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Highlights

- Prevalence of major INSTI resistance mutations is very low even through NGS.
- Combinations of minority polymorphic mutations might affect virological control.
- L74I combined with other polymorphisms seems linked to virological rebound.

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Role of low-frequency INSTI resistance mutations on virological outcomes in ART-Naïve individuals initiating second-generation integrase inhibitors

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Abstract

Objectives: This study investigated the role of low-frequency INSTI resistance mutations, detectable by next-generation sequencing (NGS), in 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 maintained virological control (controls)

under first-line regimens based on dolutegravir or bictegravir. NGS data obtained through the Illumina platform were interpreted with HIVdb algorithm v9.7. Major (MRM), accessory (ARM) and other (ORM) INSTI resistance mutations were analysed at 5%, 10%, 20% NGS cut-offs. Conditional logistic regression (CLR) was used to evaluate association between INSTI resistance and risk of VR.

Results: Among 266 PWH (90 cases, 176 controls), cases experienced VR with a median (IQR) viremia of 317 (93-6,060) copies/mL after 15 (8-28) months from ART start.

The prevalence of MRM was low (NGS cut-off 5%, 10%, 20%: 1.9%, 0.8%, 0.4%), while it was moderate for ARM (7.5%, 7.1%, 6.4%) and high for ORM (50.0%, 44.7%, and 42.1%). There was no evidence for 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 to 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.

Keywords:

HIV minority resistant variants, HIV drug resistance, next-generation sequencing, integrase inhibitors, dolutegravir, bictegravir

INTRODUCTION

Integrase strand transfer inhibitors (INSTIs) are currently recommended as the first-line combined antiretroviral therapy (cART) for human immunodeficiency virus (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 species 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 species 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 regimen for which an association was 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 indicated 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 been not thoroughly evaluated to date. We focussed 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.

METHODS

Study design

We conducted a case-control study nested within the Icona Foundation Study cohort (www.icona.org) [21], which enrolls PWH who start 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; 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 viral rebound (VR), the date of discontinuation of the INSTI drug or 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 of 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 the 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).

Ethics

All participating centres' 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).

Viral extraction and sequencing

Viral RNA was extracted using the QiAamp Viral RNA Mini Kit (QIAGEN) 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) on the MiSeq platform (Illumina). NGS data were analyzed through 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 categorization as major resistance mutations (MRM), accessory resistance mutations (ARM) and other resistance mutations (ORM). 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 whole initial regimen and separately also for only the nucleos(t)ide RT inhibitor (NRTI) backbone. Specifically, each drug was considered fully active in case of resistance level <3. Entire regimens and NRTI backbones were considered fully active when all drugs of the combinations were fully active.

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 the 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 the exposure. The role of minority variants exclusively detected through NGS was also explored restricting analyses on INSTI mutations detected with frequency ranges 5-20% or 10-20%.

Separate uni-multivariable models were built to evaluate the specific role of MRM, ARM and ORM. 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 .

RESULTS

Participants' characteristics

Overall, NGS was successful for 266 out 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 to controls, cases were more likely women, with heterosexual transmission route, with black ethnicity, lower baseline CD4+ T cell counts, and higher baseline plasma HIV-1 RNA (Table 1). 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 (IQR 8-28) after starting the first-line regimen with a median (IQR) viral load of 317 (93-6,060) 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. Noteworthy, among 86 out of 90 participants with available follow-up after VR, 91.8% of them resuppressed (of whom about half without therapy change).

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

At baseline, the proportion of individuals harbouring any MRM 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 for

a difference in the prevalence of both MRM and ARM detected between cases and controls (Figure 1, panel A-B) was found. Differently, the proportion of individuals harbouring any ORM 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 (Figure 2, Panel A). The proportion of individuals with only one ORM was similar between cases and controls (Figure 2, Panel B). By contrast, the proportion of individuals with ≥ 2 ORM was higher among cases compared to controls at NGS set at 5% (Figure 2, Panel B). Multiple ORM were never observed together with other MRM or ARM, except for one case in whom we detected the copresence as mixture of the L74M ARM and L74I ORM. The most observed mutational pattern included L74I mutation (Table 2). In particular, the combination of L74I with other ORM (such as M50I or S230N) was more likely observed in cases compared to controls regardless of the NGS threshold used. A higher prevalence of this combination in cases was observed also 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 NGS setting (cases vs. controls, 97.8% vs. 100%, $P=0.114$).

