



Role of low-frequency integrase strand transfer inhibitor resistance mutations on virological outcomes in antiretroviral therapy-naïve individuals initiating second-generation integrase inhibitors

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ABSTRACT

Objectives: This study investigated the role of low-frequency integrase strand transfer inhibitor (INSTI) resistance mutations, detectable by next-generation sequencing (NGS), at predicting virological rebound (VR) among people with HIV (PWH) starting second-generation INSTI-based first-line regimens.

Methods: This case-control study compared PWH (retrieved from the ICONA cohort; www.icona.org) who experienced VR (cases) with those who maintained virological control (controls) under first-line regimens based on dolutegravir or bictegravir. NGS data obtained through the Illumina platform were interpreted using the HIVdb algorithm version 9.7. Major (MRM), accessory (ARM), and other (ORM) INSTI resistance mutations were analysed at 5%, 10%, and 20% NGS cut-offs, respectively. Conditional logistic regression was used to evaluate the association between INSTI resistance and risk of VR.

Results: Among 266 PWH (90 cases, 176 controls), cases experienced VR with a median (interquartile range) viremia of 317 (93–6060) copies/mL after 15 (8–28) months from antiretroviral therapy start. The prevalence of MRM was low (NGS cut-off 5%, 10%, 20%: 1.9%, 0.8%, 0.4%, respectively), while it was moderate for ARM (7.5%, 7.1%, 6.4%) and high for ORM (50.0%, 44.7%, 42.1%). There was no evidence of a difference in prevalence of ≥ 1 MRM, ARM, or ORM between cases and controls. At 5% NGS cut-off, the prevalence of ≥ 2 ORM was higher in cases compared with controls. After adjusting for confounders, including HIV-1 subtype, ≥ 2 ORM detected as minority variants remained associated with VR risk.

Conclusion: Our findings suggest that combinations of low-frequency ORM may increase the risk of VR in individuals starting dolutegravir or bictegravir-based regimens. Further studies are needed to better understand these findings.

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1. Introduction

Integrase strand transfer inhibitors (INSTIs) are currently recommended as the first-line combined antiretroviral therapy (cART) for HIV infection due to their exceptional efficacy, safety, and tolerability [1–3]. However, although effective, first-generation INSTIs such as raltegravir (RAL) and elvitegravir (EVG) have a low genetic barrier to resistance, which can lead to the emergence of drug-resistant HIV strains in case of virological failure [4,5]. As a consequence, second-generation INSTIs such as dolutegravir (DTG) and bictegravir (BIC) are currently the preferred options for people with HIV (PWH) who start a first-line cART [1–3], offering a higher genetic barrier to the development of drug resistance [6–8]. Despite this, whether minority INSTI resistance mutations are able to impair the clinical and virological outcomes of INSTI-based regimens in drug-naïve PWH is still unclear and surveillance of resistance prior to ART initiation remains a cornerstone [9]. HIV drug resistance is typically identified through Sanger sequencing, which detects only quasiespecies present in at least 15–25% of the viral population. However, today more advanced and sensitive sequencing techniques such as next-generation sequencing (NGS) are more broadly used and can also help to identify quasiespecies with a lower frequency [10–13].

In ART-naïve PWH, the prevalence of INSTI-associated mutations detected as majority variants is so far very low (<1%) [11,14–19]. This low prevalence indicates that these mutations might not significantly affect the efficacy of first-line INSTI-based treatments.

To date, the role of HIV-1 minority variants for predicting virological response to first-line therapy remains unclear, except for minority resistant variants to non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens, for which an association has been demonstrated [10,20]. Recent studies using NGS have shown that INSTI resistance mutations can be present as minority variants in ART-naïve PWH, escaping detection through conventional Sanger sequencing [11,19]. However, these studies also indicate that the prevalence of circulating major INSTI minority variants is low (<4%) [11,19].

To our knowledge, the association between detection of INSTI minority variants and risk of virological failure in ART-naïve individuals who initiated second-generation INSTI-based regimens has not been thoroughly evaluated to date. Here, we focused not only on major INSTI mutations but also minor as well as other changes in the INSTI regions currently not identified as conferring resistance to INSTI drugs.

2. Methods

2.1. Study design

We conducted a case-control study nested within the ICONA Foundation Study cohort (www.icona.org) [21], which enrolled PWH who started ART when ART-naïve in >65 infectious disease clinics across Italy. To be included in the study, PWH had to satisfy the following criteria: i) availability of a stored plasma sample before treatment start (baseline), ii) having started a first-line regimen containing BIC or DTG, and iii) having achieved plasma HIV-1 RNA ≤ 50 copies/mL on the initial regimen. Follow-up time accrued from the date of viral suppression to the date of experiencing virological rebound (VR), the date of discontinuation of the INSTI drug, or the last available viral load, whichever occurred first. VR was defined as the occurrence of two consecutive plasma HIV-1 RNA measurements >50 copies/mL or a single measurement >1000 copies/mL after the achievement of virological success under first-line cART (HIV-1 RNA ≤ 50 copies/mL). Participants who experienced VR were defined as cases. Controls were PWH of the cohort who maintained virological success up to the time

at which VR was observed in the matched case. Virological success was defined as where participants remained persistently with viraemia undetectable (plasma HIV-1 RNA ≤ 50 copies/mL) or experiencing sporadic viral blips (defined as a plasma HIV-1 RNA value ranging between 51 and 1000 copies/mL preceded and followed by another value below the assay limit of 50 copies/mL). We used two controls per case, and cases and controls were matched for the exact INSTI drug used in the initial regimen (BIC or DTG).

2.2. Ethics

All participating centre Institutional Review Boards approved the ICONA Foundation Study. To participate in the cohort, each PWH signed a consent form to comply with the ethical standards of the committee on human experimentation and the Helsinki Declaration (last amendment October 2013).

