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Transmitted drug resistance mutations and trends of HIV-1 subtypes in treatment-naïve patients: A single-centre experience

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ABSTRACT

Objectives: Transmitted drug resistance (TDR) and HIV-1 genetic diversity may affect treatment efficacy and clinical outcomes. Here we describe the circulating viral subtypes and estimate the prevalence of drug resistance among antiretroviral therapy (ART)-naïve patients attending Sapienza University Hospital (Rome, Italy) from 2006–2017.
Methods: Genotypic resistance testing (GRT) was performed on 668 ART-naïve patients for integrase (n = 52), protease and reverse transcriptase (n = 668) sequences.
Results: Twenty-one different HIV-1 subtypes and circulating recombinant forms (CRFs) were identified. Subtype B was the most common (67.1%), followed by CRF02_AG (8.4%), and subtypes C and F (both 6.0%). A significantly increase in the proportion of non-B strains ($P < 0.001$) and the rate of non-Italian patients was observed over time. The overall prevalence of TDR was 9.4% (NRTI, 4.2%; NNRTI, 5.8%; and PI, 1.0%) and was higher in subtype B strains. Transmitted INSTI mutations (Q148H and G140S) responsible for high-level resistance to raltegravir and elvitegravir and intermediate resistance to dolutegravir and bictegravir were found, for the first time, in two individuals. Minor or accessory INSTI mutations were detected in 17.3% of patients. No significant decrease in the prevalence of TDR was documented over time.
Conclusion: The significant increase in non-B subtypes suggests that the molecular epidemiology of HIV-1 is changing. Detection of a major INSTI mutation in two ART-naïve patients highlights the importance of performing GRT before commencing treatment. This finding and the lack of a significant reduction in TDRs underline the importance of continuous surveillance of resistance mutations.

1. Introduction

The use of antiretroviral therapy (ART) for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in developed countries has been accompanied by an increase in transmitted drug resistance (TDR) in ART-naïve patients [1], limiting the choice of first-line antiretroviral drugs [2,3]. The emergence of TDR due to ART expansion represents a serious public-health issue because TDRs may affect treatment efficacy and may negatively affect an individual's prognosis. Despite the fact that Italian and European guidelines on the use of antiretroviral agents and the diagnostic-clinical management of HIV-1-infected persons recommend performing genotypic resistance testing (GRT) prior to ART initiation [4–6], the prevalence of TDR mutations has remained stable at approximately 8–10% in these countries over the years [7].

In addition, since integrase strand transfer inhibitors (INSTIs) are part of recommended first-line regimens for the treatment of HIV-1 infection, INSTI mutation surveillance has gained importance in order to optimise the efficacy of therapy. Primary INSTI resistance is still rare, but reports of TDRs to these drugs are emerging [8–11]. None the less, baseline resistance testing to this class is still not routinely performed and currently only a few studies have evaluated the prevalence of INSTI mutations in

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ART-naïve patients [12–14]. To date, the presence of pre-treatment INSTI resistance among ART-naïve HIV-1-infected patients in Italy was never reported.

Moreover, several authors have reported epidemiological changes, such as an increasing trend of non-B HIV-1 subtypes and circulating recombinant forms (CRFs) in Europe, Australia and North America [15–18]. This increase underlines that geographical patterns in subtype distribution are changing over time owing to migration and population mixing [15,16]; this phenomenon is clinically relevant because these changes can have an impact on pathogenesis, resistance pathways, disease progression, diagnosis and vaccine development [17].

The aim of this study was to examine temporal changes in HIV-1 subtype diversity and to evaluate the prevalence of TDRs among newly diagnosed ART-naïve HIV-1-infected individuals in Umberto I Sapienza University Hospital (Rome. Italy) in 2006–2017.

2. Methods

2.1. Study population

A retrospective study was conducted on 720 genotypic resistance tests (668 *pol* sequences and 52 integrase sequences) from 668 ART-naïve patients attending Umberto I Sapienza University Hospital from 2006–2017. For each patient, GRT performed for routine clinical purposes at diagnosis or prior to the start of therapy was considered. Baseline demographic data for the studied individuals are summarised in Table 1.

Informed consent was obtained according to the standards of the local ethics committee. All information, including virological, clinical and demographic data, was recorded in an anonymous database.

