U N I V E R S I T A ’ D E G L I S T U D I D I M I L A N O

Facoltà di Medicina e Chirurgia

DOTTORATO DI RICERCA IN MALATTIE INFETTIVE

Evaluation of virological response to antiretroviral therapy in patients carrying HIV-1 non-B subtypes according to baseline mutational patterns

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Index

Abstract

OBJECTIVES: Notwithstanding the growing proportion of HIV-1 non-B subtypes in Europe, the impact of their genetic background on response to antiretroviral therapy is still unclear. The aim of this study was to compare response to protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) containing regimens in patients carrying non-B or B clades with matched resistance mutation patterns.

METHODS: We analyzed HIV-1 *pol* sequences of 1,108 patients stored in the ARCA (Antiretroviral Resistance Cohort Analysis) database and obtained before treatment. Response to therapy was defined as viral load suppression below 50 HIV-1 RNA copies/ml at week 12. By evaluating the combination of major resistance mutations, genotype coding generated 35 and 12 different vectors for PI or NNRTI treatments.

RESULTS: The proportion of subjects achieving virological suppression was comparable in patients with non-B or B variants stratified for treatment status (51.5% vs. 41.5% in naïve and 46.7% vs. 38.7% in experienced patients) and regimens including PIs (46.9% vs. 39.7%) or NNRTIs (56.7% vs. 40%). No difference in response to therapy in patients with non-B and B HIV-1 was observed in any matched genotype with respect to treatment combination. When B vs. specific non-B clades (C, F1, CRF02_AG) were compared, the only difference was a better response of CRF02_AG compared to B clade (75.0% vs. 36.7%; p=.012).

CONCLUSIONS: Response to PI- and NNRTI-based therapy is comparable in patients carrying non-B or B subtype matched for HIV-1 *pol* genotype. Further clade-specific studies are advisable to investigate possible minor effects on response to treatment.

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Introduction

The origin and variability of HIV

HIV-1 pandemic is a relevant challenge for global health nowadays, with more than 35 million people living with this infection [1]. The virus was originated in West and Central Africa from a zoonotic transmission of simian immunodeficiency virus (SIV) from non-human primates. HIV type 1 (HIV-1) groups M, N, O and P and HIV type 2 (HIV-2) groups A-H were generated by independent zoonotic transmission events. HIV-1 group M, the pandemic branch of HIV, originated from SIVcpz in the chimpanzee *Pan troglodytes troglodytes* [2].

After transmission to humans at the beginning of the century, HIV-1 group M probably diversified into genetic subtypes (named A-D, F-H and J-K) during the first decades of the 20th century [3]. Recombinants between subtypes have been designated as circulating recombinant forms (CRFs) if fully sequenced and found in three or more epidemiologically unlinked individuals. At present, 54 different CRFs have been described. Recombinants are defined unique recombinant forms (URFs) if not meeting these criteria [4].

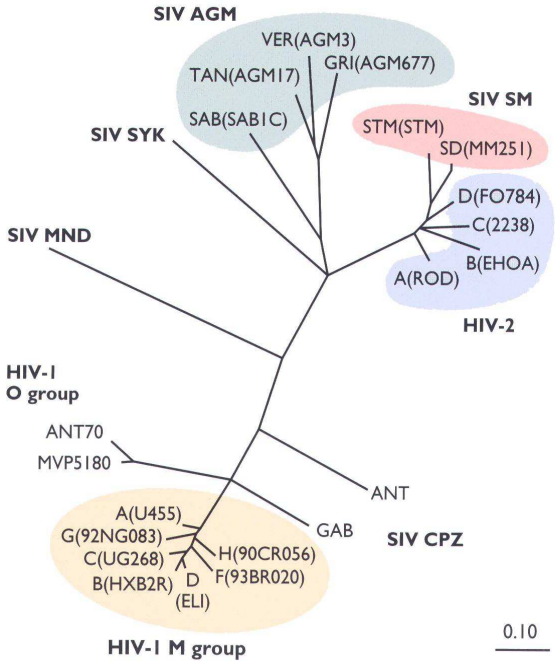

Figure 1. Phylogenetic tree showing relationships between SIV, HIV-2 and HIV-1.

Once URFs are transmitted with new infections they can originate new CRFs. Some CRFs, such as CRF01_AE and CRF02_AG, were formed early on in the epidemic in Central Africa and subsequently spread to other regions where they have played important roles in regional epidemics as well as globally. Other CRFs were generated elsewhere in the world.

Recombination is ongoing in many places in the world where different subtypes and CRFs co-circulate continuously giving rise to URFs.

