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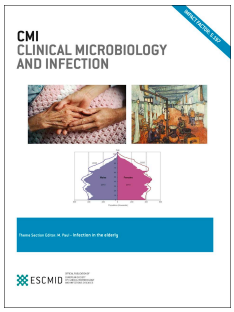

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PII: S1198-743X(20)30580-2

DOI: https://doi.org/10.1016/j.cmi.2020.09.028

Reference: CMI 2248

To appear in: *Clinical Microbiology and Infection*

Received Date: 24 January 2020

Revised Date: 25 August 2020

Accepted Date: 15 September 2020

Please cite this article as: Lai A, Franzetti M, Bergna A, Saladini F, Bruzzone B, Di Giambenedetto S, Di Biagio A, Lo Caputo S, Santoro MM, Maggiolo F, Parisi SG, Rusconi S, Gianotti N, Balotta C, Marked decrease in acquired resistance to antiretrovirals in latest years in Italy, *Clinical Microbiology and Infection*, https://doi.org/10.1016/j.cmi.2020.09.028.

Marked decrease in acquired resistance to antiretrovirals in latest years in Italy

A. Lai1†*, M. Franzetti2†, A. Bergna1, F. Saladini3, B. Bruzzone4, S. Di Giambenedetto5, A. Di Biagio6, S. Lo Caputo7, M. M. Santoro8, F. Maggiolo9, S. G. Parisi10, S. Rusconi1, N. Gianotti11, C. Balotta1

1 Department of Biomedical and Clinical Sciences 'L. Sacco', University of Milan, Milan, Italy; 2 Infectious Diseases Unit, 'A. Manzoni' Hospital, Lecco, Italy; 3 Department of Medical Biotechnologies, University of Siena, Siena, Italy; 4 Hygiene Unit, Policlinico San Martino Hospital, Genoa, Italy; 5 Institute of Clinical Infectious Diseases, Catholic University of the Sacred Heart of Rome, Rome, Italy; 6 Infectious Diseases Clinic, Department of Health Sciences, University of Genoa, San Martino Hospital-IRCCS, Genoa, Italy; 7 Clinic of Infectious Diseases, University Hospital Policlinico, University of Bari, Bari, Italy. 8 University of Rome "Tor Vergata", Rome, Italy; 9 Clinic of Infectious Diseases, Ospedali Riuniti, Bergamo, Italy; 10 Department of Molecular Medicine, University of Padua, Padua, Italy; 11 Infectious Diseases, IRCCS San Raffaele, Milan, Italy

† Contributed equally to this work.

*Corresponding Author:
Dr Alessia Lai, PhD: Department of Biomedical and Clinical Sciences "Luigi Sacco", Infectious Diseases and Immunopathology Section, University of Milan, Via G.B. Grassi 74, 20157 Milan, Italy – Phone (+39) 0250319775, Fax: (+39) 0250319768 – e-mail: alessia.lai@unimi.it

Abstract

Objectives: Aim of this study was to evaluate acquired drug resistance in Italy in the 2009-2018 period.
Methods: We analyzed 3,094 treatment patients who failed antiretrovirals from the Italian ARCA database who received a genotypic test after 6 months of treatment. Drug resistance mutations were identified through IAS-USA tables and the Stanford HIVdb algorithm. The global burden of acquired resistance was calculated among all subjects failing antiretroviral failure. Time trends and correlates of resistance were analyzed by standard statistical tests.
Results: Patients with non-European origin, as well as non-B subtypes, increased significantly from 11.5% (103/896) to 19.2% (33/172) and from 13.1% (141/1,079) to 23.8% (53/223), respectively, overtime. Overall, 14.5% (448/3,094), 12.1% (374/3,094) and 37.8% (1,169/3,094) of patients failed first, second and later lines, respectively. According to both IAS and HIVdb, in the study period resistance to any class, NRTIs, NNRTIs and PIs declined significantly. INSTI resistance declined significantly from 31% (36/116) to 20.8% (41/197) according to HIVdb but not to IAS.
Divergent data were highlighted regarding proportion of non-European patients carrying any, PI and INSTI resistance using IAS tables compared to the Stanford HIVdb algorithm as the former failed to detect a decrease of resistance while the latter indicate a reduction of 1.6-, 5- and 1.8-fold resistance for such drug classes.
In the multivariate analysis the risk of resistance increased in patients with a larger number of treatment lines and higher viremia and decreased in those starting therapy in the last biennium of the study.
Conclusions: A marked reduction in drug resistance was observed over 10 years, compatible with higher genetic barrier and potency of new antiretrovirals. Nonetheless, concerns remain for subjects with non-B subtypes when using mutation lists instead of interpretation systems because of the extensive polymorphism of protease region.