2.3. Viral extraction and sequencing

Viral RNA was extracted using the Qiamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. The NGS on HIV-1 protease, reverse transcriptase and integrase regions was performed through the AD4SEQ HIV-1 Solution v2 commercial kit (Arrow Diagnostics S.r.l, Genoa, Italy) on the MiSeq platform (Illumina, San Diego, CA USA). NGS data were analysed using the Stanford HIVdb algorithm (HIVdb version 9.7, <https://hivdb.stanford.edu>), setting a coverage of >100 reads per position as previously reported [22] and according to manufacturer instructions. INSTI resistance mutations were evaluated according to the HIVdb categorisation as major resistance mutations (MRMs), accessory resistance mutations (ARMs), and other resistance mutations (ORMs). NGS cut-off thresholds of 5%, 10%, and 20% were used to determine the prevalence of resistance and define the exposures of interest in separate model evaluations.

HIVdb was also used to calculate the genotypic susceptibility score (GSS) of the entire initial regimen and also separately for only the nucleos(t)ide reverse transcriptase inhibitor (NRTI) backbone. Specifically, each drug was considered fully active in the case of resistance level <3. Entire regimens and NRTI backbones were considered fully active when all drugs in the combinations were fully active.

2.4. Statistical analysis

A descriptive analysis of the characteristics of the population included in the case-control study, overall and after stratifying for cases and controls, was performed. Conditional logistic regression (CLR) analysis for matched case-control studies was performed to evaluate the association between the baseline characteristics of the participants as well as baseline INSTI resistance detected through NGS.

The association between the detection of minor INSTI resistance pre-ART and risk of VR was assessed by CLR analysis using different NGS thresholds (5%, 10%, and 20%) to define exposure. The role of minority variants exclusively detected through NGS was also explored by restricting analyses to INSTI mutations detected with frequency ranges of 5–20% or 10–20%.

Separate uni-multivariable models were built to evaluate the specific role of MRMs, ARMs, and ORMs. Sensitivity analyses including only cases with plasma HIV-1 RNA >200 copies/mL were performed.

All statistical analyses were conducted using SPSS (v. 26.0, SPSS Inc., Chicago, IL, USA), with a two-sided significance threshold of ≤ 0.05 .

Table 1
Baseline participant characteristics.

Variable	Overall (N = 266) ^a	Cases (N = 90) ^a	Controls (N = 176) ^a	Odds ratio (95% CI)	P-value
Age, years, median (IQR)	40 (31–51)	40 (30–51)	39 (31–51)	1.0 (0.9–1.0)	0.905
Male, n (%)	222 (83.5)	66 (73.3)	156 (88.6)	1.8 (1.1–2.9)	0.011
Ethnicity, n (%)					0.013
Caucasian	209 (78.6)	64 (71.1)	145 (82.4)	1.0	
Hispanic	27 (10.2)	8 (8.9)	19 (10.8)	1.0 (0.5–2.0)	
Black	25 (9.4)	18 (20.0)	7 (4.0)	2.4 (1.4–4.0)	
Other/unknown	5 (1.9)	0 (0.0)	5 (2.8)	0.0 (0.0–nd)	
Italian nationality, n (%)	192 (72.2)	57 (63.3)	135 (76.7)	0.7 (0.4–1.0)	0.061
Mode of HIV transmission, n (%)					0.078
Men who have sex with men	141 (53.0)	38 (42.2)	103 (58.5)	1.0	
Heterosexual	88 (33.1)	42 (46.7)	46 (26.1)	0.8 (0.4–1.7)	
Drug abuse	8 (3.0)	1 (1.1)	7 (4.0)	1.4 (0.6–2.9)	
Transgender	6 (2.3)	1 (1.1)	5 (2.8)	0.4 (0.0–2.8)	
Unknown	23 (8.6)	8 (8.9)	15 (8.5)	0.5 (0.1–3.8)	
Subtype ^b , n (%)					0.039
B	162 (60.9)	42 (46.7)	120 (68.2)	1.0	
CRF02_AG	21 (7.9)	13 (14.4)	8 (4.5)	2.4 (1.3–4.5)	
A ^c	15 (5.6)	8 (8.9)	7 (4.0)	2.1 (1.0–4.4)	
C	13 (4.9)	6 (6.7)	7 (4.0)	1.8 (0.8–4.2)	
Other	55 (20.7)	21 (23.3)	34 (19.3)	1.5 (0.9–2.5)	
CD4 ⁺ T cells, cells/mm ³ , median (IQR)	284 (80–476)	149 (55–391)	327 (98–529)	0.9 (0.8–1.0)	0.016
HIV RNA, log ₁₀ copies/mL, median (IQR)	5.1 (4.6–5.6)	5.3 (4.7–5.8)	5.0 (4.5–5.5)	1.4 (1.1–1.9)	0.014
Calendar year of ART start, median (IQR)	2017 (2016–2019)	2017 (2016–2019)	2017 (2016–2019)	1.0 (0.9–1.2)	0.773
Time between sampling and ART start, days, median (IQR)	5 (0–22)	5 (0–18)	6 (0–24)	1.0 (1.0–1.0)	0.335

^a NGS was successful in 266 of 270 samples available, including all 90 cases and 176 controls; therefore, 86 cases were matched with two controls and four cases with only one control.

^b Sub-typing was determined by using both the automatic tool COMET (<https://comet.lih.lu/>) and phylogenetic analysis. Specifically, a maximum likelihood phylogenetic tree was constructed using IQ-TREE2 (v2.1.3) with 1000 bootstrap replicates.

^c A1 (n = 8, 53.3%); A3 (n = 3, 20.0%); A6 (n = 4, 26.7 %).nd, not determined.

