J Antimicrob Chemother 2015; **70**: 3087–3095
doi:10.1093/jac/dkv205 Advance Access publication 30 July 2015

Journal of Antimicrobial Chemotherapy

Virological failure to raltegravir in Spain: incidence, prevalence and clinical consequences

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Received 3 February 2015; returned 1 May 2015; revised 9 June 2015; accepted 18 June 2015

Objectives: The objective of this study was to evaluate the incidence, prevalence and clinical consequences of virological failure (VF) to raltegravir-based regimens in Spain.

Methods: A multicentre, retrospective, observational study was performed in 10 tertiary hospitals (January 2006 to June 2013). The study included HIV-1-infected patients with loss of virological suppression (LVS; two consecutive HIV-1 RNA ≥50 copies/mL) while receiving raltegravir. VF and low-level viraemia (LLV) were defined as two consecutive HIV-1 RNA ≥200 copies/mL and 50 to <200 copies/mL, respectively. Integrase strand-transfer inhibitor resistance was investigated at LVS. During the 48 weeks following LVS, recorded data included clinical characteristics, treatment discontinuations, AIDS-associated events and deaths. Effectiveness of therapy following LVS was evaluated by ITT and PP. Multivariate regression was used to assess predictors of efficacy.

Results: Of the 15009 HIV-infected patients in participating centres, 2782 (18.5%) had received raltegravir-based regimens. Of those, 192 (6.9%), 125 (4.5%) and 67 (2.4%) experienced LVS, VF and LLV, respectively. The incidence of VF was 1.8 (95% CI, 1.5–2.1) per 100 patients/year. The prevalence of VF was 4.5% (95% CI, 3.8%–5.3%). Integrase-associated mutations were found in 78.8% of patients with integrase genotyping results available. High-level resistance to dolutegravir was not observed. Salvage therapy failed in 34.1% of patients; progression to AIDS/death occurred in 8.3% during the first year following LVS. The latter was associated with intravenous drug use, time on raltegravir and lower CD4+ count nadir in patients who started raltegravir-based treatments as salvage regimens.

Conclusions: VF with raltegravir is infrequent, but often associated with major clinical complications in treatment-experienced patients.

Introduction

Integrase strand-transfer inhibitors (INSTIs) have gained importance in the clinical management of HIV infection. Raltegravir, the first INSTI to be used in clinical practice, is one of the preferred drugs for both initial ART and infection by triple-class drug-resistant HIV-1 owing to its good efficacy and safety profiles.[1–5] Based on efficacy results, elvitegravir has been approved for treatment-naive patients in a fixed-dose combination tablet with tenofovir, emtricitabine and cobicistat.[6–10] More recently, dolutegravir, a second-generation drug, was also approved for use in treatment-naive patients and in patients with triple-class drug resistance, including those with integrase resistance.[11–13]

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The first-generation INSTIs raltegravir and elvitegravir have a low genetic barrier to resistance and extensive cross-resistance between both agents has been reported.[14–16] As with other drugs, maintenance of INSTIs in the presence of active viral replication leads to the accumulation of multiple resistance mutations and increased cross-resistance.[17] Raltegravir resistance has been detected in up to 68% of patients experiencing failure to raltegravir in clinical trials.[18] Raltegravir resistance develops through three distinct mutational pathways defined by the presence of one signature mutation in the integrase catalytic domain (Y143, Q148 or N155), usually with at least one accessory mutation, which often restores fitness.[3,18–20] In addition, broad cross-resistance to all currently used INSTIs was recently described in raltegravir-failing patients harbouring G118R and F121Y mutations.[21] Dolutegravir-containing regimens seem to be the best option for salvage therapy after failure to first-generation INSTIs.[12] Nevertheless, although viruses with the 155H mutation remain susceptible to dolutegravir, the presence of mutations at position 148 alongside one or two additional mutations may impair viral susceptibility to this drug,[22,23] thus highlighting the importance of early withdrawal of the drug to reduce INSTI selective pressure during virological failure.

Data on the antiviral efficacy of INSTI-based ART and patterns of INSTI-associated drug resistance mutations come mainly from *in vitro* studies and clinical trials. However, in a recent review of the results of all clinically indicated integrase genotyping tests performed at a US national referral laboratory from 2009 through to 2012, approximately one in six genotypes harboured significant INSTI resistance, with the Q148 and N155 pathways being equally common and dolutegravir susceptibility preserved.[24] Reports of transmission of INSTI resistance to newly HIV-infected patients,[25,26] coupled with the more frequent use of INSTIs,[6,8,27] point to the need for continued surveillance of INSTI resistance. A significant number of patients treated with INSTIs (mainly raltegravir) to date initiated these drugs as part of a salvage regimen and had limited treatment options. Thereby, virological failure to raltegravir in advanced salvage ART might be associated with severe clinical complications. Other INSTIs, such as elvitegravir or dolutegravir, have recently become commercially available in Spain, although experience with them is very limited. This study aimed to describe the incidence, prevalence and clinical consequences of virological failure to raltegravir-based regimens in Spain.

