

HIV-1 Pol Gene Polymorphism and Antiretroviral Resistance Mutations in Treatment-Naive Adult Patients in French Guiana Between 2006 and 2012

Edith Darcissac, Mathieu Nacher, Leila Adriouch, Alain Berlioz-Arthaud, Rachida Boukhari, Pierre Couppié, Félix Djossou, Damien Donato, Myriam El Guedj, Anne Lavergne, et al.

▶ **To cite this version:**

Edith Darcissac, Mathieu Nacher, Leila Adriouch, Alain Berlioz-Arthaud, Rachida Boukhari, et al.. HIV-1 Pol Gene Polymorphism and Antiretroviral Resistance Mutations in Treatment-Naive Adult Patients in French Guiana Between 2006 and 2012. AIDS Research and Human Retroviruses, 2016, 32 (8), pp.801 - 811. 10.1089/aid.2016.0048 . pasteur-01583613

HAL Id: pasteur-01583613
https://riip.hal.science/pasteur-01583613

Submitted on 7 Sep 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

HIV-1 pol gene polymorphism and antiretroviral resistance mutations in treatment-naïve adult patients in French Guiana between 2006 and 2012.

Edith Darcissac,[1] Mathieu Nacher,[2,3] Leila Adriouch,[2,*] Alain Berlioz-Arthaud,[4,*] Rachida Boukhari,[5,*] Pierre Couppié,[6,*] Felix Djossou,[7,*] Damien Donato,[1,*] Myriam El Guedj,[2,*] Anne Lavergne,[1,*] Emmanuelle Papot,[6,*] Jean-François Pouliquen,[1,*] Edouard Tanguy,[1,*] Vincent Vantilcke,[8,*] and Vincent Lacoste[1]

* alphabetically ordered

[1]Laboratoire des Interactions Virus-Hôtes, Institut Pasteur de la Guyane, 23 avenue Pasteur, BP6010, 97306 Cayenne cedex, Guyane Française
[2]Hôpital de Jour Adultes, Centre Hospitalier de Cayenne "Andrée Rosemon", rue des Flamboyants, BP6006, 97306 Cayenne cedex, Guyane Française
[3]Centre d'Investigation Clinique - Epidemiologie Clinique (CIC-EC) Antilles Guyane, INSERM CIE 802, Centre Hospitalier de Cayenne "Andrée Rosemon", rue des Flamboyants, BP6006, 97306 Cayenne cedex, Guyane Française
[4]Laboratoire de Biologie Médicale, Institut Pasteur de la Guyane, 23 avenue Pasteur, BP6010, 97306 Cayenne cedex, Guyane Française
[5]Service de Biologie Médicale, Centre Hospitalier de l'Ouest Guyanais "Franck Joly", Saint Laurent du Maroni, Guyane Française
[6]Service de Dermatologie, Centre Hospitalier de Cayenne "Andrée Rosemon", rue des Flamboyants, BP6006, 97306 Cayenne cedex, Guyane Française
[7]Unité des Maladies Infectieuses et Tropicales, Centre Hospitalier de Cayenne "Andrée Rosemon", rue des Flamboyants, BP6006, 97306 Cayenne cedex, Guyane Française
[8]Hôpital de Jour Adultes, Centre Hospitalier de l'Ouest Guyanais "Franck Joly", Saint Laurent du Maroni, Guyane Française

Correspondence to: Vincent Lacoste, PhD, Laboratoire des interactions Virus-Hôtes, Institut Pasteur de la Guyane, 23 avenue Pasteur, 97300 Cayenne, French Guiana, Phone # +594 594 29 58 17, Fax # + 594 594 29 31 37 (email: vlacoste@pasteur-cayenne.fr)

Running head: HIV-1 subtypes and genotypes in French Guiana

Abstract (147 words)

Little information is available on the molecular epidemiologic profile of HIV-1 in French Guiana, the French department with the highest HIV/AIDS incidence. To follow the evolution of HIV-1 diversity, we carried out a molecular analysis of HIV-1 isolates from 305 treatment-naïve patients between 2006 and 2012. Protease (PR) and reverse-transcriptase (RT) sequences were obtained for subtype characterization, polymorphism analysis and identification of drug resistance mutations. Of 305 HIV-1 strains, 95.1% were subtype B viruses. The overall prevalence of transmitted drug-resistance mutations (TDRM) was 4.6% (14/305), ranging from 1.9% to 7.1% depending on the year. This study shows a low level of HIV-1 genetic diversity and a moderate prevalence of TDRM with no evidence of an increasing trend over the study period. Nevertheless, the strong genetic polymorphism observed on both genes may be of concern for long-term treatment of people living with HIV-1 and thus deserves continuous monitoring.