Introduction

By the end of last century, acquired drug resistance (ADR) to antiretrovirals had become a major concern, mostly due to the use of suboptimal regimens with limited potency and/or poor adherence to treatment [1][2][3]. Thereafter, the incidence of ADR started to decrease in resource-rich countries as a consequence of (i) availability of new classes of antiretroviral drugs with increased potency, (ii) single tablet regimens facilitating adherence and (iii) early genotypic resistance testing and switch of combination therapy in patients failing treatment, limiting further accumulation of resistance. Higher genetic barrier drugs have played a major role in this trend including boosted protease inhibitors (PIs), particularly darunavir (DRV), second-generation integrase strand transfer inhibitors (INSTIs), i.e. dolutegravir (DTG) and non-nucleoside reverse inhibitors (NNRTIs) such as etravirine (ETR). However, while a marked reduction in the rate of ADR has been reported in countries with access to novel drugs and genotypic resistance testing [4][5][6][7][8], limited data are available on the impact of increased circulation of non-B subtypes on ADR, particularly in countries receiving migration flows from low-resource settings.

Drug resistance testing remains a cornerstone of ART, therefore, monitoring circulation of drug resistance is crucial for public health and prevention strategies [9]. Thus, continuous surveillance is required to identify factors leading to drug resistance and closely follow patients at risk for development drug resistance.

In latest years, there has been increased number of immigrants from Africa, South America and Eastern Europe and this may have complicated the picture of HIV ADR. This population may have been exposed to suboptimal treatment in the country of origin, facilitating emergent drug resistance, and most often harbor non-B subtypes carrying natural polymorphisms that may confound genotypic resistance interpretation, particularly in the protease and integrase regions [10], [11], [12].

In the present study, we analyzed the temporal trends of ADR and investigated factors favoring ADR in a nationwide database of Italian HIV patients in the 2009-2018 period.

Methods

Patients included in the study were adult HIV-infected individuals enrolled at 21 Italian clinical centers during the 2009–2018 period and participating to the ARCA observational cohort (Antiviral Response Cohort Analysis, www.dbarca.net) Written informed consents had been obtained by patients at enrolment. ARCA was approved by the Regional Ethical Committee of Tuscany (Comitato Etico Area Vasta Toscana Sudest) and subsequently by the Ethical Committees of participating centers. Cases were selected based on availability of an HIV-1 genotypic test obtained while on any ART for at least 6 months and concomitant detection of HIV-1 viral load over 50 copies/ml. Self reported assessment of adherence was done for all patients.

The ART regimen considered included any combination of three or more drugs including an NNRTI and/or a PI and/or an INSTI.

Protease (PR), reverse transcriptase (RT) and integrase (IN) sequences were collected as originally provided by the contributing laboratories. Subtype was assigned using the REGA HIV-1 subtyping tool version 3.0. Drug resistance was evaluated according to the 2018 International AIDS Society (IAS) mutation list [13] and by the Stanford HIVdb algorithm (https://hivdb.stanford.edu/), version 8.6. While the former considers any mutation as associated with drug resistance, the latter defines levels of resistance as low, intermediate and high based on quality and quantity of mutations. To estimate the prevalence of resistance to individual class at treatment failure, all subjects performing a genotype resistance test were included in the analysis, as performed in previous studies not limiting the analysis to those failing specific drug classes [4].

The study period was treated as biennia, including 2009–2010, 2011–2012, 2013–2014, 2015–2016 and 2017-2018. The distribution of study subjects with regard to categorical parameters was compared using chi-square or Fisher's exact tests. Standard non-parametric tests were used to compare the median age, HIV-1 RNA levels and CD4 counts. Categorical data were analyzed using linear-by-linear associations for trends over time periods.

Multivariate analysis was performed using $\chi 2$ and logistic regression on all variables. For all analyses, an error of 5% was considered. Statistical calculations were performed using the statistical software package SPSS 17.0 (SPSS Inc., Chicago).