3. Results

3.1. Participant characteristics

Overall, NGS was successful in 266 of 270 samples available, including all 90 cases and 176 controls. First-line regimens were mostly based on a triple therapy containing DTG (triple-DTG: 85.3%; triple-BIC: 7.9%; dual DTG/lamivudine: 6.8%). Compared with controls, cases were more likely to be women, with a heterosexual transmission route, Black ethnicity, lower baseline CD4⁺ T-cell counts, and higher baseline plasma HIV-1 RNA (Table 1). The HIV-1 B subtype was less common in cases than in controls (46.7% vs. 68.2%, *P* = 0.039). Cases experienced VR in a median time of 15 months (interquartile range [IQR] 8–28) after starting the first-line regimen, with a median (IQR) viral load of 317 (93–6060) copies/mL. Specifically, cases experienced VR as follows: i) 37.8% as two consecutive plasma HIV-1 RNA, both ranging 50–200 copies/mL; ii) 23.3% as one single plasma HIV-1 RNA >1000 copies/mL; iii) 22.2% as two consecutive plasma HIV-1 RNA above 50 copies/mL; iv) 16.7% as two consecutive plasma HIV-1 RNA >200 copies/mL. Notably, among 86 of 90 participants with available follow-up after VR, 91.8% resuppressed (of whom about half were without therapy change).

3.2. Evaluation of baseline INSTI resistance and genotypic susceptibility between cases and controls

At baseline, the proportion of individuals harbouring any MRMs was very low (5%: 1.9%; 10%: 0.8%; 20%: 0.4%) and moderate for ARM (5%: 7.5%; 10%: 7.1%; 20%: 6.4%), regardless of the NGS setting used. None of the participants had more than one MRM or one ARM. No evidence of a difference in the prevalence of both MRMs and ARMs detected between cases and controls (Fig. 1A,

B) was found. Conversely, the proportion of individuals harbouring any ORMs was quite high, regardless of the NGS threshold used (5%: 50.0%; 10%: 44.7%; 20%: 42.1%). Specifically, L74I was more likely to be detected in cases, while S230N was more prevalent in controls (Fig. 2A). The proportion of individuals with only one ORM was similar between cases and controls (Fig. 2B). By contrast, the proportion of individuals with ≥2 ORMs was higher among cases compared with controls at NGS set at 5% (Fig. 2B). Multiple ORMs were never observed together with other MRMs or ARMs, except for one case in which we detected the co-presence as a mixture of the L74M ARM and L74I ORM. The most observed mutational pattern included the L74I mutation (Table 2). In particular, the combination of L74I with other ORMs (such as M50I or S230N) was more likely observed in cases compared with controls, regardless of the NGS threshold used. A higher prevalence of this combination in cases was also observed after stratifying for B and non-B subtypes (Supplementary Table 1). According to GSS, most cases and controls carried a fully susceptible viral strain to the first-line regimens received regardless of NGS setting (cases vs. controls, 97.8% vs. 100%, *P* = 0.114).

Using multivariable CLR analysis, the presence of ≥2 ORMs detected with 5%, 5–20%, and 10% thresholds was more than twofold significantly associated with the risk of VR (Table 3). This twofold increase was also observed at the >20% threshold, although the association was not statistically significant (Table 3). The detection of ≥1 MRMs was not associated with case-control status regardless of the NGS threshold used, although there was large uncertainty around estimates given the low prevalence of the exposure (Table 3). The detection of ARMs was associated with case-control status only at the 5–20% threshold (Table 3).

Sensitivity analyses restricted to cases experiencing VR with plasma HIV-1 RNA >200 copies/mL (48 cases and 96 controls included) confirmed that the detection of ≥2 ORMs exclusively as

Table 2

Combinations of multiple other resistance mutations detected among cases and controls.

ORM combinations	Cases (N = 90)	Controls (N = 176)	OR (95% CI)	P-value
NGS set at 5%				
S230N alone	5 (5.6)	40 (22.7)	0.3 (0.1–0.7)	0.007
M50I alone	18 (20)	24 (13.6)	1.3 (0.8–2.2)	0.275
L74I alone	9 (10.0)	11 (6.3)	1.4 (0.7–2.7)	0.375
L74I + any ORM	8 (8.9)	1 (0.6)	1.2 (0.9–1.6)	0.182
L74I + M50I	2 (2.2)	0 (0.0)	3.2 (0.7–13.4)	0.119
L74I + S230N	5 (5.6)	1 (0.6)	2.6 (1.0–6.4)	0.042
L74I + M50I + S230N	1 (1.1)	0 (0)	3.0 (0.4–21.4)	0.279
M50I + S230N	1 (1.1)	4 (2.3)	0.6 (0.1–4.2)	0.596
E138D alone	2 (2.2)	1 (0.6)	2.1 (0.5–8.7)	0.323
S119R alone	1 (1.1)	2 (1.1)	1.0 (0.1–7.1)	0.986
S119R + S230N	1 (1.1)	2 (1.1)	1.0 (0.1–7.2)	0.991
S119R + M50I	1 (1.1)	0 (0)	3.0 (0.4–21.4)	0.279
S119R + E138D	1 (1.1)	0 (0)	3.0 (0.4–21.4)	0.279
V151I alone	1 (1.1)	0 (0)	3.0 (0.4–21.4)	0.279
NGS set at 10%				
S230N alone	3 (3.3)	39 (22.2)	0.2 (0.1–0.6)	0.004
M50I alone	13 (14.4)	21 (11.9)	1.2 (0.6–2.1)	0.637
L74I alone	10 (11.1)	11 (6.3)	1.5 (0.8–2.8)	0.261
L74I + any ORM	6 (6.7)	1 (0.6)	2.7 (1.2–6.4)	0.020
L74I + M50I	3 (3.3)	1 (0.6)	2.3 (0.7–7.3)	0.163
L74I + S230N	2 (2.2)	0 (0)	3.2 (0.7–13.4)	0.119
L74I + M50I + S230N	1 (1.1)	0 (0)	3.0 (0.4–21.4)	0.279
M50I + S230N	1 (1.1)	4 (2.3)	0.6 (0.1–4.2)	0.596
E138D alone	2 (2.2)	1 (0.6)	2.1 (0.5–8.7)	0.323
S119R + S230N	1 (1.1)	2 (1.1)	1.0 (0.1–7.2)	0.991
S119R alone	1 (1.1)	1 (0.6)	1.5 (0.2–10.6)	0.697
S119R + M50I	1 (1.1)	0 (0)	3.0 (0.4–21.4)	0.279
V151I alone	1 (1.1)	0 (0)	3.0 (0.4–21.4)	0.279
NGS set at 20%				
S230N alone	4 (4.4)	38 (21.6)	0.2 (0.1–0.7)	0.006
M50I alone	12 (13.3)	18 (10.2)	1.2 (0.7–2.2)	0.537
L74I alone	10 (11.1)	11 (6.3)	1.5 (0.8–2.8)	0.261
L74I + any ORM	4 (4.4)	1 (0.6)	2.6 (0.9–7.2)	0.077
L74I + M50I	2 (2.2)	1 (0.6)	2.0 (0.5–8.4)	0.330
L74I + S230N	0 (0)	4 (2.3)	0.0 (0.0–96.9)	0.435
L74I + M50I + S230N	2 (2.2)	0 (0)	3.2 (0.7–13.4)	0.119
E138D alone	2 (2.2)	1 (0.6)	2.1 (0.5–8.7)	0.323
S119R + S230N	1 (1.1)	2 (1.1)	1.0 (0.1–7.2)	0.991
S119R alone	1 (1.1)	1 (0.6)	1.5 (0.2–10.6)	0.697
S119R + M50I	1 (1.1)	0 (0)	3.0 (0.4–21.4)	0.279
V151I alone	1 (1.1)	0 (0)	3.0 (0.4–21.4)	0.279

