

# Prevalence of integrase inhibitor resistance mutations in Austrian patients recently diagnosed with HIV from 2008 to 2013

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## Abstract

**Purpose** Treatment guidelines often do not advocate testing for integrase inhibitor resistance associated mutations (IRAM) before initiation of first line ART given the extremely low prevalence of mutations found in older surveillance studies. We aimed to describe the prevalence of IRAM in Austrian patients recently diagnosed with HIV in the 5 years following introduction of integrase inhibitors and to analyse trends and factors associated with their detection.

**Methods** Samples of antiretroviral treatment (ART) naïve patients recently diagnosed with HIV in Austria between 2008 and 2013 were analysed for the existence of IRAM and drug penalty scores were calculated to estimate response to drugs. Demographic and virological data were extracted from a database. Descriptive and comparative statistics were used.

**Results** A total of 303 samples were analysed. 78 % were male and mean age was 38 years. Overall prevalence of IRAM was 2.3 %. Six percent had at least potentially low-level resistance to raltegravir or elvitegravir, versus 1 % for dolutegravir. One primary mutation was observed (F121Y) in a patient sample from 2012 leading to 5–10-fold reduced susceptibility to raltegravir and elvitegravir. Two patients carried the accessory mutations E138K and G140A, respectively, where both lie on the Q148 pathway. No temporal trend of IRAM prevalence was observed ( $p = 0.16$ ).

**Discussion** Primary IRAM are still rarely found despite the increasing use of INSTI in Austria, but there is a potential for reduced susceptibility to these drugs in selected patients. Routine resistance testing seems prudent to avoid the consequences including accumulation of further mutations and therapeutic failure.

**Keywords** HIV · Integrase inhibitor · Resistance · INSTI · IRAM

## Introduction

Integrase strand transfer inhibitors (INSTI) are the latest approved drug class to treat HIV infection. They exhibit antiretroviral activity by blocking the integration of HIV viral DNA into host cells. Raltegravir (RAL) was the first approved INSTI in Austria in 2008 and was initially used as a component of salvage regimens. Its promising efficacy and excellent tolerability has soon led to its introduction into first line regimens [1]. The later approved elvitegravir (EVG) and more recently dolutegravir are once daily options with excellent tolerability as well as potency and in the case of dolutegravir a significantly higher genetic barrier [2, 3].

Given these properties, which make INSTI ideal components of antiretroviral regimens, its use has greatly increased. However, resistance to the first generation integrase inhibitors RAL and EVG can arise rapidly as a result of single mutations or combinations of mutations in the integrase gene and cross resistance has been described [4–6]. At least 1–2 % of treatment naïve patients and 19 % of treatment experienced patients started on a new RAL based regimen which will develop an emergent primary integrase inhibitor resistance associated mutation (IRAM) after

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5 years [7, 8]. Given the potentially large pool of INSTI resistant strains an increasing possibility of transmission of IRAM seems highly possible.

In Europe primary drug resistance rates to reverse transcriptase inhibitors [NRTIs and NNRTIs and to a lesser extent protease inhibitors (PIs)] have been remarkably stable at rates of around 8 %. [9] Yet data on primary INSTI resistance associated mutations (IRAM) are scant. Previous studies were limited by sample size, geographic diversity and confinement to earlier time periods and have so far failed to demonstrate occurrence of primary IRAM in INSTI naïve patients [10–12].

As a consequence, treatment guidelines often do not advocate testing for IRAM before initiation of first line ART. However, given the increasing use of these drugs this situation may well have changed and emerging drug resistant strains may endanger therapeutic successes. We aimed to determine the prevalence of IRAM in Austria in the 5 years following introduction of INSTI and to analyse temporal trends.

## Methods

For this retrospective cross-sectional analysis patients who received a first diagnosis of HIV infection between 2008 and 2013 and who had a HIV drug resistance test performed at the Department of Virology in Vienna within 3 months thereafter were selected. The Department of Virology is the national HIV reference laboratory and therefore confirms new diagnoses of HIV infections and performs drug resistance analysis for a large part of HIV infected patients in Austria. Unused material is cryo-conserved for subsequent retesting.

Leftovers of the first 49–53 EDTA blood samples received from patients with new diagnosis of HIV infection in each year from 2008 to 2013 were thawed and retested for the presence of IRAM. In case of insufficient material or invalid analysis the subsequent sample was selected. One single sample per patient was used.