Results

Patient characteristics

Overall, 3,094 PR/RT and 1,058 IN sequences from an equal number of patients were available. Patient characteristics by year of genotyping are reported in Table 1. Globally, 449 (14.5%), 374 (12.1%) and 2271 (73.4%) patients failed first, second and further lines, respectively.

Gender distribution did not differ in the whole period of study. The majority of immigrants carried a non-B subtype (n=233, 68.1%). Non-European subjects, as well as non-B subtypes, increased significantly from 11.5% (103/896) to 19.2% (33/172) (*p=.002*) and from 13.1% (141/1,079)to 23.8% (53/223) (*p= <.001*) respectively. Gender was differently distributed as males predominated among those with B subtypes (55%, n=60 *vs.* 45%, n=49; *p=.001*), while females were more prevalent among individuals carrying non-B subtypes (64.4%, n=150 *vs.* 35.6%, n=84; *p=.001*).

Among non-Europeans patients with known risk factor, heterosexuals (HEs) predominated (134/175, 76.6%) and females accounted for 58.2% (n=199). Analogously, among patients carrying non-B subtype with known risk factor, HEs were largely prevalent (n=248, 82.3%) but gender was equally distributed (50.2%, n=255 *vs.* 49.8%, n=253). Median age significantly increased, while median CD4 cell counts and median HIV-1 RNA levels did not differ over time.

Drug experience and resistance evaluation

Globally, cART included NRTIs+NNRTIs in 19.7% (n=608), NRTIs+PIs in 58% (n=1,796), NRTIs+NNRTIs+PIs in 2.1% (n=66), NNRTIs+PIs in 4.3% (n=132), NRTIs INSTIs in 12.9% (n=496), NNRTIs+INSTIs in 2.9% (n=112) and PIs+INSTIs in 12.3% (n=476). While NRTIs+PIs decreased from 63.9% to 41.7% (*p<.001*) in the studied biennia NRTIs+INSTIs and PIs+INSTIs increased from 7.3% to 33.8% (p<.001) and from 8.3% to 12.1% (p<.001), respectively. The frequency of use of NRTIs+NNRTIs, NNRTIs+PIs, NRTIs+NNRTIs+PIs and NNRTIs+INSTIs remained stable. Regarding INSTIs the use of RAL decreased from 100% to 39.5%, while ELV and DTG increased from 0% to 18.6% and to 42.5%, respectively.

According to both IAS tables and Stanford HIVdb algorithm, resistance to at least one class increased (*p=.019*), while resistance to two and three class significantly decreased (*p<.001* and *p=.013*) overtime (Table 1).

Considering the IAS list of mutations, overall resistance to any class was 67.8% (n=2,098) and decreased from 75.1% (n=810) to 64.6% (n=114) (*p<.001*) in the 2009-2018 interval. As shown in Figure 1 (part A) resistance to NRTIs, NNRTIs and PIs declined significantly from 61.7% (n=666) to 43.5% (n=97), from 44.7% (n=482) to 40.4% (n=90) and from 36.4% (n=393) to 30% (n=67), respectively, while that to INSTIs slightly decreased from 33.6% (n=39) to 27.4% (n=54) (*p=.068*). However, by using the Stanford HIVdb interpretation system, i.e. evaluating intermediate to high resistance, the prevalence of resistance was lower declining from 65.9% (n=711) to 45.3% (n=101) for any resistance, from 58.1% (n=627) to 34.5% (n=77) for NRTIs, from 35.5% (n=383) to 31.4% (n=70) for NNRTIs, from 27.6% (n=298) to 11.2% (n=25) for PIs. Of note, INSTI resistance declined significantly from 31% (n=36) to 20.8% (n=41) (Figure 1, part B).

When analyzing by IAS tables the prevalence of drug resistance according to country of origin, a higher frequency of any and PI resistance was observed in non-Europeans compared with Europeans (Table 2). Although the two interpretation systems indicated comparable rates of NRTI and NNRTI resistance in European individuals, divergent data were shown according to IAS tables for any, PI and INSTI resistance. Indeed, 1.6-, 5- and 1.8-fold reductions were detected for non-European subjects when using HIVdb. Accordingly, similar figures were obtained for the proportion of any, PI and INSTI resistance in non-B subtypes by IAS *vs.* HIVdb (79.7% *vs* .45.5%, 64.2% *vs.* 12.6% and 39.9% *vs.* 29.4%, respectively).