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

Sensitivity analyses restricted on cases experiencing VR with plasma HIV-1 RNA >200 copies/mL (48 cases and 96 controls included) confirmed that the detection of ≥ 2 ORM exclusively as minority variants in the frequency window 5-20% was associated with a 3 fold increased risk of VR at univariable (Odd Ratio [95% C. I.]: 3.240 [1.165-9.013], $P=0.024$) and also at multivariable

analysis, although the association was not significant at the 5% level (Adjusted odd ratio [95% C.I.]: 3.043 [0.888-10.429], P=0.077) (Supplementary Table 2).

DISCUSSION

In this case-control study we estimated the prevalence of HIV-1 INSTI resistance mutations and their role to predict VR to first-line regimens based on DTG or BIC in PWH. Most of the observed rebounds were transient episodes of low-level viremia, followed 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 the NGS analysis set at the 20% threshold (at Sanger-like sensitivity) confirms 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 in general the estimates of the prevalence of MRM and ARM across studies in ART-naïve populations when NGS data were interpreted using cut-off <20% were very similar [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 minority variant in the 5-20% window was exclusively observed in cases.

This analysis, as far as we know, evaluated for the first time ever the complete list of INSTI resistance mutations reported in the Stanford algorithm, including also the ORM which are associated with a score of zero for BIC and DTG (such as L74I) or are not included in their score (such as M50I, S119R, E138D, V151I, S230N). In the context of these type of resistance mutations, our analysis identified a high prevalence of polymorphisms (about 40%-50%) both in cases and

controls, regardless of different NGS cut-offs used. When we analysed the specific ORM, L74I was more likely to be observed in cases compared to controls. We also found that in cases there was a higher proportion of participants with at least two ORM. By exploring the 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 a 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 does not affect the outcomes of breakthrough experiments across HIV-1 subtype A6 and, also, 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 INSTI major resistance mutations [26]. In our analysis we did not find any MRM in combination with L74I, while the combination of this mutation with other ORM appeared to be associated with the risk of virological failure. This phenomenon might be explained by an 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 separate analyses stratified by HIV-subtype (B vs. non-B viruses, Supplementary Table 1). By restricting the analyses on cases experiencing VR at viremia >200 copies/mL, sensitivity analyses confirmed that the presence of ≥ 2 ORM (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 before a first-line regimen based on second generation INSTI might be useful to identify individuals at higher risk of losing virological control.

Our study has several limitations. Firstly, we cannot rule out unmeasured or residual confounding. Mutations outside integrase region (such as *env* or 3'PPT) have been shown to be associated with

risk of virological failure of INSTI-based regimen [27,28]. We only sequenced *pol* gene regions in our stored samples. Second, no significant evidence was found indicating that major INSTI resistance mutations contribute to virological failures; this is probably due to low prevalence of the exposure and consequently low statistical power. Furthermore, our data demonstrate that minority variants detected at the 5% threshold harbouring multiple ORM 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 which indicate a debatable reliability in detecting resistance at this NGS range [22]. Unfortunately, neither resistance tests performed around the time of VR or stored plasma samples were available for the cases, thus we could not evaluate the extent of minority variant outgrowth at VR, 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 might have been underestimated.

In conclusion, our findings suggest that virological rebound 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 before 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 ORM in different settings and better understand the mechanism by which these mutations may affect virological outcome of DTG/BIC-based regimens.

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Declarations of competing interest

The authors have no conflict of interest related to this manuscript.