OR, odds ratio; ORM, other resistance mutation.

Table 3

Conditional logistic regression models to evaluate the odds of experiencing virological failure of dolutegravir/bictegravir-based first-line therapy according to baseline integrase strand transfer inhibitor resistance.

INSTI resistance detected	Frequency, n (%)		Unadjusted		Adjusted	
	Cases (N = 90)	Controls (N = 176)	P-value	OR (95% CI)	P-value	AOR (95% CI)
≥1 MRM						
NGS ≥5% ^a	1 (1.1)	4 (2.3)	0.568	0.563 (0.078–4.047)	0.816	0.785 (0.103–6.011)
NGS 5–20% ^a	1 (1.1)	3 (1.7)	0.758	0.734 (0.102–5.275)	0.964	0.953 (0.122–7.449)
NGS ≥10% ^a	1 (1.1)	1 (0.6)	0.724	1.427 (0.198–10.261)	0.781	1.343 (0.168–10.759)
NGS 10–20% ^a	1 (1.1)	0 (0.0)	0.279	2.974 (0.414–21.383)	0.544	1.958 (0.224–17.115)
NGS ≥20% ^b	0 (0.0)	1 (0.6)	–	–	–	–
≥1 ARM						
NGS ≥5% ^a	7 (7.8)	13 (7.4)	0.925	1.038 (0.478–2.254)	0.931	1.037 (0.460–2.339)
NGS 5–20% ^a	3 (3.3)	0 (0.0)	0.058	3.095 (0.963–9.947)	0.037	3.797 (1.081–13.339)
NGS ≥10% ^a	6 (6.7)	13 (7.4)	0.860	0.928 (0.404–2.134)	0.829	0.908 (0.379–2.179)
NGS 10–20% ^a	2 (2.2)	0 (2.2)	0.120	3.132 (0.743–13.193)	0.095	4.002 (0.786–20.377)
NGS ≥20% ^a	4 (4.4)	13 (7.4)	0.451	0.68 (0.249–1.856)	0.515	0.573 (0.107–3.067)
≥1 ORM						
NGS ≥5% ^c	48 (53.3)	85 (48.3)	0.526	1.144 (0.754–1.736)	0.549	1.145 (0.736–1.781)
NGS 5–20% ^c	12 (13.3)	9 (5.1)	0.059	1.803 (0.978–3.324)	0.100	1.698 (0.903–3.193)
NGS ≥10% ^c	39 (43.3)	80 (45.8)	0.787	0.944 (0.62–1.436)	0.681	0.909 (0.578–1.431)
NGS 10–20% ^c	3 (3.3)	4 (2.3)	0.681	1.274 (0.402–4.041)	0.924	0.943 (0.284–3.135)
NGS ≥20% ^c	36 (40.0)	76 (43.2)	0.684	0.916 (0.599–1.4)	0.711	0.919 (0.586–1.441)

(continued on next page)

Table 3 (continued)

INSTI resistance detected	Frequency, n (%)		Unadjusted		Adjusted	
	Cases (N = 90)	Controls (N = 176)	P-value	OR (95% CI)	P-value	AOR (95% CI)
≥2 ORM						
NGS ≥5% ^c	12 (13.3)	7 (4.0)	0.024	2.020 (1.095–3.728)	0.008	2.394 (1.254–4.571)
NGS 5–20% ^c	6 (6.7)	0 (0.0)	0.008	3.113 (1.353–7.162)	0.038	2.685 (1.056–6.826)
NGS ≥10% ^c	9 (10.0)	7 (4.0)	0.113	1.757 (0.876–3.522)	0.036	2.171 (1.052–4.479)
NGS 10–20% ^c	3 (3.3)	0 (0.0)	0.060	3.027 (0.954–9.601)	0.204	2.318 (0.633–8.494)
NGS ≥20% ^c	6 (6.7)	7 (4.0)	0.427	1.406 (0.607–3.255)	0.089	2.162 (0.889–5.255)

^a Adjusted for: calendar year of ART start, viral load at ART start (per 1 log₁₀ increase), CD4 cell count at ART start (per 100 cells increase), sex (female vs. male), ethnicity (Black, Hispanic-Latino, other/unknown vs. white [dummy]), HIV-1 subtype (CRF02_AG, A, C, others, vs. B [dummy]), genotypic susceptibility score of backbone regimen (nonsusceptible vs. susceptible).

