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Cohort profile: PRESTIGIO, an Italian prospective registry-based cohort of people with HIV-1 resistant to reverse transcriptase, protease, and integrase inhibitors

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Abstract

Purpose: The PRESTIGIO Registry was established in 2017 to collect clinical, virological and immunological monitoring data from people living with 4-class drug resistant HIV (4DR-PLWH). Key research purposes include the evaluation of residual susceptibility to specific antiretrovirals and validation of treatment and monitoring strategies in this population.

Participants: The PRESTIGIO Registry collects annual plasma and peripheral blood mononuclear cell samples and demographic, clinical, virological, treatment and laboratory data from PLWH followed at 39 Italian clinical centers and characterized by intermediate-to-high genotypic resistance to ≥1 nucleoside reverse transcriptase inhibitors (NRTIs), ≥1 non-nucleoside reverse transcriptase inhibitors (NNRTIs), ≥1 protease inhibitors (PIs), plus either intermediate-to-high genotypic resistance to ≥1 integrase strand transfer inhibitors (INSTIs) or history of virological failure to an INSTI-containing regimen. To date, 229 people have been recorded in the cohort. The most of data are collected from the date of first evidence of 4-class drug resistance (baseline) with some pre-baseline information obtained retrospectively, samples are collected from the date of enrollment in the registry.

Findings to date: The cohort has been used to assess i) prognosis in terms of survival or development of AIDS or non-AIDS-related clinical events; ii) long-term efficacy and safety of different antiretroviral regimens; iii) virological and immunological factors predictive of clinical outcome and treatment efficacy, especially through analysis of plasma and cell samples.

Future plans: The registry can provide new knowledge on how to implement an integrated approach to study PLWH with documented resistance to the 4 main antiretroviral classes, a population with a limited number of individuals characterized by a high degree of frailty and complexity in therapeutic management. Such knowledge would benefit both the individual PLWH and society.

Registration: The PRESTIGIO Registry is registered on ClinicalTrials.gov (NCT04098315).

Strengths and limitations of this study

- The first cohort specifically including people living with 4-class drug-resistant HIV (4DR-PLWH).
- The cohort is readily available for research projects: the data sources (clinical centers) have already been linked and data have been collected in order to enable easier and simpler querying across source systems.
- Annual collection of cryopreserved plasma and peripheral blood mononuclear cell samples for research purposes.
- Given the rare condition of HIV 4-class drug resistance, the cohort includes a limited number of individuals despite the multicenter design.
- Incomplete information before evidence of 4-class drug resistance.

Introduction

Continuous progress in antiretroviral therapy (ART) has resulted in a high rate of virological suppression and a consequent improvement in life expectancy for individuals with human immunodeficiency virus (HIV).[1-5] Nevertheless, heavily treatment-experienced (HTE) people living with HIV (PLWH) have a history of previous virological failures on different antiretroviral regimens and limited treatment options, because of multidrug resistance and/or drug intolerance.[6,7] ART options can also be limited by drug–drug interactions or drug toxicity, due to the need of both prophylaxis or treatment for opportunistic infections and concomitant therapies for multiple comorbidities, that are common in HTE PLWH,[8,9] especially with aging.[10] This fragile group often needs complex, unusual and asymmetrical regimens (mixture of *quaque die* and *bis in die* schedules or oral, subcutaneous and intravenous administrations) with a high pill burden, possibly leading to a suboptimal adherence, which in turn increases the risk of virological failure with emergent resistance and worsens the prognosis.[11,12]

Even though there is no consensus in defining HTE individuals, these PLWH generally have two or less antiretroviral classes available for use, with limited fully active antiretroviral drugs within each class,[13] and represent a group at increased risk of low adherence and clinical progression. Therefore, establishing cohorts aimed to characterize this population better appears essential.

Among these difficult-to-treat individuals, those who harbor a 4-class drug-resistant (4DR) virus, characterized by resistance to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and integrase strand transfer inhibitors (INSTIs), represent a particular group with a very high burden of disease and very limited therapeutic options.[14] In terms of epidemiology, the prevalence of HIV 4-class drug resistance on a global scale is unknown: a prospective study in North Carolina estimated this prevalence at approximately 1% of PLWH, with a slight increase since 2007.[15] Recent Italian studies quantified subjects with 4DR HIV at about 2% of treatment-experienced PLWH in 2011-2018.[16] According to the most recent WHO report on HIV drug resistance (2014-2020), virological suppression (defined

as HIV RNA <1000 copies/mL) was ≥90% among adults on ART for ≥48 months in only 6/14 middle- and low-income countries with available data; in virologically non-suppressed PLWH on a NNRTI-based regimen for ≥48 months, high-level resistance to efavirenz or nevirapine was estimated to range from 50 to 95%, resistance to tenofovir from 5 to 56%.[17] However, among individuals failing a dolutegravir-based regimen, NRTI resistance was reported in 2-91% (data available from 4 countries only) and INSTI resistance in 0 cases (data available from Zambia only).[17] Cases of 3-class drug resistance (NRTIs, NNRTIs, and PIs) have been described in Sub-Saharan Africa, with a high prevalence (>20%) in the setting of virological failure to a second-line PI-containing regimen.[18-20] Furthermore, resistance to INSTIs has been documented in a high percentage of individuals failing INSTI-containing regimens,[21] suggesting that 4-class drug resistance might be increasing in low-income countries.

The PRESTIGIO Registry is an Italian open multicenter cohort comprising routinely collected data and samples in 4DR-PLWH; the cohort is a valuable tool for studying individual characteristics, including clinical, virological and molecular patterns associated with disease progression. Although 4-class drug resistance might be considered a rare condition in PLWH from high-income countries, further studies are needed to assess the temporal evolution of this phenomenon, improve the management of 4DR individuals with a multidisciplinary approach, and evaluate the efficacy of the newest ART options. Furthermore, results from the PRESTIGIO Registry could be particularly beneficial for middle- and low-income countries, especially with the widespread use of INSTI-based regimens, both in the first-line setting and in more advanced lines of therapy. Therefore, this paper describes the new opportunities in healthcare research emerging from the PRESTIGIO cohort.

Cohort description

Study design

The PRESTIGIO Registry is an ongoing Italian, observational, prospective, multicenter cohort collecting biological samples and data on clinical, laboratory, treatment, and virological

characteristics of 4DR-PLWH. At first evidence of 4-class drug-resistance (baseline), individuals are informed about the Registry, after which they can either sign a specific written informed consent or opt out. Follow-up accrues from baseline until death, loss to follow-up, or patient's withdrawal of the consent.

Plasma and peripheral blood mononuclear cell samples are collected annually for each person, starting from the date of enrollment, cryopreserved and stored in a biobank (BioRep, www.biorep.it). Clinical, laboratory, treatment, and virological data are annually collected since the date of 4-class drug resistance evidence; some data before the baseline are backlogged retrospectively (figure 1). An online electronic case report form (eCRF) is available and dedicated health staff (within each center) manually inputs the requested information.

Study setting

The PRESTIGIO Registry was established in December 2017; currently, there are 39 participating Infectious Diseases Clinics, located throughout Italy [23 in Northern, 8 in Central, 4 in Southern Italy, and 4 in Sicily and Sardinia (figure 2)].

Study population

The PRESTIGIO cohort includes PLWH who are (1) 18 years or older; (2) harboring a 4DR strain. Four-class drug resistance is defined as intermediate or high-level resistance to at least 1 NRTI, at least 1 NNRTI, at least 1 PI, and at least 1 INSTI, according to the Stanford algorithm (version 9.4.1, hivdb.stanford.edu) and considering cumulative data from all the RNA-based genotypic resistance tests performed for each person. In case of unavailability of any integrase genotype, a documented virological failure (2 consecutive HIV-1 RNA detections ≥50 copies/mL, or 1 detection ≥1000 copies/mL) to an INSTI-containing regimen is accepted as an inclusion criterion.

To date (14th March 2023), 229 PLWH have been included, of whom 189 (82.5%) are currently in active care, 30 (13.1%) have died and 10 (4.4%) have been lost to follow-up (table 1; table 2; figure 3). At the last available visit, the median age was 58.3 years, 27.5% were women at birth, 91.7% Italian, with HIV-1 infection for a median of 29.8 years. About a quarter (24.5%) stated intravenous

drug use as mode of transmission, a quarter (24.5%) were men who have sex with men or bisexual men, and a quarter (23.6%) stated heterosexual mode of transmission. Noteworthy, 9.2% were mother-to-child transmissions. All were on ART (median ART duration 25.8 years), with a >3-drug regimen in 41.5% of cases; INSTIs were contained in 85.2% of the current regimens, PIs in 73.4%, NRTIs in 72.9% and NNRTIs only in 31%. Interestingly, at the last visit, maraviroc was used in 21% of the regimens, fostemsavir in 7.4%, lenacapavir in 3.9%, ibalizumab in 3.1%, and enfuvirtide in 2.6%. Despite 4-class drug-resistance, 51.5% of individuals reached and maintained a stable virological suppression (HIV-1 RNA <50 copies/mL for ≥6 months, without any subsequent failures); at last available visit, HIV-1 RNA was <50 copies/mL in 70.7% of cases, with a median $CD4^+$ T-cell count of 533 cells/μL.

As what concerns virological aspects, at the last visit 163/176 (92.6%) PLWH harbor a subtype B virus, 90/184 (48.9%) a CCR5-tropic strain (table 2); in particular, among these 90 individuals, tropism different from CCR5 had never been detected in 41.1%. According to cumulative data from genotypic resistance testing, the most common resistance-associated mutations for each class were M184V for NRTIs [205/229 (89.5%)], K103N for NNRTIs [121/229 (52.8%)], L90M for PIs [99/229 (43.2%)], and G140S for INSTIs [78/175 (44.6%), of which 74/78 (94.9%) in combination with Q148H] (online supplementary figure 1). Among the 54 PLWH with no available integrase genotype, 75.9% were included in the registry after a virological failure to a raltegravir-containing regimen, 10.5% to a dolutegravir-containing regimen, and 0.6% to an elvitegravir/cobicistat-containing regimen.

Study objectives

The primary objectives of the PRESTIGIO Registry include: (1) the clinical characterization of the population with 4DR HIV at baseline; (2) the quantification of incidence and prevalence of acquired immunodeficiency syndrome (AIDS)- and non-AIDS-defining clinical events; (3) the evaluation of long-term efficacy of different ART regimens; (4) the evaluation of ART adherence and related outcomes; (5) the description of 4DR-PLWH in terms of immunological, virological and

inflammatory parameters; (6) the description of genotypic and phenotypic resistance evolution, especially after new virological failures; (7) the use of new tools to describe the resistance patterns in 4DR-PLWH.