Introduction

In French Guiana, the HIV/AIDS epidemic is a major public health problem. Indeed, despite a decline in the number of new AIDS diagnoses in the 2003–2013 period, the incidence of new HIV diagnoses is much higher than in mainland France (83 and 91 new diagnoses of HIV-1 infection per 100,000 inhabitants in 2012 and 2013, respectively, versus 17 in mainland France in 2012), making French Guiana the most affected French department.[1] In addition, the geographical position of French Guiana, the multi-ethnicity of the population, and other socioeconomic factors (precariousness, immigration, sexual behaviors) suggest that the profile of the HIV-1 epidemic may evolve given that other subtypes may be imported. In this particular context, there has been only one published study characterizing HIV-1 subtypes present in French Guiana, showing that subtype B predominated, with 95% of 210 patients tested infected with this subtype.[2] The present study aimed to provide a more accurate view of the HIV-1 epidemic in French Guiana. This study covered a 7-year period, from 2006 to 2012, and aimed to i) define the diversity of HIV-1 subtypes circulating in our cohort of untreated adult patients and ii) identify virus(es) harboring transmitted drug-resistance mutations, their prevalence and temporal trends.

Material and methods

This study was a retrospective analysis of 305 treatment-naïve HIV-1 patients followed in French Guiana between 2006 and 2012. Samples came from two hospitals: Cayenne (Centre Hospitalier Andrée Rosemond, CHAR) and Saint Laurent du Maroni (Centre Hospitalier de l'Ouest Guyanais, CHOG). These two centers give specialized care for approximately 75% of the known HIV-infected patients in French Guiana.

For each patient, the variables extracted were: age, sex, plasma HIV-1 RNA load at the time of sampling (or within a 3-month timeframe before or after sampling), CD4 cell count at the time of sampling (or within a 3-month timeframe before or after sampling), antiretroviral (ARV) treatment at the time of sampling and hospital.

The inclusion criteria were: HIV-1-positive patients, aged 18 years or older, naïve of ARV treatment, with a CD4 cell count equal to or above 350/mm^3, and for whom a genotypic resistance test had been requested by the physician at the time of the diagnosis (all patients are expected to get an initial pretreatment genotype). This CD4 threshold corresponds to the threshold for initiation of HIV-1 treatment under the 2010 WHO antiretroviral treatment guidelines. This threshold was also slightly above the median CD4 count at the time of diagnosis (301/mm^3) (M. Nacher, personal communication) and was a compromise between having sufficient patient numbers to obtain precise estimates (a CD4 count at 500 cells/mm^3 would have eliminated over 75% of the patients) and analyzing the new patients with presumably the most recent infections. Patients for whom the concomitant analysis of the protease (PR) and reverse transcriptase (RT) sequences was not possible were excluded.

This study was approved by the Clinical Research Committee from the Institut Pasteur (CoRC # 2014-16) and was conducted in accordance with French laws and regulations. No research-specific blood collection was performed for the study. All analyzed data resulted from diagnostic procedures required for the follow-up of HIV-1 infected patients. The epidemiologic data required for the study were extracted directly from eNadis®, a computerized medical record for specialty care and monitoring of patients infected with HIV-1 or hepatitis. All patients were informed of the possible use of data entered in eNadis® for epidemiological and clinical research and gave their written consent.

The eNadis® database is approved by the Commission Nationale de l'Informatique et des Libertés, the French regulatory authority for computerized databases.

Protease and reverse-transcriptase gene sequences were recovered from the Institut Pasteur de la Guyane. They were generated as follows: blood was collected in ethylene diamine tetraacetic acid tubes and plasma was separated and stored at −80°C until RNA extraction. RNA was isolated from 1 mL of plasma with the Nuclisens® EasyMag® (bioMérieux, Marcy l'Etoile, France) as recommended by the manufacturer. RNA was then reverse-transcribed to cDNA and amplified for partial reverse-transcriptase and protease genes using the ANRS (Agence Nationale de Recherche sur le Sida) consensus sets of primers (http://www.hivfrenchresistance.org/ANRS-procedures.pdf). The amplified products were sent to Beckman Coulter Genomics (http://www.cogenicsonline.com) for direct sequencing on both strands. Chromatograms were verified, analyzed, and interpreted using CEQ 2000XL software (Beckman Coulter Inc., Fullerton, CA, USA). For both PR and RT sequences, the sense and antisense strands of each fragment were aligned and compared to the wild-type HIV-1 virus HXB2 reference sequence (GenBank accession number K03455).

The PR and RT nucleotide sequences obtained were aligned using MEGA5 software with known reference sequences of group M pooled from the HIV-1 database (http://hiv.lanl.gov/). The phylogenetic trees were inferred using the Maximum Likelihood method based on the HKY85 + G model of nucleotide evolution. Subtypes were also identified in parallel on pol (PR+RT) sequences using the Calibrated Population Resistance (CPR) tool in the Stanford Database (http://cpr.stanford.edu) with the last updated version 6.0.

To predict the susceptibility to available PIs, NRTIs and NNRTIs, PR and RT sequences were analyzed using the French ANRS HIV-1 genotypic drug-resistance algorithm version 24, updated in September 2014 (http://www.hivfrenchresistance.org/2014/Algo2014.pdf). This analysis determined the impact of all resistance-related mutations on the susceptibility of the virus to ARV, including polymorphisms that were not included in the analysis for the prevalence of TDR.

The WHO list for surveillance of DRMs was used to estimate the prevalence of TDR.[3] The overall prevalence of TDR was defined as the percentage of isolates carrying any mutation indicative of TDR. To determine if there was a significant temporal change in the prevalence of transmitted DRMs, the linear trend chi-square was used.

