AIDS Reviews 2006;8:98-107

Efficacy of Antiretroviral Therapy in Individuals Infected With HIV-1 Non-B Subtypes

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Abstract

The impact of HIV-1 subtype on clinical outcome following exposure to antiretroviral therapy is currently not well known. Natural polymorphisms are often present in HIV-1 non-B subtypes at positions known to be associated with drug resistance in clade B viruses. These changes might influence the emergence of drug-resistant viruses, modifying drug susceptibility and/or the virus replicative capacity. Moreover, different pathways may lead to drug resistance according to HIV-1 clade. Finally, the influence of subtype on the performance of phenotypic assays and in the interpretation of algorithms for genotypic resistance is currently a matter of debate. All these aspects explain why the response to antiretroviral therapy might vary in subjects infected with different HIV-1 clades. **(AIDS Reviews 2006;8:98-107)**

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Key words

Non-B subtypes. Antiretroviral therapy. Drug resistance. Genetic variability.

Introduction

The genetic diversity of the HIV-1 population in a given infected host (quasispecies) results from a high rate of virus replication paired with a low fidelity of the reverse transcriptase (RT) enzyme, which lacks proofreading function. At least nine subtypes (A, B, C, D, F, G, H, J, K), sub-subtypes (within A and F), and many more intersubtype recombinant forms (CRF and URF, circulating and unique recombinant forms, respectively) have been described within HIV-1 group M[1,2]. Infections with non-B subtypes and recombinant variants are responsible for nearly 90% of the current worldwide epidemic, with subtype C, A, CRF01_AE and CRF02_AG variants being the most prevalent[3]. Although HIV-1 clade B remains the most frequent variant in North America and Western Europe, a growing and rapid spreading of other subtypes has been recognized in these regions within recent years.

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Natural variability in HIV-1 genes

Genetic variability across HIV-1 subtypes involves genes for viral enzymes targeted by antiretroviral (ARV) drugs. These are *pol* genes (protease, PR and reverse transcriptase, RT) as well as the gene coding for the transmembrane envelope glycoprotein gp41. Those HIV-1 proteins are targets for protease inhibitors (PI), RT inhibitors and fusion inhibitors, respectively. The frequency and pattern of mutations producing resistance to these compounds may differ among HIV-1 subtypes, and hypothetically could influence therapeutic outcome[4]. The genetic diversity of the PR and RT genes in non-B viruses has been assessed in drug-naive individuals[5-7]. Specific polymorphisms have been observed at 37 PR and 41 RT positions in non-B subtypes, some of them being polymorphic also in clade-B viruses[6].

Amino acid substitutions occur at high frequencies in certain non-B viruses[5-7] at positions associated with drug resistance in clade B (http://www.iasusa.org, updated in March 2006). Moreover, substitutions at five PR and one RT positions, not known to be associated with drug resistance, were recently found to be significantly linked to drug

exposure in some non-B clades[6]. At the PR, they were present at positions 6 and 64 (subtype C), 15 (CRF02_AG), 19 (subtype F), 37 (subtype A), and 64 (CRF01_AE); at the RT, only a change at residue 102 was found to be associated with drug exposure in subtype C[6].

Moreover, subtype-specific polymorphisms at four PR and three RT positions known to be associated with drug resistance in clade B represent the consensus sequence for some non-B variants. At the PR, this is the case for K20I in subtype G and CRF02_AG, M36I in most non-B clades and recombinants, V82I in subtype G and CRF14_BG, and I93L in subtype C[6]. At the RT, these changes are A98S in CRF14_BG, V179I in subtype A, and K238R in CRF01_AE[6].

Resistance mutations to non-nucleoside RT inhibitors (NNRTI) are more frequently recognized in subtype C than B viruses[8,9]. In one study in which nearly 2,000 isolates (1299 B and 556 non-B) from the trans-European CATCH study of treatment-naive patients were examined; the non-B sequences had 20% (subtypes C, D, and CRF01_AE) to 70% (G, J and CRF02_AG) more minor PR substitutions as compared to those carrying clade-B viruses ($p < 0.001$)[10]. All single non-B subtypes contained more minor PR substitutions, which could influence the susceptibility to indinavir (IDV), nelfinavir (NFV), atazanavir (ATZ), and ritonavir (RTV) ($p < 0.001$). Conversely, subtype-B sequences generally harbored more minor PR changes relevant to saquinavir (SQV) and lopinavir/ritonavir (LPV/r) ($p < 0.001$)[10]. Of note, PR changes K20I and M36I have been shown to be the strongest predictor of virologic rebound under PI-based regimens in some studies conducted in patients infected with non-B viruses[11,12]. In one of these reports, preexisting PR mutations at codons 10 and 36 were found in 39.3 and 40%, respectively, of drug-naive individuals who subsequently failed therapy[12].