In brief, HIV-1 RNA was extracted from the plasma specimens by the Nuclisens Easy Maq kit (Biomérieux) and subjected to a reverse transcriptase-polymerase chain reaction (RT-PCR). For integrase resistance analysis an in-house protocol was used covering amino acids 1–274. Reference sequence for nt numbering was HXB2. A part of the primers used in the in-house was previously published [13]. RT and protease resistance testing was performed covering the amino acids 1–333 and 1–99, respectively. Population sequencing was done using BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems) and was run on a 3130x genetic analyser (life technologies).

Sample size calculations were performed based on the assumption that a total sample size of 300 patient samples was sufficient to detect within a 97.5 % confidence interval a true prevalence of primary INSTI resistance in Austria of below 1.5 %, if no resistance mutation was found. A prevalence of 1.5 % was estimated as the threshold below which resistance mutations would occur at a negligible rate and routine INSTI resistance testing would not be warranted.

Integrase, protease and reverse transcriptase sequences were analysed separately using the Stanford University HIV Drug Resistance Database genotypic resistance interpretation algorithm (HIVdb algorithm, version 7.0.1, <http://hivdb.stanford.edu>).

Sequence data was analysed and drug penalty scores (DPS) were calculated using the same algorithm. This algorithm estimates the level of resistance to a specific drug by adding up penalty scores conferred by individual resistance associated mutations. A score of 0–9 indicates susceptibility, a score of 10–14 potential resistance, 15–29 low level resistance, 30–59 intermediate resistance, and  $\geq 60$  high level resistance [14]. Results were linked in an electronic database with demographic variables and virological data. Demographic variables included sex and age at the time of analysis. Virological data included viral load where available, viral subtype and results of previous testing for resistance to reverse transcriptase inhibitors and protease inhibitors performed on the same sample. The dataset was anonymized prior to statistical analysis.

Ethical approval for the performance of this study was granted by the Ethics committee of the Medical University, Vienna.

The 2015 IAS Drug resistance list was used as reference to define primary and accessory drug resistance mutations for INSTI and reverse transcriptase inhibitor (RTI) as well as major and minor resistance mutations for PI [15]. Primary INSTI resistance mutation included T66I, E92Q, F121Y, Y143C/H/R, S147G, Q148H/K/R, and N155H. Accessory mutations included L74M, T97A, E138A/K, G140A/S, and R263K.

Student's *t* test-test and Wilcoxon Rank Sum tests were used to compare continuous variables between groups. Chi-squared and Fisher's exact tests were used to compare the categorical characteristics between groups. A binomial exact estimation was used to calculate the confidence intervals for prevalences. Kappa Interrater agreement test was used to calculate the agreement between various resistance mutations. A Chi-squared test for linear trend was used to estimate assess a temporal trend for occurrence of IRAM and mutations to other drug classes.

STATA v13.1 (College Station, Texas, US) was used for all analyses and a two sided *p* value of 0.05 was considered as significant.

## Results

A total of 303 patient samples were analysed. 78 % of patients were male and mean age was 38 years. HIV subtype B was most common (62 %) followed by subtype C (12 %). Median viral load was  $1 \times 10^5$  HIV RNA copies/ml and 17 % of patients had a viral load  $>1 \times 10^6$  HIV RNA copies/ml most likely indicating acute HIV infection (Table 1).

**Table 1** Patient characteristics

Mean age (years, SD)	38 (12)
Male sex ( <i>n</i> , %)	235 (77.6)
Year of sample ( <i>n</i> , %)	
2008	49 (16.2)
2009	51 (16.8)
2010	51 (16.8)
2011	50 (16.5)
2012	53 (17.5)
2013	49 (16.2)
Viral load categories (HIV RNA copies/ml, <i>n</i> , %)	
$<1 \times 10^4$	7 (6.7)
$1 \times 10^4 < 5 \times 10^4$	23 (21.9)
$5 \times 10^4 < 1 \times 10^5$	20 (19.1)
$1 \times 10^5 < 1 \times 10^6$	37 (35.2)
$>1 \times 10^6$	18 (17.1)
Viral subtype ( <i>n</i> , %)	
A	22 (7.3)
B	188 (62.1)
C	35 (11.6)
Other	58 (19.1)
Major/primary drug resistance present ( <i>n</i> , %)	
NRTI	4 (1.3)
NNRTI	20 (6.6)
PI	5 (1.7)
INSTI	1 (0.3)

Major or primary drug resistance mutations were found in 30 patients (9.9 %). 20 individuals (6.6 %) had resistance to NNRTI. The most common NNRTI resistance mutation was E138A ( $n = 12$ ), followed by K103N ( $n = 6$ ). Four (1.3 %) patients had drug resistance mutations to NRTI where M41L was most common ( $n = 2$ ). Five patients (1.7 %) had major mutations to protease inhibitors. The mutation L90M was most common ( $n = 2$ ).

