J Antimicrob Chemother 2017; **72**: 2083–2088
doi:10.1093/jac/dkx090 Advance Access publication 23 March 2017

Journal of Antimicrobial Chemotherapy

Drug resistance mutations in HIV-2 patients failing raltegravir and influence on dolutegravir response

Silvia Requena[1], Ana Treviño[1], Teresa Cabezas[2], Rosa Garcia-Delgado[3], María José Amengual[4], Ana Belén Lozano[2], María Peñaranda[5], Juan Manuel Fernández[2], Vicente Soriano[6] and Carmen de Mendoza[1]* on behalf of the Spanish HIV-2 Study Group†

[1]Puerta de Hierro University Hospital, Majadahonda, Madrid, Spain; [2]Hospital de Poniente, Almería, Spain; [3]Fundación Jiménez Díaz, Madrid, Spain; [4]Corporación Sanitaria Parc Taulí, Barcelona, Spain; [5]Son Espases Hospital, Palma de Mallorca, Spain; [6]La Paz University Hospital & Autonomous University, Madrid, Spain

*Corresponding author. E-mail: cmendoza.cdm@gmail.com
†Members are listed in the Acknowledgements section.

Received 5 December 2016; returned 10 January 2017; revised 20 February 2017; accepted 28 February 2017

Background: A broader extent of amino acid substitutions in the integrase of HIV-2 compared with HIV-1 might enable greater cross-resistance between raltegravir and dolutegravir in HIV-2 infection. Few studies have examined the virological response to dolutegravir in HIV-2 patients that failed raltegravir.

Methods: All patients recorded in the HIV-2 Spanish cohort were examined. The integrase coding region was sequenced in viraemic patients. Changes associated with resistance to raltegravir and dolutegravir in HIV-1 were recorded.

Results: From 319 HIV-2-infected patients recorded in the HIV-2 Spanish cohort, 53 integrase sequences from 30 individuals were obtained (20 raltegravir naive and 10 raltegravir experienced). Only one secondary mutation (E138A) was found in one of the 20 raltegravir-naive HIV-2 patients. For raltegravir-experienced individuals, the resistance mutation profile in 9 of 10 viraemic patients was as follows: N155H + A153G/S (four); Y143G + A153S (two); Q148R + G140A/S (two); and Y143C + Q91R (one). Of note, all patients with Y143G and N155H developed a rare non-polymorphic mutation at codon 153. Rescue therapy with dolutegravir was given to 5 of these 10 patients. After >6 months on dolutegravir therapy, three patients with baseline N155H experienced viral rebound. In two of them N155H was replaced by Q148K/R and in another by G118R.

Conclusions: A wide repertoire of resistance mutations in the integrase gene occur in HIV-2-infected patients failing on raltegravir. Although dolutegravir may allow successful rescue in most HIV-2 raltegravir failures, we report and characterize three cases of dolutegravir resistance in HIV-2 patients, emerging variants Q148K and Q148R and a novel change G118R.

Introduction

HIV-2 is a retrovirus that mostly infects people living in West Africa. Compared with HIV-1, the clinical course of HIV-2 infection is generally characterized by a longer asymptomatic stage, lower plasma HIV-2 RNA levels and lower mortality.[1,2] However, progression to AIDS does occur and HIV-2-infected patients should start ART at any time.[3]

To date, no randomized trials addressing the question of when to start ART or the choice of initial or second-line therapy for HIV-2 infection have been completed.[4] For this reason, although HIV-2 management follows HIV-1 recommendations, specific issues should be considered. First, HIV-2 is intrinsically resistant to HIV NNRTIs.[5] Second, for protease inhibitors, darunavir, lopinavir and saquinavir seem to be the most active against HIV-2, while others, such as fosamprenavir or atazanavir are less active.[6,7] Third, resistance-associated mutations are uniformly more frequently selected in HIV-2-infected patients while on therapy.[8] Finally, genotypic algorithms used to predict drug resistance in HIV-1 may not be applicable to HIV-2 because pathways and mutational patterns leading to resistance may differ between HIV types.[9]