Cohort variables and electronic case report form

Variables at baseline include: (1) socio-demographic data (date of birth, sex at birth, country of birth, ethnicity, employment); (2) lifestyle information (height, weight, smoking habit, alcohol use, recreational drug use); (3) HIV-related clinical characteristics [mode of HIV transmission, date of last negative HIV test, date of first positive HIV test, date of ART start, date of highly active ART start (defined as a combination of drugs from at least 2 classes), date of 4-class drug resistance, all the available RNA- and DNA-based genotypic resistance tests (including viral tropism and/or subtype characterization), $CD4^+$ lymphocyte nadir with date, HIV-1 RNA before ART start, HIV-1 RNA, $CD4^+$ and $CD8^+$ T-cell count]; (4) hepatitis B virus and hepatitis C virus co-infections; (5) complete blood count and blood chemistry examinations (complete blood cell count, plasma levels of glucose, triglycerides, total/HDL-/LDL-cholesterol, creatinine, transaminases, direct and indirect bilirubin); (6) ongoing antiretrovirals and concomitant drugs (with starting date, dosages, schedules and routes of administration); (7) AIDS defining conditions and non-AIDS-related clinical events (including malignancies of any type, diabetes, arterial hypertension requiring treatment, major adverse cardiovascular events, chronic liver or kidney diseases, osteoporosis and related fractures, neurocognitive disorders, chronic obstructive pulmonary disease, etc.); (8) sexually transmitted infections (STIs).

Data collected during follow-up include: (1) an update of hepatitis co-infections; (2) further RNA- and DNA-based genotypic resistance tests, HIV-1 RNA, $CD4^+$ and $CD8^+$ T-cell count determinations; (3) new complete blood chemistry; (4) modifications in ART or concomitant therapies (including also date and cause of discontinuation for each drug); (5) vaccinations; (6) incident AIDS- and non-AIDS-related clinical events with or without hospitalization; (7) incident STIs (online supplemental table 1).

Standard procedures to insert data into an eCRF (trials-ice2.advicepharma.com/PRESTIGIO/) have been defined and the staff dedicated to record information have been trained with specific data management training courses.

Participant data are entered after creating a unique pseudonym for each participant; the pseudonym is a progressive eight-digit code [center code (3 digits), dash and individual's code (4 digits)]. All the collected data are managed anonymously.

The eCRF access is regulated by a permission-based security methodology that limits access to study data based on the user ID. Permissions are carefully maintained to allow only the required level of access to study data. User IDs are required to change password on a regular basis. All eCRF data and other critical study data are fully audit trail enabled, so that all changes to the data can be monitored and/or recovered, and secured via a decentralized daily backup. Every precaution has been taken to ensure that computer confidentiality is maintained.

Patient and public involvement

Until November 2022, a representative from the community of PLWH was co-opted to the Steering Committee, together with representatives from the main (those with at least 10 4DR-PLWH) participating centers of the PRESTIGIO Registry, and was involved in the approval of all the proposed studies. The inclusion of a new member of the community in the Steering committee has been foreseen for the next months.

Findings from the past 6 years to present

Studies on clinical and immunological characteristics of 4DR-PLWH. Considering the high risk of death and comorbidities in individuals with three-class drug-resistant HIV,[12,22] Galli *et al.* performed a retrospective cohort study on 148 4DR-PLWH from the PRESTIGIO Registry followed for a median follow-up of 47 months, to assess the incidence of clinical events and death in this fragile population.[14] This investigation showed a high burden of disease in 4DR population, with a markedly

increased incidence of AIDS- (2.65/100 person-years-of-follow-up) and non-AIDS-related clinical events (4.71/100 person-years-of-follow-up) and death for any cause (1.76/100 person-years-of-follow-up); further studies to better characterize the burden of disease in the PRESTIGIO cohort are ongoing. For example, we recently used retrospective data from the Registry to evaluate the occurrence of sexually transmitted infections (STIs) in 178 4DR-PLWH and found a non-negligible incidence of bacterial infections (1.3/100 person-years-of-follow-up in men, 0 in women), together with the occurrence of viral infections (first diagnosis of genital Herpes Simplex Virus in 3.8% of men and 2.2% of women, first diagnosis of Human Papilloma Virus in 8.3% of men and 6.5% of women).[23] Although all bacterial sexually transmitted infections were diagnosed when HIV-1 RNA was <200 copies/mL, these findings highlight the need for strict HIV viremia monitoring, accurate ART adherence and STI prevention counseling in the population with 4DR HIV.

In light of the known association of inflammatory biomarkers with morbidity and mortality in the general population with HIV,[24-31] we performed a cross-sectional study to evaluate inflammation, immune activation, microbial translocation, and T-cell exhaustion in 4DR-PLWH.[32] Comparing age-, sex-, and smoking habit-matched 30 viremic 4DR individuals from the PRESTIGIO Registry, 30 non-viremic 4DR-PLWH from the PRESTIGIO Registry, and 20 non-viremic non-4DR subjects (from the MODAt study)[33], we found that a higher inflammatory burden was associated with HIV multidrug resistance, viremia and a previous cancer diagnosis. Furthermore, T cells were more activated and 'exhausted' in viremic than non-viremic 4DR individuals. These findings stress the need for further investigations to better characterize immune dysregulation in the 4DR fragile population and to evaluate new therapeutic approaches with an impact on inflammation, and potentially morbidity and mortality.

Virological studies on 4DR strains. Biological samples collected in PRESTIGIO were used to evaluate the residual susceptibility of 4DR viral strains to some already approved or investigational antiretroviral drugs. Particularly, Santoro *et al* analyzed 22 samples from 17 4DR-PLWH who previously failed twice-daily raltegravir-based or twice-daily dolutegravir-based regimens, obtaining

genotypic and phenotypic data, which confirmed that bictegravir and dolutegravir retain activity against most isolates derived from this fragile HTE population.[34] Similarly, Saladini *et al.* evaluated the phenotypic susceptibility to NNRTIs from 22 viremic 4DR-PLWH from the PRESTIGIO Registry: doravirine appeared to be a valid option for some 4DR-PLWH and its activity seemed to be inferred with fair accuracy by the Stanford HIVdb algorithm.[35] As what concerns molecules with new mechanisms of action, Saladini *et al.* investigated the genotypic and phenotypic susceptibility to temsavir in a panel of samples collected from 24 4DR individuals (79% previously exposed to maraviroc or enfuvirtide): temsavir resistance-associated mutations were detected only in three cases and there was no impact of viral tropism and/or exposure to other entry inhibitors on fostemsavir susceptibility.[36] Analogously, Rusconi *et al.* analyzed samples from 24 4DR-PLWH, showing that only 33% harbored a phenotypically R5-tropic virus, but in these cases leronlimab maintained a full activity despite the presence of extensive drug resistance and heavy treatment experience.[37] In addition, leronlimab susceptibility did not appear to be significantly altered by previous or current exposure to maraviroc. Similar studies can be performed for other recently approved or investigational antiretroviral drugs.

Finally, the role of next-generation sequencing (NGS) in genotypic drug resistance testing has been explored. Armenia *et al.* used NGS to evaluate HIV-DNA and HIV-RNA mutational load of drug resistance and APOBEC-related mutations in 20 virologically failing individuals enrolled in the PRESTIGIO Registry.[38] The study concluded that using NGS in HIV-DNA and HIV-RNA together with measurement of APOBEC editing might help to identify HTE individuals with multidrug resistance who are more prone to experience virological failure. Furthermore, in a recently published case of an HTE woman from the PRESTIGIO Registry, RNA-based NGS, performed at virological failure, was used to exclude the presence of minority resistance-associated mutations and, together with available prior genotypic resistance tests, clinical history, and adherence issues, to select an effective antiretroviral regimen with a low pill burden.[39] In light of this evidence, the PRESTIGIO Study Group is performing further studies on the use of NGS in clinical practice.

Studies on treatment strategies for 4DR-PLWH. Given the need for optimizing ART in HTE individuals, Castagna *et al.* used the data from the PRESTIGIO Registry to perform a cohort study evaluating the virological efficacy of dolutegravir 50 mg *bis in die* in 190 virologically failing PLWH with previous exposure to first-generation INSTIs.[40] The estimated probability of virological failure was limited (17%, 33%, and 52% at 12, 36, and 60 months since baseline, respectively), highlighting a favorable long-term efficacy of dolutegravir 50 mg twice daily associated with an optimized background regimen in HTE failing subjects, with an INSTI-resistant virus.

Retrospective data from the Registry also showed that a small group of 10 4DR-PLWH who underwent ART simplification with a high genetic barrier 2-drug regimen (containing darunavir/ritonavir *bis in die* and/or dolutegravir 50 mg *bis in die*), for any reason, was able to maintain a long-term suppression in 90% of cases.[41] These findings suggest that a high genetic barrier 2-drug regimen might represent an effective option in carefully selected PLWH with four-class drug resistance who need simplification; larger studies on the possibility of simplifying complex regimen in this fragile population are ongoing in the PRESTIGIO cohort.

Collaborations

Any HIV care provider from a center participating in the PRESTIGIO Registry may propose a project to the Steering Committee. After approval of the proposal, anonymized data and/or samples from the Registry will be made available to the study proponent, together with the support of a Statistical and Monitoring Team.

The PRESTIGIO Registry is also available to collaborate with national and international cohorts or centers with high expertise in specific fields (e.g., virology, immunology, reservoir quantification, molecular biology, etc.). In particular, given the limited prevalence of HIV 4-class drug resistance in high-income countries, collaboration with other cohorts of individuals with multidrug-resistant viral strains may help to characterize this population better and to draw more easily generalizable conclusions. In addition, for many clinical, immunological and virological studies, a control group of

PLWH without 4-class drug resistance but with long HIV infection and ART duration could be useful, to estimate the effect of multidrug resistance. Finally, in addition to clinical and treatment characterization, highly specialized studies may be useful not only to better understand the mechanisms underlying this population's frailty but also to offer innovative and targeted treatment approaches.

Future plans

The PRESTIGIO Registry provides a unique asset to study long-term treatment, comorbidities, and drug resistance patterns in people with multidrug-resistant HIV in Italy. On one side, 29% of 4DR-PLWH still have unsuppressed viral load, requiring new antiretroviral treatment options. On the other hand, non-viremic individuals with complex ART regimens require safe and robust simplification strategies to minimize toxicity and maximize adherence. Furthermore, attempts are made to conduct virological studies on drug resistance mechanisms and the possibility of using new tools to predict treatment success. Finally, with our aging fragile cohort, studies on comorbidities and their underlying molecular mechanisms become more important, especially to better characterize the disease burden in this population and assess the role of long-term ART exposure and past or current uncontrolled viral replication. In this sense, also study of quality-of-life in these fragile individuals could be useful to their correct management.

Strengths and limitations

The main strength of the PRESTIGIO Registry is its unicity: to our knowledge, it is the first cohort that specifically includes 4DR-PLWH. Furthermore, the registry is already available for research projects: clinical centers enrolling 4DR individuals have been linked, patient pathways have been identified, and data have been centralized to enable convenient querying. Accuracy, completeness, and consistency of data from the date of the first evidence of 4-class drug resistance are high, especially for genotypic resistance tests, virological and immunological information, antiretroviral

treatment and clinical events. Also, the availability of cryopreserved PBMC and plasma samples collected annually allows to conduct virological and immunological studies to better characterize this population. The multicenter design overcomes the limitations of single center studies on this small population, even though the number of individuals included in the cohort remains limited, due to the rarity of HIV 4-class drug resistance in high-income countries. Some variables are sparsely available in the PRESTGIO Registry, which currently limits their use for research purposes, but efforts to retrieve this information or obtain it through laboratory analysis (e.g. tropism and subtype) are under way. Another limitation is the lack of data before the first evidence of 4DR (except for genotypes), although extra information from a limited number of individuals can be easily added by the enrolling centers for specific studies.

