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Genotypic HIV-1 tropism determination might help to identify people with exhausted treatment options and advanced disease

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Objectives: To evaluate HIV-1 tropism in 1382 combined antiretroviral therapy (cART)-experienced patients failing therapy to characterize those with exhausted therapeutic options.

Methods: HIV-1 genotypic tropism was inferred through Geno2Pheno by estimating the false-positive-rate (FPR) values. Cumulative resistance and drug activity were evaluated by Stanford algorithm.

Results: Overall, median (IQR) CD4 count (cells/mm^3) nadir and at last genotypic resistance test (GRT) available were 98 (33–211) and 312 (155–517), respectively. Considering HIV-1 tropism, 30.5% had X4/dual-mixed strains (FPR $\leq$5%: 22.2%; FPR 5%–10%: 8.3%). By stratifying according to tropism, by decreasing FPR, a significant decrease of CD4 nadir and at last GRT was observed. The proportion of individuals with CD4 count <200 cells/mm^3, who were perinatally infected and with a long treatment history significantly increased as FPR levels decreased. Regarding resistance, 933 (67.5%) individuals accumulated at least one class resistance, with 52.7%, 48.2%, 23.5% and 13.2% of individuals showing resistance to NRTIs, NNRTIs, PIs and INIs; while 23.2%, 27.2%, 14.3% and 2.8% harboured resistance to 1, 2, 3 and 4 classes, respectively. Individuals with FPR $\leq$5% showed a significantly higher level of resistance to PIs, NRTIs and INIs compared with others. The proportion of individuals harbouring strains susceptible to $\leq$2 active drugs was only about 2%; nonetheless, this proportion doubled (4.6%) in patients infected with FPR $\leq$5%.

Conclusions: Our findings showed that a small proportion of cART failing individuals have limited therapeutic options. However, tropism determination might help to identify people who have accumulated a high level of resistance and have a greater risk of advanced disease.

Introduction

The availability of highly effective and safe antiretroviral (ARV) drugs for the management of human immunodeficiency virus type 1 (HIV-1) infection has considerably lengthened the life span of HIV-infected individuals, but these drugs require a lifelong administration. Despite the overwhelming success achieved using combined antiretroviral therapy (cART), some HIV-infected individuals continue to fail therapy. This is, in part, due to drug resistance,[1,2] whose management still requires an appropriate diagnostic and therapeutic approach. In fact, although a dramatic drop of the overall HIV-1 drug resistance at failure has been achieved in recent years, HIV multidrug resistance still remains a persistent silent epidemic of clinical concern.[2,3] In this context, heavily treatment-experienced (HTE) patients in particular represent a fragile population for whom only limited therapeutic options might be

available. Because of this, tropism testing is generally performed as it is a requirement when the use of a CCR5 antagonist is being considered.[4,5] Some studies[6–12] had previously attempted to determine the HIV-1 co-receptor usage among treatment-experienced HIV-1-infected individuals. To date, the association of viral tropism with immunological parameters and perinatal HIV transmission among failing patients is well documented.[12–18] However, most of these studies had a limited sample size and more recent data might be also useful. Moreover, in cART-failing patients, resistance to three or more drug classes is low but still constantly present, therefore they have limited treatment options.[3] There is also a scarcity of data on the characterization of HIV-1 tropism among these multidrug resistant (MDR) HIV-infected individuals. Thus, the aim of this study was to evaluate the HIV-1 tropism (according to false-positive rate levels) in a large cohort of cART-failing patients to characterize those with exhausted treatment options.

Patients and methods

Study population

HIV-1-infected drug-experienced individuals failing cART with at least one available plasma genotypic resistance test (GRT) for protease/reverse transcriptase and gp120-V3 were analysed. Integrase GRTs were also considered in the analysis when available. Only those patients whose therapeutic history was completely available were included in the study.

Ethics

This is a retrospective observational study, conducted on data collected for clinical purposes. All data used in the study were previously anonymized, according to the requirements set by the EU Regulation 2016/679 and by the Italian Data Protection Code. Written informed consent for medical procedures/interventions performed for routine treatment purposes was collected from each patient. The research was conducted on anonymous samples in accordance with the principles of the Declaration of Helsinki and the Italian Ministry of Health. All information, including virological and clinical data, was recorded in an anonymized database.