Intersubtype differences in the PR and RT genes are in the range of 10-12% at nucleotide level, and 5-6% at amino acid level[13]. They can influence the biochemical and biophysical microenvironment of these viral enzymes[14]. Moreover, these differences among subtypes might also determine the spectrum of mutations that develop further under selective drug pressure. In this review we will examine in detail the effect of natural HIV-1 variability on drug resistance and clinical response to ARV therapy.

Clinical impact of natural polymorphisms in distinct HIV clades

Substitutions in residues located in drug-targeted proteins may accelerate the selection of drug-resistant viruses when present as natural polymorphisms, and therefore compromise the mid/long-term efficacy of therapy. Differential effects of polymorphisms may be seen as well on the virus replicative capacity and/or in the selection of alternative pathways for drug resistance (Table 1).

Table 1. Influence of natural polymorphisms on HIV-1 properties and monitoring

- Speed for selecting single drug-resistance mutations
- Modulate the degree of resistance
- Impaired susceptibility or hypersusceptibility *in vitro*
- Virus replicative capacity
- Pathways for acquiring resistance
- Response to specific antiretroviral drugs
- Interpretation algorithms for resistance
- Performance of resistance assays

Faster emergence of drug-resistant viruses

Genetic variability across HIV-1 variants may lead to a more rapid emergence of drug resistance under drug pressure and, consequently, to earlier treatment failure. Although minor PR amino acid changes by themselves do not seem to impair drug susceptibility, they could enhance resistance by improving the replicative capacity of the resistant virus. Moreover, minor PR mutations could affect (increase or decrease) the genetic barrier for resistance[10,15]. Accordingly, those subtypes naturally showing the largest number of minor PR substitutions could have the lowest genetic barrier for resistance. If confirmed, this information may influence decisions for choosing first-line therapy.

Tipranavir resistance

One example of the decrease of the genetic barrier to reach PI resistance was observed for tipranavir (TPV) response. Several mutations at the PR gene, including at residues 20 and 36, would increase the TPV mutational score in clades A, C, F, and G subtypes[16], decreasing the genetic barrier to acquire TPV resistance. As a consequence, the susceptibility to TPV and its clinical efficacy would be lower in patients infected with those viruses, being particularly evident

with subtype G[16]. Interestingly, M36I appears along with K20I in the majority of viruses belonging to clades G and CRF02_AG[2,5,7]. M36I is also naturally present in 12% of clade-B viruses, but in up to 80% of non-B variants[7]. Thus, isoleucine (I) at residue 36 may be considered as a genetic marker for HIV-1 group M non-B subtypes[7]. The presence of I36 has already been linked to a higher rate of treatment failure[12]. Moreover, it may facilitate the selection of L90M in patients failing PI therapy[17].

Nelfinavir resistance

Drug-resistant mutations in some non-B viruses may appear at sites not known to be involved with resistance such as M89I/V[18,19]. While 89L predominates in subtype B, 89M is the most prevalent in other viral strains. Substitutions M89I/V at PR alone, previously associated with PI experience only in subtypes C, F, and G, but not in subtype B viruses , did not confer reduced susceptibility to NFV. However, when combined with L90M, it reduced the susceptibility to NFV in comparison to strains with L90M alone[18]. Thus, the presence of M89I/V changes would accelerate the appearance of NFV resistance *in vivo* in those clades where present[18], having an important effect on NFV reduced susceptibility in the presence of L90M, the primary resistant mutation for PI.

Nevirapine resistance

In Uganda, a study concluded that mutations associated with nevirapine (NVP) resistance were detected more frequently at 6-8 weeks postpartum in women with subtype D than in women with subtype A[20]. As a consequence, NVP resistance could arise faster in subjects carrying subtype-D viruses. This finding could be explained by natural RT polymorphisms still unidentified, or by a faster progression to AIDS in subjects carrying clade D with respect to those infected with subtype-A variants recently reported[21,22].