Trends of major resistance mutations

We observed in RT region a significant decrease overtime of M184V (from 48.8% to 22.9%, *p=<.001*) and TAMs (from 41.4% to 22%, *p=<.001*) associated to NRTI resistance; a significant decrease was also present for K103N (from 19.5% to 12.1%, *p=<.001*) while a significant increase was found for Y181C (from 8.2% to 13.9%, *p=.019*), associated to NNRTI resistance.

Regarding PR region we found a decrease of M46I (from 14.2% to 4.5%, *p=<.001*), I47V (from 4.2% to 1.3%, *p=.003*), I54L (from 5.2% to 1.3%, *p=.018*), I54M (from 2.9% to 0.9%, *p=<.001*), L76V (from 2.3% to 0.9%,

p=.001), V82A (from 8.3% to 1.8%, *p=<.001*), V82T (from 2% to 0.9%, *p=.003*), V82L (from 0.6% to 0%, *p=.040*), I84V (from 11.3% to 4.5%, *p=<.001*), N88S (from 0.5% to 0%, *p=.038*) and L90M (from 13.5% to 7.2%, *p=<.001*).

In the IN region we observed the decrease of G140S (from 12.9% to 0%, *p=.003*), Y143C (from 1.7% to 0.5%, *p=.015*), Q148H (from 13.3% to 2.5%, *p=<.001*), and the increase of S147G (from 0% to 4.1%, *p=.004*) and Q148R (from 2.6% to 6.1%, *p=.013*).

Predictors of acquired resistance

Table 3 shows the univariate and multivariate logistic regression analysis investigating predictors of treatment emerging resistance to PR and RT inhibitors. The multivariate analysis indicated an increased risk of resistance for patients with a higher number of treatment lines (*p<.001*) and with higher levels of plasma viremia, namely 3 to 4 and 4 to 5 log as compared to <3 log (*p<.001* and *p=.001,* respectively). A reduced probability of resistance was detected in patients failing a regimen in the last biennium of the study (*p<.001*) and among injecting drug users (IDUs) compared to HEs (*p=.027*). In the subset with INSTI therapy, the multivariate analysis confirmed an increased risk of resistance for patients with a larger number of treatment lines *(p=.001*) and 3-4 and 4-5 log plasma viremia compared with <3 log (*p=.012* and *p=.015*, respectively), and a reduced risk for MSM (*p=.032*). Moreover, older patients were associated with a higher risk of ADR (*p=.003*) (data not shown).

Comparable results were obtained in the multivariate logistic regression analysis when considering subtype instead of country of origin (OR for any resistance in non B vs. B subtypes 1.08, p=.121, 95% Confidence Interval 0.84-1.39) and time on ART instead of number of previous lines (OR for any resistance per 1 more year on antiretroviral therapy 1.10, p<.001, 95% Confidence Interval 1.08-1.12): in both cases the remaining correlates of ADR did not change their significance in the multivariate model.

Discussion

HIV drug resistance is recognized as a relevant worldwide issue and the estimation of its burden and evolution remains mandatory. Moreover, Mediterranean countries have been the destination of substantial migration from Sub Saharan Africa, South America and Eastern Europe in the last decade. This implied the entry and the spread of different viral variants with specific polymorphisms and different pathways to resistance. In this scenario, despite a declining use of resistance testing seen in the study period, we observed that around 55% of genotypes obtained at treatment failure had resistance to at least one of the drug classes included in the regimen. Our estimates are comparable to those obtained from other European cohorts although the Swiss cohort reported lower incidence of ADR in recent years, [6] [14]. As reported in other studies [7], globally resistance to NRTIs was more frequent than that to NNRTIs, PIs and INSTIs (46.2%, 31.9%, 20.6% and 27.2%, respectively).

We found that drug resistance prevalence for all classes in subjects failing cART decreased from 2009 to 2018 suggesting a relevant role of new cART regimens that are more tolerable and potent. The most important class resistance drop was observed for all the antiretroviral classes from the first to the second biennium of study, without a stable reduction in the following biennia. The most important decline was observed for NRTIs (from 58.1% to 34.5%) but even resistance to INSTI showed a significant decline (from 31% to 20.8%) despite a peak in 2013-2014 (35.6%). Indeed, it is well known that emerging INSTI resistance is not uncommon in patients who fail a first-line RAL- or EVG-based regimen [15][16], while it is rarely observed in patients failing regimens based on DTG with high genetic barrier [17][18].