Genotyping and viral tropism determination

Plasma genotypic resistance test results for protease, reverse transcriptase and integrase were obtained through commercially available kits (ViroSeq HIV-1 Genotyping System, Abbott Molecular, Des Plains, IL, USA; Trugene-HIV-1 Genotyping-Kit, Bayer HealthCare LLC, Tarrytown, NY, USA) and/or a homemade system, as previously described.[19,20] All V3 sequences were obtained using a homemade protocol for Sanger sequencing, also as previously described.[21] HIV-1 tropism was inferred through Geno2Pheno (G2P, https://coreceptor.geno2pheno.org/), by estimating the false-positive rate (FPR) values. In patients for whom more than one V3-GRT was available, the lowest FPR value available in the patients' history was considered to determine the tropism. G2P was set at an FPR of ≤10% to determine viral tropism as recommended.[22] Thus, individuals with an FPR >10% were considered to be harbouring R5 tropic viruses, while those with an FPR ≤10% were considered infected with X4/dual-mixed tropic viruses. FPR values in X4/dual-mixed tropic specimens were further stratified into two levels: ≤5% and 5%–10%, according to previous observations, highlighting that X4-tropic variants are present with the highest percentage for FPR values below 5% and are independently associated with an increased risk of virological failure to maraviroc-containing regimens.[23–26] Moreover, an additional analysis was performed by stratifying FPR values >10% in two ranges: 10%–60% and >60% according to the previous observations that for FPR >60%, only R5-tropic viruses are found.[23] Therefore, the characteristics of patients analysed were evaluated in the overall dataset and according to the following FPR ranges: (i) ≤5%, 5%–10% and >10% and (ii) ≤5%, 5%–10%, 10%–60% and >60%.

Drug resistance evaluation

Resistance mutations were evaluated using the Stanford resistance list 2019 (HIVdb version 8.9-1, https://hivdb.stanford.edu/). For each individual, the plasma cumulative resistance was evaluated by considering the resistance detected in all available plasma GRTs. The class-resistance was defined by the presence of at least one major resistance mutation to protease inhibitors (PI), nucleos(t)ide reverse transcriptase inhibitor (NRTI), non-NRTI (NNRTI) and integrase inhibitor (INI, when integrase GRT was available). MDR individuals were defined as those in whom viral resistance had accumulated to at least three drug classes among those mentioned above.

Evaluation of drug activity by cumulative genotypic susceptibility score

The calculation of the genotypic susceptibility score (GSS) for each drug among those listed in the Stanford HIV database version 8.9-1 was carried out using the Stanford algorithm by imputing all cumulative mutations detected in previous GRTs (https://hivdb.stanford.edu/hivalg/by-mutations/). Each drug was considered fully susceptible or not susceptible according to algorithm results. Concerning INIs, patients for whom an integrase GRT was not available were considered infected with viruses susceptible to this class, if they never failed or were never previously exposed to INIs. This assumption was based on the fact that in Italy the prevalence of primary INI resistance in INI-naive individuals (either drug-naive or drug-experienced) is very low (≤1%).[27–29]

With regards to entry inhibitors, patients who harboured R5 viral strains (FPR ≥10%) were considered infected by viruses susceptible to maraviroc. Patients that had never taken enfuvirtide (T20) in their treatment history were considered infected by strains susceptible to this drug.[30] Based on all these considerations, patients were defined as having exhausted treatment options when found to be harbouring strains susceptible to ≤2 ARV drugs.

Statistical analysis

All the analyses were performed using the software package SPSS version 20.0 for Windows (SPSS Inc., Chicago, Illinois). The associations between different FPR levels and demographic, viro-immunological, therapeutic and resistance parameters were investigated by Chi-Squared for trend or Kruskal-Wallis test, as appropriate. The significance level was set at $P < 0.05$.

Results

Patient characteristics

The demographic, viro-immunological and therapeutic characteristics of patients at the last GRT are reported in Table 1. Overall, 1382 cART-failing individuals with a median (IQR) time on therapy of 10 (4–16) years were analysed. The median (IQR) age was 46 (39–52) years, and most of them were males (68.1%). The most common HIV transmission routes were the heterosexual route (32.4%) and drug abuse (31.6%). Notably, 3.7% of the individuals were perinatally infected and had a median (IQR) age of 24 (16–28) years. Regarding HIV-1 subtype, most of the individuals were infected with B subtype (78.6%).