Impaired or enhanced drug susceptibility *in vitro*

The spread of HIV-1 strains with reduced susceptibility to ARV drugs is a major cause of treatment failure. Thus, drug-resistance testing has become an important tool in the management of HIV-infected individuals undergoing ARV therapy[23]. More recently it has been recommended to all drug-naive patients before initiating treatment. Both genotype and phenotype appear to be useful for determining the susceptibility of HIV-1 to ARV drugs[24].

Phenotypic assays examine *in vitro* the relative susceptibility of a virus to different drug concentrations, and have been designed and performed mostly on subtype-B strains. Most phenotypic assays use HIV-1 viruses generated by recombination between PCR-amplified RT and/or PR products from patient's viruses and a subtype-B proviral clone with a deletion of the RT and/or PR genes. Phenotypic susceptibility testing of non-B viruses with natural polymorphisms in drug-targeted proteins (RT, PR, or gp41) are required to confirm whether HIV-1 non-B variants and intersubtype recombinants are fully susceptible to current ARV *in vitro*. The influence of the genetic backbone in drug susceptibility *in vitro* is currently unknown. Due to the increasing global spread of non-B subtypes, it remains crucial to determine the performance of all commercial drug-resistance assays testing non-B *pol* or *gp41* genes in the corresponding genetic background to compare IC_{50} values for inhibitors. The obtained results could lead to a better understanding of the clinical relevance of these baseline polymorphisms in ARV drug susceptibility *in vitro*. These natural substitutions in drug-targeted genes can function in two directions, either decreasing or increasing viral susceptibility to drugs.

Reduced susceptibility to antiretroviral drugs

It is known that although loss of susceptibility to PI or RTI is largely associated with the selection of mutations in genes codifying the drug-targeted enzymes, other changes may occur at other genes, including *gag*[25-28]. Moreover, the PI or RTI resistance could also be caused by changes in viruses other than those classically described as causing PI or RTI resistance in subtype B, as previously mentioned.

Protease inhibitors

Studies using different phenotypic assays have assessed the susceptibility of HIV-1 non-B viruses to different PI, demonstrating PI-susceptibility in up to 60-97% of non-B samples[29-33]. However, non-B viruses from drug-naive subjects show higher IC_{50} values for some PI with respect to clade-B variants of 3-40%, depending on the study[29-32]. For instance, some clade-G viruses from drug-naive subjects with decreased sus-

ceptibility to different PI with respect to susceptible subtype-B strains presented 2.9-fold resistance to amprenavir (APV)[31], 3.9-fold resistance to both NFV and RTV[31], or up to fivefold for RTV[33].

The effect of some prevalent polymorphisms at some non-B clades and recombinants was assessed performing infectious HIV-1 clones carrying K20I, M36I or K20I/M36I natural polymorphisms[32]. Interestingly, an improved viral replicative capacity under drug pressure was observed in those carrying 20I and/or 36I with respect to the wild-type clone, reducing the susceptibility to SQV and IDV. The observed IC_{50} values for those drugs were 2-3.5 fold higher than wild type[32], suggesting that additional substitutions within the PR might compensate in clinical samples for the reduced PI susceptibility caused by both substitutions observed in the mutant molecular clones. Likewise, a correlation of 20I/36I with decreased SQV and IDV activity needs to be examined in subtype G or CRF02_AG infections (carrying both 20I and 36I) under therapy.

Another PR change (E35D) was associated with decreased susceptibility of CRF02_AG to NFV[19]. Others authors reported that HIV-1 CRF02-AG variants from drug-naive West-African patients naturally carrying I20 and I36 at PR, were markedly less susceptible (more resistant) to PI than B viruses in the following order: NFV=LPV>IDV>RTV>APV[34]. The PR CRF02_AG pocket presents a significant distortion affecting the insertion of NFV with respect to clade-B PR, explaining the lower effect of NFV over these recombinants. This finding suggests implications for the combination of PI during the introduction of HAART in West Africa. Whether any PI is clinically effective in West-Africans carrying CRF02_AG variants remains unclear to date.

Reverse transcriptase inhibitors

With respect to RTI, Palmer, et al. reported similar RTI susceptibility in subtypes A, B, C, and CRF01-AE viruses, except in subtype-D specimens which had higher levels of resistance to zidovudine (AZT), lamivudine (3TC), didanosine (ddI), and NVP[35]. Subtype-F specimens also showed similar susceptibility to RTI, nucleoside and nonnucleoside RT inhibitors[36,37].