^b Model not performed as MRMs were present in only 1 case at NGS set at 20%.

^c Adjusted for: calendar year of ART start, viral load at ART start (per 1 log₁₀ increase), CD4 cell count at ART start (per 100 cells increase), sex (female vs. male), ethnicity (Black, Hispanic-Latino, other/unknown vs. white [dummy]), HIV-1 subtype (CRF02_AG, A, C, others, vs. B [dummy]), genotypic susceptibility score of entire regimen (nonsusceptible vs. susceptible).

ARM, accessory resistance mutation; NGS, next-generation sequencing; INSTI, integrase strand transfer inhibitor; MRM, major resistance mutation; ORM, other resistance mutation.

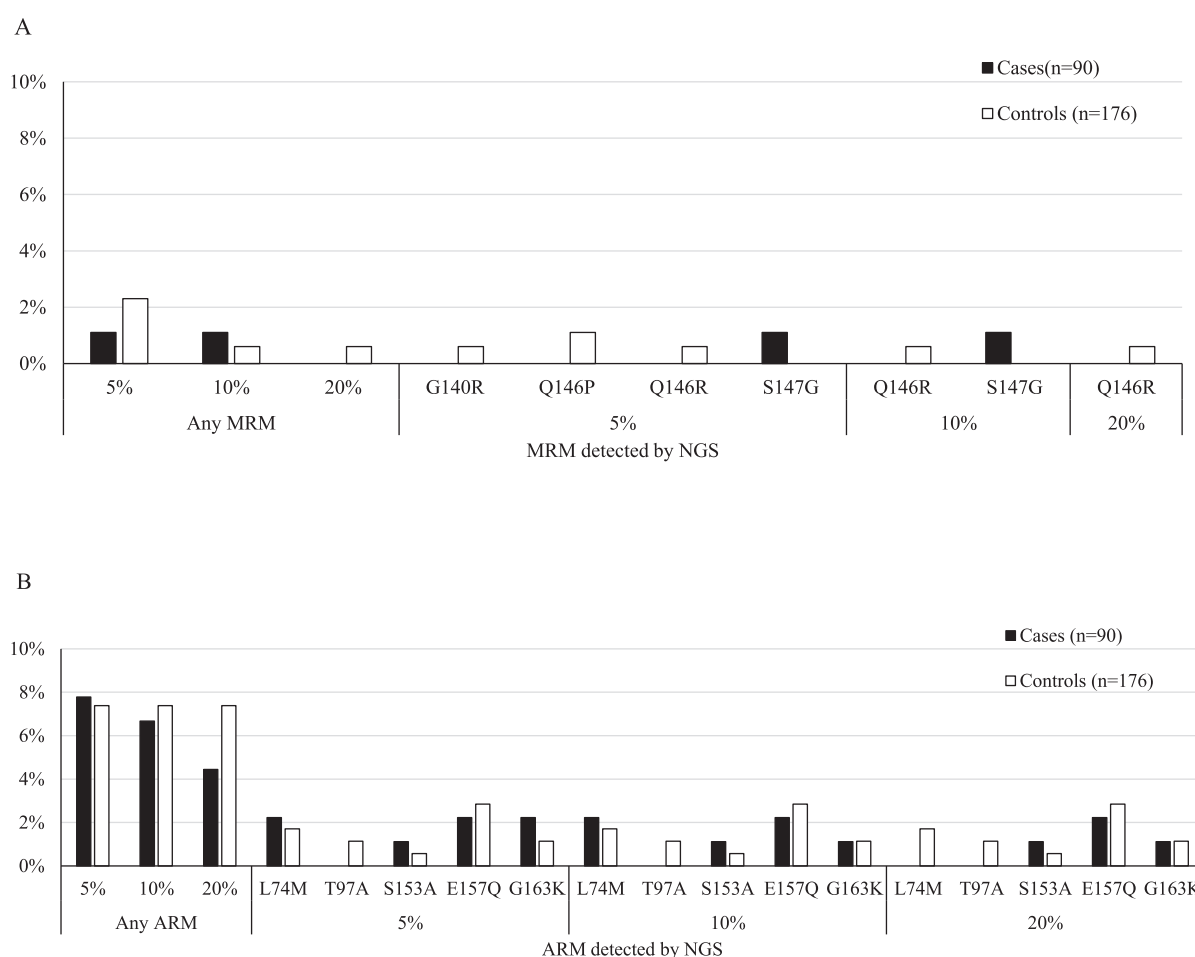


Fig. 1. Prevalence of HIV-1 major and accessory integrase strand transfer inhibitor resistance mutations (major resistance mutations [MRMs] and accessory resistance mutations [ARMs]) in cases and controls according to different next-generation sequencing (NGS) cut-offs. A) Prevalence of any MRM. B) Prevalence of any ARM. The following mutations according to HIVdb 9.7 (<https://hivdb.stanford.edu/dr-summary/comments/INSTI/>) were tested: MRMs (T66AIK, E92GVQ, G118R, F121CY, E138KAT, G140RSAC, Y143ACKGHR, P145S, Q146PRL, S147G, Q148HKRN, V151L, N155HT, R263K); ARMs (A49G, H51Y, L74FM, V75A, Q95K, T97A, T122N, A128T, G149A, V151A, S153YFA, E157Q, G163RK, S230R, D232N).

minority variants in the frequency window 5–20% was associated with a threefold increased risk of VR at univariate (odds ratio [95% CI]: 3.240 [1.165–9.013], $P = 0.024$) and multivariable analysis, although the association was not significant at the 5% level (adjusted odds ratio [95% CI]: 3.043 [0.888–10.429], $P = 0.077$) (Supplementary Table 2).