The latest family of antiretrovirals approved for the treatment of HIV infection, the integrase strand transfer inhibitors (INSTIs), has shown that all approved integrase inhibitors are effective against HIV-2 isolates.[10,11] We previously discussed the short-term efficacy of dolutegravir in a few HIV-2 patients that failed raltegravir.[12] However, there is limited information about the long-term

effectiveness of these antiretrovirals in real life as well as the mutational pathways leading to HIV-2 resistance to raltegravir, elvitegravir or dolutegravir.[9,13]

The aim of this study was to analyse retrospectively the mutational patterns emerging in HIV-2-infected patients failing raltegravir and the impact of these changes on subsequent dolutegravir therapy in a group of HIV-2 individuals living in Spain.

Methods

The Spanish HIV-2 national register is a publicly funded database that collects information from all individuals diagnosed with HIV-2 infection in Spain since year 1989. A centralized repository of stored clinical samples, including PBMCs and plasma, functions in parallel and was used for the current study.[14]

Plasma HIV-2 RNA was quantified using a non-commercial real-time PCR assay. The region amplified was the long terminal repeat with primers and probes described elsewhere.[15] Both HIV-2 group A and B are reliably detected with this assay.

Amplification of sequences of the integrase gene was attempted in plasma specimens. For HIV-2 RNA extraction, plasma was processed as indicated on the RNA extraction kit used (QIAamp DNA Mini Kit; QIAGEN, Germany). Reverse transcription was carried out for the integrase gene, followed by a nested PCR. The amplicon length was 843 bp and included the integrase region that contained known resistance-associated amino acid substitutions. Primers and conditions have been previously described.[11] The product was purified (QIAquick PCR Purification Kit; QIAGEN) and, finally, a sequencing PCR was performed (BigDye Terminator v1.1, v3.1 5X Sequencing Buffer, Applied Biosystems, UK and BigDye Terminator v1.1 Cycle, Applied Biosystems, USA). Bulk Sanger sequencing was carried out using the 3100-Avant Genetic Analyzer (Applied Biosystems, USA). Sequences were analysed with SeqScape v2.5 using ROD HIV-2 as the reference strain.

All changes recorded in the integrase gene were considered. Drug resistance-associated changes along with compensatory drug resistance mutations were considered using the information available from HIV-1. Mutations at codons 51, 66, 74, 92, 95, 97, 114, 121, 128, 138, 140, 143, 145, 146, 147, 148, 151, 153, 155, 157, 163, 230 and 263 were recorded following the Stanford HIV-1 drug resistance database and the 2011 International AIDS Society—USA panel mutation list.[16,17]

Results

From a total of 319 HIV-2-infected patients recorded in the HIV-2 Spanish cohort until December 2015, 53 integrase sequences from 30 individuals were obtained. They belonged to 20 raltegravir-naive and 10 raltegravir-experienced patients. Only patients with available plasma samples and detectable plasma viraemia were included in the study.

Overall, 23 (76.7%) were males who had acquired HIV-2 infection mainly by heterosexual contact (73%). Most were from Sub-Saharan Africa (70%) but five (16.7%) were native Spaniards. The main characteristics of the study population are recorded in Table 1.

Integrase sequence analyses in 20 raltegravir-naive individuals identified only one secondary mutation (E138A) in one subject. There is no information on the impact of this change in isolation at baseline on virological response to raltegravir. Our patient started treatment with a raltegravir-containing regimen with achievement of undetectable viraemia that has lasted for >2 years.

A total of 33 sequences were analysed from 10 patients that failed raltegravir-containing regimens at different time points. The median time on raltegravir therapy was 13 months (IQR = 9–19.5). The main characteristics of these patients are recorded in Table 2. At failure, median HIV-2 RNA was 2.9 log copies/mL (IQR = 2.3–3.5). All but one displayed integrase resistance-associated mutations. Resistance patterns were as follows: N155H + A153G/S (four patients); Y143G + A153S (two); Q148R + G140A/S (two) and Y143C + Q91R (one). Of note, all patients with mutations at codons 143 and 155 developed a non-polymorphic mutation at codon 153. Table 3 records all changes found in the nine patients with integrase resistance mutations. In addition, all patients showed distinct drug resistance mutations at the reverse transcriptase and protease regions (data not shown).