Footnotes

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AnCa reports personal fees for advisory boards, speaker panels and educational materials from Gilead Sciences, ViiV Healthcare, Janssen-Cilag, Merck Sharp & Dohme, and Theratechnlogies. All other authors: no potential conflicts.

Patient and public involvement: Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Cohort description section for further details.

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Data availability statement

Data can be made available upon reasonable request.

Ethics statements

Patient consent for publication

Not applicable.

Ethics approval

The PRESTIGIO Registry was approved by the Ethic Committee of the coordinating center (IRCCS San Raffaele Scientific Institute, protocol number 41/int/December 2017) and by Ethic Committees of the participating Centers.

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Table 1. Demographic, socio-economic and lifestyle description of the PRESTIGIO Registry at last available visit (freezing date: 14th March 2023)

Characteristics	**Overall (n=229)**
Age (years)	58.3 (53.6 – 61.6)
Gender at birth	
Female	63 (27.5%)
Male	166 (72.5%)
Birth region	
Italy	210 (91.7%)
Europe except Italy	8 (3.5%)
Africa	8 (3.4%)
South America	2 (0.9%)
Asia	1 (0.4%)
Ethnicity	
Caucasian	218 (95.2%)
Black	9 (3.9%)
Hispanic	2 (0.9%)
Smoking habit	
Yes	92 (40.2%)
No	58 (25.3%)
Previous	38 (16.6%)

	Unknown	41 (17.9%)
Reason for not being in active care		
	Dead	30 (13.1%)
	Emigrated/Lost to follow-up	10 (4.4%)

Data reported as frequency (percentage) or median (interquartile range), as appropriate.

Table 2. Virological, immunological and treatment description of the PRESTIGIO Registry at the last available visit (freezing date: 14th March 2023)

Characteristics	Overall (n=229)
Mode of HIV transmission	
Heterosexual	54 (23.6%)
Men who have sex with men/Bisexual	56 (24.5%)
Intravenous drug use	56 (24.5%)
Mother to child	21 (9.2%)
Unknown/Other	42 (18.3%)
Years since HIV diagnosis	29.8 (25.3 – 33.7)
Years since evidence of 4-class drug resistance	7.7 (4.8 - 10.2)
HIV-1 RNA (copies/mL)	
<50	162 (70.7%)
50 – 199	28 (12.2%)
200 – 999	14 (6.1%)
≥1000	25 (10.9%)
HIV-1 RNA <50 copies/mL for ≥6 months without subsequent virological failure	118 (51.5%)
$CD4^+$ T-cell count (cells/μL)	533 (330.5 – 794)
$CD4^+$ T-cell count (cells/μL)	
<200	33 (14.4%)
200 – 349	31 (13.5%)

	350 – 499	43 (18.8%)
	≥500	122 (53.3%)
$CD8^+$ T-cell count (cells/μL)		892.5 (664.5 - 1260)
$CD4^+/CD8^+$ ratio		0.63 (0.33 - 0.90)
$CD4^+$ nadir (cells/μL)		82 (17 - 183)
HIV-1 subtype		
	B	163 (71.2%)
	C	1 (0.4%)
	F	4 (1.7%)
	G	1 (0.4%)
	Circulating recombinant forms	7 (3.1%)
	Missing	53 (23.1%)
Tropism		
	CCR5-tropic	90 (39.3%)
	CXCR4-tropic or dual mixed	94 (41.0%)
	Missing	45 (19.7%)
CCR-5 tropism detected in all tests performed		37 (16.2%)
On ART		229 (100%)
ART duration (years)		25.8 (21.9 – 28.8)
Calendar year of ART start		

	<1998	148 (64.6%)
	≥1998	81 (35.4%)
Number of antiretrovirals in the current regimen		
	≤3	134 (58.5%)
	4 - 5	89 (38.9%)
	6 - 7	6 (2.6%)
NRTI-containing regimens		167 (72.9%)
NNRTI-containing regimens		71 (31%)
PI-containing regimens		168 (73.4%)
INSTI-containing regimens		195 (85.2%)
Maraviroc-containing regimens		48 (21%)
Enfuvirtide-containing regimens		6 (2.6%)
Fostemsavir-containing regimens		17 (7.4%)
Ibalizumab-containing regimens		7 (3.1%)
Lenacapavir-containing regimens		9 (3.9%)

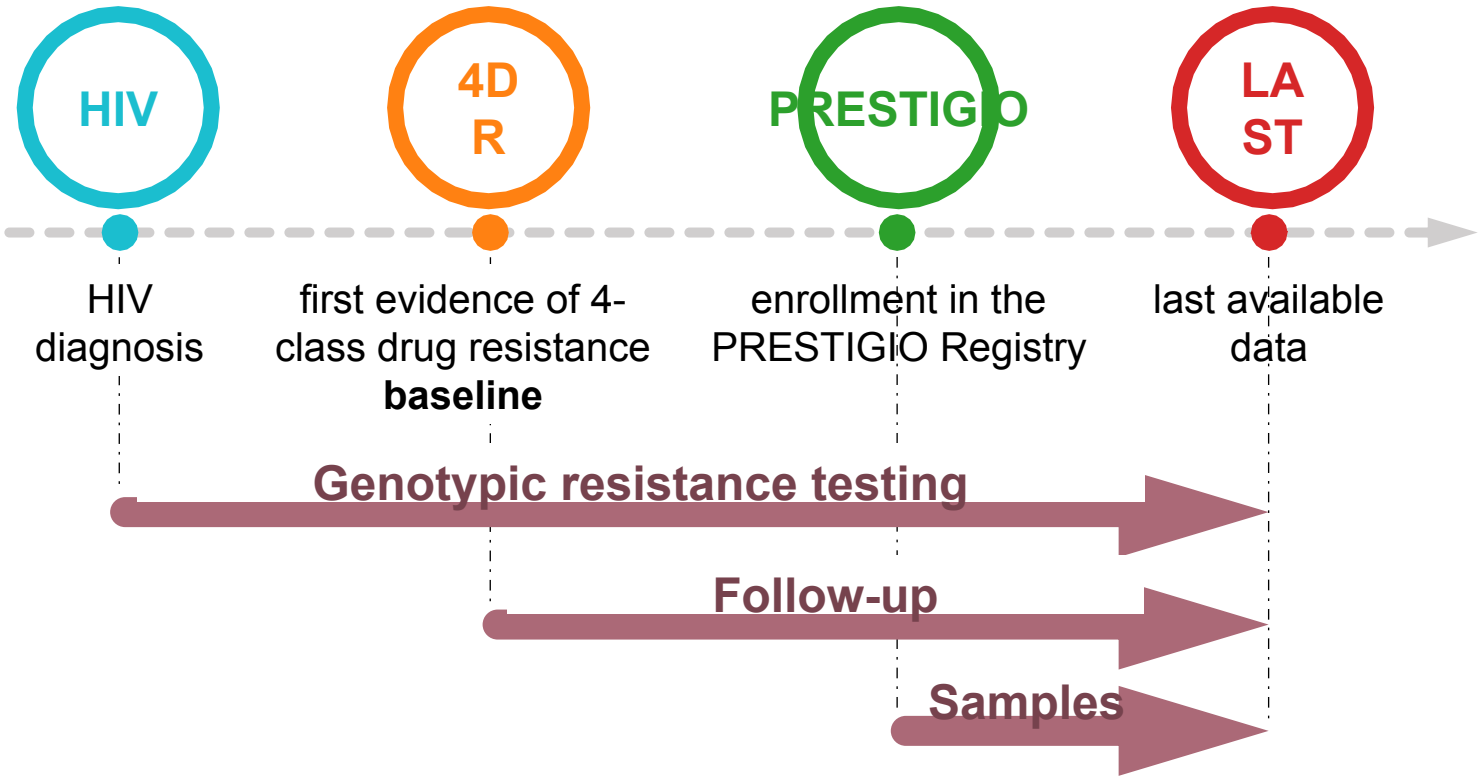
Data reported as frequency (percentage) or median (interquartile range), as appropriate. ART: antiretroviral therapy; HIV: human immunodeficiency virus; INSTI: integrase strand transfer inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.

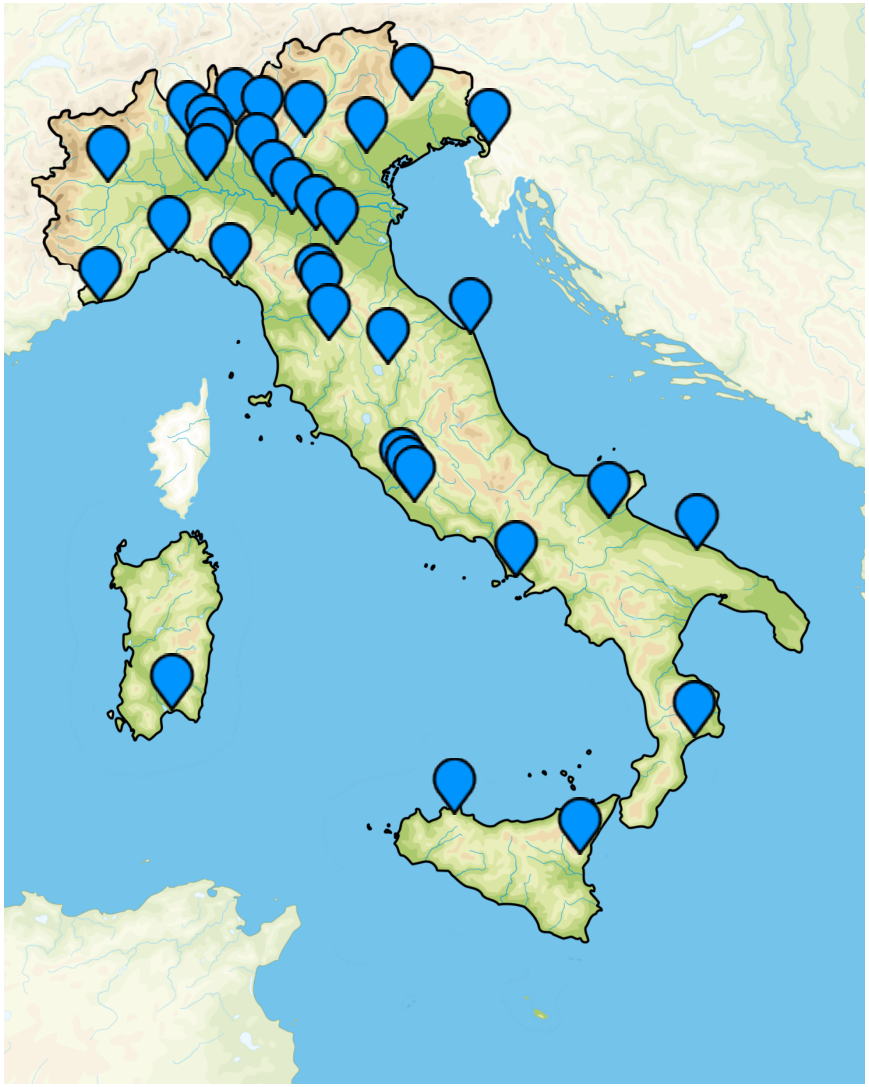
Figure captions.