4. Discussion

In this case-control study, we estimated the prevalence of HIV-1 INSTI resistance mutations and their role at predicting VR in first-line regimens based on DTG or BIC in PWH. Most of the observed rebounds comprised transient episodes of low-level viremia, fol-

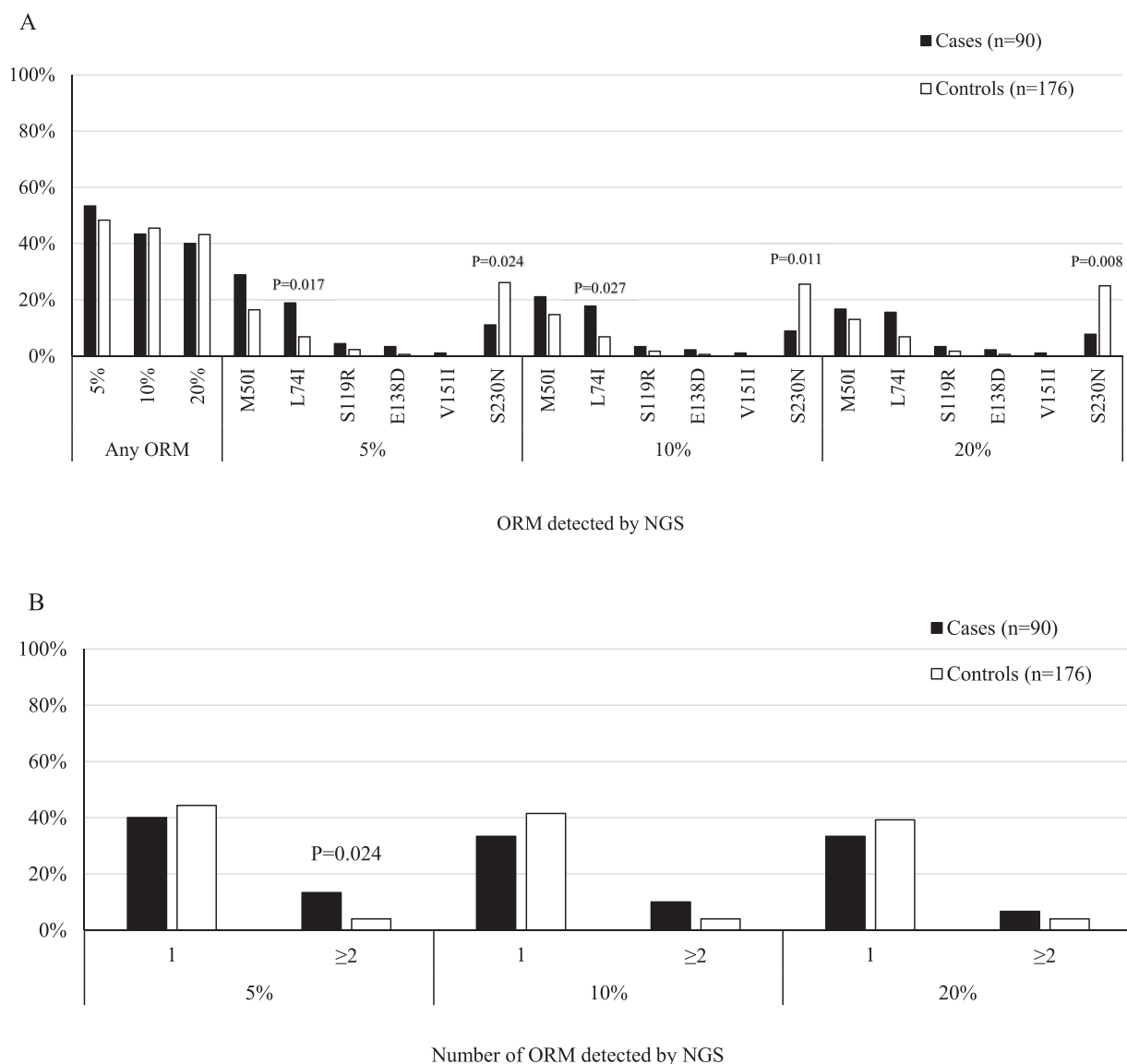


Fig. 2. Prevalence of other integrase strand transfer inhibitor resistance mutations (ORMs) in cases and controls according to different next-generation sequencing (NGS) cut-offs. A) Prevalence of any ORM. B) Prevalence of at least one or two ORMs. Statistically significant differences ($P < 0.05$) between cases and controls according to Conditional logistic regression (CLR) univariable models are reported in the graph. Only one participant (one case) harboured three ORMs (M50I, L74I, S230N) detected at next-generation sequencing set at 5% and 10%. The following mutations according to HIVdb 9.7 (<https://hivdb.stanford.edu/dr-summary/comments/INSTI/>) were tested: M50I, L74I, S119R, E138D, V151I, S230N.

lowed by virological suppression in more than 90% of individuals, thus confirming the high efficacy of first-line regimens based on second-generation INSTI after initial achievement of viral suppression.

Our estimates obtained from NGS analysis set at the 20% threshold (at Sanger-like sensitivity) confirmed that the prevalence of major INSTI resistance mutations is low ($<1\%$) and similar to that found in other treatment-naïve populations [11,14–19]. Also as previously observed, the prevalence of ARM was moderate (in the 6–8% range) according to the specific NGS threshold used [11]. More generally, estimates of the prevalence of MRMs and ARMs across studies in ART-naïve populations were very similar when NGS data were interpreted using a cut-off $<20\%$ [11,19], despite the fact that the list of considered mutations was not standardised and heterogeneous NGS methods and thresholds were used.

In this context of prevalence, no significant evidence indicating that major INSTI resistance mutations contribute to virological failures was found, whereas even though rarely observed, the

presence of ≥ 1 ARM detected as a minority variant in the 5–20% window was exclusively observed in cases.

