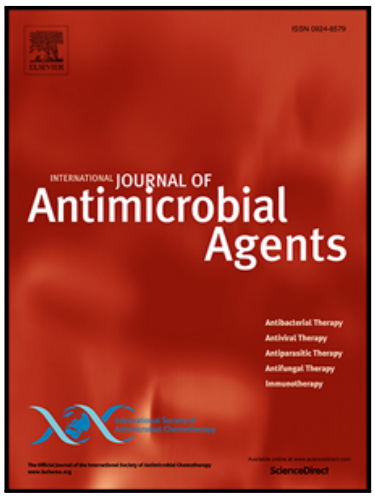

Prevalence and factors associated with HIV-1 multidrug resistance over the past two decades in the Italian Arca database

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Highlights

- Despite successful ART, HIV+ people could develop multi-class drug resistance (MDR)
- Data about the prevalence of HIV-MDR are scanty and results are unclear
- A significant decline of HIV-MDR prevalence over the 1998-2018 was observed
- Today, HIV-MDR is still present, although at a lower rate
- Management of patients infected by MDR viruses is a critical issue in HIV therapy

Prevalence and factors associated with HIV-1 multidrug resistance over the past two decades in the Italian Arca database

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Running title: HIV-MDR in ART-experienced patients

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Abstract

Despite successful antiretroviral therapy, HIV-infected people could develop multi-class drug resistance (MDR). Here we aimed to explore the prevalence of HIV-1 drug resistance over the past two decades by focusing on HIV-MDR and its predictors. This is a retrospective study of ART-experienced HIV-infected patients with at least one plasma genotypic resistance test (GRT) available, collected from 1998 to 2018 from the ARCA database. Temporal trend of resistance to any drug class was evaluated by considering all GRTs. Prevalence and predictors of HIV-MDR were analyzed by taking into account cumulative GRTs. Among 15,628 isolates from 6,802 patients, resistance to at least one drug class decreased sharply from 1998 to 2010 (1998-2001:78%; 2008-2010:59%; $p<0.001$) and then remained relatively constant at around 50% over 2011-2018, with a proportion of isolates with HIV-MDR also stable (around 9%).

By evaluating factors associated with cumulative HIV-MDR, in the multivariable model male gender, sexual and vertical transmission, number of previous PIs, NRTIs, NNRTIs, previous exposure to INSTI, enfuvirtide and maraviroc and co-infection with HBV were associated with an increased risk of HIV-MDR. By contrast, a Nadir CD4 ≥200 cells/mm^3 starting ART from 2008 and co-infection with HCV were associated with a lower risk of HIV-MDR. In conclusion, this study revealed that HIV-1 drug resistance has been stable since 2011 despite its dramatic decrease over the last two decades. HIV-MDR is still present, although at a lower rate, suggesting the need for continuous surveillance and accurate management of HIV-infected ART-experienced patients.

1. Introduction

HIV-1 drug resistance (HIV-DR) affects the ability of a drug or a combination of drugs to block virus replication, representing one of the major issues in the effective treatment of HIV infection [1–3]. In fact, HIV-DR can substantially reduce therapeutic choices of antiretroviral therapy (ART) and increase the chance of virological failure and clinical progression [4]. Despite the impressive advances in the field of ART, even the efficacy of the latest licensed drugs can be undermined by the accumulation of resistance-associated mutations selected by previous regimens [5]. In addition, both the emergence and the spread of drug resistance must be carefully monitored to avoid the choice of ineffective regimens; in fact, they may be an obstacle for future therapeutic strategies aimed at viral eradication [3,6].

In high income countries, a minority of HIV patients harbour multi-class drug resistance (MDR) viruses that have reduced susceptibility to almost all drugs, which makes it difficult to optimize therapy to halt viral replication in these patients [7,8]. HIV-MDR infection has been associated with a higher risk of clinical progression and death [9]. Currently, the management of patients infected by MDR viruses is one of the most critical issues in HIV therapy. The latest available report on HIV-DR in Europe analyzed data from the 1997-2008 timeframe, which does not contain information about resistance to integrase strand transfer inhibitors (INSTIs); however, it reports a reduced risk of resistance over time [10]. Consistently, two different studies of Italian and Portuguese resistance Cohorts, respectively, reported a similar decrease in mutations associated with resistance to nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-NRTIs (NNRTIs) and protease inhibitors (PIs) during the 2003-

2012 and 2001-2006 periods, respectively [11,12]. A dramatic decrease in acquired resistance (including resistance against INSTIs) was observed during the 1999-2013 period in ART-experienced patients from the Swiss HIV Cohort; this supports the hypothesis that HIV-DR could be virtually stopped thanks to potent new therapies and close monitoring [13].

Until now, data regarding the prevalence of HIV-MDR are scanty and results are unclear and misleading due to the different settings and MDR definitions of the studies; thus, it is difficult to make comparisons and draw conclusions [11,12,14].