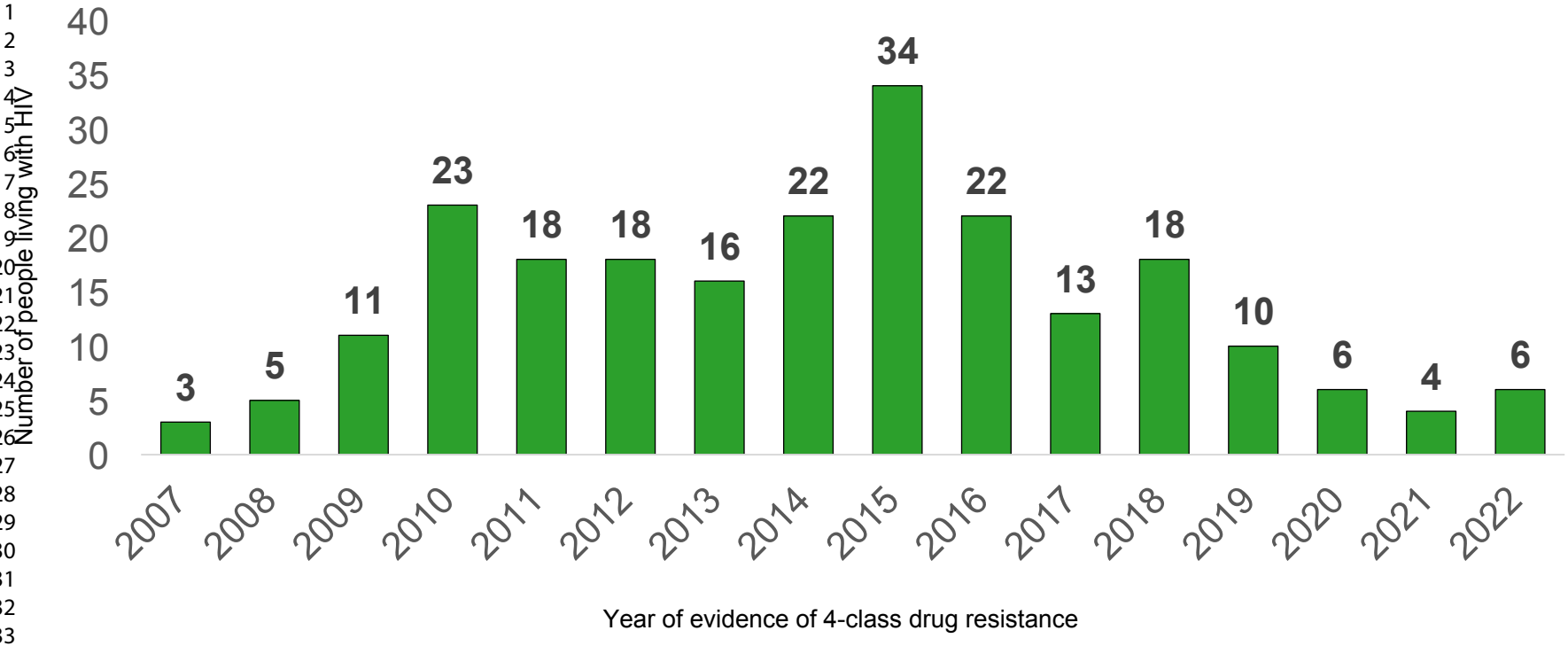
Figure 1. PRESTIGIO timeline. Genotypic resistance tests are collected since the first available from HIV diagnosis. Follow-up for clinical, laboratory, treatment, and virological data accrues from the first evidence of 4-class drug resistance (baseline). Plasma and cell sample collection on an annual basis starts at the date of enrollment in the registry.

Figure 2. The 39 Infectious Diseases Clinics participating in the PRESTIGIO Registry, located throughout Italy.

Figure 3. Year of evidence of 4-class drug resistance.


HIV
4D
R
PRESTIGIO
LA
ST
HIV diagnosis
first evidence of 4-class drug resistance
baseline
enrollment in the PRESTIGIO Registry
last available data
Genotypic resistance testing
Follow-up
Samples




Number of people living with HIV
40
35
30
25
20
15
10
5
0
3
5
11
23
18
18
16
22
34
22
13
18
10
6
4
6
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
Year of evidence of 4-class drug resistance

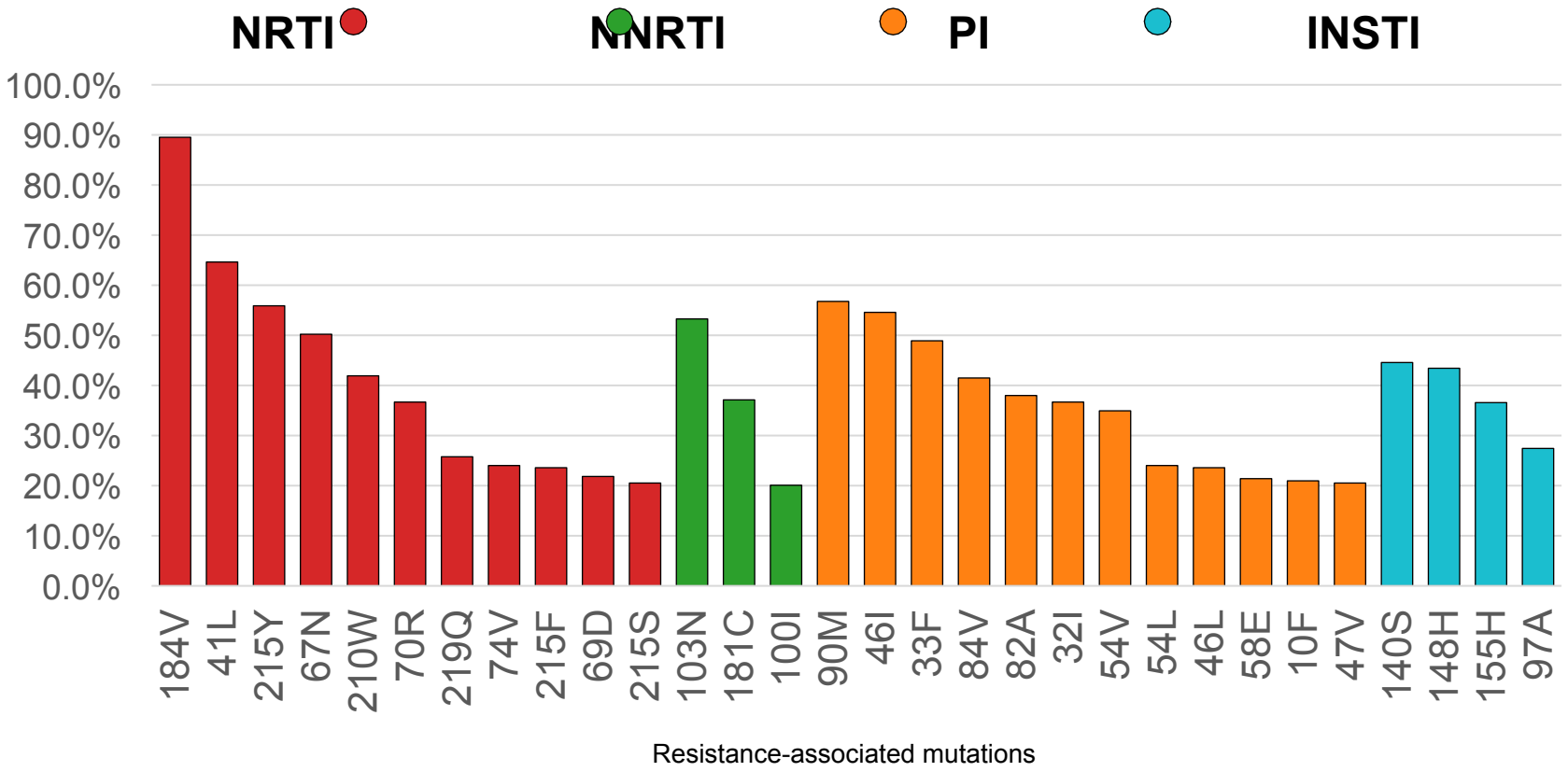
Online supplemental table 1. Summary of variables included in the PRESTIGIO Registry from the date of evidence of 4-class drug resistance.

Socio-demographic data	
General	date of birth, sex at birth, country of birth, ethnicity
Lifestyle	height, weight, smoking habit
HIV-specific	mode of HIV transmission, date of last negative HIV test, date of first positive HIV test, date of 4-class drug resistance
Laboratory results	
HIV-specific	HIV-1 RNA (copies/mL), HIV-1 RNA before ART start (copies/mL) $CD4^+$ T-cell count (cells/µL, %), $CD8^+$ T-cell count (cells/µL, %), $CD4^+/CD8^+$ ratio, $CD4^+$ nadir (cells/µL) RNA- and DNA-based genotypic resistance testing (*collected since the first test available, also before evidence of 4-class drug resistance*) subtype, viral tropism (*collected since the first available, also before evidence of 4-class drug resistance*)
Other	hepatitis B and C serologies, HBV DNA, HCV RNA complete blood cell count plasma glucose, triglycerides, total/HDL-/LDL-cholesterol, creatinine, transaminases, direct and indirect bilirubin
Treatment	
Antiretroviral therapy	date of antiretroviral therapy start, date of highly active antiretroviral therapy (defined as a combination of drugs from at least 2 different classes) start

	regimens from baseline to the last available visit (including start and stop dates, dose and mode of administration, reason for switch)
Other medications	specific drugs (including start and stop dates, dose and mode of administration, reason for stop)
Vaccines	date and type of vaccinations
AIDS diagnoses	date and type of AIDS events
Comorbidities	non-AIDS malignancies, diabetes, arterial hypertension requiring treatment, major adverse cardiovascular events, chronic liver or kidney diseases, osteoporosis and related fractures, neurocognitive disorders, chronic obstructive pulmonary disease, etc. (including dates of diagnosis and eventually resolution)
Hospitalizations	dates and reason for hospitalization
Pregnancies	dates of pregnancy, date of delivery, etc.
Sexually transmitted infections	date of diagnosis, type, and treatment of sexually transmitted infections
Death	date and reason of death

Online supplemental figure captions.

Online supplemental figure 1. Resistance-associated mutations present in ≥20% of 4DR-PLWH from the PRESTIGIO Registry. All RNA-based genotypic resistance tests through Sanger sequencing were considered cumulatively. Percentages evaluated on 229 PLWH for NRTI, NNRTI, and PI resistance-associated mutations, on 175 PLWH with at least one available integrase genotype for INSTI resistance-associated mutations. INSTI: integrase strand transfer inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; 4DR-PLWH: people living with 4-class drug-resistant HIV.