Results

In the context of our diagnostic activity, we performed thousands of HIV-1 genotypic resistance tests prescribed by Cayenne and Saint Laurent hospitals between 2006 and 2012: 745 were performed for HIV-1-positive drug-naïve patients. Among them, 305 patients (40.9%) had a CD4 count ≥350 cells/mm^3 and met all inclusion criteria. Most of them were from Cayenne Hospital (79.0%, n=241) and the rest were from Saint Laurent Hospital (21.0%, n=64). With the exception of viral load, which was only obtained for 96.4% of the patients (n=294/305), equally for females (97.6%; n=163/167) and males (94.9%, n=131/138), the rest of the collected data (age, sex, CD4 count and naïve ARV status) were obtained for all patients.

Age ranged from 19 to 77 years with a median age of 36 years. The median viral load (log_{10} copies/mL) was 4.16 (range, 1.32–7.00) and the median CD4 count (cells/mm^3) was 500 (range, 350–2901). The characteristics of the study population are presented in Table 1. There were as many males as females. Females, who are systematically screened during pregnancy, tended to be younger and to have higher CD4 counts and lower viral loads.

HIV-1 subtype determination showed that subtype B accounted for 95.1% of the samples studied (n=290/305), followed by F (1.3%, n=4), A (0.3%, n=1), C (0.3%, n=1) and K (0.3%, n=1) subtypes and B/F (0.3%, n=1) intersubtype recombinants. Among the circulating recombinant forms (CRF), CRF02_AG (1.6%, n=5) and CRF01_AE (0.7%, n=2) were identified.

All sequences were analyzed for polymorphism, which was defined as any amino acid change compared to the subtype B consensus sequence HXB2 (Tables 2 and 3). For PR, alignment of the 290 subtype B sequences showed that overall, 61 of 99 (61.6%) amino acid positions presented at least one polymorphism each. In particular, V3 proved to be highly polymorphic with 99.7% of sequences mutated, followed by L63 (80.3%), S37 (75.5%), R41 (58.3%), V77 (40.7%), E35 (30.0%), I15 (29.7%), T12 (27.6%), M36 (26.6%) and I64 (26.2%). Other polymorphic positions were present in less than 25% of cases (Table 2). RT sequences were also highly polymorphic with 151 of 250 (60.4%) amino acid positions presenting at least one polymorphism each. Substitutions found in more than 25% of the study subjects occurred at the L214 (69.0%), E122 (66.2%), Q207 (62.8%), R211 (60.0%), T200 (54.8%), V245 (40.0%), S162 (39.0%), I135 (34.1%), V35 (28.6%), D177 (28.6%) and I178 (25.5%) positions (Table 3). Polymorphic positions on PR and RT of the other subtypes, for which we have far fewer isolates, are not listed here but are presented in Tables 2 and 3, respectively.

Drug-resistance mutations for PR and RT are presented in bold in Tables 2 and 3, respectively. We determined that 288 out of the 305 patients (94.4%) had viruses harboring DRMs on PR and/or RT. PI mutations were detected in 92.8% of isolates (n=283/305). The most commonly identified were L63P (45.9%), V77I (39.3%), I15V (29.8%) and M36I (27.5%) followed by I62V (24.3%), H69Q (16.7%) and D60E (14.1%). Additional low prevalence (<10%) PI-DRMs were found (Table 2). These substitutions corresponded to secondary mutation positions to PIs. Major mutations, such as M46I, V82A and L90M, which affect the resistance to ARV drugs, were found at a low prevalence (0.3% each, n=1). NRTI and NNRTI mutations were detected in 24.9% of patients (n=76/305). A98S (8.2%) and V179I (7.2%) were the two most commonly observed. Additional RTI-DRM positions were identified but at a lower prevalence, below 2% (Table 3). Many of these low prevalence mutations – K101E, K103N, E138A, M184I, Y188H, G190E and M230I – correspond to major mutations associated with resistance to one or more ARV drug.

Of the 305 patients tested, 18 exhibited viruses with drug resistance mutations to at least one ARV drug. All of them were of the B subtype. There were 66.7% females (n=12) and 33.3% males (n=6). However, this difference was not statistically significant (P=0.3, χ^2 test). Table 4 presents the genotypic profile of the 18 isolates. Three isolates presented a mutation to a PI: two to IDV and one to NFV. Two others presented substitutions at polymorphic positions whose combination confers a resistance to TPV for the first one and to SQV for the second one. These five isolates presented mutations to only one PI and none to RTI. Two isolates presented NRTI mutations (T69D + K219Q) associated with a resistance to DDI without resistance to any other class of ARV. Eight isolates bore NNRTI-resistant mutations:

five to EFV+NVP, two to RPV and one to EFV+NVP+RPV. These eight isolates only presented NNRTI mutations. The last three isolates had mutations associated with resistance to NRTI FTC+3TC plus another NNRTI resistance: EFV+NVP or RPV or EFV+NVP+RPV. No isolate had mutations to the three classes of drugs, NRTI, NNRTI and PI.