The aim of this study was to assess the prevalence of HIV-1 drug resistance over the past two decades, i.e., from 1998 to 2018, with particular focus on HIV-MDR among isolates from ART-experienced HIV-1 infected people in a multicenter Italian network. We also aimed to identify the predictors associated with HIV-MDR.

2. Materials and Methods

2.1 Study population

This was a retrospective, observational, multicenter study of HIV-1 infected adult patients retrieved from the Antiviral Response Cohort Analysis (ARCA, https://www.dbarca.net/) database, which is a national repository used for non-profit research purposes. Data collection was approved by the local Ethics Committees and written informed consent was obtained from all patients prior to their participation.

The study included ART-experienced HIV-1 infected individuals with at least one plasma genotypic resistance test (GRT) performed for routine clinical purposes at failure by using Sanger sequencing. In particular, sequences of protease, reverse

transcriptase and integrase (when available) obtained in the period between January 1998 and December 2018 were analyzed.

2.2 Evaluation of drug resistance over time

Resistance to 1, 2 and ≥3 drug classes among NRTIs, NNRTIs, PIs or INSTIs was evaluated in the isolates over the years according to the presence of at least one major resistance mutation (MRM) paneled by the Stanford HIV Drug-resistance database 2019 (HIVdb version 8.8). MDR was defined as the occurrence of at least one MRM against at least three of the following four drug classes: NRTIs, NNRTIs, PIs or INSTIs. Resistance to INSTIs was explored in isolates for whom integrase GRTs were performed after 2008, i.e., the year the first INSTI raltegravir was approved.

2.3 Evaluation of prevalence and factors associated with cumulative multi drug-class resistance

For patients with more than one GRT, cumulative class-resistance was calculated by considering the mutations in the last GRT recorded and combining all available resistance mutations ever identified in past GRTs; resistance to 1, 2 and ≥ 3 drug classes was defined as above. Factors associated with cumulative MDR (based on the above-mentioned definition) were evaluated.

2.4 Statistical analysis

All statistical analyses were performed using a standard software package (Stata, version. 15; StataCorp College Station, Texas, USA). P-values <0.05 were considered statistically significant.

The prevalence of resistance to any drug class over time was estimated in all GRTs by dividing the two decades studied roughly into three-year periods. Potential differences over the periods in the prevalence of resistance to 1, 2 and ≥3 drug classes were evaluated with the Chi-squared test for trend. The analyses were performed by retaining all available GRTs. We performed several sensitivity analyses to confirm the robustness of the results obtained by this approach, by considering one GRT per patient per three-year period in three different ways: i) retaining the first GRT; ii) retaining the last GRT; iii) retaining the GRT with the highest resistance (in case of more than one GRT having the same number of resistances, the last one was considered).

HIV-MDR was evaluated based on cumulative resistance. Patients with HIV-MDR were compared to those with 0, 1 or 2 drug-class resistance. Differences between categorical variables were assessed using the Chi square test and differences between continuous variables were assessed using the non-parametric Mann-Whitney U test, when appropriate.

Predictors of cumulative HIV-MDR were investigated by fitting univariable and multivariable logistic regressions. The following variables were considered: sex, age, nationality, risk factor, HIV-1 subtype, HCV co-infection, HBV co-infection, zenith viremia, nadir CD4 cell count, AIDS events, year of first treatment start, number of PIs, NRTIs, NNRTIs and INIs previously administered, previous exposure to INI, maraviroc (MVC) and enfuvirtide T20, center.

For these specific analyses, patients were divided into two groups: the HIV-MDR group, which included subjects harboring viruses with ≥3 drug-class resistance, and <3 HIV-DR group, which pooled all other subjects with 0, 1 or 2 drug-class resistance.

Multiple Imputation by Chained Equations (MICE) was used to enter missing data for the following variables: HBV seropositivity, HCV seropositivity, nationality and risk factor.

Sensitivity analyses were performed to verify whether application of the logistic regression model was solid in predicting HIV-MDR risk factors. Three separate multivariable logistic models were run each of them with a different reference group: I) subjects with no resistance, II) subjects with 1 drug-class resistance, II) subjects with 2 drug-class-resistance.

3. Results

3.1 Prevalence of resistance to any drug class in all isolates over two decades

We collected 15,628 GRTs from 6,802 patients. Overall, 31% of GRTs showed no resistance, whereas the prevalence of resistance to 1, 2 and ≥3 classes was 23%, 29% and 17%, respectively. A significant decline in resistance to 1, 2 and ≥3 drug classes was observed over the two studied decades ($p<0.001$); specifically, the percentage of HIV-MDR (≥3 drug class resistance) was halved from 17% to 9% ($p<0.001$) (Figure 1).

Consistently, the percentage of GRTs without any resistance doubled over time, from 22% in the 1998-2001 period to 50% in the 2011-2018 period. The prevalence of resistance to any drug class significantly decreased specifically in the 1998-2010 period, but remained constant settling at around 50% from 2011 to 2018. In particular,

HIV-MDR significantly decreased from 17% in 1998-2001 to 13% in 2008-2010 (p<0.001), but remained nearly unchanged at 9% over the second decade (p=0.731) (Figure 1).