Looking at the immunological and virological parameters, the median (IQR) CD4 cell count (cells/mm^3) nadir and at the last GRT available were 98 (33–211) and 312 (155–517), respectively;

Table 1. Characteristics of 1382 HIV-1-infected individuals failing cART, overall and stratified according to false-positive rate (FPR) levels

Characteristics	Overall ($N = 1382$)	FPR levels (tropism)[a] ≤5% (X4/DM) ($N = 307$, 22.2%)	5%–10% (X4/DM) ($N = 114$, 8.3%)	>10% (R5) ($N = 961$, 69.5%)	*P* value[b]
Male, *n* (%)	941 (68.1)	214 (69.7)	77 (67.5)	650 (67.6)	0.519
Age, years, median (IQR)	46 (39–52)	46 (38–52)	46 (41–54)	46 (39–52)	0.526
Risk factor, *n* (%)					
Homosexual	256 (18.5)	57 (18.6)	27 (23.7)	172 (17.9)	0.609
Heterosexual	448 (32.4)	96 (31.2)	45 (39.5)	307 (31.9)	0.945
Drug abuser	437 (31.6)	93 (30.3)	31 (27.2)	313 (32.6)	0.359
Sexual[c]	84 (6.1)	**12 (3.9)**	**1 (0.9)**	**71 (7.4)**	**0.009**
Perinatal	51 (3.7)	**19 (6.2)**	**2 (1.8)**	**30 (3.1)**	**0.024**
Other/unknown	106 (7.7)	30 (9.8)	8 (7.0)	68 (7.1)	0.140
HIV-1 subtype, *n* (%)					
B	1085 (78.6)	**253 (82.4)**	**93 (81.6)**	**739 (76.9)**	**0.031**
CRF02_AG	79 (5.7)	16 (5.2)	6 (5.3)	57 (5.9)	0.615
F	58 (4.2)	15 (4.9)	1 (0.9)	42 (4.4)	0.943
C	53 (3.8)	**4 (1.3)**	**3 (2.6)**	**46 (4.8)**	**0.004**
Other	107 (7.7)	19 (6.2)	11 (9.6)	77 (8.0)	0.374
Number of years on cART, median (IQR)	10 (4–16)	10 (5–16)	11 (6–17)	10 (4–15)	0.131
Nadir CD4 count, cells/mm^3, median (IQR)	98 (33–211)	**45 (8–128)**	**108 (23–234)**	**116 (47–225)**	**<0.001**
CD4 count at last GRT, cells/mm^3, median (IQR)	312 (155–517)	**256 (87–441)**	**311 (125–517)**	**334 (179–550)**	**<0.001**
Individuals with CD4 < 200 cells/mm^3 at last GRT, *n* (%)	432 (31.4)	**127 (41.5)**	**39 (34.5)**	**266 (27.8)**	**<0.001**
Zenith viraemia, $\log_{10}$ (copies/mL), median (IQR)	5.43 (4.93–5.72)	5.46 (5.03–5.76)	5.44 (5.05–5.73)	5.42 (4.87–5.71)	0.259
Viraemia at last GRT, $\log_{10}$ (copies/mL), median (IQR)	3.59 (2.48–4.68)	3.42 (2.42–4.45)	3.73 (2.44–4.89)	3.63 (2.51–4.7)	0.360
Calendar year of GRT testing, *n* (%)					
<2010	262 (19.0)	54 (17.6)	18 (15.8)	190 (19.8)	0.322
2010–12	314 (22.7)	68 (22.2)	22 (19.3)	224 (23.3)	0.568
2013–15	437 (31.6)	95 (30.9)	42 (36.8)	300 (31.2)	0.901
>2015	369 (26.7)	90 (29.3)	32 (28.1)	247 (25.7)	0.198
History of exposure to enfuvirtide, *n* (%)	74 (5.4)	**32 (10.4)**	**1 (0.9)**	**41 (4.3)**	**<0.001**
History of exposure to maraviroc, *n* (%)	120 (8.7)	30 (9.8)	3 (2.6)	87 (9.1)	0.981
Number of regimens received at last GRT, *n* (%)					
1	214 (15.5)	**30 (9.8)**	**17 (14.9)**	**167 (17.4)**	**0.001**
2–5	635 (45.9)	142 (46.3)	52 (45.6)	441 (45.9)	0.923
6–10	360 (26.1)	83 (27.0)	31 (27.2)	246 (25.6)	0.588
>10	173 (12.5)	**52 (16.9)**	**14 (12.3)**	**107 (11.1)**	**0.009**