2.2. Genotyping and analysis of drug resistance mutations

GRT from plasma samples (*pol* and integrase genes) was performed until 2015 using a TruGene® HIV-1 Genotyping Kit (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) and TruGene® Core Reagent (Siemens Healthcare Diagnostics Inc.) as previously described [19,20]; subsequently, a ViroSeq™ HIV-1 Genotyping System (Celera Diagnostics, Alameda, CA, USA) and ViroSeq™ HIV-1 Integrase RUO Genotyping Kit (Celera Diagnostics) were used according to the manufacturer's instructions.

Table 1
Characteristics of antiretroviral therapy-naïve HIV-1-infected patients included in the study (N = 668).

Characteristic	*n* (%) or median [IQR]
Male sex	506 (75.7)
Age at diagnosis (years)	38 [31–48]
CD4$^+$ T-cell count (cell/mm^3)	340 [148–570]
HIV-RNA load (log copies/mL)	4.71 [4.1–5.3]
Country of origin	
Italy	465 (69.6)
Foreign country	203 (30.4)
Route of transmission	
MSM/bisexual	295 (44.2)
Heterosexual	290 (43.4)
Injecting drug user	59 (8.8)
Other	24 (3.6)
Viral subtype	
A	19 (2.8)
B	448 (67.1)
C	40 (6.0)
F	40 (6.0)
CRF02_AG	56 (8.4)
Other	65 (9.7)

IQR, interquartile range; MSM, men who have sex with men.

The HIV-1 subtypes and CRFs were determined according to the Stanford University HIV Drug Resistance Database HIVdb Program v.8.5 (https://hivdb.stanford.edu/hivdb).

TDR mutations were defined as the presence in the *pol* region of at least one major mutation included in the International AIDS Society (IAS) list [21] and/or the Stanford University HIV Drug Resistance Database HIVdb Program v.8.5. Regarding INSTI mutations, all mutations reported in the IAS and Stanford lists were considered.

HIV-1 strains were defined as resistant if carrying at least one TDR mutation. The overall prevalence was defined as the percentage of patients infected with a virus carrying any drug resistance mutation (DRM). The prevalence of TDR for the different drug classes [nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and INSTIs), was defined as the percentage of patients infected with a virus carrying any DRM associated with each drug class.

2.3. Phylogenetic analysis

Phylogenetic relationships were analysed by constructing a maximum-likelihood phylogenetic tree using IQ-TREE [22]. The best substitution model (GTR + I+G) was selected by analysis of sequences with the Models tool in MEGA. Tree reliability was assessed by setting bootstrap replicates to 1000. Bootstrap values >70% were considered significant. The tree was rooted with midpoint rooting and was edited using FigTree v.1.4.0.

To investigate the demographic history of an ART-naïve couple (subtype B), Bayesian calculations were performed by calibrating a molecular clock using known sequence sampling times with the Bayesian Markov chain Monte Carlo (MCMC) method implemented in BEAST v.1.8.2 (http://beast.bio.ed.ac.uk) [23,24]. Independent MCMC runs were conducted for at least 100×10^6 generations and sampled every 10 000 steps for each molecular clock model. Convergence of the MCMC was assessed by calculating the effective sample size (ESS) for each parameter. Only parameter estimates with ESS values of >250 were accepted. The maximum clade credibility (MCC) tree was obtained from the tree's posterior distributions, after a 10% burn-in, with Tree-Annotator software v.1.8.2 included in the BEAST package [23,24].

2.4. Statistical analysis

Categorical variables for the studied subjects were compared by the χ^2 test. To evaluate potential differences in trends over time, χ^2 test for trends was used. A P-value of <0.05 was considered statistically significant. All analyses were performed using PASW Statistics for Windows v.18.0 (SPSS Inc., Chicago, IL, USA) and Epi Info v.7.2.2.6 (CDC, Atlanta, GA, USA).

3. Results

3.1. Patient characteristics

Between 2006 and 2017, 668 HIV-1-infected ART-naïve individuals underwent GRT. The demographic characteristics of these subjects are shown in Table 1. The patients were primarily male (75.7%), of Italian origin (69.6%), with a median age at diagnosis of 38 years [interquartile range (IQR) 31–48 years]. The most common risk factors were homosexual (44.2%) and heterosexual contact (43.4%). At the time of GRT, the median CD4$^+$ T-cell count was 340 cells/mm^3 (IQR 148–570 cells/mm^3) and the median plasma HIV-RNA level was 4.71 $\log_{10}$ copies/mL (IQR 4.1–5.3 $\log_{10}$ copies/mL).