When we considered separately resistance to 3 and 4 drug classes we observed the emergence of resistance mutations to all 4 drug classes (NRTIs, NNRTIs, PIs and INSTIs) starting from 2008, concurrently with the licensing of INSTIs. The prevalence of 4 drug-class resistance was around 0.7% in 2008 and increased to 2% (p<0.001) in 2018; but taking into account only the 2011-2018 period it did not change (p=0.183) (data not shown).

All the results of this analysis were confirmed by the sensitivity analyses described in the Materials and Methods section (data not shown).

We repeated the analysis by stratifying all GRTs based on subtype (B [n=13,905, 89%] versus non-B [n=1,723, 11%]). Overall, both trends of resistance over time were very similar to that found in the totality of GRTs (Figure 1), though a general lower percentage of GRTs with resistances was observed in non-B subtype group versus subtype B group (51.2% versus 71.8%, p<0.001) (data not shown).

3.2 Evaluation of cumulative HIV-MDR and associated factors

When we evaluated cumulative resistance of the 6,802 patients included in the study, 1,408 (20.7%) of them harbored an MDR virus. As reported in Table 1, when compared to other patients with less drug-class or no class resistance, patients with HIV-MDR were older, mostly male, more frequently Italian and primarily infected with HIV-1 B subtype. As expected, these patients showed a more complex clinical profile (with a

lower CD4 nadir, higher viremia zenith and higher frequency of AIDS events) and a longer treatment history and had received a higher number of antiretroviral drugs and antiretroviral regimens.

According to the multivariable logistic regression model, male gender (versus female; adjusted Odds Ratio [95% Confidence Interval]: 1.28 [1.16-1.40]), sexual and mother-to-child transmission (versus heterosexual: AOR 1.63 [1.30-2.05] and 10.63 [7.30-15.47], respectively), number of previous PIs, NRTIs, NNRTIs exposure (per 1 increase: AOR 1.64 [1.58-1.70], 1.11 [1.07-1.14], 1.86 [1.75-1.98], respectively), previous exposure to INSTI, T20 and MVC (AOR 1.58 [1.32-1.89], 2.12 [1.73-2.58], 4.19 [3.02-5.81], respectively) co-infection with HBV (AOR 1.19 [1.08-1.30]), AIDS event (AOR 1.43 [1.26-1.63]) were factors associated with an increased risk of HIV-MDR. Conversely, a nadir CD4 ≥200 cells/mm^3 (versus <200: AOR 0.73 [0.68-0.79]), starting ART from 2008 (versus <2008: AOR 0.44 [0.34-0.56]) and co-infection with HCV (AOR 0.85 [0.77-0.94]) were associated with a lower risk of HIV-MDR (Table 2).

With sensitivity analyses, we basically observed similar results, confirming that independent predictors of HIV-MDR were male gender, sexual and vertical transmission, co-infection with HBV, experience of AIDS events and previous exposure to several antiretrovirals, by contrast, favorable associations were HCV co-infection, higher nadir CD4 cell count and starting ART from 2008 (Supplementary Tables 1-3).

4. Discussion

In the present study we analyzed a large cohort of ART-experienced patients retrieved from an Italian database who had at least one sample genotype test available from

1998 to 2018. First, we evaluated the overall trend of prevalence of 0, 1, 2 and ≥3 drug-class resistance mutations taking into account all genotype tests collected from the database. We found that overall drug resistance to any drug class tended to significantly drop over the study period; specifically, a two-thirds (from 78% to 48%) reduction occurred from 1998 to 2018, as a result of good management of HIV infection and progressive improvement of antiretrovirals in terms of potency, efficacy, tolerability and genetic barrier, together with rapid intervention in cases of virological failure [15–17]. Notably, the significant decrease in resistance to at least one class referred specifically to the period ranging from 1998 to 2010; by contrast, it remained stable at around 50% from 2011 to 2018. With regard to MDR, even though the percentage of isolates with MDR decreased significantly, i.e., from 17% to 9%, it has remained stably settled at around 9% from 2011 to 2018.

Consistently, a different Italian study [18] showed a similar trend in the same time period, albeit the proportion of subjects with HIV-MDR was lower around 5%) in the period 2011-2018. This discrepancy could be due to the fact that in that study the authors evaluated data from clinical centers located primarily in central Italy, whereas our study included more heterogeneous data collected from different clinical centers located in almost all Italian regions.

We found a significantly declining trend in HIV-MDR when we considered the newly acquired resistance over a 6-year timeframe (2001-2006) based on data deriving from a Portuguese resistance database [12].

Of note, Gill et al. reported a decline in the prevalence of drug resistance over the study period (1995-2014) in HIV-infected patients living in southern Alberta (Canada)

[14]; however, using different approaches they showed that the estimate prevalence may change due to variations in the number of patients tested annually, using either the entire population or only subjects with GRT as the denominator and using either last or cumulative GRT.