Entry inhibitors

With respect to the fusion inhibitor in clinical use, enfuvirtide (ENF), phenotypic drug susceptibility testing is scarce. As a consequence, it remains unclear whether the genetic variability in the gp41 region found across HIV-1 subtypes may influence their susceptibility to ENF *in vivo*. In group-O viruses, extensive genetic variability within the *gp41* gene paradoxically does not seem to compromise the antiviral activity of ENF[38], while it reduces the response to T-1249, another fusion inhibitor, not in clinical use[39]. Some studies have shown that the ENF-binding domain seems to be highly conserved across distinct HIV-1 variants[40,41]. However, phenotypic data have only rarely been reported[42,43]. Furthermore, gp41 substitutions outside the ENF interaction site including HR2 can also significantly impact on susceptibility to T20[44-46]. Recently it has been shown that HIV-1 subtypes A, C, D, F, G, J and recombinant CRF02_AG viruses seem to be susceptible to ENF *in vitro,* despite showing a significant genetic variability in the target gp41 viral region[47]. Thus, the benefit of ENF *in vivo* should be expected regardless of the HIV-1 clade.

With respect to the influence of HIV-1 subtype variability on phenotypic resistance to coreceptor antagonists, blocking HIV-1 entrance to cells needs further investigation.

Hypersusceptibility

Certain substitutions at various PR positions may mean little for subtype B, while conferring greater susceptibility to PI in other viral strains. There, IC_{50} values < 0.5 compared to wild type are seen[19,32,48]. LPV hypersusceptibility *in vitro* has been observed in drug-naive patients infected with subtype-C viruses[48], and hypersusceptibility to NFV *in vitro* for CRF02_AG recombinant viruses[19]. Using Virco's phenotype test, the hypersusceptibility to NFV in CRF02_AG was correlated with a K70R genotype ($p < 0.01$)[19]. While K70 appeared in clade B as a wild-type residue, 70R was wild type in CRF02_AG. By contrast, another PR change (E35D) was associated with decreased susceptibility of CRF02_AG to NFV[19].

It was recently also described that 10 out of 16 recombinant viruses carrying non-B PR (mostly clades G and A) from drug-naive individuals presented a certain hypersusceptibility (IC_{50} values ranging 0.1-0.4 x wild type) from one to four PI. A high percentage of hypersusceptible samples among the 16 tested non-B samples were found for APV (44%), LPV (44%), IDV (37.5%), SQV (25%), and NFV (6.25%)[32].

An essential question that remains unanswered is if the higher susceptibility of these HIV-1 strains compared with subtype B will lead to a better response to NFV, IDV or other PI. To date no clinical research support that hypothesis and such studies must be interpreted with great caution. Clinical data will have to

show whether the increased *in vitro* susceptibility of several PI (mostly APV, LPV, IDV and SQV) translates into treatment benefit in subjects infected with viruses harboring those polymorphisms. Further investigation must verify a link between the higher sensitivity-phenomenon and genetic variation in the *PR* gene that are often typical for non-B subtypes.

Natural polymorphisms influencing the virus replicative capacity

Although minor changes do not directly impair viral susceptibility to PI, they may promote resistance by enhancing viral replication capacity. Non-B differ from B in fitness (replicative capacity) assessed *in vitro*, and in binding affinities for PI *in vitro*[34]. Interestingly, hypersusceptibility ($IC_{50} < 0.5$) to some PI has been associated with mutations at polymorphic sites within PR associated with a reduced replicative capacity[49,50]. This supports the view that variations in PR sequence and function could be directly responsible for differences in fitness among strains in primary infection[50,51].

Some natural polymorphisms in non-B PR may increase the viral replication capacity in the absence of drugs and could therefore be of critical epidemiologic and clinical relevance. The impact of M36I and K20I PR changes, present in most G clades and CRF02_AG viruses[2,5,7], on the viral replicative capacity both in the absence and presence of PI, has been recently described[32]. In the absence of drug, infectious molecular clones carrying M36I change replicated more rapidly than wild type or the double-mutant K20I/M36I clones[32].