Twenty-one different subtypes and CRFs were identified. HIV-1 subtype B was the most commonly detected (67.1%; 448/668), followed by CRF02_AG (8.4%; 56/668), and subtypes C and F (both 6.0%; 40/668).

Over the 12-year study period, there was a significant increase in non-B subtype infection (13.6% in 2006 vs. 51% in 2017; $P < 0.001$) (Fig. 1a) as well as an increase in the foreign-born population ($P = 0.043$). Higher percentages of non-B infections occurred among persons born abroad (55.7%) compared with those born in Italy (22.7%) ($P < 0.001$). Nevertheless, an increase in the rate of Italian patients infected with a non-B virus (9.0% in 2006 vs. 27.0% in 2017; $P < 0.001$) was also found (Fig. 1b).

3.2. Prevalence of pol drug resistance mutations

GRT before initiation of ART was available in 668 patients. From 2006–2017, the overall prevalence of patients with at least one TDR *pol* mutation was 9.4% (63/668). Of the 668 patients, 42 (6.3%) had one drug class mutation, 11 (1.6%) had more than one mutation associated with resistance to one class drug, and 10 (1.5%) harboured multiclass resistant virus. Specifically, the rate of dual-class TDRs was 0.9% (6/668) for NRTI + NNRTI and 0.4% (3/668) for NRTI + PI. Only one patient, infected with subtype F1, had a virus with triple-class drug resistance. Overall, resistance to NNRTIs was most common (5.8%; 39/668), followed by NRTIs (4.2%; 28/668) and PIs (1.0%; 7/668). As expected, the prevalences of TDRs to NNRTIs and NRTIs were significantly higher than the prevalence of TDRs to PIs ($P < 0.001$).

The most frequent NNRTI-associated mutation was K103N, detected in 13/668 patients (1.9%), followed by E138G/K/Q (7/668; 1.0%), V108I (6/668; 0.9%) and Y181C (5/668; 0.7%). For NRTI resistance, thymidine analogue mutations (TAMs) occurred more frequently, with T215D/S mutations detected in 10 patients (1.5%), followed by D67N (6/668; 0.9%) and M41L (4/668; 0.6%). PI-related mutations were rare; M46I and I54M/L/V were each detected in was found in three individuals (0.4%) (Fig. 2).

Despite the presence of some fluctuations over the years, the overall prevalence of TDR remained at around 9.4% and did not significantly change over time (Fig. 3).

The detection rate of TDRs in patients infected subtype B was higher than in those infected with non-B subtypes (11.6% vs. 6%; $P = 0.03$).

3.3. Prevalence of integrase strand inhibitor mutations

HIV-1 integrase mutation data were available for 52 ART-naïve patients collected between 2009 and 2017. Most of them were infected with subtype B (71.2%; 37/52).