Following raltegravir failure, five patients were subsequently treated with dolutegravir along with an optimized background of at least two additional antiretroviral drugs (patients 2, 3, 6, 7 and 8). All were treated with either darunavir/ritonavir (four patients) or

Table 1. Main characteristics of the HIV-2 study population

	Raltegravir naive	Raltegravir experienced	Total
N	20	10	30
Male, *n* (%)	15 (75)	8 (80)	23 (76.7)
Age (years), median (IQR)	48 (39–54)	50 (39–55)	48 (40–54)
Country of origin, *n* (%)			
Africa[a]	14 (70)	7 (70)	21 (70)
Spain	4 (20)	2 (20)	6 (20)
Portugal	1 (5)	1 (10)	2 (6.7)
others	1 (5)	—	1 (3.3)
CD4 count (cells/mm^3), median (IQR)	276 (60–371)	176 (40–249)	210 (52–350)
HIV-2 RNA (log copies/mL), median (IQR)	3.2 (2.2–3.8)	2.9 (2.3–3.5)	3.1 (2.3–3.8)
HIV-2 subtype, *n*			
A	15	7	22
B	4	2	6
unknown	1	1	2

[a]African countries (the absolute number for each country is recorded between parentheses): Guinea Bissau (8), Senegal (6), Cape Verde (2), Mali (1), Gambia (1), Ivory Coast (1), Mauritania (1) and unknown (2).

Table 2. Main baseline characteristics of HIV-2 patients that failed raltegravir

Patient	Gender	Age (years)	Country of origin	Year of diagnosis	ARV combination with RAL	Baseline HIV-2 RNA (log copies/mL)	Baseline CD4 count (cells/mm^3)	Months on RAL
1	M	42	Guinea Bissau	1991	TDF + FTC	ND	ND	13
2	M	52	Africa	1998	ZDV + 3TC	5.05	128	11
3	M	55	Spain	2011	DRV/r + TDF	4.06	114	6
4	F	44	Portugal	2007	TDF + FTC	2.46	371	11
5	M	53	Senegal	2008	DRV/r + MVC	3.84	60	36
6	M	28	Cape Verde	1990	DRV/r + MVC	3.15	24	20
7	M	64	Spain	2009	ZDV + 3TC + ATV/r	4.42	240	17
8	M	48	Guinea Bissau	2006	TDF + FTC + DRV/r	ND	ND	7
9	M	57	Mali	1996	TDF + 3TC + ATV/r	50	109	19
10	F	31	Guinea Bissau	1985	TDF + MVC + DRV/r	50	308	36

M, male; F, female; ND, not done; ARV, antiretroviral; RAL, raltegravir; TDF, tenofovir; FTC, emtricitabine; 3TC, lamivudine; ZDV, zidovudine; DRV, darunavir; ATV, atazanavir; MVC, maraviroc; r, ritonavir.

Table 3. Resistance patterns in the integrase region in HIV-2-infected patients that failed raltegravir

Patient	HIV-2 subtype	HIV-2 RNA (log copies/mL)	Mutation pathway	Resistance-associated mutations						
				I84	Q91	E92	T97	A119	G140	A153
1	A	2.9	Y143G		R		A	T		S
2	A	3.8	Y143G/C		R/Q	E/Q	A/T	T		S
3	B	4.4	Y143C		R					S
4	A	2.3	Q148R						A	
5	A	2.4	Q148R						S	
6	A	2.9	N155H	V						G
7	B	3.2	N155H	V						G
8	A	3.2	N155H	V		Q	A			S
9	A	2.3	N155H							G