Abbreviations: cART, combined antiretroviral therapy; FPR, false positive rate; GRT, genotypic resistance test.
[a]Tropism was determined by Geno2Pheno algorithm: X4/DM with FPR ≤10%. In patients for whom more than one V3-GRT was available ($N = 537$), the lowest value of FPR was considered.
[b]Differences among all FPR levels were evaluated by using Chi-Squared for trend or Kruskal-Wallis test, as appropriate. Parameters significantly associated with FPR levels ($P < 0.05$) are shown in bold.
[c]Includes: bisexuals, transsexuals and people for whom their sexual behaviour was not specified.

whereas the median (IQR) viral load ($\log_{10}$ copies/mL) zenith and at the last GRT available were 5.43 (4.93–5.72) and 3.59 (2.48–4.68), respectively. Concerning previous drug exposure, 120 (8.7%) and 74 (5.4%) individuals were previously exposed to maraviroc and enfuvirtide, respectively.

By considering HIV-1 viral tropism, 421 (30.5%) patients were infected with X4/dual-mixed strains (FPR ≤ 10%): specifically, 307 (22.2%) had an FPR ≤5% and 114 (8.3%) had an FPR in the range of 5%–10% (Table 1). 961 (69.5%) patients were infected with R5-tropic strains [FPR 10%–60%, $n = 663$ (48.0%); FPR ≥60%, $n = 298$ (21.5%)].

By stratifying the population characteristics according to viral tropism, we have observed that with decreasing FPR values (from >10% to ≤5%), a significant decrease of the following

variables was found: CD4 cell count nadir [median (IQR) cells/mm^3: from 116 (47–225) to 45 (8–128), $P<0.001$]; CD4 cell count at last GRT available [median (IQR) cells/mm^3: from 334 (179–550) to 256 (87–441), $P<0.001$]; the proportion of HIV-1 subtype C-infected individuals (from 4.8% to 1.3%, $P=0.004$); the number of individuals who experienced only one regimen (from 17.4% to 9.8%, $P=0.001$) (Table 1). By contrast, the proportion of individuals infected with HIV-1 B subtype, with a CD4 cell count at last GRT of <200 cells/mm^3, who were perinatally infected, and experienced >10 previous regimens or were exposed to enfurvirtide, significantly increased with a decreasing of FPR levels (Table 1, $P<0.05$). No association between FPR levels and zenith or plasma viral load at the last GRT was found.

Resistance profile overall and stratified according to false positive rate levels

Regarding drug resistance, 933 (67.5%) individuals accumulated at least one class resistance among NRTIs, NNRTIs, PIs or INIs. NRTI resistance was the most prevalent (52.7%), followed by resistance to NNRTIs (48.2%), PIs (23.5%) and INIs (13.2%, Figure 1a). By considering FPR levels, compared with individuals with FPR 5%–10% and FPR >10%, individuals with FPR ≤5% showed a significantly higher prevalence of resistance to NRTIs, PIs and INIs (Figure 1b). No significant difference in the NNRTI resistance prevalence was observed among the three groups of FPR levels.

Considering the cumulative class resistance, we observed that 23.2% of the overall population harboured a resistant virus to 1 class, while 27.2% had resistance to 2 classes and 17.1% harboured MDR (14.3% to 3 classes and 2.8% to 4 classes; Figure 2a).