In light of these results, it should also be considered that in our study there was a decreasing number of GRTs during the period analyzed, starting from 82% (12877/15628) in the 1998-2010 period and decreasing to 18% (2751/15628) in the 2011-2018 period. These results could be due to the introduction of more potent and tolerable antiretrovirals in 2008 (such as INSTIs), which led to a reduction of virological failures resulting in fewer genotype tests.

Here, we also show that from 2008 resistance to the INSTI class has emerged (due to its concurrent licensing), resulting in the emergence of 4 drug-class resistance. However, the prevalence of 4 drug-class resistance in the final period (i.e., 2011-2018) has remained stable at around 1-3%.

Our study also focused on predictors of MDR. For this purpose, we considered the cumulative GRTs from each patient and assumed that the emergence of drug resistance-associated mutations is lifelong and irreversible [19,20], although it may temporarily disappear due to the loss of selective drug pressure after a regimen switch [21–23]. Since all drug resistance mutations might not be observed in patients with multiple treatment failures in the last GRT, thus leading clinicians to choose a sub-optimal regimen [24], we assessed the burden of all resistance by considering the complete resistance history.

Concerning this point, study showed that cumulative genotype estimates HIV drug resistance prevalence at a higher level than the last genotype and thus it may be more accurate for selecting the optimal ART-regimen among HIV-infected patients experiencing multiple treatment failures [25]. Consistently, Garcia et al. showed that using cumulative genotype to predict genotypic sensitivity scores (GSS) offers a more comprehensive evaluation of the burden of resistance; in fact, this approach can be beneficial in the selection of the best salvage treatment for treatment-experienced patients [26,27].

As expected, evaluating the risk factors associated with HIV-MDR showed that more extensive ART-exposure was associated with higher risk of developing an MDR virus; vertical transmission was also significantly associated with HIV-MDR. This latter result is in agreement with data in the literature which suggest that adolescents or adults with perinatally acquired HIV might find it difficult to maintain adherence to life-long therapy. In fact, perinatally infected children are usually seen by pediatric providers and at the time of transition to the adult health care setting they are likely to have advanced disease, physical and neurocognitive deficits and drug side effects [28,29]. Moreover, since many perinatally infected children are highly treatment experienced, they might have extensive drug resistance making it difficult to select effective antiretroviral drug regimens.

Co-infection with HBV was also associated with an increased risk to develop HIV-MDR. Consistently, the longer exposition to agents against HBV (such as entecavir, telbivudine and lamivudine) used often in the past in monotherapy strategies during

HIV/HBV co-infection, could select different mutations in HIV, owing their capacity to sub-optimally inhibit HIV replication [30,31]

By contrast, we showed that starting therapy from 2008 was associated with a lower risk of developing MDR. This finding is in line with the previous one and with a Swiss study [13] and suggests that from 2008 optimal combined ART regimens, even with the introduction of INSTIs, together with rapid intervention in the case of virological failures, improved clinical outcomes leading to a reduction of drug resistance prevalence. As expected, nadir CD4 count ≥200 cells/mm^3 was a protective factor associated with HIV-MDR, whereas AIDS events were associated with higher risk of HIV-MDR. The negative association between higher nadir CD4 count and HIV-MDR suggests that subjects were more likely to start ART during the early phase of HIV infection, thus limiting the viral replication and in turn the frequency of newly acquired drug resistance [32]. Notably, in this study HCV seropositivity also seemed to be a protective factor associated with HIV-MDR. Concerning to this point, patients with HIV/HCV co-infection are more likely to show poor treatment compliance or low rates of adherence to ART [33]. Therefore, a lower exposition could determine a less selective pressure from antiretroviral drugs over time and, consequently, less selection of circulating resistant virus strains. Consistently, we found a strong association between HCV infection and injection drug users who are well known to have a low rate of adherence [34].

We also hypothesized that the lower risk of HIV-MDR in HCV co-infected patients could be a misleading result determined by an immortal time bias which most of the time could result in a distortion of observed effects (biased association). In this study, since

we evaluated the drug resistance accumulation over time, it is possible that HCV-negative patients had lower probability to be discontinued in the cohort (they have survived longer), and, consequently, they were more likely to accumulate mutations in the studied period respect to HVC positive patients.

The strengths of the present study are the large sample size, the wide calendar time span analyzed and the use of cumulative GRT. In fact, as mentioned above, several studies indicated that cumulative GRT is more accurate in interpreting HIV drug resistance and, therefore, is more precise for selecting the appropriate regimen in subjects with multiple treatment failures [9,25]. However, our study has some limitations. First, the study does not incorporate the entire Italian population due to the lack of several centers in southern Italy. Nevertheless, our results are very similar to those obtained in another Italian study. Moreover, due to the recent introduction of INSTIs in clinical practice the prevalence of integrase resistance was evaluated in a subset of isolates for whom integrase GRTs were requested.