NRTI
NNRTI
PI
INSTI
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Percentage of 4DR-PLWH
184V
41L
215Y
67N
210W
70R
219Q
74V
215F
69D
215S
103N
181C
100I
90M
46I
33F
84V
82A
32I
54V
54L
46L
58E
10F
47V
140S
148H
155H
97A
Resistance-associated mutations

BMJ Open

Cohort profile: PRESTIGIO, an Italian prospective registry-based cohort of people with HIV-1 resistant to reverse transcriptase, protease, and integrase inhibitors

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Cohort profile: PRESTIGIO, an Italian prospective registry-based cohort of people with HIV-1 resistant to reverse transcriptase, protease, and integrase inhibitors

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Abstract

Purpose: The PRESTIGIO Registry was established in 2017 to collect clinical, virological and immunological monitoring data from people living with 4-class drug resistant human immunodeficiency virus (4DR-PLWH). Key research purposes include the evaluation of residual susceptibility to specific antiretrovirals and validation of treatment and monitoring strategies in this population.

Participants: The PRESTIGIO Registry collects annual plasma and peripheral blood mononuclear cell samples and demographic, clinical, virological, treatment and laboratory data from PLWH followed at 39 Italian clinical centers and characterized by intermediate-to-high genotypic resistance to ≥1 nucleoside reverse transcriptase inhibitors (NRTIs), ≥1 non-nucleoside reverse transcriptase inhibitors (NNRTIs), ≥1 protease inhibitors (PIs), plus either intermediate-to-high genotypic resistance to ≥1 integrase strand transfer inhibitors (INSTIs) or history of virological failure to an INSTI-containing regimen. To date, 229 people have been recorded in the cohort. The most of data are collected from the date of first evidence of 4-class drug resistance (baseline) with some pre-baseline information obtained retrospectively, samples are collected from the date of enrollment in the registry.

Findings to date: The open-ended cohort has been used to assess i) prognosis in terms of survival or development of acquired immunodeficiency syndrome (AIDS) or non-AIDS-related clinical events; ii) long-term efficacy and safety of different antiretroviral regimens; iii) virological and immunological factors predictive of clinical outcome and treatment efficacy, especially through analysis of plasma and cell samples.

Future plans: The registry can provide new knowledge on how to implement an integrated approach to study PLWH with documented resistance to the 4 main antiretroviral classes, a population with a limited number of individuals characterized by a high degree of frailty and complexity in therapeutic management. Given the scheduled annual updates of PLWH data, the researchers who collaborate in the registry can send study proposals at any time to the Steering Committee of the Registry, which

evaluates every 3 months whether the research studies can be conducted on data and biosamples from the Registry and whether they are aimed at a better understanding of: a specific health condition, the emergence of comorbidities, the effect of potential treatments or experimental drugs that may have an impact on disease progression and quality-of-life. Finally, the research studies should aim to be inclusive, innovative, and in touch with the communities and society as a whole.

Registration: The PRESTIGIO Registry is registered on ClinicalTrials.gov (NCT04098315).

Strengths and limitations of this study

- The registry specifically includes people living with human immunodeficiency virus (HIV) and documented 4-class drug resistance.
- The cohort is readily available for research projects: the data sources (clinical centers) have already been linked and data have been collected in order to enable easier and simpler querying across source systems.
- Annual collection of cryopreserved plasma and peripheral blood mononuclear cell samples for research purposes.
- Limited representativeness of subtype non-B viral strains and non-Italian natives with HIV.
- Incomplete information before evidence of 4-class drug resistance.

Introduction

Continuous progress in antiretroviral therapy (ART) has resulted in a high rate of virological suppression and a consequent improvement in life expectancy for individuals with human immunodeficiency virus (HIV). [1-5] Nevertheless, heavily treatment-experienced (HTE) people living with HIV (PLWH) have a history of previous virological failures on different antiretroviral regimens and limited treatment options, because of multidrug resistance and/or drug intolerance. [6,7] ART options can also be limited by drug–drug interactions or drug toxicity, due to the need of both prophylaxis or treatment for opportunistic infections and concomitant therapies for multiple comorbidities, that are common in HTE PLWH, [8,9] especially with aging. [10] This fragile group often needs complex, unusual and asymmetrical regimens (mixture of *quaque die* and *bis in die* schedules or oral, subcutaneous and intravenous administrations) with a high pill burden, possibly leading to a suboptimal adherence, which in turn increases the risk of virological failure with emergent resistance and worsens the prognosis. [11,12]

Even though there is no consensus in defining HTE individuals, these PLWH generally have two or less antiretroviral classes available for use, with limited fully active antiretroviral drugs within each class, [13] and represent a group at increased risk of low adherence and clinical progression. Therefore, establishing cohorts aimed to characterize this population better appears essential.

Among these difficult-to-treat individuals, those who harbor a 4-class drug-resistant (4DR) virus, characterized by resistance to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and integrase strand transfer inhibitors (INSTIs), represent a particular group with a very high burden of disease and very limited therapeutic options. [14] In terms of epidemiology, the prevalence of HIV 4-class drug resistance on a global scale is unknown: a prospective study in North Carolina estimated this prevalence at approximately 1% of PLWH, with a slight increase since 2007. [15] Recent Italian studies quantified subjects with 4DR HIV at about 2% of treatment-experienced PLWH in 2011-2018. [16] According to the most recent World Health Organization (WHO) report on HIV drug resistance

(2014-2020), virological suppression [defined as HIV ribonucleic acid (RNA) <1000 copies/mL] was ≥90% among adults on ART for ≥48 months in only 6/14 middle- and low-income countries with available data; in virologically non-suppressed PLWH on a NNRTI-based regimen for ≥48 months, high-level resistance to efavirenz or nevirapine was estimated to range from 50 to 95%, resistance to tenofovir from 5 to 56%. [17] However, among individuals failing a dolutegravir-based regimen, NRTI resistance was reported in 2-91% (data available from 4 countries only) and INSTI resistance in 0 cases (data available from Zambia only). [17] Cases of 3-class drug resistance (NRTIs, NNRTIs, and PIs) have been described in Sub-Saharan Africa, with a high prevalence (>20%) in the setting of virological failure to a second-line PI-containing regimen. [18-20] Furthermore, resistance to INSTIs has been documented in a high percentage of individuals failing INSTI-containing regimens, [21] suggesting that 4-class drug resistance might be increasing in low-income countries.

The PRESTIGIO Registry is an Italian open multicenter cohort comprising routinely collected data and samples in 4DR-PLWH; the cohort is a valuable tool for studying individual characteristics, including clinical, virological and molecular patterns associated with disease progression. Although 4-class drug resistance might be considered a rare condition in PLWH from high-income countries, further studies are needed to assess the temporal evolution of this phenomenon, improve the management of 4DR individuals with a multidisciplinary approach, and evaluate the efficacy of the newest ART options. Furthermore, results from the PRESTIGIO Registry could be particularly beneficial for middle- and low-income countries, especially with the widespread use of INSTI-based regimens, both in the first-line setting and in more advanced lines of therapy. Therefore, this paper describes the new opportunities in healthcare research emerging from the PRESTIGIO Registry.

Cohort description

Study design

The PRESTIGIO Registry is an ongoing Italian, observational, prospective, open-ended, multicenter cohort collecting biological samples and data on clinical, laboratory, treatment, and virological characteristics of 4DR-PLWH.

The recruitment period started in December 2017 and is still ongoing. From this date, individuals with 4-class drug resistance are informed about the Registry, after which they can either sign a written informed consent or opt out. Follow-up accrues from the first evidence of 4-class drug resistance (baseline) until death, loss to follow-up, or patient's withdrawal of the consent.

Plasma and peripheral blood mononuclear cell samples are collected annually for each person, starting from the date of enrollment, cryopreserved and stored in a biobank (BioRep, www.biorep.it). Clinical, laboratory, treatment, and virological data are annually collected (by the end of January) since the enrollment; data between the first evidence of 4-class drug resistance and the date of enrollment and some pre-baseline data are backlogged retrospectively (figure 1). An online electronic case report form (eCRF) is available and dedicated health staff (within each center) manually inputs the requested information.

Registry setting

The PRESTIGIO Registry was established in December 2017; currently, there are 39 participating Infectious Diseases Clinics, located throughout Italy [23 in Northern, 8 in Central, 4 in Southern Italy, and 4 in Sicily and Sardinia (figure 2)].

The PRESTIGIO Registry is coordinated by the Principal Investigator and a Steering Committee designed to direct and supervise the research activities. The Steering Committee is composed of experts in different areas of infectious diseases.

The Principal Investigator is responsible for coordinating all the activities of the Registry within the coordinating and the participating centers. The Steering Committee of the Registry is responsible for: i) overseeing the monitoring and data quality control procedures; ii) promoting inclusion into the Registry; iii) further developing the Registry study protocol (e.g., by considering additional components); iv) making decisions on the collected biosamples in future follow-up visits.

Study population

The PRESTIGIO Registry includes PLWH who are (1) 18 years or older; (2) harboring a 4DR strain. Four-class drug resistance is defined as intermediate or high-level resistance to at least 1 NRTI, at least 1 NNRTI, at least 1 PI, and at least 1 INSTI, according to the Stanford algorithm (version 9.4.1, hivdb.stanford.edu) and considering cumulative data from all the RNA-based genotypic resistance tests performed for each person. In case of unavailability of any integrase genotype, a documented virological failure (2 consecutive HIV-1 RNA detections ≥50 copies/mL, or 1 detection ≥1000 copies/mL) to an INSTI-containing regimen is accepted as an inclusion criterion.

To date (14th March 2023), 229 PLWH have been included, of whom 189 (82.5%) are currently in active care, 30 (13.1%) have died and 10 (4.4%) have been lost to follow-up (table 1; table 2; figure 3). At the last available visit, after a median follow-up of 7.7 years, the median age was 58.3 years, 27.5% were women at birth, 91.7% Italian, with HIV-1 infection for a median of 29.8 years. About a quarter (24.5%) stated intravenous drug use as mode of transmission, a quarter (24.5%) were men who have sex with men or bisexual men, and a quarter (23.6%) stated heterosexual mode of transmission. Noteworthy, 9.2% were mother-to-child transmissions. All were on ART (median ART duration 25.8 years), with a >3-drug regimen in 41.5% of cases; INSTIs were contained in 85.2% of the current regimens, PIs in 73.4%, NRTIs in 72.9% and NNRTIs only in 31%. Interestingly, at the last visit, maraviroc was used in 21% of the regimens, fostemsavir in 7.4%, lenacapavir in 3.9%, ibalizumab in 3.1%, and enfuvirtide in 2.6%. Despite 4-class drug resistance, 51.5% of individuals reached and maintained a stable virological suppression (HIV-1 RNA <50 copies/mL for ≥6 months, without any subsequent failures); at last available visit, HIV-1 RNA was <50 copies/mL in 70.7% of cases, with a median $CD4^+$ T-cell count of 533 cells/μL.