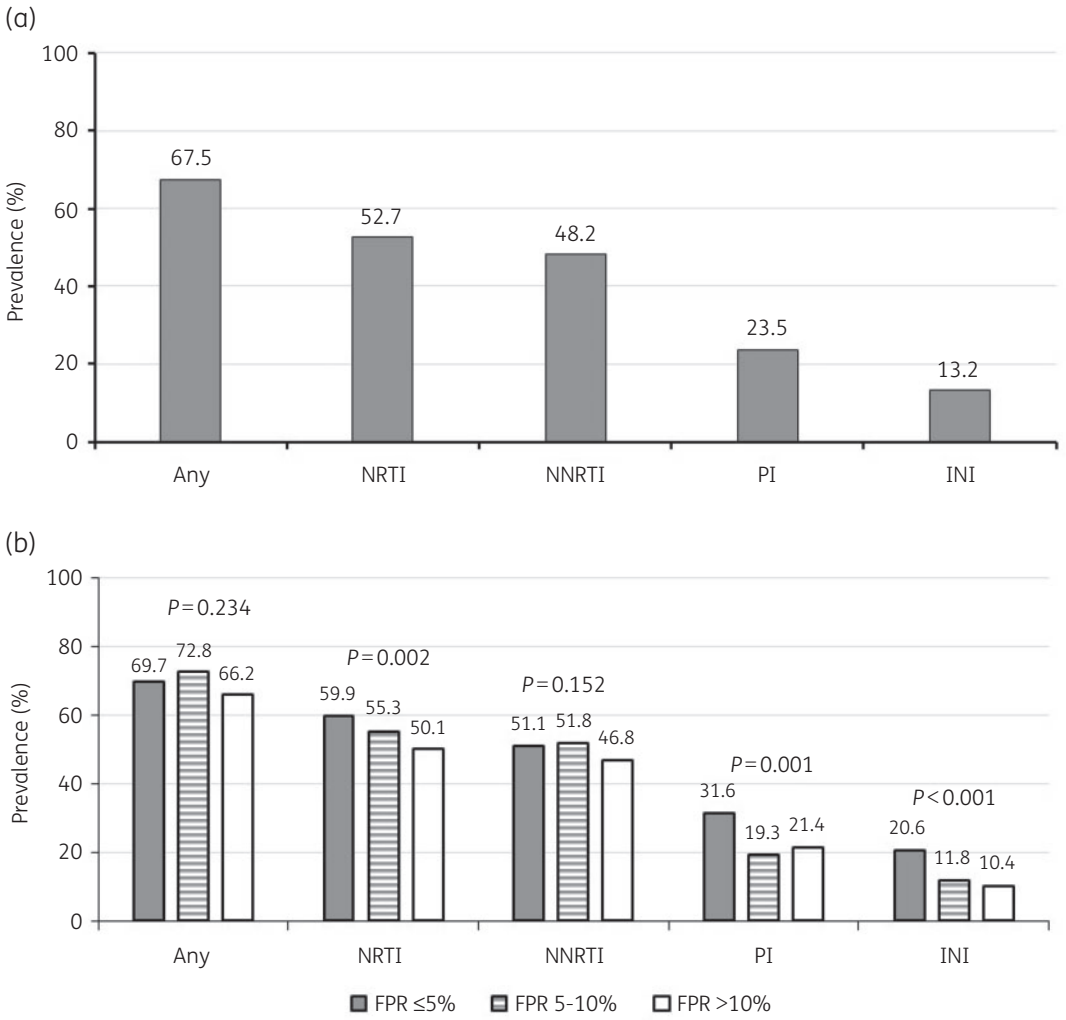

Figure 1. Prevalence of cumulative resistance. (a) Prevalence of cumulative resistance to NRTIs, NNRTIs, PIs and INIs in the overall population. (b) Prevalence of cumulative resistance to NRTIs, NNRTIs, PIs and INIs according to false-positive rate (FPR) levels. *P* values were calculated using chi-squared for trend. Integrase GRT was available for 780 patients. INI, integrase inhibitor; NNRTI, non-NRTI; NRTI, nucleos(t)ide reverse transcriptase inhibitor; PI, protease inhibitor.