The frequency of resistance mutations varied significantly over time. Thymidine analogue mutations (TAMs), known to reduce viral susceptibility to NRTIs, halved from 41.4% to 22% in the study interval. This decline may be explained by decreased use of zidovudine and stavudine, which primarily tend to select TAMs [19]. Likewise, K103N, mutation present in patients failing regimens containing first-generation NNRTIs, present in 15.4% of cases showed a significant decrease together with introduction of second-generation NNRTIs. However, also M184V, typically selected by common-used lamivudine and emtricitabine, halved in the study period (from 50% to 23%). Although we did not address the relation between such decrease and viral load in patients showing or lacking this mutation the self reported adherence to treatment may imply that the emergence of M184V is constrained when lamivudine and/or emtricitabine are accompanied by potent antiretrovirals at high genetic barrier [18].

Patients who started therapy more recently have a lower prevalence of ADR compared to individuals who started at earlier calendar years. Similar findings were observed in Switzerland [5], Canada [6] and in a multicentre European cohort [4] suggesting that patients who initiated treatment in more recent years do not acquire drug resistance due to the overall efficacy of new regimens. Subjects who experienced a larger number of regimens showed an increased risk of ADR compared to those at first or second failure. Prolonged treatment history and repeated failures are major drivers of emergent resistance to all classes including INSTIs, even though recent data demonstrated no impact of previous NRTI resistance mutations on response to therapy based on NRTIs plus a high genetic barrier INSTI such as DTG or BIC [20].

In contrast to previous Italian data [21], we observed a reduced risk of emergent resistance for MSM and IDUs compared with HEs. This might be consequent to focused prevention strategies concerning the evolution of drug resistance and medical surveillance in historical high-risk categories.

Our data did not show an association of country of origin and non-B subtype with an increased risk of ADR. Moreover, even though the occurrence of polymorphisms may increase the prevalence of any resistance mutation among non-European subjects who are more frequently carrying non B subtypes, the role of such a correlate does not seem relevant for the emergence of ADR impacting on clinical outcomes [10].

Of note, we observed a considerable discrepancy between ADR frequencies obtained using IAS tables and Stanford HIVdb algorithm interpretation. While relevant for clinical practice, this divergence could be expected because IAS solely indicates mutations and their potential on resistance, while HIVdb quantitatively translates the number and type of mutations and their combinations into levels of drug resistance. A number of studies demonstrated that the grading of resistance as low, intermediate or high is more accurate to evaluate the impact on response to therapy [22]. Furthermore, the high frequency of mutations detected for PIs and INSTIs by IAS may be explained by the presence of natural polymorphisms in non-B subtypes which can contribute to resistance in the presence of major mutations but do not have an impact on their own [23], [24], [25].

Our study presents some limitations. The most important is the lack of information regarding the total amount of patients who failed therapy in each biennium and specifically the number of those who did not received a genotypic test. In addition, it was not possible to evaluate the correlation between different drug regimens and development of resistance. Another limit of our work is the choice to evaluate the burden of antiretroviral resistance regardless of the drug regimen ongoing at time of failure; this perspective may reduce the capability to detect the evolution of ADR inside specific treatment groups and antiviral class exposure [21]. Nevertheless, the strategy adopted had the purpose to obtain an estimate of the burden of class resistance in all subjects failing antiretroviral therapy, as previously performed [4]. In conclusion, we demonstrated that the emergence of HIV-1 drug resistance has been decreasing in last decade in Italy with the introduction of new drugs and updated treatment strategies. Although the rate of treatment emergent resistance is hopefully expected to further decrease in the future, our findings supports the continuing need for ADR monitoring at treatment failure, particularly in the context of ongoing migration from areas where non-B subtypes are dominant and the most effective therapies may still remain not largely available for some time in low income countries. Furthermore, to optimize the care of immigrants treated with antiretrovirals, infectious disease physicians may carefully consider that results of genotype test may need of interpretation through algorithms that take into account specific subtypes.

Figure legend

Figure 1: Time trends of resistance to different antiretroviral drug classes according to at least one mutation by IAS tables (A) and intermediate and high resistance level by Stanford HIVdb algorithm (B).