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Access to data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

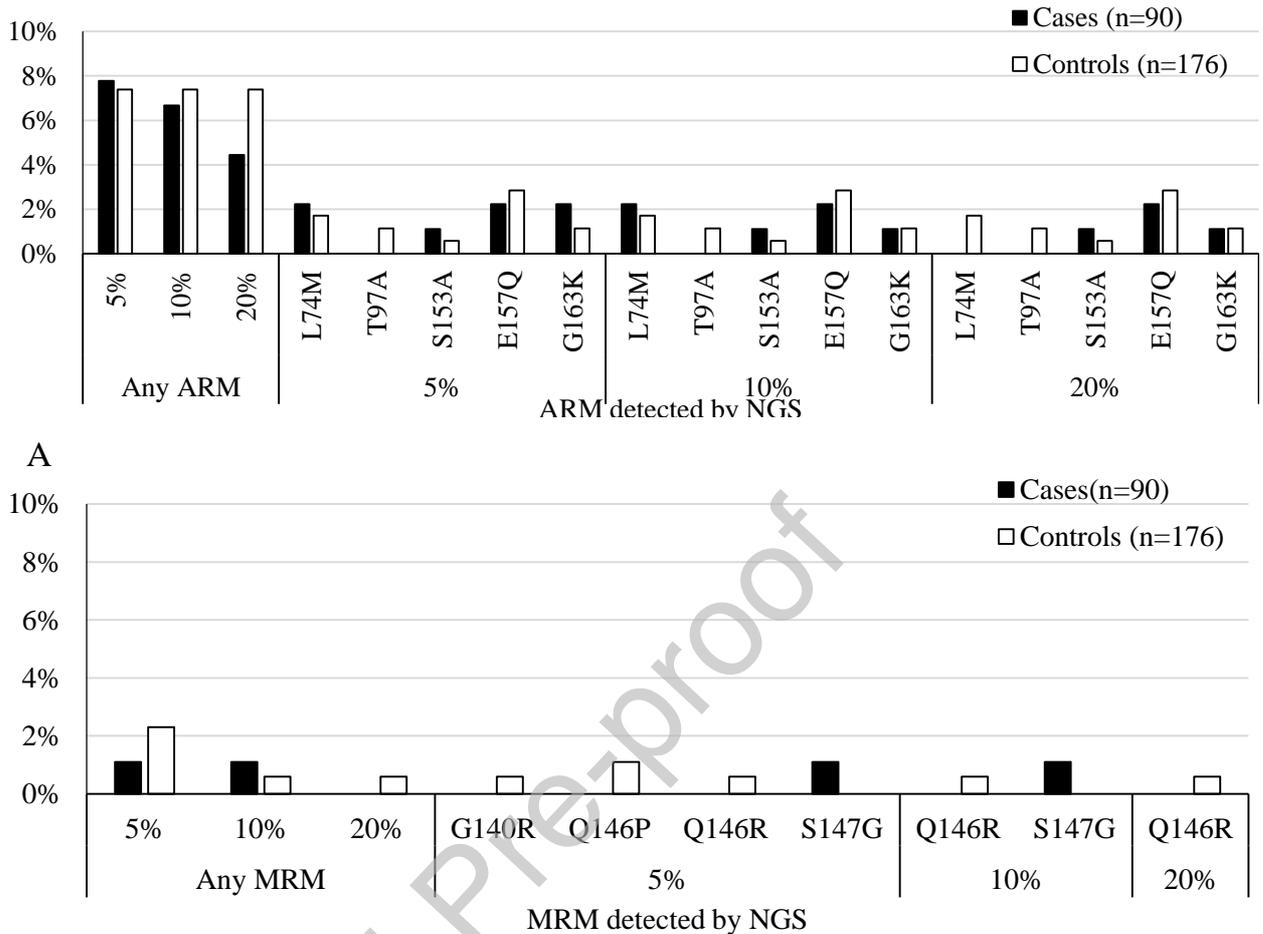
Figure 1

Figure 1. Prevalence of HIV-1 major and accessory INSTI resistance mutations (MRM & ARM) in cases and controls according to different NGS cut-offs. A) Prevalence of any MRM). B) Prevalence of any ARM. The following mutations according with HIVdb 9.7 (<https://hivdb.stanford.edu/dr-summary/comments/INSTI/>) were tested: MRM (T66AIK, E92GVQ, G118R, F121CY, E138KAT, G140RSAC, Y143ACKGHR, P145S, P146PRL, S147G, Q148HKRN, V151L, N155HT, R263K); ARM (A49G, H51Y, L74FM, V75A, Q95K, T97A, T122N, A128T, G149A, V151A, S153YFA, E157Q, G163RK, S230R, D232N).

