravir and dolutegravir and *in vitro* by elvitegravir and dolutegravir, reducing raltegravir, dolutegravir and elvitegravir susceptibility by ∼2-fold, 2-fold and 3- to 5-fold, respectively.[19,20]

Therefore, as long as transmitted InSTI resistance remains at negligible levels, there is no clinical need to perform integrase genotyping before initiating InSTI therapy. However, continued surveillance is key to informing clinicians and policymakers about when baseline genotyping should be systematically recommended. It is essential to perform integrase gene genotyping in subjects failing InSTI therapy, as new InSTIs with alternative resistance profiles are under development and subjects should not be kept on failing InSTI regimens.

In conclusion, no signature InSTI-resistant variants were circulating in Europe before introducing InSTIs. However, polymorphisms that could contribute to InSTI resistance were not rare. As InSTI use becomes more widespread, continuous surveillance of primary InSTI resistance is warranted. This study provides an extensive assessment of primary InSTI resistance based on a representative sample of the European epidemic and is a robust baseline comparator for future InSTI surveillance, which will be key to modelling the kinetics of InSTI resistance patterns of transmission in Europe in the coming years.

Acknowledgements

SPREAD programme investigators

Austria: E. Puchhammer-Stöckl (National Coordinator), M. Sarcletti, B. Schmied, M. Geit and G. Balluch. Belgium: A.-M. Vandamme (National Coordinator), J. Vercauteren, I. Derdelinckx, A. Sasse, M. Bogaert, H. Ceunen, A. De Roo, S. De Wit, F. Echahidi, K. Fransen, J.-C. Goffard, P. Goubau, E. Goudeseune, J.-C. Yombi, P. Lacor, C. Liesnard, M. Moutschen, D. Pierard, R. Rens, Y. Schrooten, D. Vaira, L. P. R. Vandekerckhove, A. Van den Heuvel, B. Van Der Gucht, M. Van Ranst, E. Van Wijngaerden, B. Vandercam, M. Vekemans, C. Verhofstede, N. Clumeck and K. Van Laethem. Bulgaria: D. Beshkov (National Coordinator) and I. Alexiev. Croatia: S. Zidovec Lepej (National Coordinator) and J. Begovac. Cyprus: L. G. Kostrikis (National Coordinator), I. Demetriades, I. Kousiappa, V. Demetriou and J. Hezka. Czech Republic: M. Linka (National Coordinator), L. Machala and M. Maly. Denmark: C. Nielsen (National Coordinator), L. B. Jørgensen, J. Gerstoft, L. Mathiesen, C. Pedersen, H. Nielsen, A. Laursen and B. Kvinesdal. Finland: K. Liitsola (National Coordinator), M. Ristola, J. Suni and J. Sutinen. Germany: O. Hamouda (National Coordinator), C. Kücherer, T. Berg, P. Braun, G. Poggensee, M. Däumer, J. Eberle, H. Heiken, R. Kaiser, H. Knechten, K. Korn, H. Müller, S. Neifer, B. Schmidt, H. Walter, B. Gunsenheimer-Bartmeyer and T. Harrer. Greece: D. Paraskevis (National Coordinator), A. Hatzakis, E. Magiorkinis, E. Hatzitheodorou, C. Haida, A. Zavitsanou, G. Magiorkinis, M. Lazanas, M. Chini, N. Magafas, N. Tsogas, V. Paparizos, S. Kourkounti, A. Antoniadou, A. Papadopoulos, P. Panagopoulos, G. Poulakou, V. Sakka, G. Chryssos, S. Drimis,

P. Gargalianos, M. Lelekis, G. Chilomenos, M. Psichogiou, G. L. Daikos, H. Sabatakou, G. Panos, G. Haratsis, T. Kordossis, A. Kontos, G. Koratzanis, M. Theodoridou, G. Mostrou and V. Spoulou. Luxembourg: J. C. Schmit (National Coordinator), D. Struck, R. Hemmer, V. Arendt, T. Staub, F. Schneider and F. Roman. The Netherlands: A. M. J. Wensing (National Coordinator), C. A. B. Boucher (National Coordinator), D. A. M. C. van de Vijver, A. van Kessel, P. H. M. van Bentum, K. Brinkman, E. L. op de Coul, M. E. van der Ende, I. M. Hoepelman, M. van Kasteren, J. Juttmann, M. Kuipers, N. Langebeek, C. Richter, R. M. W. J. Santegoets, L. Schrijnders-Gudde, R. Schuurman and B. J. M. van de Ven. Norway: B. Åsjö (National Coordinator), A.-M. Bakken Kran, V. Ormaasen and P. Aavitsland. Romania: D. Otelea (National Coordinator), S. Paraschiv and A. M. Tudor. Republic of Serbia: M. Stanojevic (National Coordinator), D. Jevtovic and D. Salemovic. Slovakia: D. Stanekova (National Coordinator), M. Habekova, M. Mokras and P. Truska. Slovenia: M. Poljak (National Coordinator), M. Lunar, D. Babic, J. Tomazic, L. Vidmar, T. Vovko and P. Karner. Spain: B. Clotet (National Coordinator), F. Garcia, P. Domingo, M. J. Galindo, C. Miralles, M. A. del Pozo, E. Ribera, J. A. Iribarren, L. Ruiz, J. de la Torre, F. Vidal, F. Garcia, R. Paredes and CoRIS investigators.