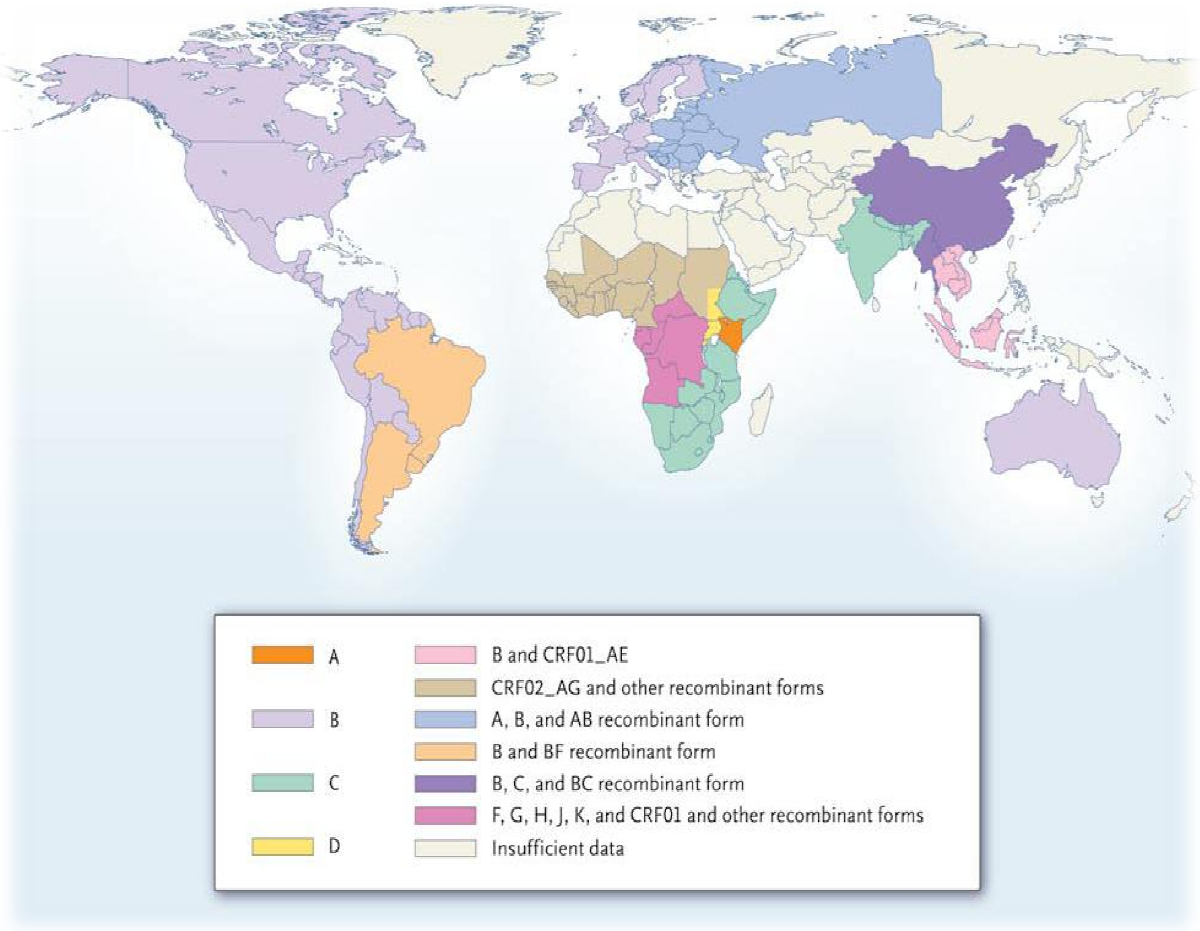

Figure 2. Distribution of main HIV-1 subtypes and recombinants in different geographical areas worldwide. (Figure reproduced from Ref. 5).

In the second half of the 20th century the global spread of HIV-1 group M resulted in a differential global distribution of HIV-1 subtypes and recombinants, as shown in Figure 1.

Notably, the circulation of non-B clades has recently increased in previously subtype B-restricted areas such as Western Europe and North America, mainly due to immigration from other regions of the world [5].

The analysis of HIV-1 heterogeneity has shown a high inter-subtype nucleotide sequence divergence in *gag, pol* and *env* genes [6]. This genetic variability of HIV results from recombination and the high mutation rates of the reverse transcriptase enzyme, which lacks a proof-reading mechanism, and to the high rates of viral replication. Also within a single individual viral sequences can differ by up to 10% [7] and genetic variation within a subtype is generally in the order of 8-17%, sometimes reaching more important proportions, as high as 30%. Variation between subtypes is usually between 17 and 35% but can be up to 42% [7].

The high variability may lead to specific differences among different subtypes in pathogenesis, infectivity, resistance to antiretrovirals and response to therapy.

The impact of HIV variability

The origin and molecular epidemiology of HIV, as far as their potential impact on disease progression, have recently been comprehensively reviewed [8, 9]. HIV-1 variability is crucial in describing the size and features of the pandemic, since groups, subtypes and recombinants differ in both epidemic size and geographical spread (Figure 1). It is uncertain whether the differential strain distribution can be accounted

for by founder effects alone or if intrinsic biological properties of the different HIV variants have played a role in their differential spread. Nevertheless, it is somehow described an impact of HIV diversity on cell biology, transmission, pathogenesis and clinical management. In addition, a key role of escape mutations in the immune response to HIV has been described and challenges to HIV vaccine development have risen from HIV diversity [10].

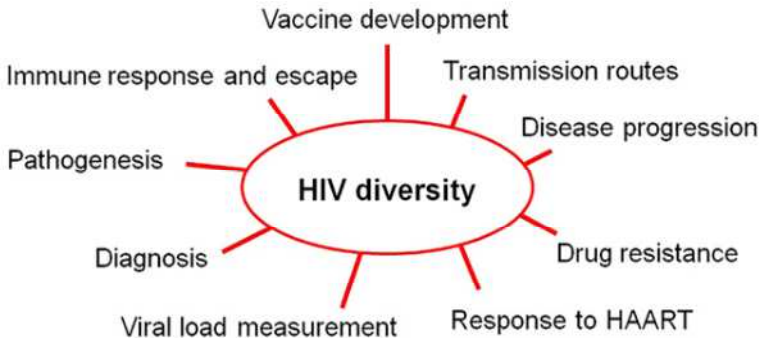

Figure 3. The role of HIV diversity: aspects of HIV infection which are affected by the viral genetic variability (Figure reproduced from Ref. 9).

A recent study found that human genetic variation, together with demographic variables, accounted for only 22% of the variability in HIV viral load and disease progression rates. Other factors, including environmental and viral factors, seem to be responsible for the remaining 78% of divergence in the clinical progression of the disease [11].

While these effects have been shown within subtype B infected populations, subtypes may play a significant role in determining viral load setpoint, considering the large genetic variability between subtypes. Indeed, cohort studies performed in areas where different subtypes co-circulate have provided some insights. According to recent studies, subtype C appeared to have a higher viral load and lower CD4

counts than those infected with subtype A [12], while CRF02_AG seems to have a higher viral load than other subtypes [13].
Some independent African studies indicated that subtype D infection is associated with faster disease progression to death than subtype A in populations where these subtypes co-circulate [14, 15]. This reduced survival was found in association with lower CD4 counts in subtype D infection rather than subtype A [14. 15]. Moreover, among individuals with advanced immunosuppression, subtype D was also associated with higher rates of dementia compared to subtype A [16].
As a possible consequence of different pathological properties, studies from Uganda and Kenya found significant decreases in the prevalence of subtype D and increases in subtype A frequency overtime [17, 18]. The changes in these proportions may have an explanation in both the faster disease progression and lower rate of heterosexual transmission of subtype D.

HIV variability and response to therapy

Previous studies did not show significant variations in virologic and immunologic responses to highly active antiretroviral therapy (HAART) between HIV-1 subtype B and all non-B subtypes grouped together. No differences in achieving viral load suppression following treatment did emerge in limited comparisons between subtype B and specific non-B variants such as clades C, A, D and CRF02_AG [19-23].
Moreover, information regarding resistance to antiretroviral drugs has been mainly derived from patients infected with HIV-1 subtype B [24].
The selection of resistance to antiretroviral drugs continues to be an important problem in the treatment of HIV-infected individuals. Indeed, most major resistance mutations in subtype B are also found

in non-B subtypes, but several novel mutations occur in non-B subtypes [25].

Although most of the resistance-associated mutations have been characterized in subtype B viruses, they are also found in treatment-failing patients harboring non-B subtypes [26, 27].

Nevertheless, some differences have been documented in the pathways to resistance in different clades [28] and subtype-specific natural polymorphisms have been suggested to play a possible role in drug activity *in vitro* and *in vivo* in some studies [29].

Of note, of 67 resistance mutations found in non-B subtype, 61 were also seen in subtype B isolates, indicating that some novel mutations only occur in non-B subtypes [25]. Examples of the latter are the non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutation V106M which occurs in subtype C and CRF01_AE and the protease inhibitor (PI) mutation L891V which has been described in subtypes C, F and G [30]. The mutational pathway leading to resistance is shorter in some non-B subtypes, which may have a role in the faster development of cross-resistance and compromise second-line regimens [30]. A differential pathway leading to resistance to is well known for mutation K65R, which has a faster selection in subtype C rather than in B clade [31] and has an important role in conferring resistance to non-nucleos(t)ide reverse transcriptase inhibitors (NNRTI).

Among specific non-B subtypes some differences are also notable. In fact, nevirapine resistance mutations seem to develop more frequently in subtype D than A in a mother-to-child transmission prevention study using single-dose nevirapine [32].

Of note, among strains not belonging to group M a high proportion of group O viruses are naturally resistant to NNRTIs due to the presence of the C181Y substitution in the RT region [33].