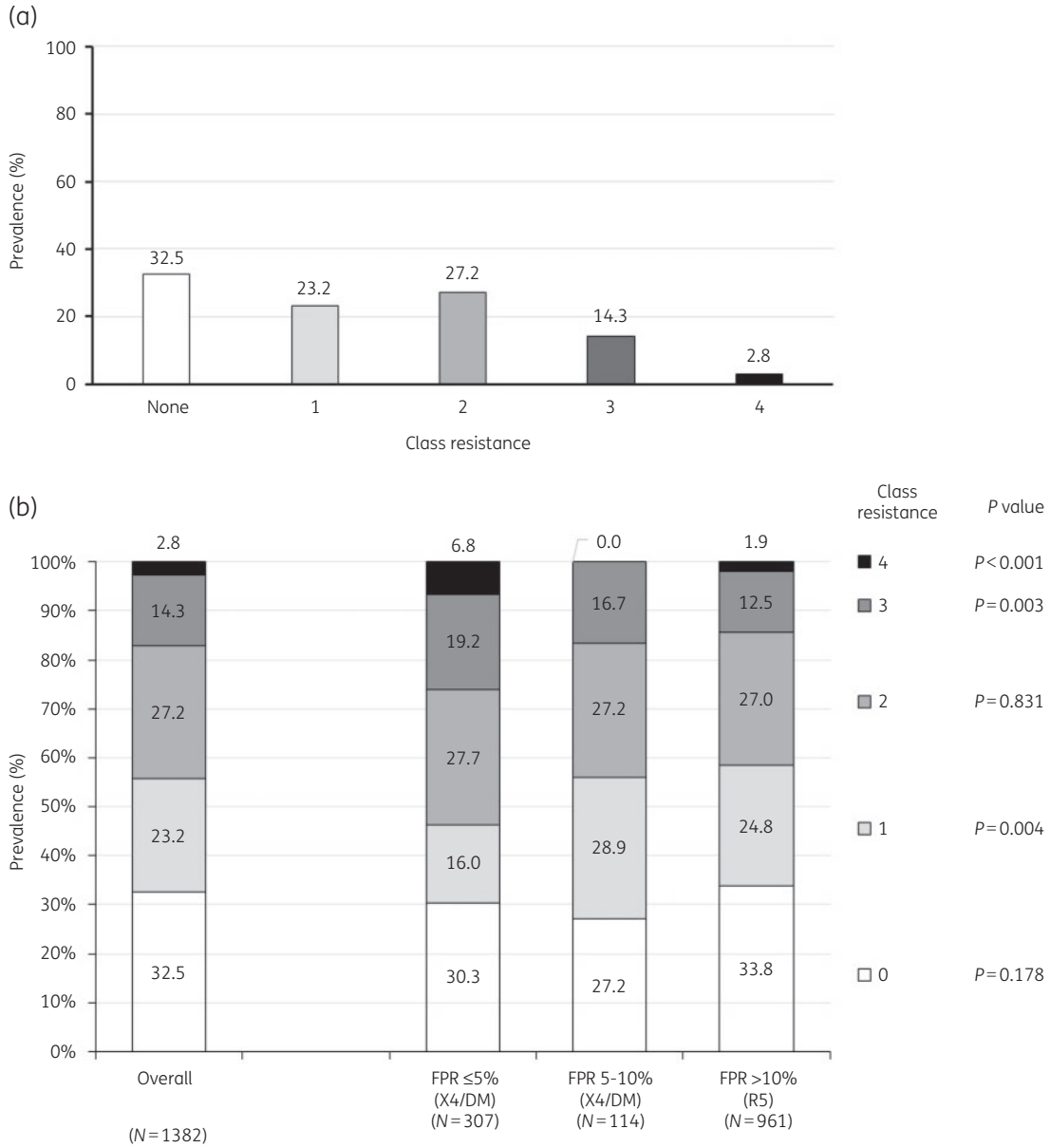

Figure 2. Prevalence of cumulative class resistance among NRTIs, NNRTIs, PIs and INIs. (a) Prevalence of cumulative class resistance in the overall population. (b) Prevalence of cumulative class resistance according to false-positive rate (FPR) levels. *P* values were calculated using chi-squared for trend. Integrase GRT was available for 780 patients. INI, integrase inhibitor; NNRTI, non-NRTI; NRTI, nucleos(t)ide reverse transcriptase inhibitor; PI, protease inhibitor.

By stratifying for FPR levels, we observed that, compared with individuals with FPR 5%–10% and FPR >10%, those with FPR ≤5% showed a significantly higher prevalence of resistance to four classes, three classes, and a significantly lower prevalence of resistance to one class (Figure 2b).

After a further stratification of R5-harbouring viruses into two groups (FPR 10%–60% and FPR >60%), no significant difference was found in terms of resistance to PIs, NRTIs, INIs, and MDR between the two strata ($P > 0.05$) (Table S1, available as Supplementary data at *JAC* Online).