Methods

Study population and data collection

The present analysis was a multicentre, retrospective, observational study. The study population included all HIV-1-infected adults who experienced loss of virological suppression (LVS; defined as a confirmed HIV-1 RNA ≥50 copies/mL) while receiving raltegravir-based regimens and who had at least one follow-up visit in Spain between January 2006 and June 2013. No patients on elvitegravir-based regimens were included, because this drug was not commercially available in Spain at the time inclusion finished, clinical trials with elvitegravir were ongoing and treatment allocation was not open. Data were retrieved from electronic medical records at 10 tertiary care centres that were representative of the geographical distribution of the Spanish HIV epidemic. Patients participating in randomized, double-blind and placebo-controlled clinical trials were only included if drug assignments were open at inclusion.

In the case of patients who had consecutive LVS to raltegravir-containing regimens, only the first regimen was considered for estimation of the incidence and prevalence of virological failure and clinical outcomes. Nevertheless, resistance data from consecutive virological failures were accepted to maximize detection of INSTI-associated mutations. Demographic, clinical and genotypic resistance data were collected at virological failure to raltegravir-based regimens (baseline) and during the 48 weeks after virological failure (at 12–24 week intervals according to clinical practice). The data collected during the follow-up period were salvage ART started, reasons for discontinuing salvage ART, records of treatment adherence when available, HIV-1 RNA levels, CD4+ cell count, AIDS-associated events and AIDS-associated and non-associated deaths.

The primary endpoint of the study was the incidence and prevalence of virological failure to raltegravir therapy. Virological failure was defined as two consecutive HIV-1 RNA measurements ≥200 copies/mL at any time prior to enrolment while receiving the first raltegravir-based regimen. Secondary endpoints included the percentage of patients whose treatment failed with low-level viraemia (LLV; defined as two consecutive HIV-1 RNA measurements between 50 and <200 copies/mL) and the proportion of patients with LVS. In addition, virological and immunological responses, rates of treatment failure and progression to AIDS or death for any reason during the following 48 weeks after raltegravir failure were also evaluated. Treatment failure was defined as any discontinuation of the salvage ART regimen. The Ethics Committee of each participating centre approved the study, which was performed according to the Declaration of Helsinki (Seoul, 2008).

Resistance analysis

In order to describe the main INSTI resistance patterns and to estimate the susceptibility to INSTI at the time of virological failure, we analysed the results of the genotypic resistance tests performed using the ViroSeq™ HIV-1 Genotyping System (Abbott Laboratories, Abbott Park, IL, USA), the TRUGENE™ HIV-1 Genotyping Kit (Bayer HealthCare, Tarrytown, NY, USA) or in-house techniques in the referral laboratory at each participating centre.

INSTI resistance was defined based on the Stanford University HIV Drug Resistance Database genotypic resistance interpretation algorithm (HIVdb Program, version 6.3.0)[28] as follows: susceptible (0–9 points), intermediate (10–59 points) and high level (≥60 points). Sequence analyses were conducted on 4 July 2013. With the aim of incorporating the latest available information on INSTI-related resistance at the time of the analysis, we added the following scores after reviewing data published in manuscripts or conference proceedings: 20 points for each of the mutation combinations 148HRK+74I and 148HRK+138T and 5 points for mutation 74I.[29]

Statistical analysis

Variables with a normal distribution were described as mean (SD) and compared using the *t*-test. Median and IQR were used to describe variables that did not follow a normal distribution, which were compared using a non-parametric test. Percentages were compared using the χ^2 test or an exact binomial test when appropriate. The incidence rate was estimated as the number of events observed divided by the time at risk of an event during the observation period (January 2006 to June 2013). Prevalence was calculated by dividing the number of cases by the number of individuals assessed. Incidence and prevalence were estimated and presented with their 95% CI, assuming that data were distributed binomially. Efficacy analyses were performed by ITT [missing equal to failure (M=F)] and PP (censoring missing data).

Multivariate logistic regression models were fitted using variables with a *P* value <0.25 in the univariate analyses to identify factors associated

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with virological failure and progression to AIDS or death in the group of patients who had initiated raltegravir owing to prior virological failure or as part of a switching strategy. ART-naive patients who started raltegravir as first-line therapy were not included in the models owing to the low number of patients available. The adjusted models were constructed using the maximum available data. In order to avoid multicollinearity when variables were correlated, the most significant covariates from the univariate model were included in the multivariate analysis. Both backward elimination and backward stepwise methods were used to obtain a multivariate model.