This analysis, as far as we know, was the first to evaluate the complete list of INSTI resistance mutations reported in the Stanford algorithm, including ORMs, which are associated with a score of 0 for BIC and DTG (such as L74I) or are not included in their score (such as M50I, S119R, E138D, V151I, and S230N). In the context of these types of resistance mutations, our analysis identified a high prevalence of polymorphisms (approximately 40–50%) in both cases and controls, regardless of the different NGS cut-offs used. When we analysed specific ORMs, L74I was more likely to be observed in cases compared with controls. We also found that there was a higher proportion of participants with at least two ORM among cases. By exploring ORM patterns, the most common included the L74I together with another ORM, regardless of NGS setting. Of note, the L74I polymorphism, highly common in the HIV-1 A6 subtype [23], has already been described as of potential concern for the virological efficacy of long-acting

cabotegravir-based treatment [24]. However, this mutation has no effect on cabotegravir susceptibility in vitro and has not affected the outcomes of breakthrough experiments across HIV-1 subtype A6 as well as B integrase genes [25]. A recent study showed that L74I confers greater replication capacity to recombinant viruses expressing HIV-1 A6 integrase when present together with other major INSTI resistance mutations [26]. In our analysis we did not find any MRMs in combination with L74I, while the combination of this mutation with other ORMs appeared to be associated with risk of virological failure. This phenomenon could be explained by increased viral fitness related to these patterns including L74I. In vitro studies are needed to confirm this hypothesis.

The association between these ORM combinations and the risk of VR appeared to be independent from key confounding factors, including HIV-1 subtype. Indeed, the magnitude of the association was even larger and still significant in the multivariable conditional regression model even after adjusting for HIV-1 subtype. Also of note, the univariable association was similar in a separate analyses stratified by HIV-subtype (B vs. non-B viruses; Supplementary Table 1). By restricting the analyses to cases experiencing VR at viremia >200 copies/mL, sensitivity analyses confirmed that the presence of ≥ 2 ORMs (exclusively detected as minority variants in the window 5–20%) was associated with an increased risk of VR. Taken together, our findings suggest that detecting minority variants through NGS-GRT prior to initiating a first-line regimen based on second-generation INSTI could be useful to identify individuals at higher risk of losing virological control.

Our study had several limitations. First, we cannot rule out unmeasured or residual confounding. Mutations outside the integrase region (such as *env* or 3'/PPT) have been shown to be associated with risk of virological failure of INSTI-based regimens [27,28], and we only sequenced *pol* gene regions in our stored samples. Second, no significant evidence was found indicating that major INSTI resistance mutations contributed to virological failures; this is probably due to the low prevalence of exposure and consequently low statistical power. Furthermore, our data demonstrated that minority variants detected at the 5% threshold harbouring multiple ORMs were associated with the risk of VR. However, variants detected between the 5% and 10% threshold should be interpreted with caution, considering our previous findings that indicated debatable reliability in detecting resistance at this NGS range [22]. Unfortunately, neither resistance tests performed around the time of VR nor stored plasma samples were available for the cases, and thus we could not evaluate the extent of minority variant outgrowth at VR, as previously observed at baseline. Last, ours is a selected sample of PWH who first achieved viral suppression with BIC/DTG-based regimens, so the prevalence of resistance pre-ART may have been underestimated.

In conclusion, our findings suggest that VR to BIC/DTG-based first-line regimens appears to be more frequent in PWH harbouring the combination of some integrase HIV-1 polymorphisms before starting ART. NGS INSTI mutation screening prior to starting first-line ART could be helpful to identify individuals at higher risk of losing virological control. Further studies are needed to further evaluate the prevalence of ORMs in different settings and better understand the mechanism by which these mutations may affect the virological outcome of DTG/BIC-based regimens.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2025.04.002.