Evaluation of drug activity by cumulative genotypic susceptibility score

Regarding the drug activity according to GSS, the overall number (IQR) of active drugs was 22 (15–26). It is notable that only about 2% ($N = 24$) of individuals had exhausted their treatment options; as expected all of them harboured MDR strains (18 with resistance to four classes and 6 with resistance to three classes). The proportion of individuals without any INI activity was 6.2% in the overall population and 7.6% in the 421 individuals infected by X4/dual-mixed strains (Table 2). By looking at drug activity according to FPR

Table 2. Drug activity by GSS among HIV-1-infected individuals failing cART, overall and stratified according to the false positive rate (FPR) levels

Characteristics	Overall (*N* = 1382)	FPR levels (tropism)[a] ≤5% (X4/DM) (*N* = 307, 22.2%)	5%–10% (X4/DM) (*N* = 114, 8.3%)	>10% (R5) (*N* = 961, 69.5%)	*P* value[b]
Number of active drugs per class, median (IQR)					
PIs[c]	8 (8–8)	**8 (2–8)**	**8 (8–8)**	**8 (8–8)**	**<0.001**
NRTIs[d]	4 (1–7)	**4 (0–7)**	**4 (2–7)**	**5 (2–7)**	**<0.001**
NNRTIs[e]	5 (1–5)	4 (0–5)	4 (2–5)	5 (1–5)	0.058
Patients without any active INI[f], *n* (%)	86 (6.2)	**28 (9.1)**	**4 (3.5)**	**54 (5.6)**	**0.011**
Individuals with ≤2 active drugs, *n* (%)	24 (1.7)	**14 (4.6)**	**0 (0.0)**	**10 (1.0)**	**<0.001**

Abbreviations: INI, integrase inhibitor; NNRTI, non-NRTI; NRTI, nucleos(t)ide reverse transcriptase inhibitor; PI, protease inhibitor.
[a]Tropism was determined by Geno2Pheno algorithm: X4/DM with FPR ≤10%. In patients for whom more than one V3-GRT was available (*N* = 537), the lowest value of FPR was considered. Parameters significantly associated with FPR levels ($P < 0.05$) are shown in bold. The calculation of GSS was carried out using the Stanford algorithm version 8.9-1.
[b]Differences among FPR levels were evaluated by using chi-squared for trend or Kruskal-Wallis test, as appropriate.
[c]PIs considered: atazanavir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, saquinavir and tipranavir.
[d]NRTIs considered: abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir disoproxil fumarate, tenofovir alafenamide fumarate and zidovudine.
[e]NNRTIs considered: doravirine, efavirenz, etravirine, nevirapine and rilpivirine.
[f]INIs considered: bictegravir, dolutegravir, elvitegravir and raltegravir.

levels, individuals with FPR ≤5% showed a significantly higher proportion of those with exhausted treatment options and those without any active INI, when compared with FPR values FPR 5%–10% and FPR >10% (Table 2). Moreover, individuals with FPR ≤5% showed a significantly lower number of active drugs among PIs and NRTIs, when compared with higher FPR levels (Table 2).

Discussion

In the present study we evaluated the HIV-1 viral tropism among cART-failing individuals tested for HIV-1 tropism for clinical purposes. Our study population was made up of individuals who were mostly highly treatment-experienced, with a median of 10 years under cART. The prevalence of X4/dual-mixed HIV-1 tropism found in our study (30.5%) is in line with those previously reported in similar settings.[11,12,31,32]

Besides tropism evaluation, we explored the levels of FPR, which provides additional information on X4 viral strains. Indeed, a low FPR (≤5%) was already associated with the presence of pure X4 strains[23] that might be present in patients with a long treatment history and at high risk of disease progression.[11,33] In our population, about 22% of the individuals harboured a strain with FPR ≤5%. In particular, a significant predominance of X4/dual-mixed strains with FPR ≤5% was found among perinatally infected individuals, as previously reported in other studies.[12–15] For these individuals, this association could be explained by the long treatment history and the possible tropism switch either as a result of the CCR5-antagonist use or viral evolution in the course of their HIV infection.[31,33–36] Moreover, the proportion of X4/dual-mixed strains with low FPR was significantly higher in patients who were previously exposed to enfuvirtide; this can be explained by the fact that these individuals showed a longer therapeutic history and lower CD4 counts (data not shown). On the other hand, we did not find an association between individuals harbouring X4 variants and a previous maraviroc exposure. This is probably because in our population, tropism testing was used to guide maraviroc administration, where basically the majority of patients who received maraviroc were R5 infected, and therefore responded to treatment without a tropism shift.