The OR and its 95% CI were also calculated. Statistical analysis was performed using SPSS version 15.0 (Chicago, IL, USA) and R (R Foundation for Statistical Computing, Vienna, Austria).[30] Differences were considered statistically significant at $P<0.05$.

Results

Incidence, prevalence and clinical outcomes

Of a total of 15009 treated HIV-infected individuals from participating Spanish care centres, 2782 (18.5%) had been exposed to raltegravir-based therapy. Of these, 17 individuals had been exposed to raltegravir more than once. Overall, 209 patients experienced LVS, 138 experienced virological failure and 71 experienced LLV (Table 1). From the 2782 patients exposed to raltegravir, 192 (6.9%) with LVS to their first raltegravir-based regimen [125 (65.1%) patients with virological failure and 67 (34.9%) with LLV] were included in this analysis. Therefore, the incidence and prevalence of virological failure to raltegravir-based treatments were 1.8 (95% CI, 1.5–2.1) virological failures per 100 patients on treatment per year and 4.5% (95% CI, 3.8%–5.3%), respectively, between January 2006 and June 2013. In addition, the prevalence of LLV and LVS was calculated as follows: [(67/2782)×100], which equals 2.4% (95% CI, 1.9%–3.0%), and [(192/2782)×100], which equals 6.9% (95% CI, 5.9%–7.9%), respectively.

Patients with LVS had a median time on INSTI treatment of 1.2 (0.7–1.9) years. Of these, 106 (55.2%) patients had started raltegravir-based regimens as part of a salvage ART regimen, 74 (38.5%) as part of switching strategies, 8 (4.2%) were ART-naive patients and 4 (2.1%) had voluntarily interrupted treatment. As part of their salvage therapy, 129 (67.2%) patients were receiving darunavir/ritonavir, 107 (55.7%) lamivudine or emtricitabine, 94 (49.0%) tenofovir and 74 (38.5%) etravirine. In addition, raltegravir was retained in 102 (53.1%) patients after virological failure. Other characteristics are shown in Table 2.

At 48 weeks of follow-up after failure of raltegravir, 87 (45.3%) patients had achieved HIV-1 RNA <200 copies/mL, 60 (31.3%) had no data because of loss to follow-up, 22 (11.5%) had interrupted their treatment voluntarily, 13 (6.8%) had experienced virological failure and 10 (5.2%) had died during follow-up. Therefore, according to the ITT efficacy analysis (M=F), 105/192 (54.7%) patients experienced treatment failure; in the PP analysis, 45/132 (34.1%) patients experienced failure. A reduction in the median (IQR) HIV-1 RNA from 3.1 (1.8–4.9) $\log_{10}$ to 2.7 (2.1–3.9) $\log_{10}$ was detected at week 48 after failure of raltegravir ($P=0.032$), as was an increase in the median (IQR) CD4+ cell count from 291 (133.8–512.3) to 362.5 (177.5–565.8) cells/mm^3 ($P<0.001$). In the multivariate analysis, the independent predictors of virological failure to the salvage regimen in the group of patients who started raltegravir-based regimens owing to prior virological failure were plasma viral load at initiation of salvage ART (OR, 2.36; 95% CI, 1.36–4.08; $P=0.002$) and estimated adherence <90% according to clinical records (OR, 13.06; 95% CI, 2.51–67.77; $P=0.002$). In the group of patients who initiated raltegravir as a switching strategy, only estimated adherence <90% was found to be associated with virological response (OR, 8.05; 95% CI, 3.38–19.18; $P<0.001$) (Table 3).

At week 48, 16 (8.3%) patients had confirmed progression to AIDS and/or had died. Of these, 11 (5.7%) experienced an AIDS-defining event and 10 (5.2%) died for any reason.

Table 1. Number of treated HIV-infected patients exposed to INSTIs and with LVS during the study period (between 2006 and 2013) in participating centres[a]

Centre	Treated HIV-infected patients	Patients exposed to INSTIs	Patients with LVS while receiving INSTIs[b]	Patients with virological failure	Patients with LLV
1	3700	690	54	38	16
2	2200	703	34	32	2
3	2119	384	25	9	16
4	1570	218	6	6	0
5	1515	223	8	7	1
6	1352	132	18	7	11
7	968	139	6	6	0
8	660	100	12	2	10
9	520	144	15	11	4
10	405	49	14	7	7
Total	15009	2782	192	125	67

[a]Participating centres: Hospital Clinic de Barcelona, Germans Trias i Pujol University Hospital, Hospital Ramón y Cajal, Gregorio Marañón Hospital, Hospital del Mar, Donostia University Hospital, La Fe University and Polytechnic Hospital, Santiago de Compostela Clinical University Hospital, Elche University General Hospital and Mataró Hospital.
[b]All patients were receiving raltegravir. None of the patients exposed to elvitegravir experienced LVS.