According to these results, the overall prevalence of transmitted DRM was 4.6% (14/305) in our cohort, ranging from 1.9% to 7.1% depending on the year (Figure 1). Based on the WHO list, four of the 18 isolates carrying a DRM to at least one drug were excluded: the two isolates (KT998118 and KT998206) that presented a PI resistance due to a combination of mutations on polymorphic positions on PR not indicative of TDR as well as the two (KT998027 and KT998252) bearing the E138A mutation given that this position is polymorphic. The prevalence of PI, NRTI, first-generation NNRTI (EFV, NVP) and RPV drug resistance mutations was 1.0%, 1.6%, 2.6% and 1.0%, respectively. There was no statistical trend over the period considered (P=0.69 for all resistance statistics and P=0.53 for NNRTI, linear trend χ^2).

Discussion

To participate in the improvement of the public health response to the HIV-1 epidemic in French Guiana, since the end of 2005 the Institut Pasteur de la Guyane has implemented the genotypic resistance test of HIV-1 against ARV. Thousands of analyses were subsequently requested by the two main hospitals in French Guiana, of which about 10% concerned drug-naïve patients. Since a large proportion of patients (33% in 2013) were diagnosed late (CD4 ≤200/mm^3), only 305 patients, accounting for 40.9% of the initial drug-naïve population, met the inclusion criteria and were included in the study.[1] Despite these

constraints, the characteristics of this cohort were similar to French Guiana's overall HIV-1 population in terms of the proportion of men and women as well as age.[1] This study thus allowed us to document the circulating HIV-1 subtypes and to present, to our knowledge, the first data on TDR in HIV-1-infected individuals in French Guiana. Given the intense migrations through the Guiana shield and beyond, these results may reflect broader regional trends.

Although migrations (80% of patients are immigrants) may lead to temporal evolution of the HIV-1 subtypes in the region, the analysis showed that subtype B still predominated, accounting for more than 95% of subtypes, as was reported 15 years ago.[2] The remaining 5% exhibited F, A, C and K subtypes, the B/F intersubtype, as well as CRF02_AG and CRF01_AE. Our proportion of subtypes was coherent with recent publications in neighboring countries showing that HIV-1 subtype B was the most prevalent in Latin America and the Caribbean, although subtype C and B/F recombinants have made significant contributions[4-6]. This stability of HIV-1 subtypes in French Guiana is in agreement with the fact that even if the migratory flux can substantially vary according to socioeconomic or political issues from one year to another, the main migratory sources (Haiti, Brazil, Suriname and Guyana) remain the same over time.

The prevalence of TDR is highly variable worldwide, with rates ranging from 0% to over 25%.[6] The highest prevalence rates are reported in countries with a long-standing use of antiretroviral therapy. In addition, during the last 10 years, the number of resistant strains has increased over time in these countries.[6, 7] We report here an average prevalence of 4.6%, which is coherent with the prevalence described in Latin America and the Caribbean,

ranging from 0% to 12% depending on the year and drug prescription patterns.[6-8] The yearly TDR prevalence in French Guiana fluctuated between 1.9% and 7.1%. Although it reached 7.1% in 2011, no significant trend was observed. However, the limited number of patients infected with a virus harboring DRM per year in our cohort (one to three per year) and the 7-year period may have been insufficient to detect a gradual increase of transmitted drug resistance.

For PR, the high degree of polymorphism at key amino acid positions known to be associated with drug resistance in HIV-1 subtype B is thus a concern for long-course PI treatment of people leaving with HIV. For NRTI, the M184I TDM, which confers high-level resistance to 3TC and FTC, was observed in three isolates. With M184V, this is the most commonly reported NRTI TDM in HIV-1 B subtype in the regions of the world where HAART has been used for years, as in French Guiana. Nevertheless, for two of the three isolates, the M184I was associated with the M230I mutation. Co-occurrence of these two mutations in the same sample suggests that they may have been generated through APOBEC3G/F editing.[9] Finally, most isolates bearing TDM (64%) presented NNRTI resistance to EFV and NVP and to RPV. Among them, the K103N mutation was observed in five isolates. These results are consistent with other studies showing that K103N is one of the most commonly transmitted mutations.[6, 7] A high correlation has been demonstrated between the prevalence of NNRTI TDM in drug-naïve patients and the ARV-treated individuals in the same region.[7, 10] Moreover, recent data pointed out the existence and the impact of low-frequency NNRTI-resistant mutations on future virologic failures.[11-14]

In conclusion, in the context of social and adherence difficulties, and of migrations from countries with a limited number of ARVs, the concern was that resistant viruses would be increasingly frequent in French Guiana. The present study shows that the prevalence of ARV resistance is consistent with other studies conducted in Latin America and that there is no evidence of an increasing trend. Nevertheless, this study also reveals a strong genetic polymorphism on both genes. Taken together these results imply that PR and RT DRMs deserve continuous attention in the future, notably in treatment-naïve individuals, in order to optimize first-line antiretroviral regimens in this territory.

Acknowledgments

This work was funded by the Institut Pasteur de la Guyane. It has also benefited from a European commission REGPOT-CT-2011-285837-STRonGer grant within the FP7. The authors wish to thank Nathalie Jolly from the Institut Pasteur Clinical Research Department (PIRC, Pôle Intégré de Recherche Clinique) for her help in the ethical aspects of the project. They also thank Ketty Bienvenu, Lidia Saint Louis, Sergine Soyon and Karine Verin for their invaluable assistance.