Table 4. Integrase sequence evolution in three HIV-2 patients that failed on dolutegravir following raltegravir failure

	Patient 6	Patient 7	Patient 8
Raltegravir-selected variants	I84V, **N155H**, A153G	I84V, **N155H**, A153G	I84V, **E92Q**, T97A, A153S, **N155H**
Dolutegravir-selected variants	K4R, K14R, V75A, **G118R**, A119T, V151I, L220F	V141I, **Q148K**, V150T, A153S, Q208H	**Q148R**

Major mutations appear in bold. Accessory mutations are underlined. All other changes have not been associated with reduced susceptibility to INSTIs so far.

atazanavir/ritonavir (one patient) along with two nucleos(t)ides. All but one achieved significant HIV-2 viral load reductions (>0.5 logs and/or to <50 HIV-2 RNA copies/mL) 6 months after beginning dolutegravir therapy and this was accompanied by a median increase of 114 CD4+ T cells/mm^3 (IQR = 76–156). After a median of 14 months on dolutegravir therapy (range = 6–34), three patients experienced viral load rebound. At that time, integrase sequence analysis showed a selection of new mutations in all cases, with disappearance of N155H and replacement by changes at amino acid Q148K/R in two of them and G118R in another one, along with other amino acid changes (Table 4). Phylogenetic analyses were performed in all longitudinal samples to confirm that all sequences belonged to the same individuals (Figure S1, available as Supplementary data at *JAC* Online).

Discussion

Three drugs within the INSTI class are currently approved for the treatment of HIV-1 infection. Despite a 40% difference in amino acid sequence between HIV-1 and HIV-2 integrases, *in vitro* data and some clinical experience suggest that raltegravir, elvitegravir and dolutegravir are effective against HIV-2.[10–13] The INSTI

mechanism of action prevents the HIV integrase from incorporating proviral DNA into the human host cell chromosomes, inhibiting the strand transfer step. This step has no human homologue, making it a specific and effective HIV drug target. Besides, drugs within this family exhibit excellent tolerability and minimal toxicity.[18]

Different clinical trials conducted in HIV-1-infected individuals have demonstrated the efficacy and safety of raltegravir,[19,20] elvitegravir[21] and dolutegravir[22] in both treatment-naive and treatment-experienced patients. In the VIKING studies, dolutegravir activity was tested in patients with genotypic raltegravir resistance.[23] Interestingly, dolutegravir demonstrated efficacy in this subset of patients except for those with changes at codon Q148 plus ≥2 additional mutations at baseline.[24]

All integrase inhibitors approved so far exhibit potent activity against HIV-2.[10,11,25] However, no randomized trials addressing the question of when to start ART or the choice of initial or second-line therapy for HIV-2 infection have been completed until now.[4] Thus, the optimal treatment strategy for HIV-2 infection has not been defined. In addition, virological failure and resistance-associated mutations to NRTIs, PIs and/or INSTIs seem to be more frequently selected in HIV-2-infected patients on therapy.[8,9,26] In our study we examined the resistance profile to INSTIs in a relatively large group of HIV-2 drug-naive individuals treated with raltegravir and in a subset of patients that subsequently was exposed to dolutegravir after failing raltegravir.

In contrast to what is seen for NNRTIs, HIV-2 does not exhibit baseline resistance mutations to INSTIs in INSTI-naive patients.[27] In our study, from 20 HIV-2 drug-naive individuals, only one showed a polymorphism, at integrase codon E138A. The change at position E138K/A has already been described as a non-polymorphic accessory resistance mutation selected in HIV-1 patients treated with raltegravir, elvitegravir or dolutegravir.[27] It is generally selected in combination with Q148 mutations. By itself it does not reduce INSTI susceptibility.[28] However, it is associated with >100-fold reduced raltegravir and elvitegravir susceptibility and up to 10-fold reduced dolutegravir susceptibility when present along with Q148 mutations.[23] In our patient, the presence of isolated E138A was not associated with raltegravir failure and this supports that it did not compromise raltegravir antiviral activity.