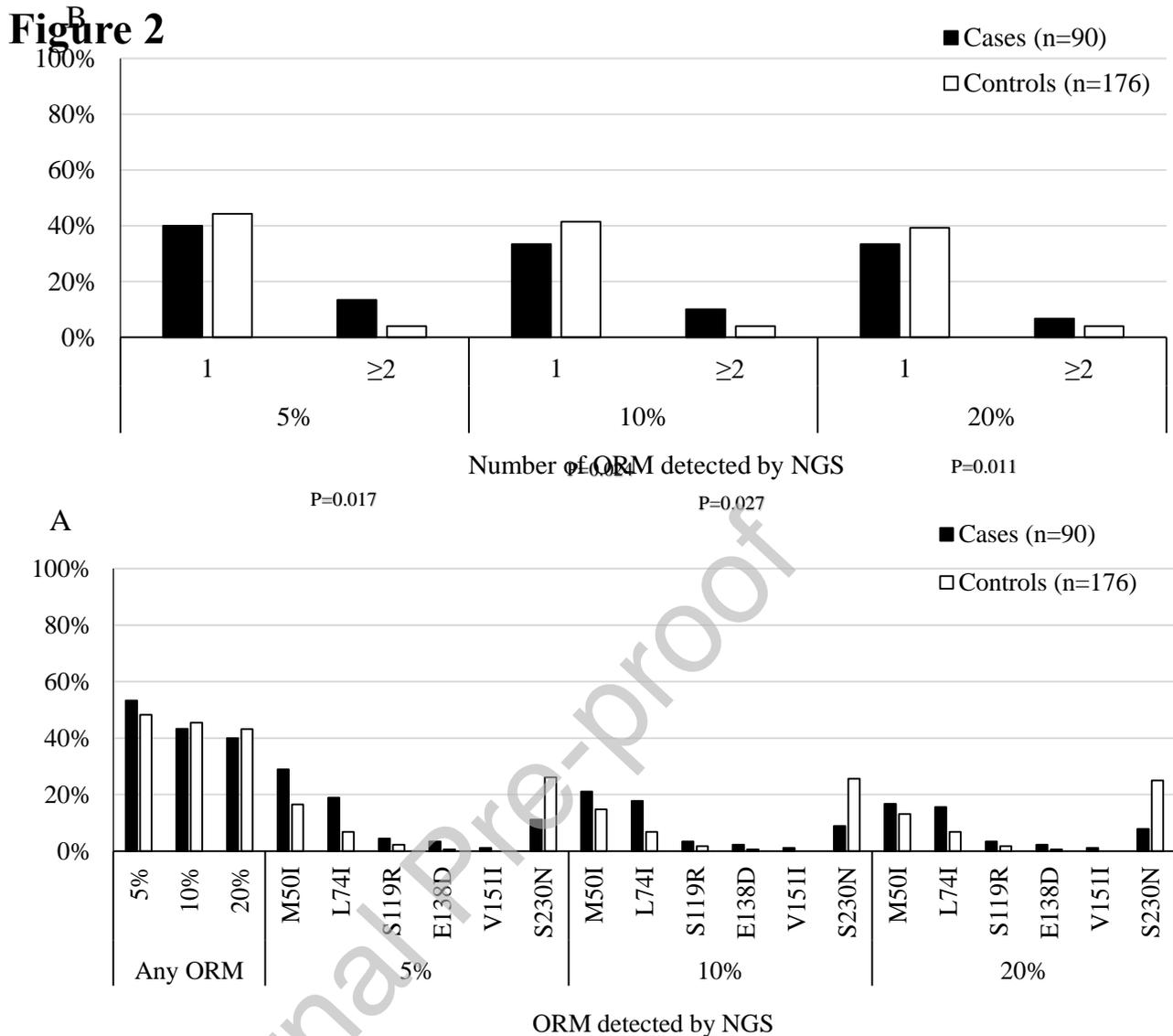


Figure 2. Prevalence of other INSTI resistance mutations (ORM) in cases and controls according to different NGS cut-offs. A) Prevalence of any ORM. B) Prevalence of at least one or at least two ORM. In the graph are reported statistically significant differences ($P < 0.05$) between cases and controls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