Although PR polymorphisms can have a direct impact on PI activity, other sterically or functionally incompatible substitutions might neutralize or modulate this viral advantage, as changes in gag cleavage sites. Interestingly, not only mutations selected upon failure under PI, but also naturally occurring polymorphisms in the PR gene would be expected to lead to adaptative (compensatory) changes in gag cleavage sites[52,53], affecting viral fitness. Thus, although some polymorphisms associated with dug resistance, such as in positions PR 36, can have an effect on viral fitness, it could be reduced or neutralized by other sterically or functionally incompatible substitutions *in vivo.*

Alternative pathways for resistance in HIV-1 non-B subtypes

Genetic variation of HIV-1 in the *pol* gene favors viral diversity and selection of drug resistance during ARV therapy. However, these changes may contribute to the selection of different resistance pathways[54,55]. Non-B subtypes follow different pathways of resistance and differ in codon use at sites critical to resistance, e.g. at positions 82 and 90 in PR, and 106 in RT.

Nelfinavir

After NFV therapy, a different selection of resistant mutations also is known among HIV-1 clades. Although the PR mutations D30N and L90M both develop in non-B viruses during NFV therapy, D30N occurs more frequently in subtype B, whereas L90M occurs more frequently in subtypes C, G, and CRF01_AE[54-57]. Thus, different pathways leading to NFV resistance seem to be chosen by those clades. This finding could be explained by polymorphic meandering at position 89, with 89L dominant in subtype B and 89M in other viral strains[57]. The preferential selection of L90M in NFV-treated, subtype-C patients could suggest that the L89M change generates a higher barrier for selection of D30N.

Indinavir

Camacho, et al. described different substitutions under drug pressure at PR codon 82 in HIV-1 subtype G compared to clade B-infected individuals failing therapy[58]. The wild codon was valine (V) in subtype B compared to isoleucine (I) in clade G viruses. Under PI pressure, substitutions were different among both clades, being more common V82A in clade-B and I82T/S/M in subtype-G viruses. The proposed reason for the different codon 82 usage by these clades could be due to different genetic barriers to PI resistance. In subtype G, the genetic barrier would be lower for I82T/S/M compared to I82A. The authors also confirmed by directed mutagenesis that viruses with I82M showed reduced susceptibility to IDV *in vitro*. Therefore, it could be considered a new resistance mutation in clade-G variants.

Tipranavir

Several polymorphisms frequently found in some non-B subtypes and recombinants are part of the list of changes included in the genotypic score for TPV[59], such as I13V, M36I and H69K[2,16]. On the other hand, the susceptibility to the drug is compromised by 82T, which is favored in clade-G viruses under PI pressure and only rarely in clade-B isolates.

Efavirenz

V106M is a signature mutation in clade C following exposure to efavirenz (EFV), and confers cross-resistance to NNRTI[60-62]. V106A is the most common substitution in clade-B viruses who have failed to EFV. This is due to the different codon use for valine in clade C and B (GTG and GTA, respectively). Clade-C viruses only need one nucleotide change to get M (ATG), while B variants need two.

Influence of HIV-1 subtypes on the interpretation of algorithms for genotypic resistance

Discordances exist when therapy response is predicted using different algorithms interpreting genotypic resistance, such as Rega 6.3, HIVDB-08/04, ANRS [07/04], and VGI 8.0). This has been attributed to specific combinations of subtype-dependent mutations[59,63]. For therapy-naive patients, especially those infected with subtypes C and G, discordances for NFV were primarily driven by the PR-mutational pattern 36I/V+63P+82I/V for subtype C, and 36I+63P+82I for subtype G. Subtype-F variants displayed more discordances for RTV in naive patients due to the presence of PR 10I/V+20R. Significant discordances for SQV and IDV were due to mutational patterns involving PR 82I+90M in treatment-experienced patients with subtype-G viruses. Subtype F showed more discordance for NFV attributable to the presence of 54V+82A+88S. For TPV, the currently available genotypic algorithm does not fully capture the effects of all mutations impacting on *in vitro* susceptibility, and efforts to improve the genotype interpretation are also required[16,59]. For the NRTI, 3TC and emtricitabine (FTC), CRF01-AE had more discordances than subtype B due to the presence of RT-mutational patterns 65R+115M and 118I+215Y, respectively[63]. No doubt HIV-1 non-B subtypes are a challenge for these systems, because these algorithms were originally designed using genos, phenos, and therapy response information was largely derived from experience with clade B.