Transparency declaration

The authors report no conflicts of interest relevant to this letter. No external funding was received for this study.

Acknowledgements

The authors would like to thank all members of the centers of the ARCA collaborative Group.

Authors' contributions

AL, MF, CB and AB conceived and designed the study. FS, BB, SDG, ADB, SLC, MMS, FM, SGP, SR and NG collected the epidemiological and viral data of patients. AL, MF, CB and AB wrote the first draft of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

References

[1] Richman DD, Morton SC, Wrin T, Hellmann N, Berry S, Shapiro MF, et al. The prevalence of antiretroviral drug resistance in the United States. AIDS (London, England) 2004;18:1393–401.

[2] C. T, J. F, C. T, N. Y. Resistance of HIV-1 to multiple antiretroviral drugs in France: A 6-year survey (1997-2002) based on an analysis of over 7000 genotypes. Aids 2003;17:2383–8.

[3] Scott P, Arnold E, Evans B, Pozniak A, Moyle G, Shahmenesh M, et al. Surveillance of HIV antiretroviral drug resistance in treated individuals in England: 1998-2000. The Journal of Antimicrobial Chemotherapy 2004;53:469–73.

[4] 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. The Journal of Infectious Diseases 2013;207:1216–20.

[5] Scherrer AU, von Wyl V, Yang W-L, Kouyos RD, Boni J, Yerly S, et al. Emergence of Acquired HIV-1 Drug Resistance Almost Stopped in Switzerland: A 15-Year Prospective Cohort Analysis. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America 2016;62:1310–7.

[6] Rocheleau G, Brumme CJ, Shoveller J, Lima VD, Harrigan PR. Longitudinal trends of HIV drug resistance in a large Canadian cohort, 1996-2016. Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 2018;24:185–91.

[7] Assoumou L, Charpentier C, Recordon-Pinson P, Grude M, Pallier C, Morand-Joubert L, et al. Prevalence of HIV-1 drug resistance in treated patients with viral load >50 copies/mL: a 2014 French nationwide study. The Journal of Antimicrobial Chemotherapy 2017;72:1769–73.

[8] Lepik KJ, Harrigan PR, Yip B, Wang L, Robbins MA, Zhang WW, et al. Emergent drug resistance with integrase strand transfer inhibitor-based regimens. AIDS (London, England) 2017;31:1425–34.

[9] Gunthard 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. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America 2019;68:177–87.

[10] Franzetti M, Violin M, Casazza G, Meini G, Callegaro A, Corsi P, et al. Human immunodeficiency virus-1 B and non-B subtypes with the same drug resistance pattern respond similarly to antiretroviral therapy. Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 2012;18:E66-70.

[11] Rhee S-Y, Taylor J, Fessel WJ, Kaufman D, Towner W, Troia P, et al. HIV-1 protease mutations and protease inhibitor cross-resistance. Antimicrobial Agents and Chemotherapy 2010;54:4253–61.

[12] Low A, Prada N, Topper M, Vaida F, Castor D, Mohri H, et al. Natural polymorphisms of human immunodeficiency virus type 1 integrase and inherent susceptibilities to a panel of integrase inhibitors. Antimicrobial Agents and Chemotherapy 2009;53:4275–82.

[13] Wensing AM, Calvez V, Gunthard HF, Johnson VA, Paredes R, Pillay D, et al. 2017 Update of the Drug Resistance Mutations in HIV-1. Topics in Antiviral Medicine 2017;24:132–3.

[14] Abela IA, Scherrer AU, Boni J, Yerly S, Klimkait T, Perreau M, et al. Emergence of drug resistance in the Swiss HIV Cohort Study under potent antiretroviral therapy is observed in socially disadvantaged patients. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America 2019.

[15] Kulkarni R, Abram ME, McColl DJ, Barnes T, Fordyce MW, Szwarcberg J, et al. Week 144 resistance analysis of elvitegravir/cobicistat/emtricitabine/tenofovir DF versus atazanavir+ritonavir+emtricitabine/tenofovir DF in antiretroviral-naive patients. HIV Clinical Trials 2014;15:218–30.

[16] Rockstroh JK, DeJesus E, Lennox JL, Yazdanpanah Y, Saag MS, Wan H, et al. Durable efficacy and safety of raltegravir versus efavirenz when combined with tenofovir/emtricitabine in treatment-naive HIV-1-infected patients: final 5-year results from STARTMRK. Journal of Acquired Immune Deficiency Syndromes (1999) 2013;63:77–85.