Aim of this study

Since the majority of data about highly active antiretroviral therapy (HAART) efficacy and resistance has been obtained from clinical trials involving mainly patients harboring subtype B virus, implications of subtype divergence in HAART efficacy still need to be studied in detail. This achievement is urgent because antiretroviral drugs are being introduced into developing countries where non-B subtypes are highly prevalent and non-B clades are expanding in previously clade B homogeneous areas, including Italy [34-39].

The aim of this study was to explore an innovative methodology to compare the impact of specific patterns of mutations on virological response to treatment in patients harboring different HIV-1 clades. Herein, we evaluated the overall and subtype specific response to PI or NNRTI containing regimens in patients carrying non-B or B clades with matched pre-therapy genotype, in a multicenter nationwide cohort.

Methods

Inclusion criteria and endpoints

Patients included in this analysis participated to the Antiretroviral Resistance Cohort Analysis (ARCA, www.hivarca.net) database and gave their informed consent to have their anonymised data stored on a central server and used for non-profit research purposes.

Patient cases were selected based on availability of baseline HIV-1 genotype obtained at maximum 12 weeks before treatment start and availability of a 12-week (range 8-16 weeks) follow-up HIV-1 RNA determination. Response to therapy was defined as viral load suppression below 50 HIV-1 RNA copies/ml at week 12. As secondary end-points HIV-1 viral load reduction between baseline and 12-week follow up and proportions of individuals reaching undetectable viral load at week 24 were also considered. Comparisons between response to treatment in subjects carrying B and non-B variants were conducted with and without matching for baseline resistance pattern and treatment (see below).

Genotypic resistance and subtype assignment

Resistance mutations were identified for IAS-USA 2011 tables [40]. Subtyping was based on a partial HIV-1 *pol* sequence about 1,000 to 1,280 nucleotides long, depending on the sequencing protocol used at the contributing laboratory. Sequences were firstly analyzed using the NCBI HIV-1 subtyping tool to discriminate between B and non-B strains.

Non-B sequences were subsequently aligned to the most recent reference dataset from Los Alamos National Laboratory website (http://hiv.lanl.gov/) using BioEdit 7.0.5 and ClustalX 1.83. The resulting alignment was analyzed with Phylip package version 3.67 (http://evolution.genetics.washington.edu/phylip.html) building a Neighbor-Joining tree based on the F84 substitution model. Reliability of the tree topology was assessed through bootstrapping using 1,000

replicate datasets. Sequences that could not be unequivocally assigned to a pure subtype or CRF were considered as possible recombinants and examined using Simplot 3.5.1.

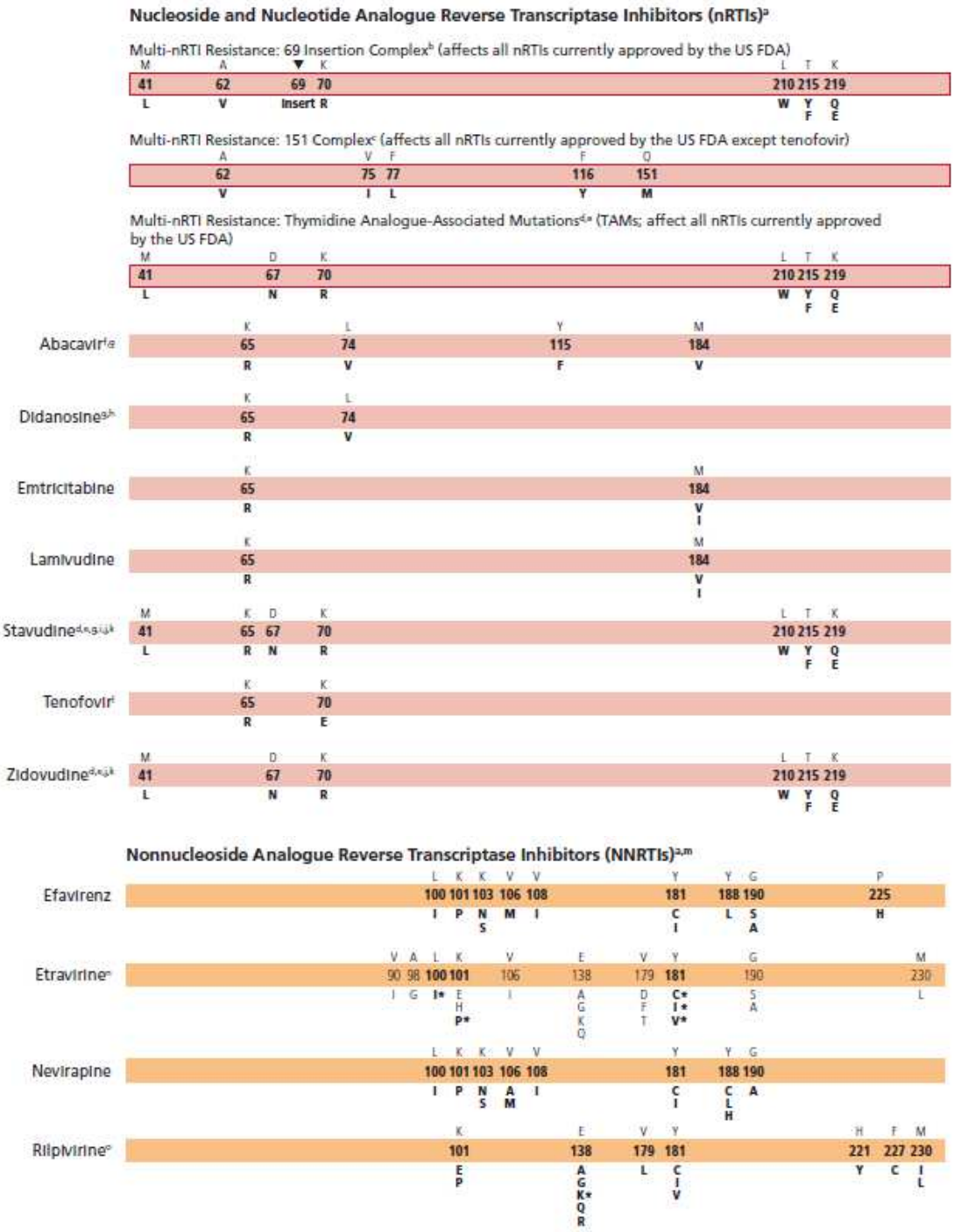

Figure 4. Mutations in the reverse transcriptase gene associated with resistance to reverse transcriptase inhibitors [40].