In conclusion, HIV-1 drug resistance in Italian ART-experienced HIV-1 infected people has been stable since 2011, despite its dramatic decrease over time, from 1998 to 2010. In particular, HIV-MDR is still present but at a lower rate. Drugs with new mechanisms of action and the appropriate strategies to regain undetectability in this setting are urgently needed.

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Declarations

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Ethical Approval: Not required.

Author contributors: FL, AG, DA, AL, AC and MMS carried out study conception and design, analysis and interpretation of data, and drafting of manuscript. FL, LT and DA carried out the statistical analysis. AD and FS participated in the study conception and design. AB carried out acquisition and management of data. FV, PC GC, BB, RG and APG contributed to the collection of the data. AD, FS, AB, FV, PC GC, BB, RG and APG carried out a critical revision of the manuscript.

The following individuals (and institutions) currently contribute clinical and laboratory data to the ARCA database initiative:

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References

[1] Wegner SA, Wallace MR, Aronson NE, Tasker SA, Blazes DL, Tamminga C, et al. Long-term efficacy of routine access to antiretroviral-resistance testing in HIV type 1-infected patients: results of the clinical efficacy of resistance testing trial. Clin Infect Dis 2004; 38:723-730.

[2] Wensing AMJ, van de Vijver DA, Angarano G, Asjö B, Balotta C, Boeri E, et al. Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. J Infect Dis 2005; 192:958-966

[3] Frenkel LM, Tobin NH. Understanding HIV-1 Drug Resistance. Ther Drug Monit 2004; 26:116-121.

[4] Di Giambenedetto S, Colafigli M, Pinnetti C, Bacarelli A, Cingolani A, Tamburrini E, et al. Genotypic Resistance Profile and Clinical Progression of Treatment-Experienced HIV Type 1-Infected Patients with Virological Failure. AIDS Res Hum Retroviruses 2008; 24:149-154.

[5] Castagna A, Ferrara M, Galli L, Comi L, Sterrantino G, Cenderello G, et al. Long-term efficacy of dolutegravir in treatment-experienced subjects failing therapy with HIV-1 integrase strand inhibitor-resistant virus. J Antimicrob Chemother 2018; 73:177-182.

[6] Günthard HF, Calvez V, Paredes R, Pillay D, Shafer RW, Wensing AM, et al. Human Immunodeficiency Virus Drug Resistance: 2018 Recommendations of the International Antiviral Society-USA Panel. Clin Infect 2019; 68:177-187.

[7] Mocroft A, Ledergerber B, Viard JP, Staszewski S, Murphy M, Chiesi A, et al. Time to virological failure of 3 classes of antiretrovirals after initiation of highly

active antiretroviral therapy: results from the EuroSIDA study group. J Infect Dis 2004; 190:1947-1956

[8] Mocroft A, Phillips AN, Friis-Møller N, Colebunders R, Johnson AM, Hirschel B, et al. Response to antiretroviral therapy among patients exposed to three classes of antiretrovirals: Results from the EuroSIDA study. Antivir Ther 2002; 7:21-30.

[9] Zaccarelli M, Tozzi V, Lorenzini P, Trotta MP, Forbici F, Visco-Comandini U, et al. Multiple drug class-wide resistance associated with poorer survival after treatment failure in a cohort of HIV-infected patients. AIDS 2005; 19:1081-1089.

[10] De Luca A, Dunn D, Zazzi M, Camacho R, Torti C, Fanti I, et al. Declining prevalence of HIV-1 drug resistance in antiretroviral treatment-exposed individuals in Western Europe. J Infect Dis 2013; 207:1216-1220.

[11] Franzetti M, Violin M, Antinori A, De Luca A, Ceccherini-Silberstein F, Gianotti N, et al. Trends and correlates of HIV-1 resistance among subjects failing an antiretroviral treatment over the 2003-2012 decade in Italy. BMC Infect Dis 2014; 14:398.

[12] Vercauteren J, Deforche K, Theys K, Debruyne M, Duque LM, Peres S, et al. The incidence of multidrug and full class resistance in HIV-1 infected patients is decreasing over time (2001-2006) in Portugal. Retrovirology 2008; 5:12.

[13] Scherrer AU, Von Wyl V, Yang WL, Kouyos RD, Böni J, Yerly S, et al. Emergence of acquired HIV-1 drug resistance almost stopped in Switzerland: A 15-year prospective cohort analysis. Clin Infect Dis 2016; 62:1310-1317.

[14] Gill VC, Lynch T, Ramazani S, Krentz HB. Reporting on the prevalence of antiretroviral drug resistance in a regional HIV population over 20 years: A word of caution. Antivir Ther 2017; 22:277-286.

[15] European AIDS Clinical Society (EACS). EACS Guidelines, Version 10.0. 2019. Available at: https://www.eacsociety.org/files/2019_guidelines-10.0_final.pdf.

[16] Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. Department of Health and Human Service (DHHS). 2019. Available at: https://aidsinfo.nih.gov/contentfil.