[17] Raffi F, Wainberg MA. Multiple choices for HIV therapy with integrase strand transfer inhibitors. Retrovirology 2012;9:110.

[18] White KL, Raffi F, Miller MD. Resistance analyses of integrase strand transfer inhibitors within phase 3 clinical trials of treatment-naive patients. Viruses 2014;6:2858–79.

[19] Larder BA, Kemp SD. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). Science (New York, NY) 1989;246:1155–8.

[20] Andreatta K, Willkom M, Martin R, Chang S, Wei L, Liu H, et al. Switching to bictegravir/emtricitabine/tenofovir alafenamide maintained HIV-1 RNA suppression in participants with archived antiretroviral resistance including M184V/I. The Journal of Antimicrobial Chemotherapy 2019;74:3555–64.

[21] 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 Infectious Diseases 2014;14:398.

[22] Paredes R, Tzou PL, van Zyl G, Barrow G, Camacho R, Carmona S, et al. Collaborative update of a rule-based expert system for HIV-1 genotypic resistance test interpretation. PloS One 2017;12:e0181357.

[23] Abecasis AB, Deforche K, Bacheler LT, McKenna P, Carvalho AP, Gomes P, et al. Investigation of baseline susceptibility to protease inhibitors in HIV-1 subtypes C, F, G and CRF02_AG. Antiviral Therapy 2006;11:581–9.

[24] Brenner BG, Lowe M, Moisi D, Hardy I, Gagnon S, Charest H, et al. Subtype diversity associated with the development of HIV-1 resistance to integrase inhibitors. Journal of Medical Virology 2011;83:751–9.

[25] Doyle T, Dunn DT, Ceccherini-Silberstein F, De Mendoza C, Garcia F, Smit E, et al. Integrase inhibitor (INI) genotypic resistance in treatment-naive and raltegravir-experienced patients infected with diverse HIV-1 clades. The Journal of Antimicrobial Chemotherapy 2015;70:3080–6.

Table 1. Characteristics of patients according to the year of genotype testing.

	2009-2010 (N=1,079)	2011-2012 (N=793)	2013-2014 (N=596)	2015-2016 (N=403)	2017-2018 (N=223)	*p*	Total (N=3,094)
Gender							
Males, n (%)	686 (63.6)	509 (64.2)	375 (62.9)	250 (62.0)	135 (60.5)	*.857*	1,955 (63.2)
Country of origin							
Europeans, n (%)	793 (88.5)	567 (86.2)	417 (86.5)	248 (83.2)	139 (80.8)	*.002*	2,164 (86.4)
Subtype							
B, n (%)	938 (86.9)	678 (85.5)	482 (80.9)	318 (78.9)	170 (76.2)	*<.001*	2,586 (83.6)
Age, median (IQR)	45 (40-50)	47 (41-51)	48 (42-52)	50 (42-54)	52 (44-55)	*<.001*	48 (42-52)
Mode of infection							
HEs	365 (33.8)	228 (28.8)	174 (29.2)	92 (22.8)	40 (17.9)	*<.001*	899 (29.1)
IDUs	341 (31.6)	266 (33.5)	166 (27.9)	100 (24.8)	46 (20.6)		919 (29.7)
MSM	136 (12.6)	88 (11.1)	43 (7.2)	31 (7.7)	16 (7.1)		314 (10.1)
Other/unknown	237 (22.0)	211 (26.6)	213 (35.8)	180 (44.7)	121 (54.2)		962 (31.1)
pVL at GRT, median (IQR), log_{10} copies/ml	3.1 (2.2-4.3)	3.2 (2.0-4.1)	3.2 (2.3-4.0)	3.0 (2.1-4.3)	3.1 (2.2-4.2)	*.593*	3.2 (2.0-4.1)
CD4 cell count at GRT, median (IQR), cells/mm^3	355 (189-505)	350 (206-520)	350 (189-547)	398 (186-585)	300 (153-523)	*.351*	321 (170-480)
One-class resistance, n (%)	271 (25.1)	215 (27.1)	173 (29.0)	123 (30.5)	66 (29.6)	*.019*	848 (27.4)
Two-class resistance, n (%)	347 (32.2)	186 (23.5)	111 (18.6)	79 (19.6)	46 (20.6)	*<.001*	769 (24.9)
Three-class resistance, n (%)	192 (17.8)	117 (14.8)	93 (15.6)	47 (11.7)	32 (14.3)	*.013*	481 (15.5)