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Table 1. Baseline participants' characteristics

Variables	Overall (N=266) ^a	Cases (N=90) ^a	Controls (N=176) ^a	Odd ratio (95% C.I.)	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
<i>Caucasian</i>	209 (78.6)	64 (71.1)	145 (82.4)	1.0	
<i>Hispanic</i>	27 (10.2)	8 (8.9)	19 (10.8)	1.0 (0.5-2.0)	
<i>Black</i>	25 (9.4)	18 (20.0)	7 (4.0)	2.4 (1.4-4.0)	
<i>Other/Unknown</i>	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
<i>Men who have sex with men</i>	141 (53.0)	38 (42.2)	103 (58.5)	1.0	
<i>Heterosexual</i>	88 (33.1)	42 (46.7)	46 (26.1)	0.8 (0.4-1.7)	
<i>Drug abuse</i>	8 (3.0)	1 (1.1)	7 (4.0)	1.4 (0.6-2.9)	
<i>Transgender</i>	6 (2.3)	1 (1.1)	5 (2.8)	0.4 (0.0-2.8)	
<i>Unknown</i>	23 (8.6)	8 (8.9)	15 (8.5)	0.5 (0.1-3.8)	
Subtype^b, n (%)					0.039
<i>B</i>	162 (60.9)	42 (46.7)	120 (68.2)	1.0	
<i>CRF02_AG</i>	21 (7.9)	13 (14.4)	8 (4.5)	2.4 (1.3-4.5)	
<i>A^c</i>	15 (5.6)	8 (8.9)	7 (4.0)	2.1 (1.0-4.4)	
<i>C</i>	13 (4.9)	6 (6.7)	7 (4.0)	1.8 (0.8-4.2)	
<i>Other</i>	55 (20.7)	21 (23.3)	34 (19.3)	1.5 (0.9-2.5)	
CD4+ T cells, cells/mm³, median (IQR)	284 (80-476)	149 (55-391)	327 (98-529)	0.9 (0.8-1.0)	0.016
HIV RNA, log₁₀ 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 for 266 out 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. ^bSubtyping was determined by using both the automatic tool COMET (<https://comet.luh.lu/>) and phylogenetic analysis. Specifically a maximum likelihood phylogenetic tree was constructed using IQ-TREE2 (v2.1.3) with 1000 bootstrap replicates. ^cA1 (n=8, 53.3%); A3 (n=3, 20.0%); A6 (n=4, 26.7%). Nd: not determinable.

Table 2. Combinations of multiple ORM detected among cases and controls

Combinations of ORM	Cases (N=90)	Controls (N=176)	OR (95% C.I.)	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
<i>L74I+M50I</i>	2 (2.2)	0 (0.0)	3.2 (0.7-13.4)	0.119
<i>L74I+S230N</i>	5 (5.6)	1 (0.6)	2.6 (1.0-6.4)	0.042
<i>L74I+M50I+S230N</i>	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
<i>L74I+M50I</i>	3 (3.3)	1 (0.6)	2.3 (0.7-7.3)	0.163
<i>L74I+S230N</i>	2 (2.2)	0 (0)	3.2 (0.7-13.4)	0.119
<i>L74I+M50I+S230N</i>	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
<i>L74I+M50I</i>	2 (2.2)	1 (0.6)	2.0 (0.5-8.4)	0.330
<i>L74I+S230N</i>	0 (0)	4 (2.3)	0.0 (0.0-96.9)	0.435
<i>L74I+M50I+S230N</i>	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