Sequence Data

The GenBank accession numbers for the sequences are KT998002 to KT998288 and KU052741 to KU052759.

Author Disclosure Statement

The authors have no competing interests to disclose.

References

1. CIRE Antilles Guyane. Infection par le VIH dans les DFA. BVS. 2014.
2. Kazanji M, Lavergne A, Pouliquen JF, et al. Genetic diversity and phylogenetic analysis of human immunodeficiency virus type 1 subtypes circulating in French Guiana. AIDS Res Hum Retroviruses. 2001;17(9):857-861.
3. Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One. 2009;4(3):e4724.
4. de Medeiros RM, Junqueira DM, Matte MC, Barcellos NT, Chies JA, Matos Almeida SE. Co-circulation HIV-1 subtypes B, C, and CRF31_BC in a drug-naive population from Southernmost Brazil: analysis of primary resistance mutations. J Med Virol. 2011;83(10):1682-1688.
5. Guimaraes PM, Ferreira JL, Coelho LP, et al. Transmitted Drug Resistance Among Recently Diagnosed Adults and Children in Sao Paulo, Brazil. AIDS Res Hum Retroviruses. 2015;31(12):1219-1224.
6. Rhee SY, Blanco JL, Jordan MR, et al. Geographic and temporal trends in the molecular epidemiology and genetic mechanisms of transmitted HIV-1 drug resistance: an individual-patient- and sequence-level meta-analysis. PLoS Med. 2015;12(4):e1001810.
7. WHO. WHO HIV drug resistance report 2012: World Health Organization Departement of HIV/AIDS. 2012.
8. Carvalho BC, Cardoso LP, Damasceno S, Stefani MM. Moderate prevalence of transmitted drug resistance and interiorization of HIV type 1 subtype C in the inland North State of Tocantins, Brazil. AIDS Res Hum Retroviruses. 2011;27(10):1081-1087.
9. Noguera-Julian M, Cozzi-Lepri A, Di Giallonardo F, et al. Contribution of APOBEC3G/F activity to the development of low-abundance drug-resistant human immunodeficiency virus type 1 variants. Clin Microbiol Infect. 2016;22(2):191-200.

10. Cambiano V, Bertagnolio S, Jordan MR, et al. Predicted levels of HIV drug resistance: potential impact of expanding diagnosis, retention, and eligibility criteria for antiretroviral therapy initiation. AIDS. 2014;28 Suppl 1:S15-23.

11. Gupta S, Lataillade M, Kyriakides TC, et al. Low-frequency NNRTI-resistant HIV-1 variants and relationship to mutational load in antiretroviral-naive subjects. Viruses. 2014;6(9):3428-3437.

12. Li JZ, Paredes R, Ribaudo HJ, et al. Impact of minority nonnucleoside reverse transcriptase inhibitor resistance mutations on resistance genotype after virologic failure. J Infect Dis. 2013;207(6):893-897.

13. Li JZ, Paredes R, Ribaudo HJ, et al. Relationship between minority nonnucleoside reverse transcriptase inhibitor resistance mutations, adherence, and the risk of virologic failure. AIDS. 2012;26(2):185-192.

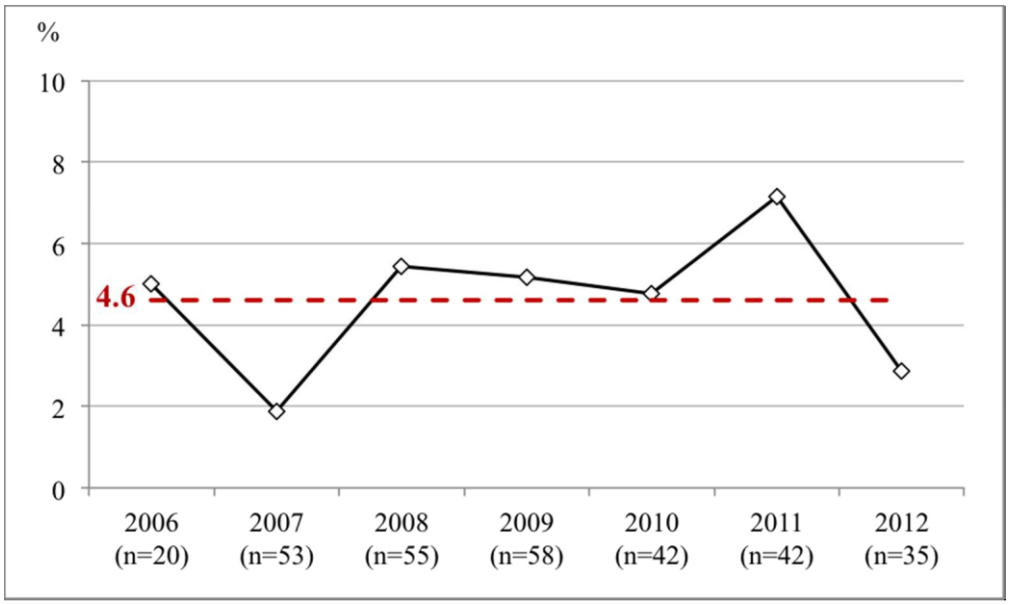
14. Li JZ, Paredes R, Ribaudo HJ, et al. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure: a systematic review and pooled analysis. JAMA. 2011;305(13):1327-1335.