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Table 2. Characteristics of patients with LVS while on treatment with raltegravir-based regimens ($n=192$)[a]

	General ($n=192$)	Patients with virological failure ($n=125$)	Patients with LLV ($n=67$)
Age (years)[b]	46.0 (42.0–51.0)	46.0 (42.0–52.0)	47.0 (42.0–50.0)
Gender			
male	142 (74.0)	87 (69.6)	55 (82.1)
female	50 (26.0)	38 (30.4)	12 (17.9)
HCV/HBV coinfection	79 (41.1)	54 (43.2)	25 (37.3)
Intravenous drug use	61 (31.8)	40 (32.0)	21 (31.3)
CDC stage			
A	33 (17.2)	19 (15.2)	14 (20.9)
B	27 (14.1)	12 (9.6)	15 (22.4)
C	67 (34.9)	45 (36.0)	22 (32.8)
unknown	65 (33.9)	49 (39.2)	16 (23.9)
Time on INSTI treatment (years)[b]	1.2 (0.7–1.9)	1.8 (0.6–1.9)	1.1 (0.7–2.1)
Nadir CD4+ count (cells/mm^3)[b]	80 (27.8–170.0)	72 (24.0–176.0)	100 (40.5–171.0)
Zenith HIV-1 RNA (log)	5.5 (4.9–5.9)	5.6 (5.0–5.9)	5.3 (4.9–5.8)
Viral tropism			
CCR5	67 (34.9)	46 (36.8)	21 (31.3)
CXCR4	14 (7.3)	10 (8.0)	4 (6.0)
dual/mix	22 (11.5)	19 (15.2)	3 (4.5)
not available	89 (46.4)	50 (40.0)	39 (58.2)
Time since HIV diagnosis (years)[b]	15.3 (10.3–19.0)	15.9 (10.7–19.5)	14.5 (9.6–17.9)
Adherence			
<90%	61 (31.8)	53 (42.4)	8 (11.9)
≥90%	93 (48.4)	43 (34.4)	50 (74.6)
not available	38 (19.8)	29 (23.2)	9 (13.4)
Reasons for starting INSTI			
virological failure	106 (55.2)	69 (55.2)	37 (55.2)
switching strategies	74 (38.5)	49 (39.2)	25 (37.3)
naive to ART	8 (4.2)	4 (3.2)	4 (6.0)
previous ART interruption	4 (2.1)	3 (2.4)	1 (1.5)

HCV, hepatitis C virus; HBV, hepatitis B virus.
[a]All values are expressed as *n* (%), unless otherwise indicated.
[b]Median (IQR).

The independent predictors in the multivariate analysis of progression to AIDS or death in the group of patients who started raltegravir-based regimens due to prior virological failure were intravenous drug use (OR, 0.11; 95% CI, 0.02–0.56; $P=0.008$), lower CD4+ cell count nadir at initiation of salvage ART (OR, 0.95 per each increase of 100 cells/mm^3; 95% CI, 0.92–0.98; $P=0.007$) and time on raltegravir (OR, 0.31; 95% CI, 0.11–0.88; $P=0.028$). No factors associated with progression to AIDS or death were found in patients who initiated raltegravir-based regimens as part of a switching strategy (Table 4).

Analysis of resistance

Of the 209 patients with LVS (including 17 patients with consecutive failures to different raltegravir-based regimens), 154 (73.7%) did not undergo genotyping. Among these patients, genotyping tests were not requested in 144 (68.9%) cases and were requested, but not amplified, in 10 (4.8%). Of the 55 patients who did undergo genotyping, the integrase gene was correctly amplified, with available results in 33 (60.0%) samples: 31 (93.9%) genotypes were obtained from patients who experienced virological failure and 2 (6.1%) from patients with LLV. Integrase-associated mutations were found in 26 (78.8%) patients who had valid integrase genotyping results available.