Overall prevalence of integrase inhibitor associated resistance mutations was 2.3 %. In the year 2012 the primary INSTI associated mutation F121Y was detected in one patient (0.3 %, 95 % CI 0.00–0.02). In this patient, no other accessory mutations to INSTI or resistance mutations to other drug classes were found. Accessory polymorphic and non-polymorphic mutations that confer decreased susceptibility to INSTI alone or in combination were found in 6 (2 %) patients. Detected mutations included T97A in 3 patients, and L74M, E138K, and G140A in 1 patient each (Table 2).

The majority of RTI resistance mutations occurred in 2010 ( $n = 4$ ) and most NNRTI resistance mutations were detected in 2012 ( $n = 5$ ). Two major PI resistance mutations were detected in 2009. There was no significant trend regarding the occurrence of NRTI ( $p = 0.42$ ), NNRTI ( $p = 0.85$ ), or major PI resistance ( $p = 0.74$ ) over the years 2008–2013. In addition, there was no significant temporal trend for the occurrence of primary or accessory INSTI resistance mutations ( $p = 0.16$ ). The presence of any IRAM was not significantly associated with male sex (OR 0.78, 95 % CI 0.14–4.30), older age (OR 0.98, 95 % CI 0.91–1.05), calendar year (OR 1.28, 95 % CI 0.80–2.03), or occurrence of any other drug resistance mutations (OR 1.41, 95 % CI 0.16–12.23) in a multivariable logistic regression.

Patients with primary or accessory INSTI resistance mutations did not differ from patients without these mutations regarding age ( $p = 0.57$ ), sex ( $p = 0.68$ ), or viral subtype ( $p = 0.82$ ). No association was found between viral

**Table 2** Patients with primary and accessory integrase inhibitor resistance associated mutations (IRAM)

Patient	Sex	Age	Subtype	Year of detection	IRAM	RTI resistance mutation	PI
1	Male	45	B	2008	T97A <sup>a</sup>	None	None
2	Male	30	B	2009	G140A <sup>a</sup>	None	A71V <sup>c</sup>
3	Female	31	CRF01_AE	2012	T97A <sup>a</sup>	None	None
4	Female	22	D	2012	L74M <sup>a</sup>	None	None
5	Male	30	B	2012	F121Y <sup>b</sup>	None	None
6	Male	48	C	2012	T97A <sup>a</sup>	None	None
7	Male	39	B	2013	E138K <sup>a</sup>	None	None

<sup>a</sup> Accessory IRAM

<sup>b</sup> Primary IRAM

<sup>c</sup> Minor PI resistance mutation

**Table 3** Integrase inhibitor associated resistance mutations (IRAM) and drug penalty scores

Year	IRAM ( <i>n</i> )		Raltegravir ( <i>n</i> )				Elvitegravir ( <i>n</i> )				Dolutegravir ( <i>n</i> )		
	Major	Accessory	Drug penalty score (DPS)										
			0–9	10–14	15–29	30–59	0–9	10–14	15–29	30–59	0–9	10–14	15–59
2008	0	1	17	0	2	0	47	1	1	0	49	0	0
2009	0	1	17	0	2	2	47	0	2	2	50	1	0
2010	0	0	0	0	1	0	50	0	1	0	51	0	0
2011	0	0	0	0	2	0	48	0	2	0	50	0	0
2012	1	3	50	1	5	1	46	3	3	1	53	0	0
2013	0	1	17	0	1	0	48	0	1	0	48	1	0

Stanford HIVdb Algorithm version 7.0.1: 0–9 susceptible, 10–14 potential low level, 15–29 low level resistance, 30–59 intermediate level resistance

load and occurrence of INSTI resistance mutations (OR 1.00,  $p = 0.98$ ).