[17] Saag MS, Benson CA, Gandhi RT, Hoy JF, Landovitz RJ, Mugavero MJ, et al. Antiretroviral Drugs for Treatment and Prevention of HIV Infection in Adults. JAMA 2018; 320:379-396.

[18] Armenia D, Di Carlo D; Philippe F, Yagai B, Borghi V, et al. HIV multi-drug resistance is still a relevant issue despite its dramatic drop over the years. J Antimicrob Chemother 2020; in course of publication (doi: 10.1093/jac/dkz554).

[19] Kouyos RD, Günthard HF. The irreversibility of HIV drug resistance. Clin Infect Dis 2015; 61:837-839.

[20] Miller V, Sabin C, Hertogs K, Bloor S, Martinez-Picado J, D'Aquila R, et al. Virological and immunological effects of treatment interruptions in HIV-1 infected patients with treatment failure. AIDS 2000; 14:2857-2867.

[21] Izopet J, Souyris C, Hance A, Sandres-Sauné K, Alvarez M, Pasquier C, et al. Evolution of Human Immunodeficiency Virus Type 1 Populations after

Resumption of Therapy following Treatment Interruption and Shift in Resistance Genotype. J Infect Dis 2002; 185:1506-1510.

[22] Verhofstede C, Van Wanzeele F, Van Der Gucht B, De Cabooter N, Plum J. Interruption of reverse transcriptase inhibitors or a switch from reverse transcriptase to protease inhibitors resulted in a fast reappearance of virus strains with a reverse transcriptase inhibitor-sensitive genotype. AIDS 1999; 13:2541-2546.

[23] Albrecht D, Zöllner B, Feucht HH, Lorenzen T, Laufs R, Stoehr A, et al. Reappearance of HIV multidrug-resistance in plasma and circulating lymphocytes after reintroduction of antiretroviral therapy. J Clin Virol 2002; 24:93-98.

[24] Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat Med Published 1999; 5:512-517.

[25] Punyacam P, Iemwimangsa N, Chantratita W, Sukasem C, Sungkanuparph S. HIV Drug Resistance Interpreted by Cumulative Versus Last Genotypes in HIV-Infected Patients with Multiple Treatment Failures. Curr HIV Res 2012; 10:271-274.

[26] Garcia F, Alvarez M, Fox Z, Garcia-Diaz A, Guillot V, Johnson M, et al. Predicting antiretroviral drug resistance from the latest or the cumulative genotype. Antivir Ther 2011; 16:373-382.

[27] Zaccarelli M, Lorenzini P, Ceccherini-Silberstein F, Tozzi V, Forbici F, Gori C, et al.

Historical resistance profile helps to predict salvage failure. Antivir Ther 2009; 14:285-291.

[28] Mofenson LM, Cotton MF. The challenges of success: Adolescents with perinatal HIV infection. J Int AIDS Soc 2013; 16:18650.

[29] Lam PK, Fidler S, Foster C. A review of transition experiences in perinatally and behaviourally acquired HIV-1 infection; same, same but different? J J Int AIDS Soc 2017; 20:21506.

[30] McMahon MA, Jilek BL, Brennan TP, Brennan TP, Shen L, Zhou Y, et al. The HBV drug entecavir - effects on HIV-1 replication and resistance, N Engl J Med 2007; 356:2614-2621;

[31] Yasutake Y, Hattori S, Tamura N, Matsuda K, Kohgo S, Maeda K, et al. Structural features in common of HBV and HIV-1 resistance against chirally-distinct nucleoside analogues entecavir and lamivudine. Sci Rep 2020; 10:3021.

[32] Palumbo PJ, Fogel JM, Hudelson SE, Wilson EA, Hart S, Hovind L, et al. HIV Drug Resistance in Adults Receiving Early vs. Delayed Antiretroviral Therapy: HPTN 052. J Acquir Immune Defic Syndr 2018; 77:484-491.

[33] Braitstein P, Justice A, Bangsberg DR, Alfonso V, Schechter MT,Hoggs RS, et al. Hepatitis C coinfection is independently associated with decreased adherence to antiretroviral therapy in a population-based HIV cohort. AIDS (London, England). 2006 Feb;20(3):323-331.

[34] Wood E, Montaner JS, Yip B, Tyndall MW, Schechter MT, O'Shaughnessy MV, Hogg RS. Adherence and plasma HIV RNA responses to highly active antiretroviral

therapy among HIV-1 infected injection drug users. CMAJ. 2003 Sep 30;169(7):656-61. PMID: 14517122; PMCID: PMC202281.

Figure legend

Figure 1. Temporal trend of resistance prevalence to any drug-class over the period 1998-2018. Analysis performed on 15,628 sequences of protease/reverse transcriptase and integrase (if available) from 6,802 ART-experienced HIV-1 infected patients. In the figure are reported p values from Chi-squared test for trend performed to evaluate potential differences in the prevalence of resistance to 1, 2 and ≥3 drug-classes across the periods defined by braces. GRTs, genotypic resistance test.