Of the integrase genotyping test samples with valid results, 19 (57.6%) harboured at least two integrase-associated mutations, 7 (21.2%) harboured single mutations and 7 (21.2%) harboured no resistance mutations. The most frequent integrase-associated mutations were 155H (36.4%), 140S (18.2%), 148H (15.2%) and 143R, 151I and 203M (12.1% each). Integrase-associated

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Table 3. Factors associated with virological failure of the salvage ART initiated following raltegravir failure

	Initiation of RAL-based ART as a salvage regimen				Initiation of RAL-based ART as a switching strategy			
	univariate analysis OR (95% CI)	*P* value[a]	multivariate analysis OR (95% CI)	*P* value	univariate analysis OR (95% CI)	*P* value[a]	multivariate analysis OR (95% CI)	*P* value
Age (years)	1.00 (0.96–1.04)	0.935	—	—	0.98 (0.93–1.04)	0.609	—	—
Male gender	0.76 (0.28–2.08)	0.607	—	—	0.33 (0.10–1.00)	0.051	—	—
HCV/HBV coinfection	0.78 (0.34–1.77)	0.554	—	—	0.92 (0.35–2.39)	0.872	—	—
Intravenous drug use	1.15 (0.49–2.70)	0.738	—	—	0.84 (0.30–2.29)	0.737	—	—
Stage C of CDC	1.05 (0.46–2.41)	0.893	—	—	0.83 (0.29–2.35)	0.728	—	—
Time on RAL treatment (years)[b]	0.90 (0.55–1.47)	0.699	—	—	0.90 (0.51–1.59)	0.739	—	—
CD4+ cell count nadir (cells/mm^3)	0.99 (0.99–1.00)	0.696	—	—	0.99 (0.99–1.00)	0.330	—	—
CD4+ at baseline (cells/mm^3)[c]	0.99 (0.99–1.00)	0.030	—	—	0.99 (0.99–1.00)	0.226	—	—
Zenith of HIV-1 RNA (log)	1.50 (0.81–2.79)	0.191	—	—	1.25 (0.75–2.08)	0.378	—	—
HIV-1 RNA at baseline (log)	1.98 (1.36–2.90)	<0.001	2.36 (1.36–4.08)	0.002	1.48 (0.95–2.32)	0.082	—	—
CCR5 viral tropism (n=67)	1.05 (0.46–2.41)	0.893	—	—	0.49 (0.16–1.52)	0.221	—	—
Time since HIV diagnosis (years)[b]	1.03 (0.96–1.11)	0.343	—	—	0.99 (0.93–1.06)	0.963	—	—
Adherence <90%	17.30 (3.76–79.50)	<0.001	13.06 (2.51–67.77)	0.002	3.91 (1.24–12.29)	0.020	8.05 (3.38–19.18)	<0.001

RAL, raltegravir; HCV, hepatitis C virus; HBV, hepatitis B virus.
[a]All analyses are ITT. The full multivariate model included age, gender, intravenous drug use as mode of HIV transmission, hepatitis C virus/hepatitis B virus coinfection, CDC stage before baseline, CD4+ cell count at baseline, increase in CD4+ cell count, CD4+ cell count nadir, viral load at baseline, zenith viral load, decrease in viral load, time since HIV diagnosis, time on treatment with raltegravir, adherence, viral tropism and reasons for raltegravir-based treatment initiation (virological failure versus switching strategy).
[b]Risk per year.
[c]Risk per each 100 cells/mm^3.

mutations at residues 148, 155 and 143 were only observed in patients with HIV-1 RNA ≥200 copies/mL. In addition, 19 (57.6%) individuals had a combination of mutations that decreased susceptibility to at least one INSTI. Mutation 155H was observed alongside another mutation in 8/33 (24.2%) genotypes; in 3/33 (9.1%) cases, 155H was combined with two other mutations. Mutations 148HKR combined with one and at least two additional mutations were observed in 5 (15.2%) and 3 (9.1%) cases, respectively. In addition, 148HKR combined with 140S and at least one other mutation was also observed in 3/33 (9.1%) cases. Combinations of mutations containing 143CHR were observed in two (6.1%) cases. Figure 1 shows the frequency of integrase-related mutations and their combinations in patients with LVS stratified according to reasons for initiation of raltegravir.

High-level resistance to raltegravir and elvitegravir was predicted in 17 (51.5%) and 15 (45.5%) cases, respectively, whereas 11 (33.3%) and 15 (45.5%) genotypes retained full susceptibility. Intermediate resistance to dolutegravir was predicted in 11 (33.3%) genotypes, whereas the remaining 22 (66.7%) showed full susceptibility to dolutegravir. High-level dolutegravir resistance was not found.

Discussion

We found an incidence rate of virological failure of 2 per 100 patients receiving raltegravir-based ART per year and a prevalence of 5% between January 2006 and June 2013. Despite an improvement in the CD4+ cell count and a decrease in the plasma HIV-1 viral load, 34% experienced a new episode of treatment failure and 7% experienced a new episode of virological failure after raltegravir failure. In addition, progression to AIDS and/or death was observed during the first year after failure in 8% of patients, mainly in heavily pretreated patients. This finding was associated with intravenous drug use, a low CD4+ cell count nadir at initiation of salvage ART and time on raltegravir in the group of patients who started raltegravir-based regimens owing to prior virological failure.