Ten HIV-2 patients experienced viral rebound while on raltegravir. All but one developed integrase resistance-associated mutations. The most frequent pattern was N155H (four cases) followed by Y143G/C (three cases) and Q148R (two cases). Similarly we found other integrase changes previously described in HIV-2 infection (I84V, E92Q, T97A, G140A/S).[12,29] *In vitro* studies have examined the activity of dolutegravir against a panel of viruses with site-directed mutations in the integrase of HIV-2 subtype A.[30] Interestingly, a few single amino acid changes (T97A, G140S, Q148H or N155H) did not impact on dolutegravir susceptibility. By contrast, changes at positions E92Q, Y143C or Q148K/R alone or in combination led to moderate to high-level resistance to dolutegravir.[30] Based on those findings, all our patients should have exhibited extensive cross-resistance to dolutegravir.

Although only two patients in our series were infected with HIV-2 subtype B, we could not find any evidence of an association between HIV-2 subtype and a specific INSTI resistance pattern. For instance, it was Y143C + Q91R + A153S and N155H + I84V + A153G, respectively, in the two subtype B patients.

Changes at integrase position 153 (A153S/G) were seen in seven HIV-2 patients failing on raltegravir. In HIV-1 infection, S153Y/F is an extremely rare polymorphism selected *in vitro* by elvitegravir and dolutegravir. On the other hand, S153Y/F has been shown to reduce raltegravir and dolutegravir susceptibility by 2-fold and elvitegravir susceptibility by 4-fold.[31,32]

Five patients were treated with dolutegravir-based combinations after raltegravir failure. All but one experienced a significant viral load reduction. Previous studies reported short-term efficacy of dolutegravir in HIV-2 patients that failed on raltegravir.[12,33] Herein we report that after a median of 14 months, virological failure occurred in three initial responders. Interestingly, all three harboured N155H + I84V + A153G/S upon failing on raltegravir. This change disappeared in all of them upon failing on dolutegravir. Two evolved to select Q148K/R and the remaining to G118R, along with other minor changes. In molecular clones, Fransen *et al.*[34] reported that N155H is gradually replaced by Y143R or Q148H/R under continued raltegravir exposure. The combination pattern G140S/Q148R seems to be the one with the highest viral fitness whereas it provides the greatest high-level raltegravir resistance.[35] Interestingly, this pattern has already been associated with reduced susceptibility to dolutegravir in HIV-1 infection.[24] We hypothesize that a similar behaviour may occur in HIV-2, supporting the evolution in resistance patterns noticed in our patients. More intriguing is patient no. 6, in whom mutation G118R was selected replacing N155H; however, this change has already occasionally been shown to reduce dolutegravir resistance in SIVmac239 and HIV-1.[36–39]

In conclusion, there is a wide repertoire of resistance mutations in the integrase gene in HIV-2-infected patients failing raltegravir and/or dolutegravir. Although dolutegravir is efficacious in most patients harbouring primary raltegravir (and elvitegravir) drug resistance mutations, our data suggest that the resistance barrier to dolutegravir could be lower in HIV-2 compared with HIV-1 infection. Considering the limited options for ART in HIV-2 infection, our results highlight the importance of prioritizing potent antiretroviral combinations in HIV-2 infection to maximize the long-term benefit of ART, with particular attention on avoiding selection of drug resistance mutations that ultimately result in cross-resistance.