Response to antiretroviral therapy in patients infected with non-B subtypes

The susceptibility of different HIV-1 subtypes to HAART is currently of interest. To date it is known that some HIV variants show natural resistance to several ARV drugs, as occurs for NNRTI in HIV-2[64] and in HIV-1 group-O viruses[65]. Group-N viruses have not been phenotypically studied in detail.

Whether subjects infected with different HIV-1 clades show the same clinical response to ARV drugs is still controversial. Whereas the response to ARV therapy overall may be independent of subtype and baseline polymorphisms[66,67], an accumulation of substitutions at positions associated with drug resistance (some of them natural polymorphism in non-B) increases the risk of treatment failure[12,68-72]. The greater the number of these mutations, the lower the sensitivity to PI. Interestingly, more than half non-B viruses from drug-naive individuals harbor three or more PI-resistance mutations with respect to 8% of individuals carrying subtype B ($p < 0.05$)[7]. NNRTI-resistant mutations were observed more frequently in subtype-C than in clade-B viruses[69,73]. Others observed that non-B sequences contained more minor substitutions impacting on IDV, NFV, ATZ, and RTV susceptibility[10], and those in subtype B to SQV and LPV/r[10].

Changes at PR codons 20, 36, and 82 influence the virologic response to LPV/r[74], and some non-B variants carry natural polymorphisms at those residues. Accordingly, others observed that PR changes K20I and M36I were the strongest predictors of virologic rebound to PI in nearly 40% of drug-naive individuals, of whom their first PI non-boosted based regimen failed[12]. Perno, et al. reported that the probability of accumulating a 90M mutation at virologic failure after the first PI-based ARV regimen was associated with the presence at baseline of minor mutation 36I and, possibly, of 10I/V[11]. Thus the presence of 36I at baseline could predict the appearance of 90M at virologic failure[11].

Table 2 summarizes some clinical studies performed to determine if the virologic and immunologic response to ARV treatment is similar in subjects carrying clade-B and non-B variants. One, including 79 African immigrants (86% carrying non-B strains, mainly A, C, and D), documented that all non-B subtypes were similarly clinically susceptible to initial HAART treatment[75]. Fully 76% had an undetectable viral load after one year. HAART included PI (95% with non-boosted PI) or NNRTI (90% NVP, 10% EFV) in 50% of cases. The ARV failure was observed in 28, 23 and 13% of subjects carrying subtypes A, D, and C, respectively. They concluded that country of origin, sex, and viral subtype had no impact on outcome of HAART. A limitation of this study is that adherence was not considered, and there were more individuals carrying subtype-B taking IDV and more with non-B getting NFV. Thus, the treatment regimen was not homogeneous in both groups of subjects carrying HIV-1 B and non-B subtypes.

Table 2. Main clinical trials comparing the virologic and immunologic responses to antiretroviral therapy in HIV-infected subjects carrying B *vs.* non-B subtypes

Reference	No. of patients	HIV-1 non-B subtypes (%)	Treatment regimen (months of follow-up)	Virologic response	Immunologic response
Frater, 2001	79	25A,11B,23C,13D,1H,1U,1AC, 1AD,3AG (86%)	HAART first regimen 12 months	Similar	Similar
Alexander, 2002	479	1A,459B,11C,2D,5CRF01_AE,1U (4.2%)	ARV 18 months	Similar	–
Nicastri, 2004	45	1A,34B,1C,3F, 2AG,2CRF02_AG,2J (24.4%)	HAART pretreated 12 months	Similar	Similar
De Wit , 2004	175	21A,56B,25C,8D,8G, 2H,1J,1CRF 01AE,22CRF02AG,22 mosaics (68%)	HAART 24 months	Similar	Lower in non-B
Bocket , 2005	416	317B, 99 non-B (not specified) (23.8%)	ARV 12 months	Similar	Similar
Bannister, 2005	684	7A, 547B, 24C, 34 other subtypes (20%)	HAART 12 months	Similar	Similar
Atlas, 2005	172	32A,44B,34C,18D,5G,19CRF01_AE (74.4%)	3 NRTI or HAART 6 months	Different across subtypes	Different across subtypes

The same authors concluded one year later that the initial virologic and immunologic responses of the African and European cohorts to HAART was similar[76]. In that study, viruses from 59/97 Africans and 80/265 Europeans were characterized at *pol* gene. All Africans carried non-B clades and recombinants, and all Europeans clade-B viruses. Both CD4+ cell-count responses and time to first undetectable viral load was similar for both groups. However, the African cohort showed the poorest longer-term virologic response, presenting an increase in viral load after nine months[76]. This may be related to poorer adherence to treatment in African subjects.