In clinical trials, virological failure to raltegravir-based regimens has been reported in up to 35% of experienced patients with triple-class drug resistance[3] and up to 10% of naive patients.[31] Nevertheless, few studies specifically evaluate the epidemiological characteristics of failure with raltegravir-based regimens in large populations. Although our study population could be subject to selection and recall biases, our estimations of 2% annual incidence of virological failure with a prevalence of 5% in Spain are lower than in the above-mentioned clinical trials, especially if we consider the increased use of raltegravir in clinical practice and the fact that most patients were ART experienced and had initiated raltegravir as part of a salvage regimen.

Clinical consequences and their management are a key concern in failure of raltegravir-based regimens. We observed that 34% of patients who initiated salvage ART regimens experienced

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Table 4. Factors associated with progression to AIDS or death during 1 year following raltegravir failure

	Initiation of RAL-based ART as a salvage regimen				Initiation of RAL-based ART as a switching strategy			
	univariate analysis OR (95% CI)	*P* value[a]	multivariate analysis OR (95% CI)	*P* value	univariate analysis OR (95% CI)	*P* value[a]	multivariate analysis OR (95% CI)	*P* value
Age (years)	0.98 (0.92–1.05)	0.603	—	—	0.98 (0.89–1.08)	0.763	—	—
Male gender	0.63 (0.15–2.69)	0.543	—	—	2.65 (0.29–23.92)	0.385	—	—
HCV/HBV coinfection	0.82 (0.22–3.03)	0.771	—	—	0.28 (0.04–1.63)	0.158	—	—
Intravenous drug use	0.30 (0.07–1.15)	0.080	0.11 (0.02–0.56)	0.008	0.18 (0.03–1.21)	0.002	—	—
Stage C of CDC	0.14 (0.02–0.71)	0.018	—	—	2.07 (0.22–18.80)	0.516	—	—
Time on RAL treatment (years)[b]	0.49 (0.16–1.47)	0.205	0.31 (0.11–0.88)	0.028	0.70 (0.17–2.88)	0.624	—	—
CD4+ cell count nadir (cells/mm^3)	0.96 (0.93–0.99)	0.015	0.95 (0.92–0.98)	0.007	0.99 (0.97–1.00)	0.131	—	—
CD4+ at baseline (cells/mm^3)[c]	0.99 (0.98–0.99)	0.006	—	—	0.99 (0.99–1.00)	0.362	—	—
Zenith of HIV-1 RNA (log)	5.69 (1.17–27.52)	0.031	—	—	1.72 (0.49–5.97)	0.391	—	—
HIV-1 RNA at baseline (log)	2.14 (1.06–4.33)	0.033	—	—	1.04 (0.56–1.92)	0.891	—	—
CCR5 viral tropism[d]	3.01 (0.60–14.99)	0.177	—	—	—	—	—	—
Time since HIV diagnosis (years)[b]	1.04 (0.92–1.18)	0.472	—	—	0.93 (0.83–1.04)	0.240	—	—
Adherence <90%	2.52 (0.52–12.07)	0.246	—	—	1.12 (0.20–6.06)	0.895	—	—

RAL, raltegravir; HCV, hepatitis C virus; HBV, hepatitis B virus.
[a]All analyses are ITT. The full multivariate model included age, gender, intravenous drug use as mode of HIV transmission, hepatitis C virus/hepatitis B virus coinfection, CDC stage before baseline, CD4+ cell count at baseline, increase in CD4+ cell count, CD4+ cell count nadir, viral load at baseline, zenith viral load, decrease in viral load, time since HIV diagnosis, time on treatment with RAL, adherence, viral tropism and reasons for initiation of raltegravir-based treatment (virological failure versus switching strategy).
[b]Risk per year.
[c]Risk per each 100 cells/mm^3.
[d]Not available for the group of patients initiating raltegravir-based ART as a switching strategy.