Acknowledgements

Members of the Spanish HIV-2 Study Group

C. Rodríguez, M. Vera and J. del Romero (Centro Sanitario Sandoval, Madrid); G. Marcaida and M. D. Ocete (Hospital General Universitario, Valencia); E. Caballero (Hospital Vall d'Hebrón, Barcelona); A. Aguilera (Hospital Conxo-CHUS, Santiago); M. J. Amengual and M. Cervantes (Corporación Sanitaria Parc Taulí, Barcelona); R. Benito and S. Algarate (Hospital Clínico Universitario Lozano Blesa, Zaragoza); R. Ortiz de Lejarazu and S. Rojo (Hospital Clínico Universitario, Valladolid); J. M. Eirós (Hospital Rio Hortega, Valladolid); C. Manzardo and J. M. Miró (Hospital Clínic-IDIBAPS, Universidad de Barcelona, Barcelona); J. García (Hospital Cristal-Piñor, Orense); E. Calderón (Hospital Virgen del Rocío, Sevilla; CIBER de Epidemiología y Salud Pública); M. Trigo, J. Diz and M. García-Campello (Complejo Hospitalario, Pontevedra); M. Rodríguez-Iglesias (Hospital Universitario Puerta del Mar, Cádiz); A. Hernández-Betancor and A. M. Martín (Hospital Insular Universitario, Las Palmas de Gran Canaria); J. M. Ramos and A. Gimeno (Hospital

Universitario, Alicante); F. Gutiérrez, J. C. Rodríguez and V. Sánchez (Hospital General, Elche); C. Gómez-Hernando (Complejo Hospitalario Virgen de la Salud, Toledo); G. Cilla and E. Pérez-Trallero (Hospital Donostia, San Sebastián); J. López-Aldeguer (Hospital La Fe, Valencia); L. Fernández-Pereira (Hospital San Pedro de Alcántara, Cáceres); J. Niubó (Ciudad Sanitaria de Bellvitge, Barcelona); N. Margall (Hospital Santa Creu i Sant Pau, Barcelona); M. Hernández, A. M. López-Lirola and J. L. Gómez-Sirvent (Hospital Universitario La Laguna, Tenerife); L. Force and M. Sauca (Hospital General, Mataró); C. Cifuentes (Hospital Son Llátzer, Palma de Mallorca); S. Pérez and L. Morano (Hospital do Meixoeiro, Vigo); C. Raya (Hospital del Bierzo, Ponferrada); A. González-Praetorius (Hospital Universitario, Guadalajara); J. L. Pérez and M. Peñaranda (Hospital Son Espases, Palma Mallorca); Silvia Hernáez-Crespo (Hospital de Basurto, Bilbao); J. M. Montejo (Hospital de Cruces, Bilbao); L. Roc and A. Martinez-Sapiña (Hospital Miguel Servet, Zaragoza); I. Viciana (Hospital Virgen de la Victoria, Málaga); T. Cabezas, A. Lozano and J. M. Fernández (Hospital de Poniente, Almería); I. García Bermejo and G. Gaspar (Hospital Universitario de Getafe, Madrid); R. García, M. Górgolas, C. Vegas, J. Blas and R. Tellez (Fundación Jiménez Díaz, Madrid); P. Miralles, M. Valeiro and T. Aldamiz (Hospital Gregorio Marañón, Madrid); F. García (Hospital Clínico Universitario, Granada); A. Suárez (Hospital Clínico San Carlos, Madrid); V. Soriano, P. Barreiro and J. Peña (Hospital, La Paz, Madrid); C. de Mendoza, S. Requena, A. Treviño, I. Carrasco, M. Cuesta, L. Benítez-Gutiérrez and V. Cuervas-Mons (Hospital Puerta de Hierro-Majadahonda, Madrid).

Funding

This work was supported in part by grants from Fundación Investigación y Educación en SIDA (F-IES); Fondo de Investigación Sanitaria-Fondos FEDER (FIS, PI13/01574; ICI14/0274, CES12/003, FI14/00264, CD14/00243).

Transparency declarations

None to declare.

Author contributions

All authors contributed to the work and saw and approved the final submission.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Azevedo-Pereira JM, Santos-Costa Q. HIV interaction with human host: HIV-2 as a model of a less virulent infection. *AIDS Rev* 2016; **18**: 44–53.

2 Kashyap B, Gautam H, Bhalla P. Epidemiology and seroprevalence of human immunodeficiency virus type 2. *Intervirology* 2011; **54**: 151–5.