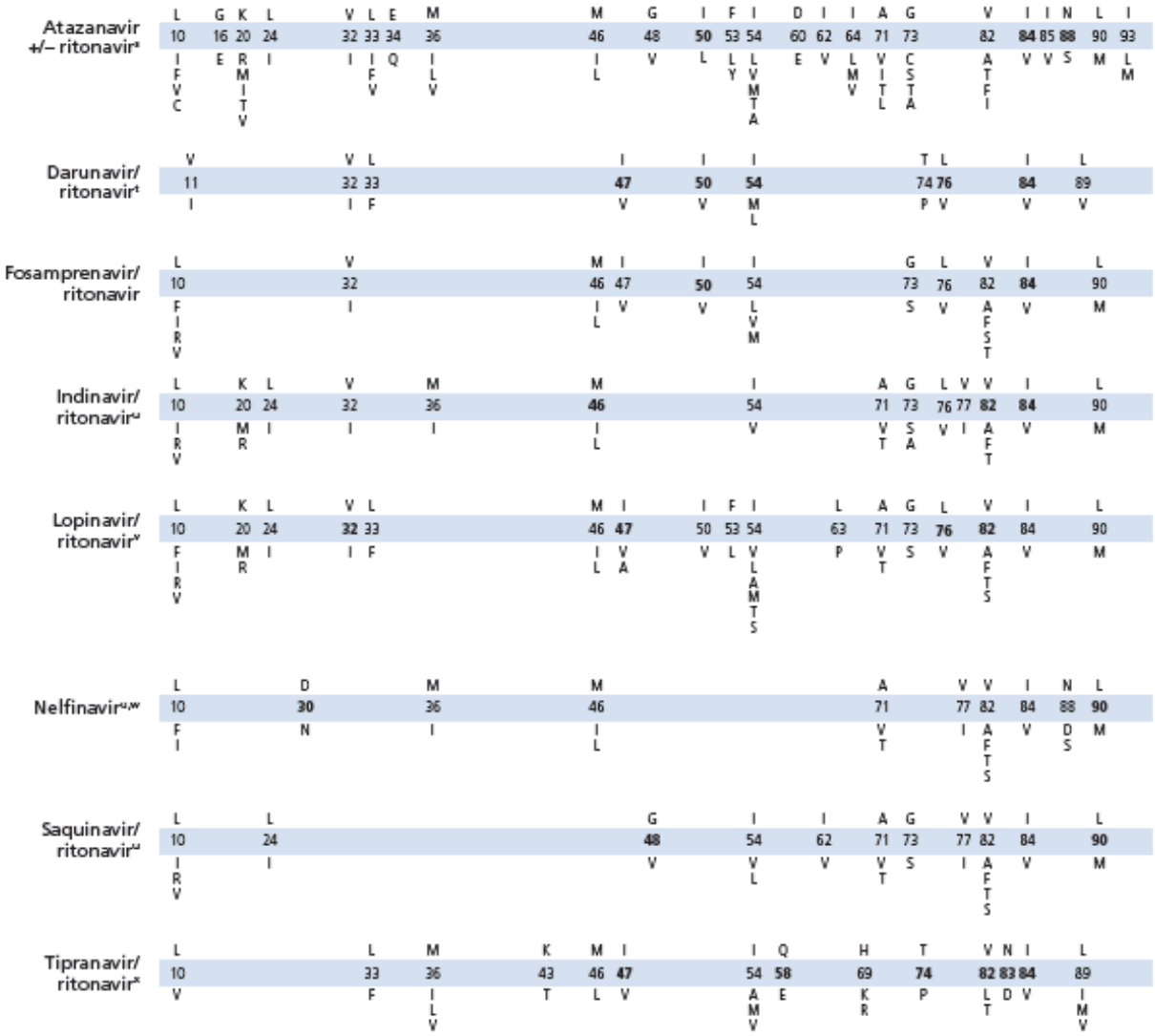

Figure 5. Mutations in the protease gene associated with resistance to protease inhibitors [40].

HIV-1 genotype and treatment coding

Response to therapy was evaluated in patients carrying either non-B or B subtypes with matched resistance patterns and treatment type. HIV-1 genotype was coded by considering the following mutations: any thymidine analogue mutations (TAMs) (M41L, D67N, K70R, L210W, T215Y/F and K219Q/E), K65R, L74I/V, Q151M, 69ins, M184I/V, any major NNRTI mutation (K103N/S/T, Y181C/I, Y188C/H/L, G190A/E/S/T)

and the number (0, 1-3, or >3) of major PI mutations (D30N, I47A/V, G48V, I50L/V, I54L/M, L76V, V82A/F/L/S/T, N88D/S, I84V, L90M). Treatments were coded as two nucleoside reverse transcriptase inhibitors (NRTIs) plus one NNRTI or two NRTIs plus one PI. Each case was then defined by the vector combining resistance pattern and treatment type (Table 1).

Table 1. Generation of vectors and response evaluation.
Treatments were coded as 2 NRTIs + NNRTI or 2 NRTIs + PI; each case was defined by the vectors combining resistance pattern and treatment. Sufficient numerous vectors allowing for statistical analysis were coded from 1 to 17.

Vectors	Mutation pattern					
	TAM	65R	74V	151M	184V	PI [a]
1	0	0	0	0	0	0
2	0	0	0	0	1	0
3	1	0	0	0	0	0
4	1	0	0	0	1	0
5	1	0	0	0	0	1
…	…	…	…	…	…	…
	TAM	65R	74V	151M	184V	NNRTI
14	0	0	0	0	0	0
15	0	0	0	0	0	1
16	0	0	0	0	1	0
17	1	0	0	0	1	0

Statistical analysis

The distribution of study subjects with regard to categorical parameters was compared using X2 or Fisher exact test. Standard non parametric methods (Wilcoxon signed-rank test) were used to compare the median

age, HIV-1 RNA levels and CD4 counts.

The crude and Mantel-Haenszel adjusted odds ratios (OR) of response to therapy with 95% confidence interval (CI) were calculated. Univariate analysis was performed using logistic regression and a subsequent multivariate analysis was done on all variables, using the same tests with a full model.

The changes in HIV-1 viral load following therapy in genotype and treatment matched cases derived from HIV-1 B and non-B cases were compared by Wilcoxon signed rank test.

In all tests, a p-value below 0.05 was considered significant.

Results

Characteristics of the population

The selection procedure generated 1,108 cases from as many HIV-1 positive individuals, who had referred to 65 clinical Centres of 13 Italian regions in the 1997-2009 period.

One hundred twenty-six individuals (11.4%) harbored non-B clades, mostly represented by CRF02_AG (n=26), F1 (n=25) C (n=20).

The distribution of non-B subtypes and CRFs in our population is shown in Figure 1.

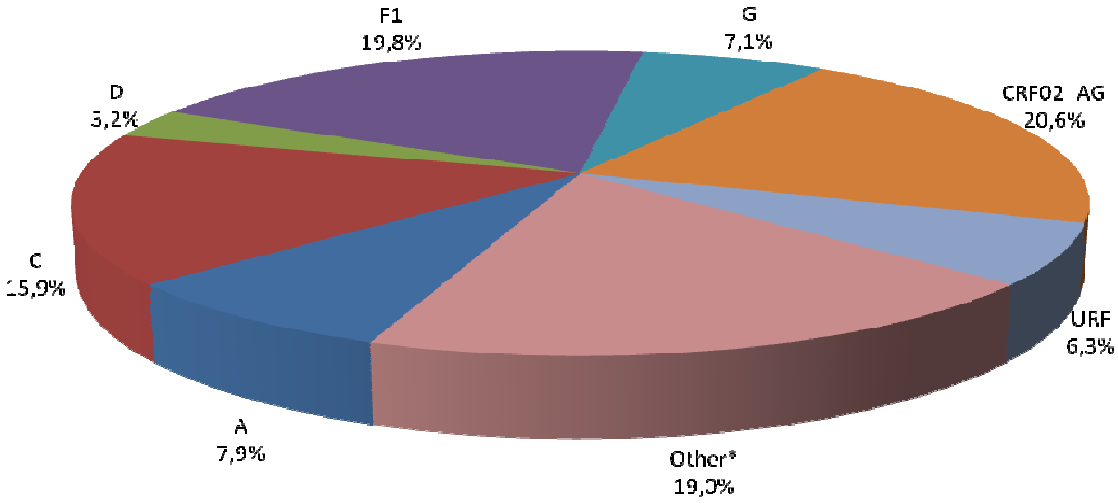

Figure 6. Distribution of non-B subtypes detected in 126 patients of the study population.