Table 1. Characteristics of patients, overall and according to the level of cumulative HIV-1 drug-class resistance at the last GRT

Variables	Overall (N=6,802)	Number of cumulative class resistance				P value[a]	P value[b]	P vaue[c]
		0 (N=1,693)	1 (N=1,415)	2 (N=2,286)	≥3 (N=1,408)			
Age, median (IQR)	44 (38-49)	43(37-49)	44 (38-49)	43 (38-48)	45 (40-51)	*<0.001*	*0.052*	*<0.001*
Male, n (%)	4,576 (67.3)	1,115 (65.9)	916 (64.7)	1,527 (66.8)	1,018 (72.3)	*<0.001*	*<0.001*	*<0.001*
Nationality, n (%)						*<0.001*	0.026	0.001
Italian	5,312 (78.1)	1,309 (77.3)	1,100 (77.7)	1,768 (77.3)	1,135 (80.6)			
Not Italian	780 (11.5)	259 (15.3)	151 (10.7)	261 (11.4)	109 (7.7)			
Unknown	710 (10.4)	125 (7.4)	164 (11.6)	257 (11.2)	164 (11.6)			
Risk factor, n (%)						*<0.001*	*<0.001*	*<0.001*
Heterosexual	2,000 (29.4)	499 (29.5)	423 (29.9)	650 (28.4)	428 (30.4)			
Homosexual	891 (13.1)	176 (10.4)	154 (10.9)	309 (13.5)	252 (17.9)			
IDU	2,219 (32.6)	518 (30.6)	500 (35.3)	760 (33.2)	441 (31.3)			
Sexual	493 (7.2)	195 (11.5)	90 (6.4)	139 (6.1)	69 (4.9)			
Vertical	96 (1.4)	6 (0.4)	21 (1.5)	34 (1.5)	35 (2.5)			
Other/unknown	1,103 (16.2)	299 (17.7)	227 (16.0)	394 (17.2)	183 (13.0)			
Subtype, n (%)						*<0.001*	*<0.001*	*0.001*
B	6,051 (89.0)	1,409 (83.2)	1,252 (88.5)	2,070 (90.6)	1,320 (93.8)			
Non-B[d]	751 (11)	284 (16.8)	163 (11.5)	216 (9.4)	88 (6.2)			
AIDS events, n (%)	941 (13.8)	185 (10.9)	184 (13.0)	322 (14.1)	250 (17.8)	*<0.001*	*<0.001*	*0.003*
HCV-infection, n (%)						0.019	*0.001*	0.053
Yes	2,798 (41.1)	684 (40.4)	636 (44.9)	944 (41.3)	534 (37.9)			
No	3,114 (45.8)	824 (48.7)	614 (43.4)	1,002 (43.8)	674 (47.9)			
Unknown	890 (13.1)	185 (10.9)	165 (11.7)	340 (14.9)	200 (14.2)			
HBV-infection, n (%)						*<0.001*	0.115	0.049
Yes	1,076 (15.8)	271 (16.0)	217 (15.3)	339 (14.8)	249 (17.7)			
No	4,354 (64.0)	1,155 (68.2)	902 (63.7)	1,450 (63.4)	847 (60.2)			
Unknown	1,372 (20.2)	267 (15.8)	296 (20.9)	497 (21.7)	312 (22.2)			

Viremia Zenith, Log median (IQR)	5.1 (4.5-5.6)	5.1 (4.5-5.6)	5.1 (4.5-5.6)	5.1 (4.5-5.6)	5.3 (4.8-5.7)	*<0.001*	*<0.001*	*<0.001*
Viremia Zenith, copies/mL, n (%)						*<0.001*	*<0.001*	*<0.001*
<100,000	2,990 (44.0)	767 (45.3)	662 (46.8)	1,067 (46.7)	494 (35.1)			
100,001-500,000	2,644 (38.9)	637 (37.6)	521 (36.8)	884 (38.7)	602 (42.8			
>500,000	1,168 (17.2)	289 (17.1)	232 (16.4)	335 (14.7)	312 (22.2)			
Nadir CD4 count, median (IQR) cells/mm^3	221 (89-376)	246 (108-408)	233 (95-394)	232 (101-382)	157 (51-303)	*<0.001*	*<0.001*	*<0.001*
Nadir CD4 count, cells/mm^3, n (%)						*<0.001*	*<0.001*	*<0.001*
<200	3,146 (46.3)	694 (41.0)	628 (44.4)	998 (43.7)	826 (58.7)			
>200	3,648 (53.6)	996 (58.8)	786 (55.5)	1,284 (56.2)	582 (41.3)			
Unknown	8 (0.1)	3(0.2)	1 (0.1)	4 (0.2)	0 (0.0)			
Year of starting first-line regimen, median (IQR)	1998 (1995-2002)	2001 (1997-2007)	1998 (1996-2002)	1997 (1995-2001)	1996 (1994-1998)	*<0.001*	*<0.001*	*<0.001*
Year of starting first-line regimen, n (%)								
<2008	6,177 (90.8)	1,349 (79.7)	1,286 (90.9)	2,166 (94.8)	1,376 (97.7)	*<0.001*	*<0.001*	*<0.001*
≥2008	625 (9.2)	344 (20.3)	129 (9.1)	120 (5.2)	32 (2.3)			
Number of previously administered regimens, median (IQR)	4 (2-8)	3 (1-5)	4 (2-6)	5 (3-8)	8 (5-12)	*<0.001*	*<0.001*	*<0.001*
Number of PIs previously administered, median (IQR)	1 (1-3)	1 (1-2)	1 (0-2)	1 (1-2)	3 (2-4)	*<0.001*	*<0.001*	*<0.001*
Number of NRTIs previously administered, median (IQR)	4 (3-5)	3 (2-4)	4 (2-5)	4 (3-5)	5 (4-6)	*<0.001*	*<0.001*	*<0.001*
Number of NNRTIs previously administered, median (IQR)	1 (0-1)	1 (0-1)	0 (0-1)	1 (0-1)	1 (1-2)	*<0.001*	*<0.001*	*<0.001*
Previous INIs exposure, n (%)	585 (8.6)	149 (8.8)	109 (7.7)	120 (5.2)	207 (14.7)	*<0.001*	*<0.001*	*<0.001*
Previous T 20 exposure, n (%)	318 (4.7)	9 (0.5)	21 (1.5)	65 (2.8)	223 (15.8)	*<0.001*	*<0.001*	*<0.001*