FIGURE 1. Transmitted drug resistance prevalence (% per year)

The dotted line represents the prevalence (%) for the 2006–2012 period

The number of patients was between 20 and 58 depending of the year

n stands for the number of patients



77x46mm (300 x 300 DPI)

TABLE 1. Characteristics of the study population

Characteristics	Female	Male	Overall
N (%)	167 (54.8%)	138 (45.2%)	305 (100%)
Age[1] (years)	34 (19–75)	38 (19–77)	36 (19–77)
CD4[1] count (cells/mm^3)	506 (350–2280)	486 (350–2901)	500 (350–2901)
Viral load[1] ($\log_{10}$ copies/mL)	3.98 (1.32–7.00)	4.38 (1.60–7.00)	4.16 (1.32–7.00)

[1] median (range)

TABLE 2. Amino acid substitutions in the HIV-1 protease of isolates from 305 patients.

A

Subtype or CRF	N	V3	T4	L5	Q7	L10	V11	T12	I13	K14	I15	G16	G17	Q18	L19
B (95.1%)	290	I289	P1	F3	K1	I9, IV1, V4	I3	A14, AE1, AS1, D2, DN1, DV1, E25, I3, K6, M1, N11, P4, R2, S5, PS1, IKN1, AIV1	V48	E1, I1, M1, N1, R30	V86	E21, EG4, R1	E3	E1, H3, L2	I43, Q4, R1, S2, T7, V8
CRF02_AG (1.6%)	5	I5				I1		S1	V5	R4	V1	E1			
F (1.3%)	4	I4													
CRF01_AE (0.7%)	2	I2				V1			V2						
A (0.3%)	1	I1							V1						
C (0.3%)	1				E1			S1							I1
K (0.3%)	1	I1									V1				
B/F (0.3%)	1	I1													

B

Subtype or CRF	N	K20	E21	D25	L33	E35	M36	S37	P39	R41	K43	K45	M46	G48
B (95.1%)	290	E1, I2, M1, R9	Q1	N1	F1, I2, V4	D86, G1	I70, IT1, L2, T3, V1	C5, D5, E3, H8, IS1, K1, N185, T3, DN2, HD1, HN1, KN2, KT1, NT1	E1, L1, Q3, S9, T3, QR1, AS1	K166, N1, KN1, KT1	R4, T1	R6	I1, V1	E2
CRF02_AG (1.6%)	5	I4				D2	I4, L1	N4		K5				
F (1.3%)	4					D4	I4	N3, D1	S1	K4				
CRF01_AE (0.7%)	2	I1				D1	I2	N1, DN1		K2				
A (0.3%)	1	I1					I1	N1		K1				
C (0.3%)	1						I1	N1		K1				
K (0.3%)	1	R1				D1	I1	N1		K1				
B/F (0.3%)	1							N1						

C

Subtype or CRF	N	I54	R57	Q58	Y59	D60	Q61	I62	L63	I64	E65	I66	C67	G68
B (95.1%)	290	V1	K27	E2	H1	E39, N1	D1, H9, HN1, L1, N7,P1	V70	P123, PS5, PST1, PT2, PA1, PH3, A14, C10, K3, AV1, E1, H15, IV1, N2, NSTY1, Q7, R11, S25, ST1, T3, V3	L12, M4, V59, LV1	D14	M1, V2	H1, S4, Y2	R1
CRF02_AG (1.6%)	5		K1					V1	P2					
F (1.3%)	4		K4			E3	N4	V2	V1, Q1		D1			
CRF01_AE (0.7%)	2													
A (0.3%)	1													
C (0.3%)	1					E1			P1		D1			
K (0.3%)	1		K1				E1	V1	P1	M1				
B/F (0.3%)	1								P1					

D

Subtype or CRF	N	H69	K70	A71	I72	G73	V75	V77	V82	N83	I85	G86	R87	N88	L89	L90	T91	Q92	I93	L97	N98	F99
B (95.1%)	290	K1, N1, Q50, R1, Y10	E5, EGR1, I4, L1, P1, Q8, R31, LP1, T17	I1, T11, V8	E7, EV1, M1, R1, T18, TV1, V35	T2	I10	I118	A1, I16	H1	V1	R2	G1, T2	T1	M6, IM1, V1	M1	I1	H1, K1, T1	L65	S1	Y1	R1
CRF02_AG (1.6%)	5	K5	R1												M5							L1
F (1.3%)	4				A1, T1			I2							M4				L2			
CRF01_AE (0.7%)	2	K2													M2							
A (0.3%)	1	K1	R1												M1							
C (0.3%)	1	K1													M1				L1			
K (0.3%)	1	Q1			V1																	
B/F (0.3%)	1																					

N: number of strains

The gray boxes concern polymorphic positions (>25%) and the boxed columns point to the positions of resistance mutations according to the French ANRS algorithm, version 24, September 2014.