*Other: non-B clades were CRF01_AE (n=5), CRF12_BF (n=4), CRF06_cpx (n=4), CRF15_01B (n=3), CRF10_CD (n=2), CRF09_cpx (n=2), CRF13_cpx (n=1), CRF20_BG (n=1), CRF28_BF (n=1), CRF31_BC (n=1).

In the overall population different proportions of subjects carrying non-B rather than B strains were observed for ethnicity (Europeans accounting for 56.3% and 79.9%, respectively, p<.0001) risk factor

(heterosexual mode of infection in 81.8% and 43.7% of patients, respectively, p<.0001), gender (males in 52% and 68.5% of cases p<.0001) and median age (35 vs. 41 years, p=0.01). No difference in baseline HIV-1 RNA levels was observed (4.6 vs. 4.6 Log copies/ml), while a difference in CD4 cell count at baseline was found in patients carrying non-B rather than B variants (201 cells/µl vs. 245 cells/µl, p=.0007).

The prevalence of non-B subtypes was higher among drug-naïve than drug experienced patients (20.7%, 66/319 vs. 60/789, respectively, p<.0001). The demographic, immunologic and virological data of the patients stratified between naïve and experienced individuals are shown in Table 1, part A.

In the overall population, the prevalence of any drug resistance was 57% (631/1,108). NRTI, NNRTI and PI-associated resistance mutations were detected in 52.0%, 28.7% and 24.3%, respectively. The prevalence of drug resistance mutations was lower in subjects carrying non-B rather than B subtypes, either considering any mutation (35.7% vs. 59.7%, p<.0001) or NRTI (33.3% vs. 54.4%, p<.0001), NNRTI (12.2% vs. 30%, p=.006) and PI (15.1% vs. 25.5%, p=.010) mutations.

No difference in the proportions of drug resistance was observed between non-B and B strains when grouped in naïve or previously experienced individuals.

Table 1 shows the characteristics of our study population according to demographic and immunovirologic parameters, for the whole population and for subjects carrying B or non-B variants.

The distribution of epidemiologic and immunovirologic parameters among 319 naïve individuals and 789 experienced individuals is shown in Table 3 and 4, respectively.

Table 2. Demographic, virological and immunological features of 1,108 patients enrolled in our cohort.

	ALL PATIENTS	B SUBTYPE	NON-B CLADE
Ethnic group, % (n)			
Europeans	77.3 (856)	79.9 (785)	56.3 (71)
Africans	3.0 (33)	0.3 (3)	23.8 (30)
Latin Americans	2.4 (27)	2.5 (24)	2.4 (3)
Asians	0.5 (6)	0.5 (5)	0.8 (1)
Others	16.8 (186)	11.4 (126)	0.2 (21)
Risk factor, % (n)			
Heterosexual sex	48 (532)	43.7 (429)	81.8 (103)
Men having sex with men	20.4 (226)	21.9 (215)	8.7 (11)
Intravenous drug use	28 (310)	31.0 (304)	4.8 (6)
Other	3.6 (40)	3.5 (34)	4.7 (6)
Gender, % (n)			
Males	68.8 (758)	68.5 (754)	52.2 (71)
Age (yr), median (IQR*)	40 (36-45)	41 (36-46)	35 (32-43)
HIV-1 RNA (Log cp/mL), median (IQR)	4.6 (3.9-5.2)	4.6 (3.9-5.2)	4.6 (4.0-5.3)
CD4 count (cells/mL), median (IQR)	237 (118-380)	245 (128-390)	201 (81-290)
Total patients, % (n)	100 (1,108)	88.6 (982)	11.4 (126)

Table 3. Demographic, virological and immunological features among 319 naïve individuals, according to subtype.

	B subtype	Non-B subtype	p
Ethnic group, % (n)			
Europeans	79.4 (579)	65.7 (34)	
Africans	0.1 (1)	26.7(16)	<.0001
Latin Americans	1.9 (14)	3.3 (2)	
Asians	0.3 (2)	0	
Others	18.2 (123)	13.3 (8)	
Risk factor, % (n)		85.0 (51)	
Heterosexual sex	38.4 (280)	5.0 (3)	
Men having sex with men	19.3 (141)	3.3 (2)	<.0001
Intravenous drug use	38.5 (281)	6.7 (4)	
Other	3.7 (27)		
Gender, % (n)			
Males	69.74 (507)	45.0 (27)	<.0001
Age (yr), median (IQR*)	41 (37-45)	36.5 (32-46)	.001
HIV-1 RNA (Log cp/mL), median (IQR)	4.3 (3.7-5)	4.3 (3.9-4.7)	n.s.
CD4 count (cells/mL), median (IQR)	262 (150-411)	209 (101-317)	.029
Total patients, % (n)	92.4 (729)	7.6 (60)	

Table 4. Demographic, virological and immunological features among 789 previously experienced patients, according to subtype.

	B subtype	Non-B subtype	p
Ethnic group, % (n)			
Europeans	81.4 (206)	56.1 (37)	
Africans	0.8 (2)	21.2 (14)	
Latin Americans	4.0 (10)	1.5 (1)	<.0001
Asians	1.2 (3)	1.5 (1)	
Others	12.6 (32)	0.2 (13)	
Risk factor, % (n)			
Heterosexual sex	58.9 (149)	78.8 (52)	
Men having sex with men	29.3 (74)	12.1 (8)	.013
Intravenous drug use	9.1 (23)	6.1 (4)	
Other	2.8 (7)	3.3 (2)	
Gender, % (n)			.046
Males	73.9 (184)	60.6 (40)	
Age (yr), median (IQR*)	41 (34-47)	35 (32-41)	.001
HIV-1 RNA (Log cp/mL), median (IQR)	5.1 (4.7-5.5)	4.9 (4.2-5.4)	.049
CD4 count (cells/mL), median (IQR)	198 (64-323)	182 (64-286)	n.s.
Total patients, % (n)	79.3 (253)	20.7 (66)	

Previous treatments and starting regimens

Drug experienced patients had a median number of 6 regimens (IQR 3-9), with a lower number of drug regimens taken in subtype non-B vs. B infected individuals (3 vs. 5 median previous regimens, p=.0008). The proportion of experienced subjects carrying a non-B clade was lower (47.6%) than that found in patients with B strains (74.2%) (p<.0001).

Complete previous treatment history was available for 424 patients. All these subjects were NRTI experienced, 53 and 109 patients had previously been administered an NNRTI- or PI-containing therapy, respectively; 232 received both NNRTIs and PIs.