GRT, Genotyping Resistance test; HEs, Heterosexuals; IDUs, Injecting Drug Users; MSM, Men who have Sex with Men; pVL, plasma Viral Load; IQR, Interquartile Range

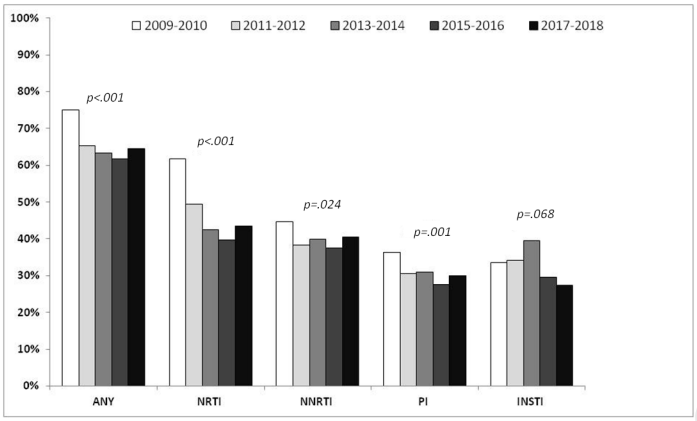
Table 2. Comparison of drug resistance frequencies between IAS tables and Stanford HIVdb algorithm results.

	IAS Tables			STANFORD HIVdb		
	Europeans	**Non-Europeans**	***p***	**Europeans**	**Non-Europeans**	***p***
Any, % (n)	67.3 (1,456)	79.2 (271)	*<.001*	57.1 (1,236)	48.2 (165)	*.002*
NRTI, % (n)	53.1 (1,150)	36.5 (125)	*<.001*	48.1 (1,041)	35.7 (122)	*<.001*
NNRTI, % (n)	42.1 (912)	35.7 (122)	*.028*	32.6 (705)	30.7 (105)	*ns*
PI, % (n)	28.9 (626)	58.5 (200)	*<.001*	22.2 (481)	12 (41)	*<.001*
INSTI, % (n)	34.6 (250)	31.4 (38)	*.557*	30.6 (221)	17.4 (21)	*.004*

Table 3. Logistic regression model of acquired HIV-1 PI, NRTI and NNRTI resistance predictors in 3,094 subjects enrolled in the ARCA cohort from 2009 to 2018.

	Univariate			Multivariate		
	OR	(95% CI)	*p*	OR	(95% CI)	*p*
GENDER, Females *vs.* Males	1.07	0.93-1.24	*.343*	0.98	0.78-1.22	*.839*
MODE OF INFECTION						
HEs	–	–	–	–	–	–
MSM	1.03	0.79-1.33	*.855*	0.70	0.45-1.07	*.099*
IDUS	0.91	0.75-1.09	*.297*	0.73	0.55-0.97	*.027*
Other/Unknown	0.74	0.61-0.90	*.002*	0.78	0.58-1.05	*.097*
AGE (per 10 years older)	1.00	1.00-1.00	*.475*	1.00	1.00-1.00	*.717*
COUNTRY OF ORIGIN, Non-Europeans *vs.* Europeans	1.43	1.14-1.80	*.002*	1.25	0.93-1.69	*.145*
HIV-RNA						
<3 Log cp/ml	–	–	–	–	–	–
3-4 Log cp/ml	1.74	1.41-2.15	*<.001*	1.69	1.30-2.19	*<.001*
4-5 Log cp/ml	1.48	1.18-1.86	*.001*	1.61	1.20-2.15	*.001*
>5 Log cp/ml	0.89	0.67-1.19	*.439*	0.80	0.55-1.16	*.241*
CD4 cell count (per 50 cells/mmc higher)	0.99	0.98-1.01	*.888*	1.01	0.99-1.03	*.110*
STUDY PERIOD (per 1 biennium higher)	0.78	0.74-0.83	*<.001*	0.77	0.70-0.85	*<.001*
NUMBER OF PRIOR ARV REGIMENS (per 1 higher)	1.10	1.09-1.12	*<.001*	1.13	1.10-1.15	*<.001*

A



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