As what concerns virological aspects, at the last visit 163/176 (92.6%) PLWH harbor a subtype B virus, 90/184 (48.9%) a CCR5-tropic strain (table 2); in particular, among these 90 individuals, tropism different from CCR5 had never been detected in 41.1%. According to cumulative data from genotypic resistance testing (RNA-based, Sanger sequencing method), the most common resistance-

associated mutations for each class were M184V for NRTIs [205/229 (89.5%)], K103N for NNRTIs [121/229 (52.8%)], L90M for PIs [99/229 (43.2%)], and G140S for INSTIs [78/175 (44.6%), of which 74/78 (94.9%) in combination with Q148H] (online supplementary figure 1). Fifty-four PLWH did not have an available integrase genotype at the time of virological failure to an INSTI-containing regimen, because the method was not available at the centers where theses individuals were in care; among them, 75.9% were included in the registry after a virological failure to a raltegravir-containing regimen, 10.5% to a dolutegravir-containing regimen, and 0.6% to an elvitegravir/cobicistat-containing regimen.

Study objectives

The primary objectives of the PRESTIGIO Registry include: (1) the clinical characterization of the population with 4DR HIV at baseline; (2) the quantification of incidence and prevalence of acquired immunodeficiency syndrome (AIDS)- and non-AIDS-defining clinical events; (3) the evaluation of long-term efficacy of different ART regimens; (4) the evaluation of ART adherence and related outcomes; (5) the description of 4DR-PLWH in terms of immunological, virological and inflammatory parameters; (6) the description of genotypic and phenotypic resistance evolution, especially after new virological failures; (7) the use of new tools to describe the resistance patterns in 4DR-PLWH.

Cohort variables and electronic case report form

Variables at baseline include: (1) socio-demographic data (date of birth, sex at birth, country of birth, ethnicity, employment); (2) lifestyle information (height, weight, smoking habit, alcohol use, recreational drug use); (3) HIV-related clinical characteristics [mode of HIV transmission, date of last negative HIV test, date of first positive HIV test, date of ART start, date of highly active ART start (defined as a combination of drugs from at least 2 classes), date of 4-class drug resistance, all the available RNA- and deoxyribonucleic acid (DNA)-based genotypic resistance tests (considering both Sanger sequencing and next-generation sequencing methods, and including data on capsid, envelope, viral tropism, and/or subtype characterization), $CD4^+$ lymphocyte nadir with date, HIV-1

RNA before ART start, HIV-1 RNA, $CD4^+$ and $CD8^+$ T-cell count]; (4) hepatitis B virus and hepatitis C virus co-infections; (5) complete blood count and blood chemistry examinations [complete blood cell count, plasma levels of glucose, triglycerides, total/high-density lipoprotein (HDL)-/low-density lipoprotein (LDL)-cholesterol, creatinine, transaminases, direct and indirect bilirubin]; (6) ongoing antiretrovirals and concomitant drugs (with starting date, dosages, schedules and routes of administration); (7) AIDS defining conditions and non-AIDS-related clinical events (including malignancies of any type, diabetes, arterial hypertension requiring treatment, major adverse cardiovascular events, chronic liver or kidney diseases, osteoporosis and related fractures, neurocognitive disorders, chronic obstructive pulmonary disease, etc.); (8) sexually transmitted infections (STIs).

Data collected during follow-up include: (1) an update of hepatitis co-infections; (2) further RNA- and DNA-based genotypic resistance tests, HIV-1 RNA, $CD4^+$ and $CD8^+$ T-cell count determinations; (3) new complete blood chemistry; (4) modifications in ART or concomitant therapies (including also date and cause of discontinuation for each drug); (5) vaccinations; (6) incident AIDS- and non-AIDS-related clinical events with or without hospitalization; (7) incident STIs (online supplemental table 1).

Standard procedures to insert data into an eCRF (trials-ice2.advicepharma.com/PRESTIGIO/) have been defined and the staff dedicated to record information have been trained with specific data management training courses.

Participant data are entered after creating a unique pseudonym for each participant; the pseudonym is a progressive eight-digit code [center code (3 digits), dash and individual's code (4 digits)]. All the collected data are managed anonymously.

The eCRF access is regulated by a permission-based security methodology that limits access to study data based on the user ID. Permissions are carefully maintained to allow only the required level of access to study data. User IDs are required to change password on a regular basis. All eCRF data and other critical study data are fully audit trail enabled, so that all changes to the data can be monitored

and/or recovered, and secured via a decentralized daily backup. Every precaution has been taken to ensure that computer confidentiality is maintained.

All data entered into the eCRF are controlled and verified at multiple levels: the eCRF has several automated control mechanisms (for example, the time difference between a visit and the date of tests associated with that visit cannot be >6 months); a Statistical and Monitoring Team verifies the appropriateness and completeness of the provided information and the data manager of the registry may request clarifications to the staff responsible for recording information of each center through queries on the eCRF platform or personal contact; in case of doubt about the validity or completeness of the recorded virological information, a Virology Team is also involved and queries are made through the eCRF platform.

To date, data monitoring has been performed online, but the PRESTIGIO Registry has planned to undertake annual on-site monitoring to check source documents and data entry since 2024.

Patient and public involvement

Until November 2022, a representative from the community of PLWH was co-opted to the Steering Committee, together with representatives from the main (those with at least 10 4DR-PLWH) participating centers of the PRESTIGIO Registry, and was involved in the approval of all the proposed studies. The inclusion of a new member of the community in the Steering committee has been foreseen for the next months.

Findings from the past 6 years to present

Studies on clinical and immunological characteristics of 4DR-PLWH. Considering the high risk of death and comorbidities in individuals with three-class drug-resistant HIV, [12,22] Galli *et al.* performed a retrospective cohort study on 148 4DR-PLWH from the PRESTIGIO Registry followed for a median follow-up of 47 months, to assess the incidence of clinical events and death in this fragile population. [14] This investigation showed a high burden of disease in 4DR population, with a markedly increased incidence of AIDS- (2.65/100 person-years-of-follow-up) and non-AIDS-related

clinical events (4.71/100 person-years-of-follow-up) and death for any cause (1.76/100 person-years-of-follow-up); further studies to better characterize the burden of disease in the PRESTIGIO cohort are ongoing. For example, we recently used retrospective data from the Registry to evaluate the occurrence of sexually transmitted infections (STIs) in 178 4DR-PLWH and found a non-negligible incidence of bacterial infections (1.3/100 person-years-of-follow-up in men, 0 in women), together with the occurrence of viral infections (first diagnosis of genital Herpes Simplex Virus in 3.8% of men and 2.2% of women, first diagnosis of Human Papilloma Virus in 8.3% of men and 6.5% of women). [23] Although all bacterial sexually transmitted infections were diagnosed when HIV-1 RNA was <200 copies/mL, these findings highlight the need for strict HIV viremia monitoring, accurate ART adherence and STI prevention counseling in the population with 4DR HIV.

In light of the known association of inflammatory biomarkers with morbidity and mortality in the general population with HIV, [24-31] we performed a cross-sectional study to evaluate inflammation, immune activation, microbial translocation, and T-cell exhaustion in 4DR-PLWH. [32] Comparing age-, sex-, and smoking habit-matched 30 viremic 4DR individuals from the PRESTIGIO Registry, 30 non-viremic 4DR-PLWH from the PRESTIGIO Registry, and 20 non-viremic non-4DR subjects (from the MODAt study) [33], we found that a higher inflammatory burden was associated with HIV multidrug resistance, viremia and a previous cancer diagnosis. Furthermore, T cells were more activated and 'exhausted' in viremic than non-viremic 4DR individuals. These findings stress the need for further investigations to better characterize immune dysregulation in the 4DR fragile population and to evaluate new therapeutic approaches with an impact on inflammation, and potentially morbidity and mortality.

Virological studies on 4DR strains. Biological samples collected in PRESTIGIO were used to evaluate the residual susceptibility of 4DR viral strains to some already approved or investigational antiretroviral drugs. Particularly, Santoro *et al* analyzed 22 samples from 17 4DR-PLWH who previously failed twice-daily raltegravir-based or twice-daily dolutegravir-based regimens, obtaining genotypic and phenotypic data, which confirmed that bictegravir and dolutegravir retain activity

against most isolates derived from this fragile HTE population. [34] Similarly, Saladini *et al.* evaluated the phenotypic susceptibility to NNRTIs from 22 viremic 4DR-PLWH from the PRESTIGIO Registry: doravirine appeared to be a valid option for some 4DR-PLWH and its activity seemed to be inferred with fair accuracy by the Stanford HIVdb algorithm. [35] As what concerns molecules with new mechanisms of action, Saladini *et al.* investigated the genotypic and phenotypic susceptibility to temsavir in a panel of samples collected from 24 4DR individuals (79% previously exposed to maraviroc or enfuvirtide): temsavir resistance-associated mutations were detected only in three cases and there was no impact of viral tropism and/or exposure to other entry inhibitors on fostemsavir susceptibility. [36] Analogously, Rusconi *et al.* analyzed samples from 24 4DR-PLWH, showing that only 33% harbored a phenotypically CCR5-tropic virus, but in these cases leronlimab maintained a full activity despite the presence of extensive drug resistance and heavy treatment experience. [37] In addition, leronlimab susceptibility did not appear to be significantly altered by previous or current exposure to maraviroc. Similar studies can be performed for other recently approved or investigational antiretroviral drugs.

Finally, the role of next-generation sequencing (NGS) in genotypic drug resistance testing has been explored. Armenia *et al.* used NGS to evaluate HIV-DNA and HIV-RNA mutational load of drug resistance and apolipoprotein B messenger RNA editing enzyme catalytic polypeptide (APOBEC)-related mutations in 20 virologically failing individuals enrolled in the PRESTIGIO Registry. [38] The study concluded that using NGS in HIV-DNA and HIV-RNA together with measurement of APOBEC editing might help to identify HTE individuals with multidrug resistance who are more prone to experience virological failure. Furthermore, in a recently published case of an HTE woman from the PRESTIGIO Registry, RNA-based NGS, performed at virological failure, was used to exclude the presence of minority resistance-associated mutations and, together with available prior genotypic resistance tests, clinical history, and adherence issues, to select an effective antiretroviral regimen with a low pill burden. [39] In light of this evidence, the PRESTIGIO Study Group is performing further studies on the use of NGS in clinical practice.

Studies on treatment strategies for 4DR-PLWH. Given the need for optimizing ART in HTE individuals, Castagna *et al.* performed a retrospective study evaluating the virological efficacy of dolutegravir 50 mg *bis in die* in 190 virologically failing PLWH with previous exposure to first-generation INSTIs. [40] The estimated probability of virological failure was limited (17%, 33%, and 52% at 12, 36, and 60 months since baseline, respectively), highlighting a favorable long-term efficacy of dolutegravir 50 mg twice daily associated with an optimized background regimen in HTE failing subjects, with an INSTI-resistant virus.

