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OPEN

Emerging patterns in HIV integrase resistance

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We assessed integrase resistance in 837 treatment-experienced people with HIV (PWH) with virological failure (2022–2024) in Portugal. Major resistance mutations were found in 5.5%, with N155H and R263K being the most common. Resistance was more frequent in non-B subtypes and often co-occurred with resistance to other antiretroviral classes. Though prevalence remains low, the findings highlight the need for continued surveillance to inform treatment decisions, especially as integrase inhibitors like dolutegravir, bictegravir and cabotegravir become more widely used.

The global scale-up of antiretroviral therapy (ART) has led to significant reductions in HIV-related morbidity and mortality, with an estimated 30 million people with HIV (PWH) receiving ART by the end of 2023 [1]. Integrase strand transfer inhibitors (INSTIs), notably dolutegravir (DTG), bictegravir (BIC), and, more recently, cabotegravir (CAB) are central to most recommended first-line and second-line treatment regimens. Their potent antiviral activity, high genetic barrier to resistance and favorable tolerability profiles make them ideal anchors in modern ART. However, emerging resistance to INSTIs, particularly in treatment-experienced individuals, raises concerns for future treatment durability and virological suppression [2,3].

In this context, we conducted a retrospective longitudinal study to characterize the prevalence and mutational landscape of INSTI resistance among treatment-experienced PWH attending the Egas Moniz Hospital, a major HIV referral center in Lisbon that receives samples from central, southern and islands of Portugal. Our analysis focused on individuals who underwent next-generation sequencing (NGS) resistance testing between February 2022 and August 2024, interpreted using the Stanford HIVdb v9.4 algorithm. Variants present at at least 5% frequency were considered; those between 5 and 15% were evaluated cautiously in conjunction with the clinical and treatment history before being included in the resistance interpretation.

From a total of 2694 PWH tested during this period, 837 were ART-experienced and presented a positive resistance test result (detection of resistance mutations). Within this group, 46 individuals (5.5%) exhibited at least one major INSTI resistance mutation. The mean age of these 46 patients was 47 years (range: 28–71), and the cohort reflected a highly diverse population: 56.5% were of Portuguese origin, while the remainder came from Angola (21.7%), Brazil (13.0%), Mozambique (4.3%) and São Tomé and Príncipe (4.3%). The overview of the study group, consisting of 46 PWH, are summarized in Table 1.

Analysis of integrase resistance mutations revealed that the most frequently detected major mutations were N155H ($n = 15$, 32.6%) and R263K ($n = 14$, 30.4%), which are associated with reduced susceptibility to multiple INSTIs. E138K ($n = 11$, 23.9%), while not a primary resistance mutation, was commonly co-detected as an accessory mutation, potentially enhancing resistance in the presence of major mutations. Additional mutations included S147G ($n = 10$), G140S ($n = 7$), Q148H ($n = 7$), G118R ($n = 4$), Q148R ($n = 3$), Y143R ($n = 2$) and G140A ($n = 2$). T66I, T66K, E92Q, E138A, E138T, Y143C, Q148K and

Table 1. Overview of major integrase mutations identified in each HIV-1 individual, along with their prevalence percentage and previous antiretroviral therapy regimen.