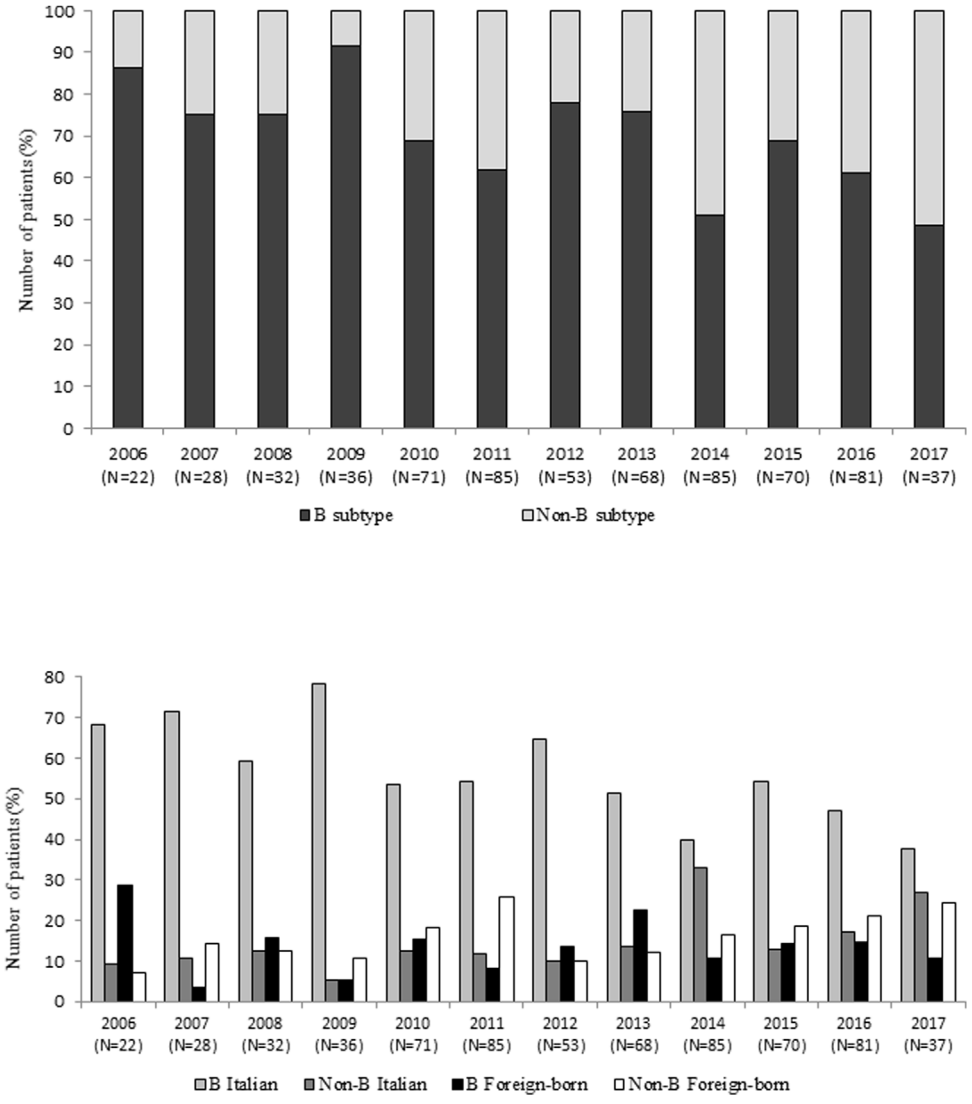

Fig. 1. Prevalence of HIV-1 B and non-B subtypes over time in (A) the overall population and (B) Italian or foreign-born patients.

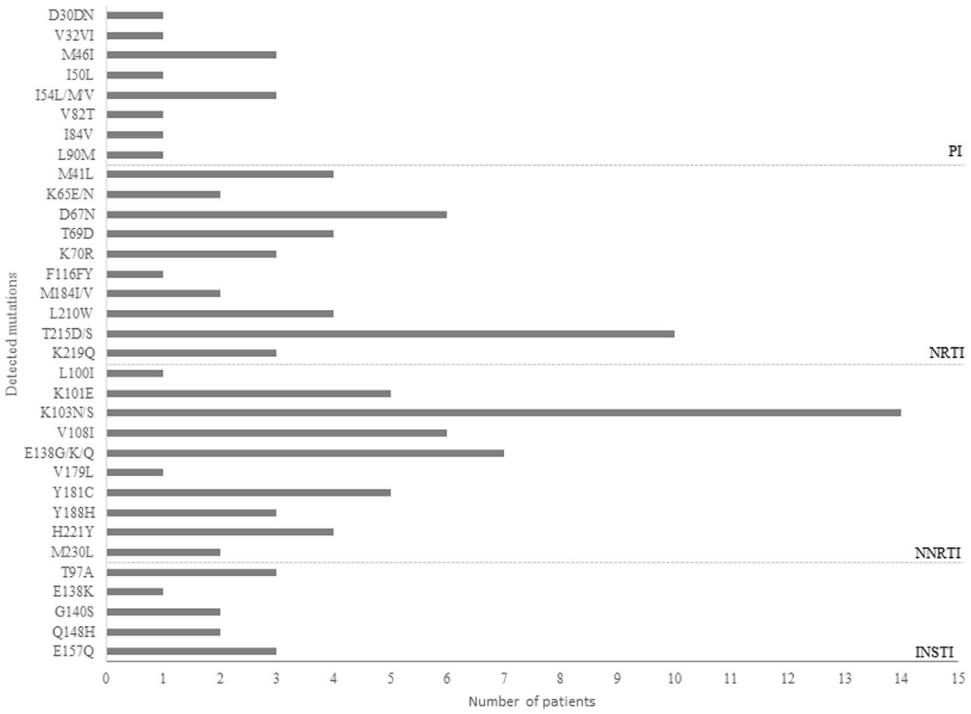

Fig. 2. Transmitted drug resistance mutations detected in antiretroviral therapy-naïve patients. PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; INSTI, integrase strand transfer inhibitor.