References

- [1] DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV Developed by the DHHS Panel on Antiretroviral Guidelines for Adults. <https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/adult-adolescent-arv/guidelines-adult-adolescent-arv.pdf> 2024 [accessed 24.12.28].
- [2] European AIDS Clinical Society (EACS), European AIDS Clinical Society (EACS) Guidelines. Version 12.1 2024, <https://www.eacsociety.org/guidelines/eacs-guidelines/>; November 2024 [accessed 24.12.28].
- [3] World Health Organization (WHO). Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, <https://iris.who.int/bitstream/handle/10665/342899/9789240031593-eng.pdf?sequence=1>; July 2021 [accessed 24.12.28].
- [4] Anstett K, Brenner B, Mesplede T, Wainberg MA. HIV drug resistance against strand transfer integrase inhibitors. *Retrovirology* 2017;14:1–16. doi:10.1186/S12977-017-0360-7/TABLES/10.
- [5] Smith SJ, Zhao XZ, Passos DO, Lyumkis D, Burke TR, Hughes SH. Integrase strand transfer inhibitors are effective anti-HIV drugs. *Viruses* 2021;13:205. doi:10.3390/V13020205.
- [6] Rathbun RC, Lockhart SM, Miller MM, Liedtke MD. Dolutegravir, a second-generation integrase inhibitor for the treatment of HIV-1 infection. *Ann Pharmacother* 2014;48:395–403. doi:10.1177/1060028013513558.
- [7] Tsiang M, Jones GS, Goldsmith J, Mulato A, Hansen D, Kan E, et al. Antiviral activity of bictegravir (GS-9883), a novel potent HIV-1 integrase strand transfer inhibitor with an improved resistance profile. *Antimicrob Agents Chemother* 2016;60:7086. doi:10.1128/AAC.01474-16.
- [8] Santoro MM, Fornabaio C, Malena M, Galli L, Poli A, Menozzi M, et al. Susceptibility to HIV-1 integrase strand transfer inhibitors (INSTIs) in highly treatment-experienced patients who failed an INSTI-based regimen. *Int J Antimicrob Agents* 2020;56. doi:10.1016/j.ijantimicag.2020.106027.
- [9] Tzou PL, Rhee SY, Descamps D, Clutter DS, Hare B, Mor O, et al. Integrase strand transfer inhibitor (INSTI)-resistance mutations for the surveillance of transmitted HIV-1 drug resistance. *J Antimicrob Chemother* 2020;75:170–82. doi:10.1093/JAC/DKZ417.
- [10] Cozzi-Lepri A, Dunn D, Tostevin A, Marvig RL, Bannedbæk M, Sharma S, et al. Rate of response to initial antiretroviral therapy according to level of pre-existing HIV-1 drug resistance detected by next-generation sequencing in the strategic timing of antiretroviral treatment (START) study. *HIV Med* 2024;25:212–22. doi:10.1111/HIV.13556.
- [11] Mbisa JL, Ledesma J, Kirwan P, Bibby DF, Manso C, Skingsley A, et al. Surveillance of HIV-1 transmitted integrase strand transfer inhibitor resistance in the UK. *J Antimicrob Chemother* 2020;75:3311–18. doi:10.1093/JAC/DKAA309.
- [12] Ávila-Ríos S, Parkin N, Swanstrom R, Paredes R, Shafer R, Ji H, et al. Next-generation sequencing for HIV drug resistance testing: laboratory, clinical, and implementation considerations. *Viruses* 2020;12. doi:10.3390/V12060617.
- [13] Metzner KJ. Technologies for HIV-1 drug resistance testing: inventory and needs. *Curr Opin HIV AIDS* 2022;17:222–8. doi:10.1097/COH.0000000000000737.
- [14] Raffaelli CS, Rossetti B, Paglicci L, Colafigli M, Punzi G, Borghi V, et al. Impact of transmitted HIV-1 drug resistance on the efficacy of first-line antiretroviral therapy with two nucleos(t)ide reverse transcriptase inhibitors plus an integrase inhibitor or a protease inhibitor. *J Antimicrob Chemother* 2018;73:2480–4. doi:10.1093/JAC/DKY211.
- [15] Guo C, Wu Y, Zhang Y, Liu X, Li A, Gao M, et al. Transmitted drug resistance in antiretroviral therapy-naïve persons with acute/early/primary HIV infection: a systematic review and meta-analysis. *Front Pharmacol* 2021;12. doi:10.3389/FPHAR.2021.718763.
- [16] McClung RP, Oster AM, Ocfemia MCB, Saduvala N, Heneine W, Johnson JA, et al. Transmitted drug resistance among human immunodeficiency virus (HIV)-1 diagnoses in the United States, 2014–2018. *Clin Infect Dis* 2022;74:1055–62. doi:10.1093/CID/CIA8583.
- [17] de Salazar A, Viñuela L, Fuentes A, Teyssou E, Charpentier C, Lambert-Niclot S, et al. Transmitted drug resistance to integrase-based first-line human immunodeficiency virus antiretroviral regimens in Mediterranean Europe. *Clin Infect Dis* 2023;76:1628–35. doi:10.1093/CID/CIA972.
- [18] Fabeni L, Armenia D, Abbate I, Gagliardini R, Mazzotta V, Bertoli A, et al. HIV-1 transmitted drug resistance in newly diagnosed individuals in Italy over the period 2015–21. *J Antimicrob Chemother* 2024;79:2152–62. doi:10.1093/JAC/DKAE189.
- [19] Baxter JD, Dunn D, Tostevin A, Marvig RL, Bannedbæk M, Cozzi-Lepri A, et al. Transmitted HIV-1 drug resistance in a large international cohort using next-generation sequencing: results from the Strategic Timing of Antiretroviral Treatment (START) study. *HIV Med* 2021;22:360–71. doi:10.1111/HIV.13038.
- [20] Mbunkah HA, Bertagnolio S, Hamers RL, Hunt G, Inzaule S, Rinke De Wit TF, et al. Low-abundance drug-resistant HIV-1 variants in antiretroviral drug-naïve individuals: a systematic review of detection methods, prevalence, and clinical impact. *J Infect Dis* 2020;221:1584–97. doi:10.1093/INFDIS/JIZ650.
- [21] Stella-Ascariz N, Arribas JR, Paredes R, Li JZ. The role of HIV-1 drug-resistant minority variants in treatment failure. *J Infect Dis* 2017;216:S847–50. doi:10.1093/INFDIS/JIX430.
- [22] Armenia D, Carioti L, Micheli V, Bon I, Allice T, Bonura C, et al. Comparison of different HIV-1 resistance interpretation tools for next-generation sequencing in Italy. *Viruses* 2024;16:1422. doi:10.3390/V16091422.
- [23] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)* 1995;57:289–300. doi:10.1111/j.2517-6161.1995.tb02031.x.
- [24] Kirichenko A, Lapovok I, Baryshev P, van de Vijver DAMC, van Kampen JJA, Boucher CAB, et al. Genetic features of HIV-1 integrase sub-subtype A6 predominant in Russia and predicted susceptibility to INSTIs. *Viruses* 2020;12. doi:10.3390/V12080838.
- [25] Orkin C, Schapiro JM, Perno CF, Kuritzkes DR, Patel P, DeMoor R, et al. Expanded multivariable models to assist patient selection for long-acting cabotegravir + rilpivirine treatment: clinical utility of a combination of patient, drug concentration, and viral factors associated with virologic failure. *Clin Infect Dis* 2023;77:1423–31. doi:10.1093/CID/CIA9370.
- [26] Jeffrey JL, Clair MS, Wang P, Wang C, Li Z, Beloor J, et al. Impact of integrase sequences from HIV-1 subtypes A6/A1 on the In vitro potency of cabotegravir or rilpivirine. *Antimicrob Agents Chemother* 2022;66. doi:10.1128/AAC.01702-21.
- [27] Hu Z, Cordwell T, Nguyen H, Li J, Jeffrey JL, Kuritzkes DR. Effect of the L74I polymorphism on fitness of cabotegravir-resistant variants of human immunodeficiency virus 1 subtype A6. *J Infect Dis* 2023;228:1352–6. doi:10.1093/INFDIS/JIAD291.