Overall, subjects beginning a PI-based HAART regimen were 883.

Among these individuals 54.5% (n=153), 36.3% (n=102) and 9.2% (n=26) carried 0, 1-3 and >3 mutations in protease region as detected before starting or changing therapy. An antiretroviral regimen containing an NNRTI was started in 225 individuals, 7 of whom had a transmitted NNRTI mutation.

Table 5. Prevalence of drug resistance before the beginning of a new HAART regimen in naïve and experienced subjects, according to subtype.

	All patients	NAÏVE PATIENTS		EXPERIENCED PATIENTS	
		B	non-B	B	Non- B
Any resistance % (n)	57.0 (631)	9.9 (25)	6.1 (4)	76.9 (561)	68.3 (41)
NRTI resistance % (n)	52.0 (576)	7.5 (19)	6.1 (4)	70.6 (515)	63.3 (38)
NNRTI resistance% (n)	28.7 (318)	3. 2 (8)	4.6 (3)	39.4 (287)	33.3 (20)
PI resistance % (n)	24.3 (269)	2.8 (7)	0	33.3 (243)	31.7 (19)
Previous drug regimens, median (IQR)	3 (0-7)	-	-	5 (3-8)	3 (1-7)
Total patients, % (n)	100 (1,108)	79.3 (253)	20.7 (66)	92.4 (729)	7.6 (60)

Virological response to HAART

In the whole study population the proportion of subjects achieving virological suppression on HAART at median week 12 was higher in individuals carrying non-B (62/126, 49.2%) rather than B variants (387/982, 39.4%, p=.035). Subgroup analysis showed no difference either among drug-naïve (34/66, 51.5% vs. 105/253, 41.5%) or pretreated individuals (28/60, 46.7% vs. 282/729, 38.7%). No difference in virological response was detected in the proportion of

individuals starting a PI-containing HAART regimen according to subtype (45/96, 46.9% vs. 309/787, 39.7%, in patients with non-B and B clades). A trend to a better virological response was observed in subjects undergoing NNRTI-based HAART carrying non-B rather than B subtypes (17/30, 56.7% vs. 78/195, 40%; p=.085).

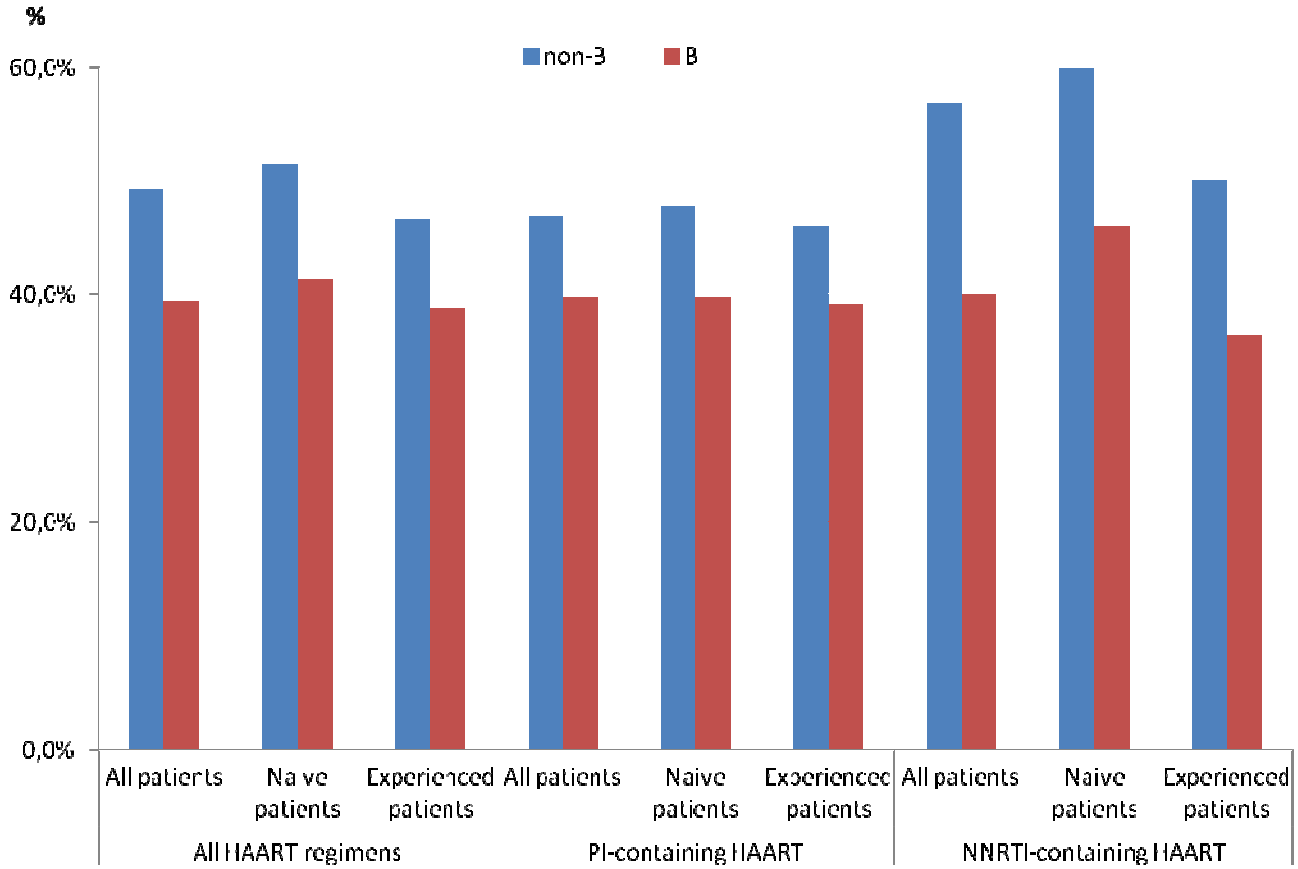

Figure 7. Prevalence of virological response to HAART at the median week 12 among naïve and experienced subjects.

No difference was detected when comparing the viral load reduction between baseline and the median week 12 between subjects harboring non-B and B subtypes when stratified between drug-naïve and experienced subjects (2.24 vs. 1.82 and 2.83 vs. 2.98 Log copies/ml,

respectively).

We investigated the response to treatment as the proportion of subjects achieving virological response at median week 24 in a subset of 777 individuals (70.1%) with an available follow up at this time-point. No difference was found when grouped together (56/89, 62.9% vs. 379/688, 55.1%), in naïve (35/49, 71.6% vs. 145/189, 76.7%) or in experienced patients (21/.40, 52.5% vs. 234/499, 46.9%).

Predictors of response to treatment

With the whole case file, predictors of a higher probability of virological suppression at week 12 in the univariate analysis included subtype (OR, for non-B variants: 1.49; 95% CI: 1.02-2.16; p=.035), viral load at baseline (OR per 1 Log higher: 0.55; 95% CI: 0.47-0.65; p<.0001), time of virological follow-up (OR per 1 week higher: 1.01; 95% CI: 1-1.11; p=.051), number of previous regimens (OR per 1 regimen higher: 0.95; 95% CI: 0.92-0.97; p<.0001) and calendar year (OR per 1 year later: 1.21; 95% CI: 1.14-1.28; p<.0001). By contrast, ethnicity, mode of transmission, gender and age, as well as NNRTI or PI-containing regimens, did not influence the outcome of therapy. Predictors of viral load below 50 copies/ml in the multivariate model were viral load at baseline (OR per 1 Log higher: 0.41; 95% CI: 0.34-0.50; p<.0001), number of previous regimens (OR per 1 regimen higher: 0.92; 95% CI: 0.89-0.95; p<.0001) and calendar year (OR per 1 year higher: 1.27; 95% CI: 1.19-1.37; p<.0001).