Retrospective data from the PRESTIGIO Registry also showed that a small group of 10 4DR-PLWH who underwent ART simplification with a high genetic barrier 2-drug regimen (containing darunavir/ritonavir *bis in die* and/or dolutegravir 50 mg *bis in die*), for any reason, was able to maintain a long-term suppression in 90% of cases. [41] These findings suggest that a high genetic barrier 2-drug regimen might represent an effective option in carefully selected PLWH with four-class drug resistance who need simplification. Moreover, in a case report on an 80-year-old HTE man from the PRESTIGIO Registry, ART was successfully simplified by reducing drug and pill burden after achievement of virological suppression, thanks to a careful evaluation of cumulative genotypic resistance testing and drug-drug interactions. [42] Larger studies on the possibility of simplifying complex regimens in this fragile population are ongoing in the PRESTIGIO cohort.

Collaborations

Any HIV care provider from a center participating in the PRESTIGIO Registry may propose a project to the Steering Committee. After approval of the proposal, anonymized data and/or samples from the Registry will be made available to the study proponent, together with the support of a Statistical and Monitoring Team.

The PRESTIGIO Registry is also available to collaborate with national and international cohorts or centers with high expertise in specific fields (e.g., virology, immunology, reservoir quantification, molecular biology, etc.). In particular, given the limited prevalence of HIV 4-class drug resistance in

high-income countries, collaboration with other cohorts of individuals with multidrug-resistant viral strains may help to characterize this population better and to draw more easily generalizable conclusions. In addition, for many clinical, immunological and virological studies, a control group of PLWH without 4-class drug resistance but with long HIV infection and ART duration could be useful, to estimate the effect of multidrug resistance. Finally, in addition to clinical and treatment characterization, highly specialized studies may be useful not only to better understand the mechanisms underlying this population's frailty but also to offer innovative and targeted treatment approaches.

Future plans

The PRESTIGIO Registry provides a unique asset to study long-term treatment, comorbidities, and drug resistance patterns in people with multidrug-resistant HIV in Italy.

On one side, 29% of 4DR-PLWH still have unsuppressed viral load, requiring new antiretroviral treatment options. On the other hand, non-viremic individuals with complex ART regimens require safe and robust simplification strategies to minimize toxicity and maximize adherence. Furthermore, attempts are made to conduct virological studies on drug resistance mechanisms and the possibility of using new tools to predict treatment success. Finally, with our aging fragile cohort, studies on comorbidities and their underlying molecular mechanisms become more important, especially to better characterize the disease burden in this population and assess the role of long-term ART exposure and past or current uncontrolled viral replication. In this sense, also study of quality-of-life in these fragile individuals could be useful to their correct management. The studies of the Registry are generally aimed to a better understanding of: a specific health condition, the emergence of comorbidities, the effect of potential treatments or experimental drugs that may have an impact on disease progression. The research studies should be also aimed to be inclusive, innovative and in touch with the communities and society as a whole.

Strengths and limitations

The main strength of the PRESTIGIO Registry is its unicity: to our knowledge, it is the first cohort that specifically includes 4DR-PLWH. Furthermore, the registry is already available for research projects: clinical centers enrolling 4DR individuals have been linked, patient pathways have been identified, and data have been centralized to enable convenient querying. Accuracy, completeness, and consistency of data from the date of the first evidence of 4-class drug resistance are high, especially for genotypic resistance tests, virological and immunological information, antiretroviral treatment and clinical events. Also, the availability of cryopreserved peripheral blood mononuclear cell (PBMC) and plasma samples collected annually allows to conduct virological and immunological studies to better characterize this population. The multicenter design overcomes the limitations of single center studies on this small population, even though the number of individuals included in the cohort remains limited, due to the rarity of HIV 4-class drug resistance in high-income countries. Some variables are sparsely available in the PRESTGIO Registry, which currently limits their use for research purposes, but efforts to retrieve this information or obtain it through laboratory analysis (e.g. tropism and subtype) are under way. Furthermore, the vast majority of the cohort was born in Italy and harbors a subtype B viral strain, presumably due to the long duration of HIV infection: most diagnoses could be dated to a period when Italy was considered a B-restricted area. However, it is possible that non-Italian-born individuals and subtype non-B viruses with 4-class drug resistance could be enrolled in the PRESTIGIO Registry in the coming years, following the trend of new diagnoses. [43] Another limitation is the lack of data before the first evidence of 4DR (except for genotypes), although extra information from a limited number of individuals can be easily added by the enrolling centers for specific studies.

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Data availability statement

Data can be made available upon reasonable request.

Ethics statements

Patient consent for publication

Not applicable.

Ethics approval

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Table 1. Demographic, socio-economic and lifestyle description of the PRESTIGIO Registry at last available visit (freezing date: 14th March 2023)

Characteristics	Overall (n=229)
Age (years)	58.3 (53.6 – 61.6)
Gender at birth	
Female	63 (27.5%)
Male	166 (72.5%)
Birth region	
Italy	210 (91.7%)
Europe except Italy	8 (3.5%)
Africa	8 (3.4%)
South America	2 (0.9%)
Asia	1 (0.4%)
Ethnicity	
Caucasian	218 (95.2%)
Black	9 (3.9%)
Hispanic	2 (0.9%)
Smoking habit	
Yes	92 (40.2%)
No	58 (25.3%)
Previous	38 (16.6%)

	Unknown	41 (17.9%)
Reason for not being in active care		
	Dead	30 (13.1%)
	Emigrated/Lost to follow-up	10 (4.4%)

Data reported as frequency (percentage) or median (interquartile range), as appropriate.

Table 2. Virological, immunological and treatment description of the PRESTIGIO Registry at the last available visit (freezing date: 14th March 2023)

Characteristics	Overall (n=229)
Mode of HIV transmission	
Heterosexual	54 (23.6%)
Men who have sex with men/Bisexual	56 (24.5%)
Intravenous drug use	56 (24.5%)
Mother to child	21 (9.2%)
Unknown/Other	42 (18.3%)
Years since HIV diagnosis	29.8 (25.3 – 33.7)
Years since evidence of 4-class drug resistance (follow-up duration)	7.7 (4.8 - 10.2)
HIV-1 RNA (copies/mL)	
<50	162 (70.7%)
50 – 199	28 (12.2%)
200 – 999	14 (6.1%)
≥1000	25 (10.9%)
HIV-1 RNA <50 copies/mL for ≥6 months without subsequent virological failure	118 (51.5%)
$CD4^+$ T-cell count (cells/μL)	533 (330.5 – 794)
$CD4^+$ T-cell count (cells/μL)	
<200	33 (14.4%)
200 – 349	31 (13.5%)

	350 – 499	43 (18.8%)
	≥500	122 (53.3%)
$CD8^+$ T-cell count (cells/μL)		892.5 (664.5 - 1260)
$CD4^+/CD8^+$ ratio		0.63 (0.33 - 0.90)
$CD4^+$ nadir (cells/μL)		82 (17 - 183)
HIV-1 subtype		
	B	163 (71.2%)
	C	1 (0.4%)
	F	4 (1.7%)
	G	1 (0.4%)
	Circulating recombinant forms	7 (3.1%)
	Missing	53 (23.1%)
Tropism		
	CCR5-tropic	90 (39.3%)
	CXCR4-tropic or dual mixed	94 (41.0%)
	Missing	45 (19.7%)
CCR-5 tropism detected in all tests performed		37 (16.2%)
On ART		229 (100%)
ART duration (years)		25.8 (21.9 – 28.8)
Calendar year of ART start		

	<1998	148 (64.6%)
	≥1998	81 (35.4%)
Number of antiretrovirals in the current regimen		
	≤3	134 (58.5%)
	4 - 5	89 (38.9%)
	6 - 7	6 (2.6%)
NRTI-containing regimens		167 (72.9%)
NNRTI-containing regimens		71 (31%)
PI-containing regimens		168 (73.4%)
INSTI-containing regimens		195 (85.2%)
Maraviroc-containing regimens		48 (21%)
Enfuvirtide-containing regimens		6 (2.6%)
Fostemsavir-containing regimens		17 (7.4%)
Ibalizumab-containing regimens		7 (3.1%)
Lenacapavir-containing regimens		9 (3.9%)

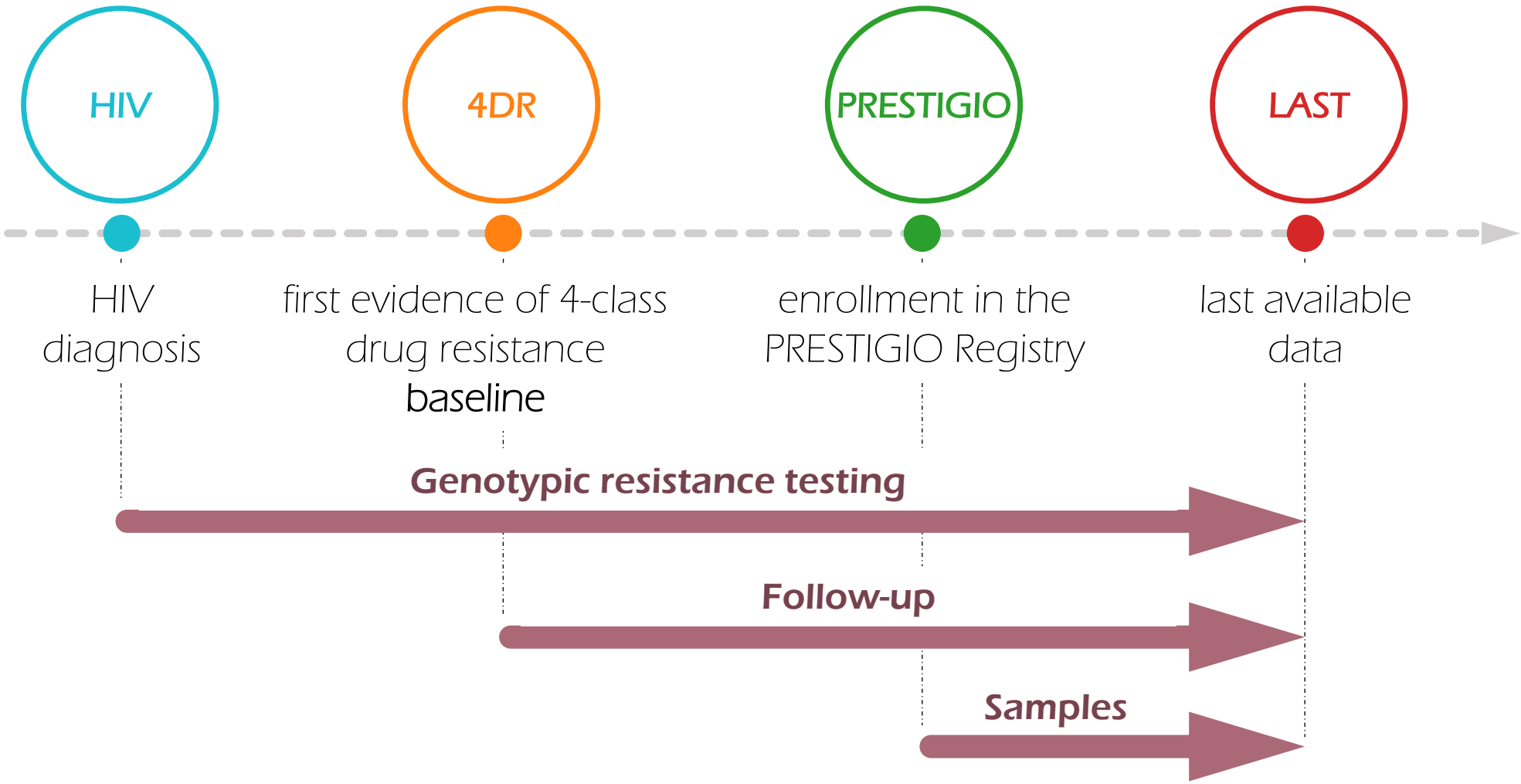
Data reported as frequency (percentage) or median (interquartile range), as appropriate. ART: antiretroviral therapy; HIV: human immunodeficiency virus; INSTI: integrase strand transfer inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.