3 Tchounga B, Ekouevi D, Balestre E *et al*. Mortality and survival patterns of people living with HIV-2. *Curr Opin HIV AIDS* 2016; **11**: 537–44.

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

5 Ren J, Bird L, Chamberlain P *et al*. Structure of HIV-2 reverse transcriptase at 2.35-Å resolution and the mechanism of resistance to non-nucleoside inhibitors. *Proc Natl Acad Sci USA* 2002; **99**: 14410–5.

6 Mahdi M, Szojka Z, Mótyán JA *et al*. Inhibition profiling of retroviral protease inhibitors using an HIV-2 modular system. *Viruses* 2015; **7**: 6152–62.

7 Balestre E, Ekouevi D, Tchounga B *et al*. Immunologic response in treatment-naïve HIV-2-infected patients: the IeDEA West Africa cohort. *J Int AIDS Soc* 2016; **19**: 20044.

8 Treviño A, de Mendoza C, Caballero E *et al*. Drug resistance mutations in patients infected with HIV-2 living in Spain. *J Antimicrob Chemother* 2011; **66**: 1484–8.

9 Charpentier C, Camacho R, Ruelle J *et al*. HIV-2EU-supporting standardized HIV-2 drug resistance interpretation in Europe: an update. *Clin Infect Dis* 2015; **61**: 1346–7.

10 Charpentier C, Larrouy L, Collin G *et al*. In-vitro phenotypic susceptibility of HIV-2 clinical isolates to the integrase inhibitor S/GSK1349572. *AIDS* 2010; **24**: 2753–5.

11 Roquebert B, Damond F, Collin G *et al*. HIV-2 integrase gene polymorphism and phenotypic susceptibility of HIV-2 clinical isolates to the integrase inhibitors raltegravir and elvitegravir *in vitro*. *J Antimicrob Chemother* 2008; **62**: 914–20.

12 Treviño A, Cabezas T, Lozano A *et al*. Dolutegravir for the treatment of HIV-2 infection. *J Clin Virol* 2015; **64**: 12–5.

13 Menéndez-Arias L, Alvarez M. Antiretroviral therapy and drug resistance in human immunodeficiency virus type 2 infection. *Antiviral Res* 2014; **102**: 70–86.

14 Treviño A, Caballero E, de Mendoza C *et al*. The burden of neglected HIV-2 and HTLV-1 infections in Spain. *AIDS Rev* 2015; **17**: 212–9.

15 Ferns R, Garson J. Development and evaluation of a real-time RT-PCR assay for quantification of cell-free human immunodeficiency virus type 2 using a Brome Mosaic Virus internal control. *J Virol Methods* 2006; **135**: 102–8.

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

17 Wensing A, Calvez V, Gunthard H *et al*. 2015 update of the drug resistance mutations in HIV-1. *Top Antivir Ther* 2015; **23**: 132–41.

18 Clercq E, Li G. Approved antiviral drug over the past 50 years. *Clin Microbiol Rev* 2016; **29**: 695–747.

19 Lennox J, DeJesus E, Lazzarin A *et al*. Safety and efficacy of raltegravir-based versus efavirenz-based combination therapy in treatment-naive patients with HIV-1 infection: a multicentre, double-blind randomised controlled trial. *Lancet* 2009; **374**: 796–806.

20 Rockstroh JK, Lennox JL, DeJesus E *et al*. Long-term treatment with raltegravir or efavirenz combined with tenofovir/emtricitabine for treatment-naive human immunodeficiency virus-1-infected patients: 156-week results from STARTMRK. *Clin Infect Dis* 2011; **53**: 807–16.

21 Sax PE, DeJesus E, Mills A *et al*. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus co-formulated efavirenz, emtricitabine, and tenofovir for initial treatment of HIV-1 infection: a randomised, double-blind, phase 3 trial, analysis of results after 48 weeks. *Lancet* 2012; **379**: 2439–48.