Patient number	INSTI-associated resistance major mutations	Substitution frequency (%)	Previous treatment	Subtype	Origin
1	R263K	NA	NA	C	NA
2	E138T, G140S, Q148H	99.04/99.29/99.28	BIC/TAF/FTC	B	Portugal
3	R263K	99.88	DTG/ABC/3TC	C	Mozambique
4	N155H	42.92	BIC/TAF/FTC	G	São Tomé e Príncipe
5	Y143R	99.65	ABC/3TC+RAL	F1	Angola
6	R263K	99.58	BIC/TAF/FTC	A1	Russia
7	N155H	NA	FTC/TDF+RAL	B	Portugal
8	N155H	NA	AZT+TDF+RAL	CRF02_AG	Angola
9	R263K	99.06	BIC/TAF/FTC	F1	Brazil
10	E138K, G140S, S147G, Q148HR, N155H	46.76/30.12/68.11/26.87(H)/ 5.09(R)/99.30	NA	B	NA
11	E92Q, S147G, N155H	8.21/81.37/91.20	NA	A1	NA
12	N155H	99.18	MVC+ETR+RAL	B	Portugal
13	E138K, G140S, Q148H	48.54/99.29/99.62	DTG/ABC/3TC	B	Brazil
14	Y143R	99.50	DRV/c/FTC/TAF+RAL	C	Portugal
15	R263K	22.71	TDF/3TC/DTG	CRF02_AG	Portugal
16	G118R	76.55	DTG/RPV	CRF06_CPX	Portugal
17	E138K, G140S, Q148H	99.41/99.30/99.99	FTC/TAF/DRV/r/DTG	B	Portugal
18	E138K, S147G, N155H	99.95/97.92/99.39	DRV/c+DTG	G	Portugal
19	R263K	99.55	3TC/DTG	CRF14_BG	Portugal
20	G118R	96.80	DTG/RPV	CRF14_BG	São Tomé e Príncipe
21	E138K, Q148K	99.75/98.05	DRV/c+DOR+DTG	C	Portugal
22	E138K, S147G, N155H	99.47/99.63/99.46	NA	B	South Africa
23	R263K	34.04	NA	C	Angola
24	N155H	98.05	FTC/TDF/DRV/c+RAL	B	Portugal
25	R263K	11.91	DTG+DRV/r	F1	Brazil
26	N155H, R263K	10.31/90.35	DTG/3TC	C	Portugal
27	E138K, N155H	6.25/99.46	DTG/3TC	CRF14_BG	Portugal
28	S147G, N155H	99.72/99.71	DTG+DRV/r	B	Portugal
29	R263K	98.89	BIC/TAF/FTC	C	Angola
30	T66K	15.60	BIC/TAF/FTC	B	NA
31	E138K, G140A, S147G, Q148R	99.77/99.71/51.66/99.80	AZT+3TC/FTC/DTG	CFR14_BG	Portugal
32	G118R, E138K	99.59/99.64	3TC/DTG	CFR14_BG	Portugal
33	R263K	99.64	TDF/FTC+DTG	C	Angola
34	S147G	99.12	TAF/FTC/EVG/c	CFR14_BG	Portugal
35	N155H	92.22	TDF/FTC+DTG	B	Portugal
36	G140S, Y143C, Q148H, N155H	74.35/21.85/74.17/23.77	TDF/FTC+RAL	B	Portugal
37	T66I, G118R, E138A	99.32/99.74/32.72	DOR+DTG	C	Mozambique
38	R263K	29.41	BIC/TAF/FTC	CFR14_BG	Portugal
39	R263K	7.24	BIC/TAF/FTC	CRF02_AG	Portugal
40	E138K, S147G, N155S	99.77/99.80/99.65	3TC/DTG	CRF57_BC	Portugal
41	G140S, Q148H	99.50/99.20	ABC+AZT+RAL	F1	NA
42	R263K	21.31	TAF/FTC+DRV/r+DTG	CRF37_cpx	Portugal
43	N155H	99.55	TDF/FTC+RAL	CRF14_BG	Portugal
44	S147G	9.93	BIC/TAF/FTC	B	Portugal
45	G140S, Q148H	62.08/63.90	TAF/FTC/DRV/RAL	B	Portugal
46	E138K, G140A, S147G, Q148R	99.51/99.74/89.26/99.50	BIC/TAF/FTC	F1	Brazil

3TC, lamivudine; ABC, abacavir; AZT, zidovudine; BIC, bictegravir; c, cobicistat; DOR, doravirine; DRV, darunavir; DTG, dolutegravir; ETR, etravirine; EVG, elvitegravir; FTC, emtricitabine; MVC, maraviroc; NA, information not available; RAL, raltegravir; RPV, rilpivirina; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil.