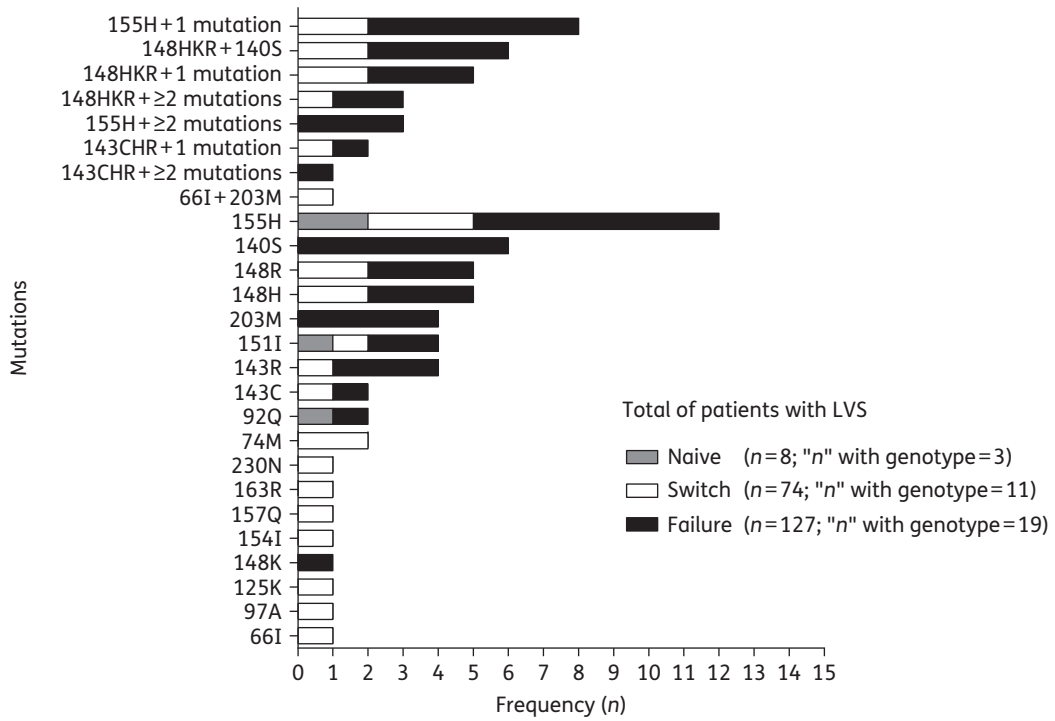


Figure 1. Frequency of integrase-related mutations and their combinations in patients with LVS and an available integrase genotyping test result according to reasons for initiation of raltegravir. Substitutions not shown were not detected.

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a new treatment failure with an 8% rate of progression to AIDS or death. Voluntary interruption was the most frequent cause of treatment failure, followed by virological failure. In addition, a considerable percentage of patients were lost to follow-up, suggesting poor adherence in this mostly ART-experienced population. Our findings are consistent with data reported elsewhere, as is the observation that poor adherence, plasma HIV-1 viral load, CD4 cell count nadir at initiation of salvage ART, time on raltegravir and intravenous drug use were associated with an increased risk of virological failure to salvage ART and progression to AIDS or death.[19,31–39]

The percentage of patients whose raltegravir-based regimen failed and who had not undergone integrase genotyping tests was high and many retained raltegravir in their salvage regimens, despite the risk of accumulating multiple integrase-associated mutations over time.[17] These findings could be explained by the fact that most patients were ART experienced and possibly had a limited number of therapeutic options. In addition, raltegravir was commercially available before integrase genotyping tests in the participating centres. Nevertheless, lack of genotyping may have important consequences in the near future in terms of accumulation of integrase mutations, loss of dolutegravir activity in salvage therapy[17,22] and increase in health-related costs. Therefore, it is important for HIV care providers to understand INSTI resistance and its consequences[24] and to establish mechanisms for surveillance of INSTI resistance.

Few data are available on the prevalence of integrase-associated mutations. In the USA, a prevalence of 15% was recently reported, as were equal representation of N155H and Q148HKR insertions and 2% of predicted high resistance to dolutegravir.[24] In our study, the high number of patients who had not undergone integrase genotyping made it difficult to estimate the prevalence of integrase-associated mutations. Nevertheless, consistent with data reported elsewhere,[18,20] we found the most frequent mutations to be 155H followed by 148HR and 140S (alone or in combination with other mutations). We also observed a predicted high level of resistance to raltegravir and elvitegravir in half of the sequences. In addition, 33% of genotypes retained intermediate susceptibility to dolutegravir, but no cases of predicted high resistance were found. Therefore, in agreement with other authors,[12,13,24] we believe that dolutegravir could be a useful component of raltegravir-based salvage regimens in routine clinical practice in Spain.

Our study is subject to a series of limitations. The retrospective, uncontrolled design and the lack of a comparison group could lead to bias or unmeasured confounding factors. The limited number of ART-naive patients available makes it difficult to perform analyses for this clinical setting. Selection and recall biases could explain the low incidence and prevalence rates of virological failure, even though most of the patients included were ART experienced and had initiated raltegravir as part of a salvage regimen. In addition, the limited number of patients whose treatment with raltegravir failed and who underwent integrase genotyping at failure, as well as the absence of a genotypic sensitivity score, could hamper assessment of the efficacy of salvage regimens.