TABLE 3. Amino acid substitutions in the HIV-1 reverse-transcriptase of isolates from 305 patients.

A

Subtype or CRF	N	P1	I2	P4	E6	V8	V10	K11	K13	P14	G15	M16	P19	K20	V21	K22	Q23	W24	L26	E28	K30	I31	K32	V35	E36
B (95.1%)	290	S3	F1, L1	H1, S1	A2, D9, K2, Q1	I3	A1	H1, IT1, N1, Q1, R2, T1	N2	Q1	R2	I1	A1	E1, N1, R32	I7	R1	H1, HP1, T1	R4	PQS1	A1	R1	T1	N2, Q1, R12	A1, AIT1, E1, I45, IT3, M4, R1, T27	D1, G1, Q2
CRF02_AG (1.6%)	5														I1					D1			R1	T5	D1
F (1.3%)	4																							K2, T2	
CRF01_AE (0.7%)	2				K1			T2							I1									T2	A1
A (0.3%)	1							T1																T1	
C (0.3%)	1				K1																			T1	A1
K (0.3%)	1													R1										T1	
B/F (0.3%)	1																							T1	

B

Subtype or CRF	N	I37	C38	T39	E40	M41	E42	K43	E44	G45	I47	S48	K49	I50	G51	P52	E53	V60	F61	I63	K64	D67	S68	T69
B (95.1%)	290	M1, N1	R1, W1	A8, K1, Q1	D23, G1	I2, L2, T1	Q1	Q1, K3	K1	E1, R2	M1	P1, T14	GR1, R35	V2	R2	L1	D3	I55	Y1	M1	R23	N2	G12, R1	A2, D2, N1, P1, S1
CRF02_AG (1.6%)	5																	I1						
F (1.3%)	4												R3											S1
CRF01_AE (0.7%)	2			K2	D1			E1									D2	I1						
A (0.3%)	1																D1	I1						
C (0.3%)	1			E1								T1												
K (0.3%)	1			I1	D1								R1					I1						
B/F (0.3%)	1			A1	D1																			

C

Subtype or CRF	N	W71	R72	E79	L80	K82	R83	Q85	F87	E89	V90	G93	H96	P97	A98	G99	L100	K101	K102	K103	K104	V106	T107	V108	V111	G112	V118
B (95.1%)	290	G1	K1		I1	R4	K3	L1	L2	G1	I2	R1	N1	L1	S25	E1	S1	E1, Q1, R5	Q12, R3	N5, R1	N2, R5	I5	S1	I2	M1	S1	I23
CRF02_AG (1.6%)	5																		R1								
F (1.3%)	4			D1																		I1					
CRF01_AE (0.7%)	2																										
A (0.3%)	1																										
C (0.3%)	1																										
K (0.3%)	1																										
B/F (0.3%)	1																										

D

Subtype or CRF	N	D121	E122	D123	R125	F130	P133	S134	I135	E138	T139	I142	Q145	N147	L149	K154	A158	F160	S162	M164	T165
B (95.1%)	290	A1, CY1, H9, HY1, Y12	A3, K174, KR1, N2, P9,Q2, T1	E49, EG1, G3, N10, NST1, R1, S4	K1	L2	H1, L1	I1	AT1, ATV2, KR1, L1, R2, T62, V30	A2, D1	A2, R1, S1, V1	GSV1, K1, L1, MV1, Q1, T10, V28	L1	T1	F1	Q1	S13, ST1	C1	A22, C76, CY1, F1, H4, N1, Y8	I1, T1	I16
CRF02_AG (1.6%)	5		K3	E1, H1, NS1					V4			V1					S1		A5		
F (1.3%)	4	Y2	K2	E1					T3, V1										C4		
CRF01_AE (0.7%)	2	H2		S1					T2			V2							Y1, H1		
A (0.3%)	1	H1		S1					T1			V1							Y1		
C (0.3%)	1			G1																	
K (0.3%)	1		P1	N1															C1		
B/F (0.3%)	1		K1	N1																	I1

E

Subtype or CRF	N	K166	I167	L168	E169	P170	F171	K173	Q174	N175	P176	D177	I178	V179	I180	Q182	M184	Y188	V189	G190	D192	E194	I195
B (95.1%)	290	N1, NY1, R11, T1	M1, T1, V1	V1	A1, D38, K1		C1, L1, Y4	A2, E7, N1, Q6, R8, T6	E3, EGKR1, H7, K2, L2, N3, R6	H1	S2	E80, G3	L32, M41, MV1	D3, DE1, I22	V2	R1	I3	H1	I5	E2, R1	EKN1, N1	D2, G1	L5, LS1
CRF02_AG (1.6%)	5							A1, AV1, I1, R1, T1	K3, N2			E5	M1										
F (1.3%)	4				D4			A2, T2	K3, R1			E1	L2										
CRF01_AE (0.7%)	2					T1		S2	K1			E2											
A (0.3%)	1							S1	R1														
C (0.3%)	1	R1						A1	K1			E1											
K (0.3%)	1							AT1	K1			E1	M1										
B/F (0.3%)	1							T1	K1			E1											