22 Dow D, Bartlett J. Dolutegravir, the second-generation of integrase strand transfer inhibitors (INSTIs) for the treatment of HIV. *Infect Dis Ther* 2014; **3**: 83–102.

23 Eron J, Clotet B, Durant J *et al*. Safety and efficacy of dolutegravir in treatment-experienced subjects with raltegravir resistant HIV-1 infection: 24-week results of the VIKING study. *J Infect Dis* 2013; **207**: 740–8.

24 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.

25 Smith R, Raugi D, Pan C *et al*. Three main mutational pathways in HIV-2 lead to high-level raltegravir and elvitegravir resistance: implications for emerging HIV-2 treatment regimens. *PLoS One* 2012; **7**: e45372.

26 Raugi D, Smith R, Ba S *et al*. Complex patterns of protease inhibitor resistance among antiretroviral treatment experienced HIV-2 patients from Senegal: implications for second-line therapy. *Antimicrob Agents Chemother* 2013; **57**: 2751–60.

27 Cavaco-Silva J, Abecasis A, Miranda AC *et al*. HIV-2 integrase polymorphisms and longitudinal genotypic analysis of HIV-2 infected patients failing a raltegravir-containing regimen. *PLoS One* 2014; **9**: e92747.

28 Jones GS, Yu F, Zeynalzadegan A *et al*. Preclinical evaluation of GS-9160, a novel inhibitor of human immunodeficiency virus type 1 integrase. *Antimicrob Agents Chemother* 2009; **53**: 1149–203.

29 Salgado M, Toro C, Simón A *et al*. Mutation N155H in HIV-2 integrase confers high phenotypic resistance to raltegravir and impairs replication capacity. *J Clin Virol* 2009; **46**: 173–5.

30 Smith RA, Raugi DN, Pan C *et al*. In vitro activity of dolutegravir against wild-type and integrase inhibitor-resistant HIV-2. *Retrovirology* 2015; **12**: 10.

31 Margot N, Hluhanich R, Jones G *et al*. *In vitro* resistance selection using elvitegravir, raltegravir and two metabolites of elvitegravir M1 and M4. *Antiviral Res* 2012; **93**: 288–96.

32 Kobayashi M, Yoshinaga T, Seki T *et al*. *In vitro* antiretroviral properties of S/GSK1349572, a next generation HIV integrase inhibitor. *Antimicrob Agents Chemother* 2011; **55**: 813–21.

33 Descamps D, Peytavin G, Visseaux B *et al*. Dolutegravir in HIV-2 infected patients with resistant virus to first-line integrase inhibitors from the French named patient program. *Clin Infect Dis* 2015; **60**: 1521–7.

34 Fransen S, Gupta S, Frantzell A *et al*. Substitutions at amino acid positions 143, 148, and 155 of HIV-1 integrase define distinct genetic barriers to raltegravir resistance *in vivo*. *J Virol* 2012; **86**: 7249–55.

35 Quashie PK, Mesplede T, Wainberg MA. Evolution of HIV integrase resistance mutations. *Curr Opin Infect Dis* 2013; **26**: 43–9.

36 Hassounah SA, Mesplède T, Quashie PK *et al*. Effect of HIV-1 integrase resistance mutations when introduced into SIVmac239 on susceptibility to integrase strand transfer inhibitors. *J Virol* 2014; **88**: 9683–92.

37 Malet I, Gimferrer Arriaga L, Artese A *et al*. New raltegravir resistance pathways induce broad cross-resistance to all currently used integrase inhibitors. *J Antimicrob Chemother* 2014; **69**: 2118–22.

38 Munir S, Thierry E, Malet I *et al*. G118R and F121Y mutations identified in patients failing raltegravir treatment confer dolutegravir resistance. *J Antimicrob Chemother* 2015; **70**: 739–49.

39 Brenner BG, Thomas R, Blanco JL *et al*. Development of a G118R mutation in HIV-1 integrase following a switch to dolutegravir monotherapy leading to cross-resistance to integrase inhibitors. *J Antimicrob Chemother* 2016; **71**: 1948–53.