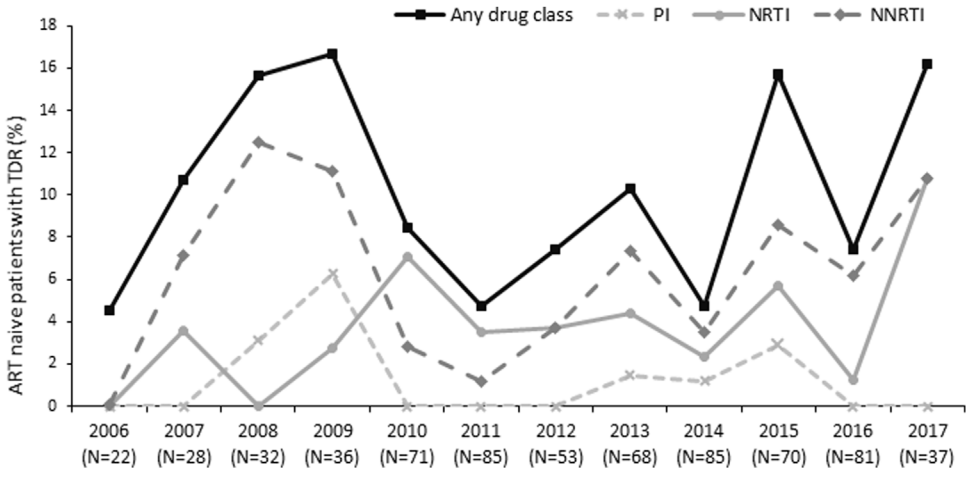

Fig. 3. Temporal trends in the yearly proportion of transmitted drug resistance (TDR) mutations among the overall antiretroviral therapy (ART)-naïve population. PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

Minor or accessory mutations were detected in 9 patients (17.3%). Among the minor mutations, T97A and E157Q were each detected in 3 patients (5.8%), G140S in 2 patients (3.8%) and E138K occurred in 1 patient (1.9%).

Two patients (3.8%) had the major Q148H mutation and the minor G140S. Interestingly, these two individuals were in an acute phase of infection when the GRT was performed; they were a couple and were both active drug users and also shared the reverse transcriptase mutations E138G, T215S, H221Y and M230L.

3.4. Phylogenetic analysis

From the maximum-likelihood tree performed on the HIV-1 subtype B sequences, five statistically supported clades were highlighted. The clusters mainly included sequences from different years. Mutated viruses were dispersed among clades, except that some viruses probably originated from the same strain.

Phylogenetic trees demonstrated that the viruses from the HIV-1-infected ART-naïve couple were almost identical. The Bayesian skyline plot (BSP) growth demographic model with a relaxed molecular clock was selected as the most appropriate to describe the evolutionary history of this virus. The evolutionary rate used for the Bayesian calculation was 0.0021 substitution sites per year. The root of the time of the most common recent ancestor (tMRCA) corresponded to January 2017 (May 2016–February 2017) indicating that the probable origin of that strain dated back to early 2017.

The maximum-likelihood phylogenetic tree of non-B subtype HIV-1 viruses showed 39 statistically supported clusters (bootstrap >70%). Specifically, CRF02_AG showed 9 clusters, subtypes CRF01_AE and F each showed 7 clusters, subtype G showed 6

clusters, subtype C showed 4 clusters, and subtypes A and CRF12_BF each showed 3 clusters.

4. Discussion

This study focused on the occurrence of TDRs in 668 HIV-1-infected ART-naïve individuals attending Umberto I Sapienza University Hospital in the period 2006–2017. This treatment-naïve population, as reported in other Italian cohorts [25–28], showed a significantly increased proportion of patients carrying non-B subtype virus and an increased rate of the foreign-born population in the last years. As expected, a higher prevalence of non-B infections was found among non-Italian patients. This is in line with data reported in several European countries during the last decade and can be explained by the waves of migrants from low-middle-income countries [28–31]. However, a significant increase of non-B infections was also found in Italian patients in more recent years. This finding, in agreement with previous observations [27,28], clearly indicates that, in conjunction with epidemiological changes, non-B strains have become endemic in the Italian population.