Previous MVC exposure, n (%)	149 (2.2)	22 (1.3)	26 (1.8)	30 (1.3)	71 (5.0)	*<0.001*	*<0.001*	*<0.001*

[a]Group ≥3 class resistance versus 0 class resistance. [b]Group ≥3 class resistance versus 1 class resistance. [c]Group ≥3 class resistance versus 2 class resistance. [d]Main specific subtypes non-B: A (n=73), C (n=94), D (n=18), F (n=134), G (n=61), H (n=2), J (n=1), K (n=2), AE (n=25), AG (n=207). GRT, genotypic resistance test; IDU, injection drug user; IQR, interquartile range; MVC, maraviroc; n, number; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-NRTIs; PIs, protease inhibitors; T20, enfuvirtide.

Table 2. Predictors of HIV-MDR. Adjusted odds ratio (AOR) of having cumulative HIV-MDR (versus <3 drug-class resistance as reference group)

Variables	AOR	95% CI		P value
		Lower	Upper	
Sex (male vs. female)	**1.28**	**1.16**	**1.40**	**<0.001**
Age (per 5 years increase)	1.01	0.99	1.03	0.426
Nationality (Non-Italian vs. Italian)	1.00	0.86	1.16	0.955
Risk factor				
Heterosexual	1			
Homosexual	1.10	0.96	1.27	0.155
IDU	0.95	0.84	1.07	0.410
Sexual	**1.63**	**1.30**	**2.05**	**<0.001**
Vertical	**10.63**	**7.30**	**15.47**	**<0.001**
Other	1.12	0.98	1.27	0.093
HIV-1 Subtype (B vs. non-B)	1.06	0.90	1.26	0.476
HCV-infection (yes vs. no)	**0.85**	**0.77**	**0.94**	**0.001**
HBV-infection (yes vs. no)	**1.19**	**1.08**	**1.30**	**<0.001**
Zenith viremia (>100000 vs. ≤100000 copies/mL)	0.97	0.89	1.05	0.415
Nadir CD4 (≥200 vs. <200 cells/mm^3)	**0.73**	**0.68**	**0.79**	**<0.001**
AIDS events (yes vs. no)	**1.43**	**1.26**	**1.63**	**<0.001**
Year of first treatment start (≥2008 vs. <2008)	**0.44**	**0.34**	**0.56**	**<0.001**

Number of PIs previously administered (per 1 increase)	**1.64**	**1.58**	**1.70**	**<0.001**
Number of NRTIs previously administered (per 1 increase)	**1.11**	**1.07**	**1.14**	**<0.001**
Number of NNRTIs previously administered (per 1 increase)	**1.86**	**1.75**	**1.98**	**<0.001**
Previous INIs exposure (yes vs. no)	**1.58**	**1.32**	**1.89**	**<0.001**
Previous T20 exposure (yes vs. no)	**2.12**	**1.73**	**2.58**	**<0.001**
Previous MVC exposure (yes vs. no)	**4.19**	**3.02**	**5.81**	**<0.001**

Significant values (p<0.05) are shown in bold. The multivariable regression model was built by adjusting for the following variables: sex, age, nationality, risk factor, HIV-1 subtype, HCV-infection, HBV-infection, zenith viremia, nadir CD4 cell count, AIDS events, year of first treatment start, number of PIs, NRTIs, NNRTIs and INIs previously administered, previous exposure to INI, MVC and T20, center. AOR, adjusted odds ratio; CI, confidence interval; IDU, injection drug user; MVC, maraviroc; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-NRTIs; PIs, protease inhibitors; T20, enfuvirtide.

Figure 1.