The presence of X4/dual-mixed strains might jeopardize the immunological status of HIV-1-infected patients as previously demonstrated in other studies.[16–18] Our findings confirm this observation; in fact, we found that individuals with low nadir CD4 counts and low CD4 counts at last GRT predominantly harboured X4/dual-mixed strains with low FPR. In contrast, no association was found between FPR levels and viral load.

Another factor that was associated with FPR levels was the HIV-1 subtype. In this regard, a recent study showed that in general, the G2P tropism determination might be challenging only for D and CRF01_AE non-B subtype where FPR cut-off adjustments are needed to avoid an excess of X4 predictions.[37] In our population, <1% of individuals were infected by these subtypes; thus, this issue does not affect our reliability of tropism determination.

Concerning the association between tropism and subtypes, we observed that non-B subtypes showed a higher proportion of R5-tropic viruses; however, contradictory reports exist in the literature on this point.[16,38,39]

In our analyses, an increased proportion of HIV-1 B subtype-infected individuals was found with decreasing FPR levels; by contrast, the proportion of C subtype-infected individuals significantly increased with increasing FPR values. This might be because HIV-1 B subtype-infected individuals had a longer treatment history compared with those infected with subtype C, which might be associated with a marked viral evolution toward X4 tropism. Moreover, the peculiarities of the conformational structure of HIV-1 subtype C in the V3 region of the envelope protein might also

explain the low propensity of this subtype to switch from R5- to X4-tropic viruses when compared with subtype B.[40]

Beyond viro-immunological and therapeutic factors, another important finding to point out is the fact that X4/dual-mixed tropism and FPR levels in our population are associated with drug resistance. In this regard, we found that, compared with individuals harbouring R5 strains (FPR >10%), those with FPR ≤5% significantly accumulated more resistance to PIs, NRTIs and INIs. This can be explained by the fact that these individuals showed a longer therapeutic history and a higher number of previous regimens received before GRT.

Moreover, the highest proportion of resistance to three and four drug classes was observed among individuals with X4/dual-mixed strains with low FPR (≤5%).

By evaluating drug genotypic susceptibility, we found that only about 2% of cART-failing individuals had ≤2 active drugs and had thus, exhausted treatment options. In addition, about 6% of individuals were not eligible to the entire class of INIs, showing resistance to all INIs of both first and second generation. In particular, the proportion of patients with exhausted treatment options and resistance to INI significantly increased with FPR ≤5% (4.6% and 9.1%, respectively, Table 2).

Given the fact that concerns about individuals with MDR are still present (mainly among those with exhausted treatments options),[3] and resistance to INI might be challenging, our findings confirm that characterizing and seeking solutions for individuals with limited therapeutic options, taking tropism determination also into account, might provide useful information, as previously described.[29,41] Thus, although the majority of cART-experienced patients harboured strains susceptible to maraviroc, particular attention should be paid to those patients showing X4/dual mixed strains with low levels of FPR. On the other hand, patients harbouring R5 strains seem less prone to accumulate high levels of resistance regardless of FPR levels. In fact, in individuals with FPR >60%, known to only harbour R5 strains,[23] no difference in resistance (and also immuno-virological parameters) was found when compared with individuals with FPR 10%–60%.

Another important piece of information provided from our study is that the majority of cART-experienced patients harbour strains susceptible to maraviroc. This is reassuring because this drug might be a valid option for difficult-to-treat individuals with particular conditions related to comorbidities and/or with ageing. In fact, several studies showed that maraviroc might be beneficial in terms of reducing atherosclerosis and improving liver fibrosis in HIV-infected individuals.[42]

In conclusion, we found that, among people failing cART, around two-thirds of individuals accumulated resistance to at least one drug class, and one-third had X4/dual-mixed strains; however, only a small proportion of them (about 2%) have very limited treatment options. HIV-1 tropism determination might be a useful tool to better identify cART-failing patients with low levels of FPR, who are more prone to accumulate a high level of resistance and have a greater risk of advanced disease. Further studies are required to overcome the limitations related to the observational nature of this study.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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