In our centre, the estimated prevalence of TDR was 9.4%, similar to the global incidence in Italy [14,32], and was significantly higher in subtype B than in non-B viruses. These data may be due to a different selective pressure exerted by antiretroviral drugs on B and non-B subtypes over time, probably because of limited ART coverage in the country of origin of non-B strains. Currently, NNRTIs remain the drug class with the highest prevalence of TDR mutations [33,34]. The frequency calculation of single resistance mutations revealed that K103N, a non-polymorphic mutation causing high-level resistance to nevirapine (NVP) and efavirenz (EFV), was the most frequently observed. This probably occurs because this mutation can be quickly transmitted due its limited effect on viral fitness [35]. The persistence of DRMs in the ART-naïve population could also be associated with high use of the drug responsible for its selection. It is known that K103N is selected in patients receiving first-generation NNRTIs such as NVP and EFV, which are drugs with a low genetic barrier. NVP was widely used in the past years especially before the introduction of the second-generation drugs rilpivirine and etravirine, whilst EFV is still recommended as an alternative in Italian and European guidelines [4–6]. Detection of K103N, despite the introduction of new drugs over the years, suggests that its persistence is not linked to continuous use of its selective agents. Similarly, among NRTI DRMs, T215 revertant mutations, such as T215D/S, and M41L were most frequently detected. These mutations belong to the TAMs pattern and are selected by thymidine analogues, a class of drugs no longer used in current therapeutic regimens. Therefore, despite a progressive reduction in drugs selecting TAMs and K103N, these mutations remain the most frequent in ART-naïve patients [16,27,28], suggesting that these viral variants are well adapted to the host and derive from individuals who have been infected for a long time and treated in the past with suboptimal therapy.

In the current study, the prevalence of INSTI TDRs was also analysed in a small number of samples (n = 52). ART containing INSTIs has become the preferred first-line regimen, as currently recommended by ART guidelines in high-income countries [4–6]. However, there is no consensus opinion regarding baseline testing for INSTI resistance, basically because INSTI resistance remains rare worldwide. This study provides important information about TDRs to INSTIs in Italy because, to the best of our knowledge, it documents for the first time the presence of major associated INSTI DRMs in Italian ART-naïve patients. A virus with Q148H and G140S mutations, responsible for a high-level of resistance to raltegravir and elvitegravir and intermediate resistance to dolutegravir and bictegravir, was identified. Interestingly, the ART-naïve patients who harboured this virus were a couple, were both intravenous drug users and were in an acute phase of infection. Phylogenetic analysis revealed that these patients harboured the same viruses and that the infection dates back to early 2017. In the literature, a few cases of transmitted INSTI resistance have been reported [8–10,36] and recently a case of transmitted INSTI resistance affecting the second-generation INSTIs dolutegravir and bictegravir [11] has been documented.

Moreover, in the small cohort in the current study, minor INSTI mutations were observed in 17.3% of analysed sequences. The prevalence of these minor and accessory substitutions was lower than that detected in another cohort [37] but higher than that reported elsewhere [13]. To date, the clinical impact of these mutations in ART-naïve patients starting an INSTI-containing regimen is unknown and prospective studies are needed to elucidate their role in affecting the genetic barrier.

In conclusion, although this study was limited by the single-centre setting, the data revealed that despite subtype B HIV-1 continuing to predominate in Italy, the percentage of non-B infections has grown in recent years, also among Italian patients. These data emphasise that the molecular epidemiology of HIV-1 in Western Europe, including Italy, is changing and underline the importance of monitoring the dynamics of HIV-1 transmission. The broad genetic diversity of HIV-1 can have important implications for public health since subtypes and CRFs can show different properties affecting their fitness, transmissibility and response to therapy [38].

This study also showed that the prevalence of TDRs appears to be stable despite the availability of newer antiretroviral drugs and recommended pre-therapy GRT, which should help to prevent virological failure and the accumulation of further DRMs.

Finally, this study, although performed on too few samples for a prevalence study, highlights the importance of implementing data regarding resistance to INSTIs in the newly diagnosed population and understanding the impact of the minor INSTI mutations in this population. Despite the significant role of INSTIs as first-line antiretroviral agents for the treatment of HIV-1 infection, the documented cases of TDRs to INSTIs should serve as a reminder that the appearance of resistance is always lurking. INSTIs have been increasingly used both for first-line and salvage ART, therefore it is reasonable to assume that the prevalence of INSTI mutations might rise over time. This study reinforces the current recommendations to perform GRT for integrase inhibitors in ART-naïve patients, especially in those presenting TDRs to other classes of drugs, in order to guide treatment decisions.

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Competing interests

None declared.

Ethical approval

Informed consent was obtained according to the standards of the local ethics committee.

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