A study in 479 drug-naive individuals (only 4.2% carrying non-B viruses) demonstrated that although baseline CD4+ cell counts were lower in individuals harboring the non-B *vs.* B subtypes ($p = 0.02$), there were no significant differences in virologic response up to 18 months[77].

Another group also did not show any difference in terms of virologic/immunologic outcome between 45 drug-experienced individuals infected with different clades (24.4% non-B variants) after only year[78]. Both groups of patients with B and non-B subtypes had an increase in CD4+ count (70 and 156 cells, respectively). Undetectable viral load (< 50 copies/ml) was reached in 28 and 55% of patients carrying clade-B and non-B viruses, respectively. However, individuals carrying non-B subtypes had lower baseline CD4+ counts, were heavily treated with significantly more NNRTI, and were less adherent to HAART, according to a self-reported questionnaire[78].

Bocker, et al. compared the effectiveness of ARV during one year in 416 naive subjects, with 99 (24%) infected with HIV-1 non-B variants[79]. They concluded that HIV-1 subtype had no impact on clinical progression, CD4+ cell count, or viral load response to first-line ARV, and in the time to undetectable viral load (147 and 168 days in the B and non-B group, respectively). Similar rates were observed for clinical progression at 12 months in the B *vs.* non-B group (12 *vs.* 13%), for the percentage of patients with undetectable viremia (44 *vs.* 42%), and for the median increase in total lymphocyte cell count (124 *vs.* 88 cells/ul). At baseline, non-B patients had a lower median CD4+ cell count than those harboring B (226 *vs.* 266 cells/ul; $p = 0.05$), but their viral load was similar[79]. However, in this study the authors did not take into account individual non-B subtypes, adherence to treatment of enrolled subjects, and specific treatment follow up. Also, they did not analyze the clinical response to specific drug regimens.

Bannister, et al. conducted a study with 684 EuroSIDA patients (24% infected with non-B clades) with similar baseline viral load and CD4+ counts[80]. They observed that the virologic response (< 500 copies/ml) after HAART of subjects carrying non-B and B clades was similar at 6-12 months after starting ARV therapy. The therapy was defined as at least three ARV drugs including a PI, a NNRTI, abacavir (ABC) or tenofovir (TDF). However, the

response rates of those achieving virologic suppression in non-B subtypes were 57% for A, 71% for C and 74% for other non-B clades and recombinants, compared to 64% for clade B. Again, adherence was not determined.

Easterbrook, et al. determined the rate of CD4+ cell decline after ARV therapy in 594 HIV+ subjects, with 186 (31%) infected with non-B clades (40A, 81C, 14D, 15 CRF02_AG and 36 other recombinants)[81]. The initial virologic response after ARV therapy in individuals carrying B and non-B clades was similar[81].

At our institution we found a similar time to reach undetectable viremia and similar CD4+ count increments at 4, 12, and 24 months following initiation of the first HAART regimen in 70 drug-naive individuals with good adherence to treatment and carrying different HIV-1 subtypes: 6A, 35B, 1C, 3D, 1F2, 20G, 1H, 1J, 1CRF02_AG, 1U. All patients had similar viremia and CD4+ counts at baseline.

An additional study revealed that ethnicity but not genetic subtype correlated with virologic response to ARV therapy (mostly PI based regimens) at six months[82]. The study included 172 patients, with 74% carrying non-B. Undetectable viremia after six months was observed in 100% of CRF01_AE, 90% of B, 85% of C, 80% of G, 77% of A and only in 67% of D. A significant increment in CD4+ count over time was observed through all subtypes except D and G, despite no statistical difference in CD4+ count between the different subtypes at baseline being found. The CD4+ count increase depended on the infecting HIV-1 subtype, being significantly higher for subtypes CRF01_AE and B compared to subtype A[82]. Undetectable viral load after six months of treatment was observed in 77% of patients of African origin, in 91% of Asians, and in 100% of Caucasians.