Table 6. Logistic regression analysis investigating possible predictors of undetectable levels of HIV1 RNA at median week 12 in naïve individuals.

[a] Other: Africans, Latin Americans, Asians or not available;
[b] Other: professional risk, transfusions, vertical transmission.

	Univariate			Multivariate		
Covariate	**OR**	**95%CI**	***p***	**OR**	**95%CI**	***p***
Subtype						
B	-	-	-	-	-	-
Non-B	1.498	0.870-2.579	0. 145	0.963	0.467-1.986	0.919
Ethnicity						
Europeans	-	-	-	-	-	-
Other[a]	2.264	1.052-4.873	0.037	2.145	0.797-5.773	0.131
Risk category						
HE	-	-	-	-	-	-
HO	0.567	0.333-0.967	0.037	1.136	0.549-2.353	0.731
IDU	0.875	0.390-1.963	0.746	1.715	0.662-4.441	0.266
Other[b]	0.547	0.133-2.247	0.403	0.130	0.014-1.241	0.076
Gender						
Male	-	-	-	-	-	-
Female	2.085	1.272-3.418	0.004	2.201	1.141-4.243	0.018
Age						
per 10 years older	1.067	0.857-1.328	0.561	1.331	0.988-1.793	0.060
Time of viral load follow-up per 1 week higher	1.099	0.994-1.216	0.065	1.174	1.032-1.336	0.015
Baseline viral load per 1 log higher	0.369	0.257-0.530	<.0001	0.335	0.216-0.512	<.0001
Starting HAART						
PI	-	-	-	-	-	-
NNRTI	0.735	0.453-1.192	0.212	0.590	0.323-1.077	0.086
Number of previous regimens per 1 regimen higher	-	-	-	-	-	-
Calendar year per 1 year higher	1.123	1.024-1.231	0.014	1.190	1.066 -1.342	0.002

Table 6. Logistic regression analysis investigating possible predictors of undetectable levels of HIV1 RNA at median week 12 in drug-experienced individuals.
[a] Other: Africans, Latin Americans, Asians or not available;
[b] Other: professional risk, transfusions, vertical transmission.

	Univariate			Multivariate		
Covariate	**OR**	**95%CI**	***p***	**OR**	**95%CI**	***p***
Subtype						
B	-	-	-	-	-	-
Non-B	1.387	0.817-2.353	0.225	1.283	0.639-2.576	0.483
Ethnicity						
Europeans	-	-	-	-	-	-
Other[a]	0.728	0.356-1.489	0.384	0.585	0.254-1.348	0.208
Risk category						
HE	-	-	-	-	-	-
HO	0.882	0.590-1.319	0.541	1.247	0.727-2.140	0.423
IDU	0.977	0.707-1.351	0.889	1.060	0.698-1.608	0.785
Other[b]	0.602	0.269-1.347	0.216	0.425	0.133-1.359	0.149
Gender						
Male	-	-	-	-	-	-
Female	1.041	0.767-1.414	0.794	0.996	0.654-1.517	0.985
Age						
per 10 years older	1.138	0.950-1.364	0.164	1.091	0.870-1.368	0.452
Time of viral load follow-up per 1 week higher	1.038	0.975-1.106	0.245	1.026	0.950-1.107	0.516
Baseline viral load per 1 log higher	0.519	0.426-0.633	<.0001	0.4230	0.338-0.548	<.0001
Starting HAART						
PI	-	-	-	-	-	-
NNRTI	1.100	0.747-1.620	0.628	1.260	0.807-1.968	0.309
Number of previous regimens per 1 regimen higher	0.942	0.910-0.975	0.0008	0.922	0.884-0.963	0.0002
Calendar year per 1 year higher	1.255	1.170-1.345	<.0001	1.352	1.233 -1.482	<.0001

HAART effect in paired *pol* genotype

We evaluated whether mutations associated to resistance differently influence the proportions of subtype B and non-B infected patients achieving an undetectable viral load at week 12 when beginning a PI- or an NNRTI-containing HAART. The procedure used to code HIV-1 genotype generated 35 and 12 vectors for PI- and NNRTI-treated patients, respectively. Table 3 shows the patterns of mutations identified and the proportions of patients with B or non-B subtypes who achieved an undetectable viral load at week 12.

Among patients on PI-based regimens, the rate of virological response was not different between subtype B and non-B virus either in the absence of drug resistance mutations (group 1) or in the presence of any of the most common mutational patterns found. Patients harboring a wild type HIV-1 genotype were the only group allowing comparisons between subtype B and specific non-B subtypes. No significant difference was observed in this group when subtype F1 (n=13) and C (n=9) were compared to subtype B (n=324) (response rates of 23.1% and 44.4% vs. 36.7%,respectively). By contrast, a better response in patients carrying CRF02_AG (n=12) compared to those with HIV-1 B clade (75.0% vs. 36.7%, p=.012) was observed.

Among patients on NNRTI-based regimens, no difference in the response rate was found between subjects harboring non-B or B subtypes when mutations were absent at baseline. Finally, HIV-1 RNA reduction at the median week 12 and the proportions of response to treatment at median week 24 in patients matched for specific mutation patterns and specific genotype did not significantly differ between non-B and B clade.

Table 7. Resistance patterns and response to treatment in patients carrying non-B and B subtypes.

[a]0: no mutations; 1: 1-3 mutations; 2: >3 mutations

[b]0: no mutations; 1: any mutations

Vectors	Mutation pattern						Subtype		Response to HAART	
	TAM	65R	74V	151M	184V	PI [a]	Non-B % (num.)	B % (num.)	Non-B % (num.)	B % (num.)
1	0	0	0	0	0	0	15.4 (59)	84.6 (324)	40.7 (24)	36.7 (119)
2	0	0	0	0	1	0	13.2 (9)	86.8 (59)	44.4 (4)	57.6 (34)
3	1	0	0	0	0	0	4.4 (4)	95.6 (86)	75.0 (3)	41.9 (36)
4	1	0	0	0	1	0	5.2 (4)	94.8 (73)	75.0 (3)	42.5 (31)
5	1	0	0	0	0	1	10.9 (7)	89.1 (57)	28.6 (2)	26.3 (15)
6	1	0	0	0	1	1	4.1 (3)	95.9 (71)	33.3 (1)	42.3 (30)
7	0	0	0	0	0	1	12.5 (2)	87.5 (14)	100 (2)	42.9 (6)
8	0	0	0	0	1	1	7.7 (1)	92.3 (12)	0 (0)	33.3 (4)
9	0	0	1	0	0	0	50 (2)	50 (2)	100 (2)	50 (1)
10	0	1	0	0	0	0	20 (1)	80 (4)	100 (1)	100 (4)
11	1	0	1	0	1	0	10 (1)	90 (9)	100 (1)	66.7 (6)
12	1	0	1	0	1	1	20 (1)	80 (4)	100 (1)	50 (2)
13	1	0	1	0	1	2	20 (1)	80 (4)	100 (1)	100 (4)
	TAM	65R	74V	151M	184V	NNRTI				
14	0	0	0	0	0	0	16.3 (24)	83.7 (123)	62.5 (15)	50.4 (62)
15	0	0	0	0	0	1	50 (1)	50 (1)	100 (1)	100 (1)
16	0	0	0	0	1	0	12.5 (2)	87.5 (14)	0 (0)	57.1 (8)
17	1	0	0	0	1	0	6.9 (2)	93.1 (27)	50 (1)	18.5 (5)