Funding

This study was funded through an investigator-initiated grant from Merck Sharpe & Dohme. It was also partially supported through 'CHAIN, Collaborative HIV and Anti-HIV Drug Resistance Network', Integrated Project 223131, funded by the European Commission Framework 7, through a CORE grant (Fonds National de la Recherche Luxembourg, C12/BM/4011111—HIV molecular epidemiology in Europe—HIVmolEpi) and through Red Española de Investigación del SIDA (RIS). M. S. was supported through a grant (no. 175024), from the Republic of Serbia Ministry of Education, Science and Technological Development.

Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Marrazzo JM, del Rio C, Holtgrave DR *et al*. HIV prevention in clinical care settings: 2014 recommendations of the International Antiviral Society-USA Panel. *JAMA* 2014; **312**: 390–409.

2 Department of Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents*. http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf.

3 Correll T, Klibanov OM. Integrase inhibitors: a new treatment option for patients with human immunodeficiency virus infection. *Pharmacotherapy* 2008; **28**: 90–101.

4 Hughes A, Barber T, Nelson M. New treatment options for HIV salvage patients: an overview of second generation PIs, NNRTIs, integrase inhibitors and CCR5 antagonists. *J Infect* 2008; **57**: 1–10.

5 Eron JJ, Rockstroh JK, Reynes J *et al*. Raltegravir once daily or twice daily in previously untreated patients with HIV-1: a randomised, active-controlled, phase 3 non-inferiority trial. *Lancet Infect Dis* 2011; **11**: 907–15.

6 Steigbigel RT, Cooper DA, Kumar PN *et al*. Raltegravir with optimized background therapy for resistant HIV-1 infection. *N Engl J Med* 2008; **359**: 339–54.

7 Cooper DA, Steigbigel RT, Gatell JM *et al*. Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. *N Engl J Med* 2008; **359**: 355–65.

8 Goethals O, Clayton R, Van Ginderen M *et al*. Resistance mutations in human immunodeficiency virus type 1 integrase selected with elvitegravir confer reduced susceptibility to a wide range of integrase inhibitors. *J Virol* 2008; **82**: 10366–74.

9 Malet I, Delelis O, Valantin M-A *et al*. Mutations associated with failure of raltegravir treatment affect integrase sensitivity to the inhibitor in vitro. *Antimicrob Agents Chemother* 2008; **52**: 1351–8.

10 Loizidou EZ, Kousiappa I, Zeinalipour-Yazdi CD *et al*. Implications of HIV-1 M group polymorphisms on integrase inhibitor efficacy and resistance: genetic and structural in silico analyses. *Biochemistry* 2009; **48**: 4–6.

11 Castagna A, Maggiolo F, Penco G *et al*. Dolutegravir in antiretroviral-experienced patients with raltegravir- and/or elvitegravir-resistant HIV-1: 24-week results of the phase III VIKING-3 study. *J Infect Dis* 2014; **210**: 354–62.

12 Underwood MR, Johns BA, Sato A *et al*. The activity of the integrase inhibitor dolutegravir against HIV-1 variants isolated from raltegravir-treated adults. *J Acquir Immune Defic Syndr* 2012; **61**: 297–301.

13 Young B, Fransen S, Greenberg KS *et al*. Transmission of integrase strand-transfer inhibitor multidrug-resistant HIV-1: case report and response to raltegravir-containing antiretroviral therapy. *Antivir Ther* 2011; **16**: 253–6.

14 Boyd SD, Maldarelli F, Sereti I *et al*. Transmitted raltegravir resistance in an HIV-1 CRF_AG-infected patient. *Antivir Ther* 2011; **16**: 257–61.

15 Low A, Prada N, Topper M *et al*. Natural polymorphisms of human immunodeficiency virus type 1 integrase and inherent susceptibilities to a panel of integrase inhibitors. *Antimicrob Agents Chemother* 2009; **53**: 4275–82.

16 Rhee S-Y, Liu TF, Kiuchi M *et al*. Natural variation of HIV-1 group M integrase: implications for a new class of antiretroviral inhibitors. *Retrovirology* 2008; **5**: 74.

17 Alcantara LCJ, Cassol S, Libin P *et al*. A standardized framework for accurate, high-throughput genotyping of recombinant and non-recombinant viral sequences. *Nucleic Acids Res* 2009; **37**: W634–42.

18 Marinello J, Marchand C, Mott BT *et al*. Comparison of raltegravir and elvitegravir on HIV-1 integrase catalytic reactions and on a series of drug-resistant integrase mutants. *Biochemistry* 2008; **47**: 9345–54.

19 Shafer RW. Rationale and uses of a public HIV drug-resistance database. *J Infect Dis* 2006; **194** Suppl 1: S51–8.

20 Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. *Clin Infect Dis* 2006; **42**: 1608–18.