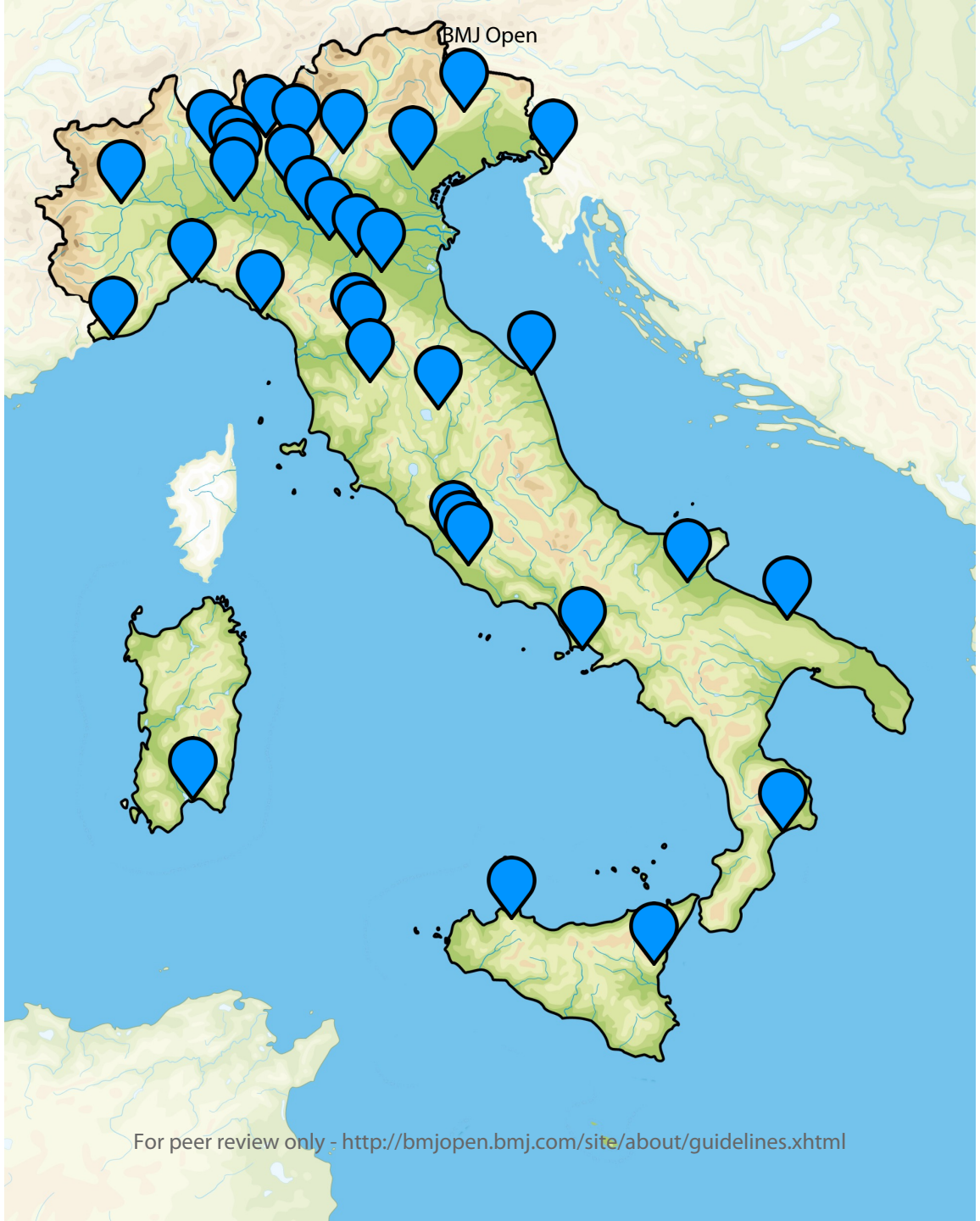
Figure captions.

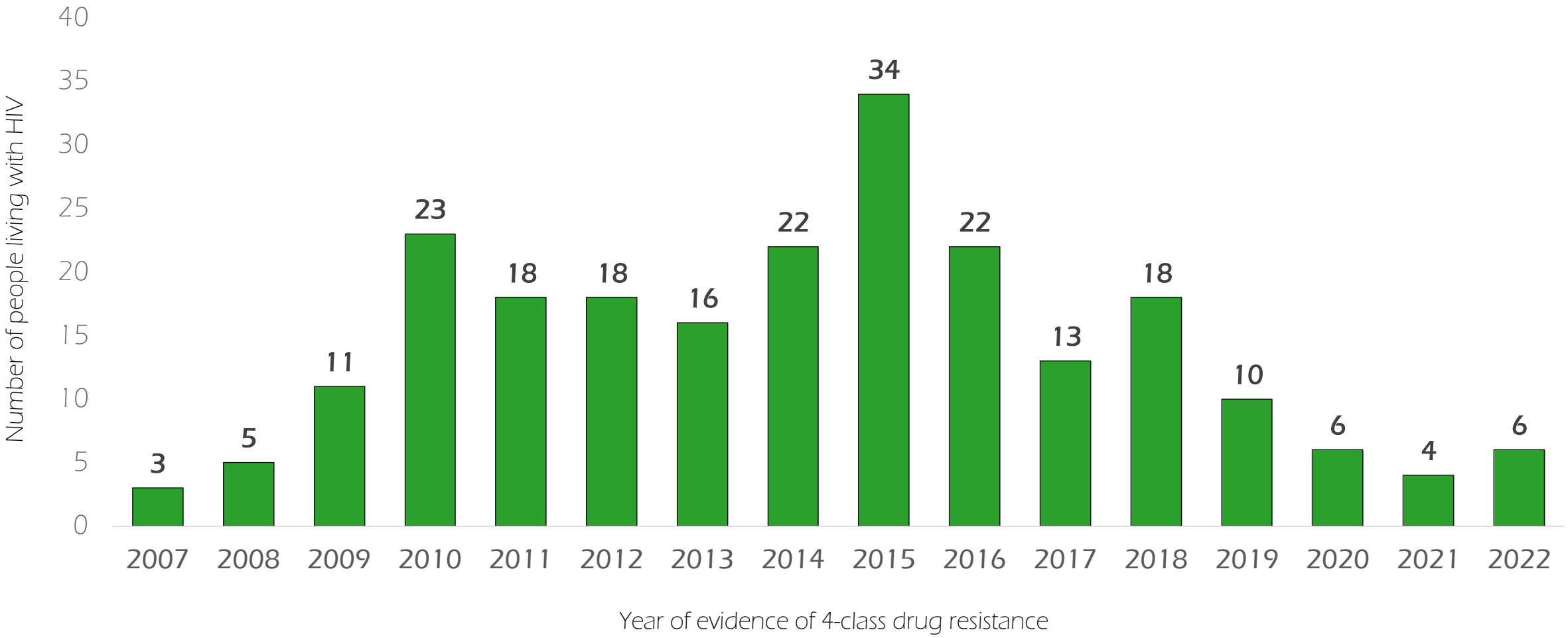
Figure 1. PRESTIGIO timeline. Genotypic resistance tests are collected since the first available from HIV diagnosis. Follow-up for clinical, laboratory, treatment, and virological data accrues from the first evidence of 4-class drug resistance (baseline). Plasma and cell sample collection on an annual basis starts at the date of enrollment in the registry.

Figure 2. The 39 Infectious Diseases Clinics participating in the PRESTIGIO Registry, located throughout Italy.

Figure 3. Year of evidence of 4-class drug resistance.


HIV
4DR
PRESTIGIO
LAST
HIV diagnosis
first evidence of 4-class drug resistance
baseline
enrollment in the PRESTIGIO Registry
last available data
Genotypic resistance testing
Follow-up
Samples





Online supplemental table 1. Summary of variables included in the PRESTIGIO Registry from the date of evidence of 4-class drug resistance.

Socio-demographic data	
General	date of birth, sex at birth, country of birth, ethnicity
Lifestyle	height, weight, smoking habit
HIV-specific	mode of HIV transmission, date of last negative HIV test, date of first positive HIV test, date of 4-class drug resistance
Laboratory results	
HIV-specific	HIV-1 RNA (copies/mL), HIV-1 RNA before ART start (copies/mL) $CD4^+$ T-cell count (cells/µL, %), $CD8^+$ T-cell count (cells/µL, %), $CD4^+/CD8^+$ ratio, $CD4^+$ nadir (cells/µL) RNA- and DNA-based genotypic resistance testing (*collected since the first test available, also before evidence of 4-class drug resistance*) subtype, viral tropism (*collected since the first available, also before evidence of 4-class drug resistance*)
Other	hepatitis B and C serologies, HBV DNA, HCV RNA complete blood cell count plasma glucose, triglycerides, total/HDL-/LDL-cholesterol, creatinine, transaminases, direct and indirect bilirubin
Treatment	
Antiretroviral therapy	date of antiretroviral therapy start, date of highly active antiretroviral therapy (defined as a combination of drugs from at least 2 different classes) start

	regimens from baseline to the last available visit (including start and stop dates, dose and mode of administration, reason for switch)
Other medications	specific drugs (including start and stop dates, dose and mode of administration, reason for stop)
Vaccines	date and type of vaccinations
AIDS diagnoses	date and type of AIDS events
Comorbidities	non-AIDS malignancies, diabetes, arterial hypertension requiring treatment, major adverse cardiovascular events, chronic liver or kidney diseases, osteoporosis and related fractures, neurocognitive disorders, chronic obstructive pulmonary disease, etc. (including dates of diagnosis and eventually resolution)
Hospitalizations	dates and reason for hospitalization
Pregnancies	dates of pregnancy, date of delivery, etc.
Sexually transmitted infections	date of diagnosis, type, and treatment of sexually transmitted infections
Death	date and reason of death

Online supplemental figure 1. Resistance-associated mutations present in ≥20% of 4DR-PLWH from the PRESTIGIO Registry. All RNA-based genotypic resistance tests through Sanger sequencing were considered cumulatively. Percentages evaluated on 229 PLWH for NRTI, NNRTI, and PI resistance-associated mutations, on 175 PLWH with at least one available integrase genotype for INSTI resistance-associated mutations. INSTI: integrase strand transfer inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; 4DR-PLWH: people living with 4-class drug-resistant HIV.