No correlation was found between occurrence of RTI and PI resistance mutations and INSTI resistance mutations ( $p = 0.94$ ). No significant association was found between presence of NRTI, NNRTI, and PI resistance mutations and sex ( $p = 0.62$ ), age ( $p = 0.51$ ), and presence of subtype B ( $p = 0.32$ ).

Seventeen patients (6 %) had a DPS  $\geq 10$  for RAL or EVG indicating at least potential low-level resistance. Three of these patients had intermediate level resistance (DPS  $\geq 30$ ) for RAL or EVG. Two patients had a DPS of 10–14 for dolutegravir indicating potential low level resistance. No patient had a DPS higher than 14 for dolutegravir (Table 3).

## Discussion

In this cross sectional analysis covering a representative sample of ART-naïve patients in Austria during a relatively large period of time after INSTI have begun to be widely used in Austria, primary IRAM were rarely detected. In fact, only one patient carried the F121Y mutation which confers intermediate resistance to RAL and EVG due to a 5–10-fold reduction in susceptibility [16].

This mutation was detected only 4 years after INSTI were introduced in Austria. By this time the integrase inhibitors RAL and EVG were already used in more than 20 % of initial antiretroviral regimens [17]. Given the limited sample size and the low prevalence our study seems to be underpowered to detect a positive time trend and to assess clear risk factors for the occurrence of IRAM. Larger studies including more recent data seem necessary to prove or disprove that such an association exists. However, the low number of primary IRAM found in our patient collective may still not be negligible. We estimated that

the true prevalence for primary IRAM could be as high as 2 % which is in the range of prevalence found for NRTI or major PI resistance mutations in our study. For both latter drug classes, testing for drug resistance mutations prior to initiating first line therapy has become the standard and is recommended by current national guidelines whereas routine testing for IRAM is currently not recommended [18]. Thus ongoing surveillance and routine testing for IRAM seems now necessary to detect a potentially increasing number of resistance mutations in the future and to avoid complications with HIV treatment. In the patient bearing the F121Y mutation, no other resistance mutations to other drug classes were detected. This suggests that combination ART including RAL or EVG might still lead to complete viral suppression given the full activity of backbone drugs. On the other hand, possibly in the light of incomplete adherence to a regimen containing first generation integrase inhibitors, accumulation of accessory INSTI mutations and evolution of viral resistance along the Y143R pathway could take place. The Y143R mutation has been associated with high level RAL and EVG resistance [19]. Particularly in combination with the mutation G118R, the F121Y mutation confers also intermediate resistance to dolutegravir [20].

The origin of the F121Y mutation seems less clear. The fact, that the F121Y mutation was detected in 2012, 4 years after INSTI were first used in Austria suggests that transmission of this mutation is possible [21]. However, in a recent study the Swiss HIV Cohort described the occurrence of the high level N155H mutation among a therapy naïve patient even before INSTI had ever been used in this country suggesting the possibility of de novo mutagenesis of IRAM [22]. It seems likely that the IRAM found in our study are also caused by polymorphic de novo mutagenesis.

In addition to the primary F121Y mutation, the polymorphic mutations T97A and L74M and the non-polymorphic accessory mutations E138K and G140A were also detected

in several patients. Both, G140 and E138A usually occur in patients receiving RAL but the latter has already been described in the pre-INSTI era. Neither of these mutations causes higher level resistance alone, however, in combination in particular with the Q148 mutation they have been associated with high level RAL and EVG susceptibility and even reduced dolutegravir susceptibility [23]. Taken together primary and accessory mutations, more than 4 % of our patients had at least low level resistance to RAL and EVG. The Europe-wide SPREAD program demonstrated the occurrence of resistance associated polymorphisms before INSTI were introduced, but data are lacking from the years thereafter [9, 23].

Although the overall prevalence of IRAM appears to be low, the existence of such resistant strains in untreated populations may not be negligible as drug resistant viruses and in particular low fitness cost strains may be self-sustained and therefore transmitted readily [24].

In conclusion, primary IRAM are still rarely found in ART-naïve individuals despite an increasing use of INSTI in Austria, but there is the possibility that reduced susceptibility may exist to these drugs in selected patients. In the absence of predictors for occurrence of IRAM routine resistance testing seems prudent to avoid the consequences including accumulation of further mutations and therapeutic failure.

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#### Compliance with ethical standards

**Conflict of interest** None.

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