The main strength of our study is that it is the first to evaluate the incidence, prevalence and clinical consequences of failure to first-generation INSTI-based regimens in Spain. Notable rates of treatment failure and progression to AIDS or death, together with the main associated risk factors, were observed after failure of raltegravir in this mostly ART-experienced, HIV-infected population. In addition, the high number of treatment failures in clinical practice in patients with no integrase genotyping data and the expected increase in the use of INSTI-based regimens and transmitted INSTI resistance[25,26] highlight the importance of surveillance of integrase mutations in other countries where INSTIs are available.

In conclusion, the incidence and prevalence rates of virological failure to raltegravir-based regimens in Spain are low. Nevertheless, treatment failures to salvage ART and progression to AIDS or death are major clinical complications in ART-experienced patients whose raltegravir-based regimens fail. The low frequency of emergent mutations conferring high-level resistance to dolutegravir in Spain could be useful when designing salvage regimens in treatment-experienced patients with prior INSTI failure. Finally, it is very important for HIV care providers to understand INSTI resistance and its consequences and to establish mechanisms for the surveillance of integrase mutations.

Acknowledgements

We are grateful to Thomas O'Boyle for editorial assistance, Aintzane Aiestarán and Nuria Pérez-Alvarez for their support in the statistical analysis and Isabel Bravo and Cristina Herrero for their assistance in coordinating and recording data.

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Funding

This work was supported in part by grants from Lluita contra la SIDA Foundation (Barcelona, Spain), the Spanish AIDS Network 'Red Temática de Investigación en SIDA' (RIS, RD12/0017) and ViiV Healthcare (Tres Cantos, Spain). M. P. was supported by a grant from the Spanish Ministry of Economy and Competitiveness. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript. The authors had full access to the data and the corresponding author had the final responsibility for submitting the manuscript for publication.

Transparency declarations

J. R. S. has received research funding, consultancy fees and lecture sponsorships from and has served on advisory boards for Abbott, Boehringer Ingelheim, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Bristol-Myers Squibb, Merck Sharp & Dohme, ViiV Healthcare and

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Pfizer. J. L. B. has received funding, consultancy fees, travel expenses and lecture sponsorships from Abbot, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohme and ViiV Healthcare. M. M. has received research funding, consultancy fees and lecture sponsorships from Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohme and ViiV Healthcare. F. G. has received research funding, consultancy fees and lecture sponsorships from Bristol-Myers Squibb, Boehringer Ingelheim, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Merck Sharp & Dohme and ViiV Healthcare. M. J. P.-E. has received research funding, consultancy fees and lecture sponsorships from and has served on advisory boards for Abbott, Boehringer Ingelheim, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Bristol-Myers Squibb and ViiV Healthcare. J. A. I. has received research funding, consultancy fees and lecture sponsorships from and has served on advisory boards for Gilead Sciences, Janssen-Cilag, Abbvie, Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Pfizer and ViiV Healthcare. L. F. has received research funding, consultancy fees and lecture sponsorships from and has served on advisory boards for Abbott, Boehringer Ingelheim, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Bristol-Myers Squibb, Merck Sharp & Dohme and ViiV Healthcare. A. A. has received research funding, consultancy fees and honoraria for participating in speaker bureaus or advisory boards from Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag and ViiV Healthcare. H. K. has received research funding, consultancy fees and lecture sponsorships from and has served on advisory boards for Abbott, Boehringer Ingelheim, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Bristol-Myers Squibb and ViiV Healthcare. M. S. has received research funding, consultancy fees and lecture sponsorships from and has served on advisory boards for Gilead Sciences, Janssen-Cilag, Bristol-Myers Squibb, Merck Sharp & Dohme and Pfizer. J. C. L. B. D. Q. has received research funding, consultancy fees and lecture sponsorships from and has served on advisory boards for Boehringer Ingelheim, ViiV Healthcare, Bristol-Myers Squibb, Abbvie, Gilead Sciences, Janssen-Cilag, Roche and Merck Sharp & Dohme. R. P. has received consulting fees from Boehringer Ingelheim, Gilead Sciences, GlaxoSmithKline, Merck Sharp & Dohme, Pfizer and ViiV Healthcare. B. C. has received research funding, consultancy fees and lecture sponsorships from and has served on advisory boards for Abbott, Boehringer-Ingelheim, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Merck Sharp & Dohme, Panacos, Pfizer, Roche and Tibotec. M. P.: none to declare.

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