Table 3. Conditional logistic regression models to evaluate the odds of experiencing virological failure to DTG/BIC based first-line according to baseline INSTI resistance

INSTI resistance detected	Frequency, n (%)		Unadjusted		Adjusted	
	Cases (N=90)	Controls (N=176)	P value	OR (95% C.I.)	P value	AOR (95% C.I.)
≥ 1 MRM						
<i>NGS ≥5%^a</i>	1 (1.1)	4 (2.3)	0.568	0.563 (0.078-4.047)	0.816	0.785 (0.103-6.011)
<i>NGS 5-20%^a</i>	1 (1.1)	3 (1.7)	0.758	0.734 (0.102-5.275)	0.964	0.953 (0.122-7.449)
<i>NGS ≥10%^a</i>	1 (1.1)	1 (0.6)	0.724	1.427 (0.198-10.261)	0.781	1.343 (0.168-10.759)
<i>NGS 10-20%^a</i>	1 (1.1)	0 (0.0)	0.279	2.974 (0.414-21.383)	0.544	1.958 (0.224-17.115)
<i>NGS ≥20%^b</i>	0 (0.0)	1 (0.6)	-	-	-	-
≥ 1 ARM						
<i>NGS ≥5%^a</i>	7 (7.8)	13 (7.4)	0.925	1.038 (0.478-2.254)	0.931	1.037 (0.460-2.339)

<i>NGS 5-20%</i> ^a	3 (3.3)	0 (0.0)	0.058	3.095 (0.963-9.947)	0.037	3.797 (1.081-13.339)
<i>NGS ≥10%</i> ^a	6 (6.7)	13 (7.4)	0.860	0.928 (0.404-2.134)	0.829	0.908 (0.379-2.179)
<i>NGS 10-20%</i> ^a	2 (2.2)	0 (2.2)	0.120	3.132 (0.743-13.193)	0.095	4.002 (0.786-20.377)
<i>NGS ≥20%</i> ^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						
<i>NGS ≥5%</i> ^c	48 (53.3)	85 (48.3)	0.526	1.144 (0.754-1.736)	0.549	1.145 (0.736-1.781)
<i>NGS 5-20%</i> ^c	12 (13.3)	9 (5.1)	0.059	1.803 (0.978-3.324)	0.100	1.698 (0.903-3.193)
<i>NGS ≥10%</i> ^c	39 (43.3)	80 (45.8)	0.787	0.944 (0.62-1.436)	0.681	0.909 (0.578-1.431)
<i>NGS 10-20%</i> ^c	3 (3.3)	4 (2.3)	0.681	1.274 (0.402-4.041)	0.924	0.943 (0.284-3.135)
<i>NGS ≥20%</i> ^c	36 (40.0)	76 (43.2)	0.684	0.916 (0.599-1.4)	0.711	0.919 (0.586-1.441)
≥ 2 ORM						
<i>NGS ≥5%</i> ^c	12 (13.3)	7 (4.0)	0.024	2.020 (1.095-3.728)	0.008	2.394 (1.254-4.571)
<i>NGS 5-20%</i> ^c	6 (6.7)	0 (0.0)	0.008	3.113 (1.353-7.162)	0.038	2.685 (1.056-6.826)
<i>NGS ≥10%</i> ^c	9 (10.0)	7 (4.0)	0.113	1.757 (0.876-3.522)	0.036	2.171 (1.052-4.479)
<i>NGS 10-20%</i> ^c	3 (3.3)	0 (0.0)	0.060	3.027 (0.954-9.601)	0.204	2.318 (0.633-8.494)
<i>NGS ≥20%</i> ^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. caucasian [dummy]), HIV-1 subtype (CRF02_AG, A, C, others, vs. B [dummy]), genotypic susceptibility score of backbone regimen (non-susceptible vs. susceptible). ^b Model not performed since MRM 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. caucasian [dummy]), HIV-1 subtype (CRF02_AG, A, C, others, vs. B [dummy]), genotypic susceptibility score of whole regimen (non-susceptible vs. susceptible).