N155S mutations were only observed once in this group of PWH. Notably, 19 individuals (41.3%) carried two or more major INSTI mutations. All individuals underwent resistance testing because of virological failure.

When considering the origin of our population surveyed, the most prevalent INSTI mutations in the 19 PWH of Portuguese origin were N155H (10/26) and R263K (6/26), and 4 out of 26 showed a combination of changes at positions 138 and 148. R263K was the most observed INSTI mutation in three out of five PWH of Angolan origin and two out of four of Brazilian origin.

Subtype analysis showed a predominance of non-B subtypes (70%), with HIV-1 subtype B identified in only 30.4% of patients. Among the non-B subtypes, subtype C, CRF14_BG, F1 and CRF02_AG were particularly represented. Although resistance appeared to be more prevalent among individuals with non-B subtypes and a non-Portuguese origin, formal statistical testing could not be performed due to the small number of cases. These trends should, therefore, be interpreted with caution.

At the time of resistance testing, the majority of patients were receiving either DTG-based ($n = 20$), BIC-based

($n=9$) or RAL-based ($n=10$) regimens. Only one individual had a TARV that included Elvitegravir (EVG), while therapeutic regimen information was unavailable for six PWH.

Each patient was analyzed for major drug resistance mutations across all three viral enzymes. Six PWH presented only major mutations in the integrase gene, while 33 of the 46 PWH had a combination of reverse transcriptase (RT) and integrase mutations.

Two individuals were excluded from final resistance prevalence estimates due to low-frequency R263K variants (5.58 and 7.24%, respectively) accompanied by clustered hypermutations characteristic of APOBEC-mediated editing, likely representing in-vitro artefacts rather than transmitted or selected resistance [4]. This underlines the importance of distinguishing true resistance mutations from sequencing noise, especially when interpreting low-abundance variants via NGS.

The clinical relevance of these findings is twofold. Firstly, although INSTI resistance remains relatively uncommon in people who have received treatment before, its emergence at a prevalence of 5.5% and an overall prevalence of 1.7% among the 2694 people tested, regardless of their treatment history, warrants continued monitoring. Secondly, the identification of complex resistance patterns involving dual or triple mutations highlights the potential for reduced efficacy of even high-barrier drugs, such as DTG and BIC. These mutations are often selected under prolonged virological failure, suboptimal adherence or insufficient drug pressure, and they may compromise future therapeutic options.

Our findings are consistent with recent international studies reporting low but increasing rates of INSTI resistance, particularly in regions where non-B subtypes predominate [3,5,6]. Given Portugal's unique epidemiological setting marked by significant HIV-1 subtype diversity and a large population of PWH from Portuguese-speaking African countries, surveillance efforts should prioritize subtype-specific resistance patterns and assess their clinical implications [7].

We cannot exclude the possibility that prior exposure to nonsuppressive ART regimens, particularly before entry into care in Portugal, may have contributed to the resistance profiles observed in individuals with nonclade B viruses.

This study has limitations, including the lack of longitudinal adherence and pharmacokinetic data to contextualize resistance selection. Still, NGS allowed detection of minority variants ($\geq$5%) often missed by Sanger sequencing, helping to identify emerging resistance and limit further mutation accumulation.

In conclusion, our study provides a comprehensive snapshot of INSTI resistance mutations among treatment-experienced PWH in Portugal during recent years. While resistance remains rare, its clinical impact may be profound, particularly in patients harboring multiple integrase mutations or infected with non-B subtypes. Continued surveillance, including routine genotypic testing and integration of resistance data into clinical decision-making, is essential to preserve INSTI efficacy and guide the design of future ART strategies.

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Conflicts of interest

There are no conflicts of interest.

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