F

Subtype or CRF	N	G196	Q197	T200	K201	I202	E203	E204	L205	R206	Q207	H208	L210	R211	G213	L214	T215
B (95.1%)	290	E32, K3, N1, R3, S3	E2, H1, P4, R2	A130, AE2, AV1, D1, E8, EV1, I11, IM1, IV1, R1, S1, V1	IN1, R1	V14	D2, G2, K1, Q1	A1, D3, DKN1, K12, N2, Q3	M1, P1, W1	G1	A10, AEKT1, AGRT1, D7, DE2, E112, EG5, EK10, G17, H1, K5, KN1, KR1, N1, R6, S1, V1	D1, Y1	F1, W1	E4, EQ1, G1, GMV1, K159, KQ2, Q3, S2, T1	F1, R2	F198, P2	C3, CDGY1, L1, N1
CRF02_AG (1.6%)	5			A5	R1						E4, G1			K4		F4	
F (1.3%)	4			I1							E3, R1			A1, K3		F2	
CRF01_AE (0.7%)	2			A1		V1					A2			K2		F1	
A (0.3%)	1			A1							A1			K1			
C (0.3%)	1	R1		A1							E1					F1	
K (0.3%)	1										E1			K1		F1	
B/F (0.3%)	1			V1							DE1			K1			

G

Subtype or CRF	N	T216	K219	K220	H221	Q222	E224	F227	L228	W229	M230	E233	L234	H235	P236	D237	K238	W239	P243
B (95.1%)	290	P1	Q2	I1	P1	L2, P3	A1, D1, K1	S1	F1, H1, N1, Q1	CG1, G1, L1	I2, R1	G13, V3	V2	P1	H12	A1, N1	E1, N1	G1	A2, H1, R1, S6, T5
CRF02_AG (1.6%)	5																		H1
F (1.3%)	4																		
CRF01_AE (0.7%)	2																		
A (0.3%)	1																		
C (0.3%)	1																		
K (0.3%)	1																		
B/F (0.3%)	1																		

H

Subtype or CRF	N	I244	V245	E248	K249	D250
B (95.1%)	290	V5	E13, GL1, I8, K19, KT1, L3, LM2, M40, N4, Q9, R3, T13	D21, G1, KN1, L1, N6, Q4	Q6, R3	E25, G1, N2, S1
CRF02_AG (1.6%)	5		Q4	N1		
F (1.3%)	4		Q1	D1		
CRF01_AE (0.7%)	2		E1, Q1	D1		
A (0.3%)	1		K1			
C (0.3%)	1		Q1			
K (0.3%)	1		Q1	D1		E1
B/F (0.3%)	1		K1	D1		E1

N: number of strains

The gray boxes concern polymorphic positions (>25%) and the boxed columns point to the positions of resistance mutations according to the French ANRS algorithm, version 24, September 2014.

TABLE 4. Characteristics of the samples presenting drug-resistance mutations.

Sample	Year	Age	Sex	CD4	VL[2]	Drug-resistance mutations[1] on		Targeted drug classes		
						PR	RT	PI	NRTI	NNRTI
KU052742	2006	32	F	423	4.33	L10I, L90M	T69N, A98S	NFV		
KT998023	2007	36	F	374	3.67	I15V	G190E			EFV+NVP
KT998027	2007	44	F	846	4.77	I15V, M36I, V77I	A98S, E138A			RPV
KT998067	2008	29	F	576	3.09	L63P	D67N, T69D, K219Q		DDI	
KT998094	2008	21	F	381	2.92	M36I, I62V	K101E, K103R			EFV+NVP+RPV
KT998106	2008	36	F	402	4.23	G16E, K20R, M36V, I62V	A98S, K103N			EFV+NVP
KT998118	2009	31	M	436	2.83	L10I, M36I, H69Q, L89M	V106I	TPV		
KT998131	2009	40	F	366	3.71	L69P, H69R	K103N, M184I, Y188H		FTC+3TC	EFV+NVP
KT998157	2009	45	M	517	1.66	G16E, V77I	K103N			EFV+NVP
KT998159	2009	39	F	500	3.46		G190E			EFV+NVP
KT998180	2010	52	M	483	4.65	L10I, I15V, I54V, L63P, H69Q, A71V, V82A		IDV		
KT998183	2010	59	F	477	4.49	M46I		IDV		
KT998206	2010	31	F	593	3.20	L10I, K20I, M36I, I62V, L63P, G73T	M41L, T215L	SQV		
KT998242	2011	22	F	426	3.72	G16E, V77I	D67N, T69D, K219Q		DDI	
KT998210	2011	31	M	684	4.72		K103N, M184I, M230I		FTC+3TC	EFV+NVP+RPV
KT998243	2011	55	M	670	4.49	A71T, V77I	V179I, M184I, M230I		FTC+3TC	RPV
KT998252	2012	64	M	396	4.28	L10IV, M36I, I62V	E138A			RPV
KT998278	2012	31	F	471	4.28		K103N, V179I			EFV+NVP

[1] According to the French ANRS algorithm, version 24, September 2014. Major resistance mutations are in bold

[2] VL, viral load (log_{10} copies/mm^3)