Discussion

Overall access to antiretrovirals through global or local treatment programs is increasing in low-income areas where non-B HIV-1 strains are highly prevalent. Although the circulation of non-B subtypes has recently increased in most European countries, response to treatment in Western countries has been mainly analyzed with subtype B viruses and in some cases compared with that of non-B subtypes considered as a single cumulative group [19, 20]. This approach may mask differences among specific subtypes in disease progression and drug susceptibility [25, 41]. Indeed, medium-term outcome of large studies in low-income countries demonstrated good virological and immunological responses to therapy, but few studies have addressed treatment response with specific subtypes [42-44]. Some reports indicated subtype-independent effects of HAART [45] while others suggested that D subtype, compared to C and A clades, negatively impacts on disease progression and response to treatment [47-49].

We studied a large population of either naïve or drug experienced HIV-1 patients who started an antiretroviral therapy guided by a baseline genotype over the last 13 years in Italy. We recently reported evidence of onward transmission of non-B variants through migration and travels among Caucasian living in Italy [36]. Other Italian studies indicated that such viral variants are spreading to heterosexuals and male homosexuals, thus non-B subtypes are no longer restricted to African ethnicity and heterosexuality [37, 38].

A high heterogeneity of group M HIV-1 clades was detected in our

study population. Of note, F1 subtype and CRF02_AG accounted for similar proportions (about 20%) of non-B strains. In previous studies the very low frequency of F1 subtypes did not allow to address their response to HAART therapy [27, 45, 49]. Overall, the prevalence of any and class-specific drug resistance mutations was lower in non-B compared to B clade in our case-file. However, no difference in TDR was detected in naive patients carrying either non-B or B subtype. This finding indicates that primary resistance is no longer restricted to patients of Caucasian ethnicity mainly infected with subtype B. An explanation of these data may be the lower frequency (about 8%) of non-B clades in drug experienced patients compared to that found in naive individuals (about 21%). The former subjects may have acquired HIV-1 infection at an earlier time point when the circulation of non-B variants, including transmission of resistant strains, was lower than that found in the last years [39].

As expected, the relative proportion of treated individuals was lower among those with a non-B clade, who received a median lower number of regimens compared to patients carrying a B subtype. By studying the overall population we observed a better response to HAART of non-B variants regardless of the class of drugs used together with an NRTI backbone. However, this difference in the virological outcome was still present but not significant when comparing patients who received an NNRTI- or PI containing regimen either in naïve or previously treated individuals, probably due to a size effect. A slightly better response was observed in patients with non-B clades on NNRTI-containing regimens. This finding is not in agreement with the results of the ACTG 5095 trial that indicated a higher risk of virological failure of efavirenz therapy in black patients with respect to white Americans [50]. Nonetheless, the multivariate analysis supported our finding demonstrating that the ethnicity does not influence the short-term response to HAART.

Moreover, a limited proportion of subjects achieved viral suppression at week 12. This result may be partly explained by i) the high proportion (more than 25%) of patients with HIV-1 viral load above 106 copies/ml and ii) the relatively short follow-up which was deliberately chosen to focus on the impact of genotype on virological response. In any case, no worse response of non-B clades was detected even analyzing the difference in HIV-RNA reduction at week 12 or the proportion of individuals reaching viral load suppression at week 24.

Due to its retrospective nature, our study has several limitations, particularly the lack of any information about adherence to treatment. It has been proposed that the maintenance of long-term adherence may be influenced by effective engagement of health care facilities [50]. Although we could not check for adherence in our database, the free access to Italian medical services and the availability of therapy for legal or illegal immigrants could have favoured their compliance to therapy regardless of their ethnicity. Moreover, an interaction between race and adherence has been only reported for an NNRTI, efavirenz, due to a genetic polymorphism of the subfamily of cytochrome P450 (CYP2B26) in blacks that leads to a decreased metabolism of this drug, relevant side effects and low adherence [50]. A number of factors possibly affecting adherence, such as ethnicity, age, gender and risk factors, were considered as possible confounders in our regression model. None of these covariates significantly resulted to impact the virological outcome of patients stratified according to treatment status and subtypes in the multivariate analysis. In addition, although CD4 cell count at baseline was higher in subjects carrying a subtype B, this parameter was not associated with different virological outcome neither in univariate nor multivariate analysis.

Furthermore, even though a genotype guided choice of regimens

warrants for avoiding suboptimal therapy in naïve patients, this may not be fully applied to experienced patients, particularly those with multiple failures. Indeed, we observed that the number of previous regimens interacts with treatment response, however, this factor was considered as a confounder in the multivariate analysis. The treatment cases included in our study span 14 years. During this period several drugs have been replaced by more potent compounds and, as expected, the more recent calendar year of treatment was a predictor of virological success.

Our findings indicate that the efficacy of NNRTI- or PI-containing HAART is similar with B and non-B subtypes matched for major resistance mutations. This suggests that HIV-1 pol minor mutations and polymorphisms do not significantly impact response to HAART with HIV-1 subtype B vs. non-B. Based upon discrepant results of different interpretation algorithms, it has been suggested that polymorphisms could impact the outcome of patients with non-B variants [51, 52]. However, the potential influence of mutations associated to minor resistance has been studied in detail in a paper analyzing amino acid protease and RT changes of about two hundred non-B clinical isolates of naïve and treated HIV-1 patients. By using the Virtual PhenotypeTM tool and linear regression analysis it has been observed that individual unreported changes not belonging to known resistance mutations are not predictors of resistance of non-B variants [27].

By comparing sequences with identical patterns of major resistance mutations, we could compare virological outcome only in a limited number of individual HIV-1 clades (F1, CRF02_AG and C). The data suggest that response to HAART for these HIV-1 variants is comparable to that detected for B subtype. These data support previous results and extend them to the F1 subtype [45]. However, achievement of viral suppression was detected in an higher proportion

of patients infected with the CRF02_AG clade, compared to B subtype, starting a PI-containing regimen with a wild type virus. Even though the number of patients carrying CRF02_AG is limited, a possible influence of clade-specific polymorphisms on the therapeutic outcome cannot be excluded, thus requiring further investigations.

Our study provides early data showing similar responses of non-B and B clades even when paired for genotype, even though extended follow-up data are required to provide conclusive results. While it cannot be ruled out that specific combinations of mutations and specific polymorphisms exert different effects on drug susceptibility with a particular clade, larger datasets, most likely derived from international cohorts, are required to address this possibility.

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