In a single-center Belgian study, other authors considering longer follow-up found a significant difference in the median CD4+ cell increase at month 24 after initial HAART treatment in drug-naive subjects (68% harboring non-B viruses)[83]. The proportion of subjects who achieved undetectable viral load values (< 400 copies/ml) at month 24 was similar in B and non-B groups (52 *vs.* 64%). However, the CD4+ cell increase at month 24 was significantly lower in the non-B group with respect to clade B, with clade A exhibiting the lower increase (+162 *vs.* +235 cells at month 24; $p < 0.03$)[83]. Accordingly, a lower CD4+ recovery following initiation of HAART was observed in HIV-2 clinical isolates *vs.* HIV-1 clade B variants over 12 months, likely due to a lower baseline viremia in HIV-2 infected subjects[84].

Future clinical studies should consider non-recombinant infections *versus* intersubtype-recombinants, widely spread in the pandemic. Accordingly, more HIV clinical trials need to be conducted in Africa, where non-B subtypes and recombinants are prevalent. African cohorts of patients on ARV therapy need to be established in order to better understand the virologic and immunologic response to each regimen of ARV[85]. Some reports have concluded that large-scale HIV treatment initiatives in sub-Saharan Africa are effective, resulting in significant and persistent clinical and immunologic benefits[86]. Other authors have observed therapeutic responses to AZT+3TC+EFV in drug-naive HIV-1 infected Ugandan patients comparable to those previously described in the western world[87]. They showed virologic suppression below detection in 86% after three months despite high baseline viremia (> 100,000 copies/ml), with a median increment of CD4+ count at 31 weeks of 183 cells. Of interest, subtypes A and D, as well as AD recombinants are predominant in Uganda.

Whether the high rate of natural polymorphisms at *pol* genes observed in HIV-1 non-B clades will have a significant clinical impact remains uncertain. Despite these discrepant tendencies, response to ARV among HIV-1-infected individuals appears regardless of the genetic subtype, at least in the short term. However, all reports suggesting a similar clinical outcome presented limitations in the study design, with the most relevant summarized in table 3. Available data on non-B subtype viruses in datasets and therapy efficacy studies on subjects carrying those variants are too small, and clinical protocols testing drug efficacy do not enroll a large enough number of subjects carrying each different clade. Some of the clinical trials performed did not study the response of drugs in the different clades, and the response to each ARV drug in each clade. It is necessary to compare patient groups with similar treatment regimens, comparable CD4+ cell counts and viremia at baseline, and a similar onset time of infection. Moreover, it is essential to unify the criteria of virologic success in those studies, depending on the performed viral load quantification assay. Long-term assessment and close monitoring of subjects infected with non-B viruses will be required to determine the actual clinical impact of PR and RT polymorphisms in HIV-1 non-B variants. Lastly, more studies of structural differences in drug-bindings between PR and RT of different clades remain necessary, as was previously demonstrated for NFV in PR of CRF02-AG recombinants[34]. It is also important to have an accurate measurement of treatment adherence, addressed better by drug levels than by questionnaire.

Table 3. Limitations of studies comparing the clinical response to antiretroviral therapy according to HIV-1 subtype

1. Clinical response mainly assessed in non-B clades as a group, without differentiating distinct subtypes.
2. Low number of patients within each different non-B subtype.
3. Few studies considering infections with HIV-1 intersubtype recombinants.
4. Short follow-up period.
5. Adherence to antiretroviral treatment not always considered.
6. CD4+ cell count and viral load at baseline not always homogeneous among subjects carrying B/non-B viruses
7. Antiretroviral regimens not homogeneous among subjects carrying B/non-B viruses.
8. Response to each antiretroviral drug is not assessed for each clade.
9. Scarce data about response to antiretroviral therapy in drug-naive *vs.* pretreated subjects in different clades.
10. No consideration of genetic factors favoring higher plasma drug levels.
11. Wide range of assays used for considering undetectable viral load.
12. Subtyping not always performed by phylogenetic analysis in the target gene for antiretroviral drugs.
13. No grouping of subjects by time of infection, which could influence the clinical response to antiretroviral therapy.
14. Very few studies using boosted protease inhibitors.

Acknowledgements

This work was supported in part by grants from Red de Investigación en SIDA (RIS) and Fundación Investigación y Educación en SIDA (IES). A. H. was funded by Fundación para la Investigación Biomédica del Hospital Ramón y Cajal and Agencia Laín Entralgo.

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