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# Prevalence of HIV-1 drug resistance in treated patients with viral load >50 copies/mL in 2009: a French nationwide study

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**Background:** Surveillance of HIV-1 drug resistance in treated patients with plasma viral load (VL) >50 copies/mL.

**Methods:** The protease and reverse transcriptase (RT) genes were systematically sequenced in samples from 756 patients with VL >50 copies/mL in 2009. The genotyping results were interpreted for each antiretroviral drug (ARV) by using the ANRS algorithm v21. Weighted analyses were used to derive representative estimates of percentages of patients. Prevalence rates were compared with those obtained in 2004 among patients with VL >1000 copies/mL.

**Results:** Sequences were obtained for 506 patients. Sequencing was successful in 45%, 80% and 96% of samples with VL of 51–500, 501–1000 and >1000 copies/mL, respectively. Resistance or possible resistance to at least one ARV was observed in 59% of samples. Overall, 0.9% of samples contained viruses resistant to all drugs belonging to at least three drug classes. All resistance prevalence rates were significantly lower in 2009 than in 2004.

**Conclusion:** In France, where 86% of patients were receiving combination antiretroviral therapy in 2009, only 15.0% of patients had a VL >50 copies/mL, suggesting that only 8.9% of treated patients could potentially transmit resistant viruses. Only 0.08% of patients harboured viruses fully resistant to at least three antiretroviral drug classes. Further studies are needed to determine whether resistance continues to decline over time.

**Keywords:** antiretroviral therapy, epidemiology, HIV-1 drug resistance, multidrug resistance, plasma HIV RNA, virological failure

## Introduction

The use of combined antiretroviral therapy (cART) has led to a marked fall in HIV/AIDS-related morbidity and mortality.[1,2] However, viruses resistant to all classes of antiretroviral drugs can be selected during treatment and can lead to virological failure.[3] Resistant viruses may be acquired at the time of primary infection or, more importantly, be selected during virological failure on cART. It is important to monitor the prevalence of drug-resistant HIV in treated patients, both to limit the spread of resistance and to determine the proportion of patients requiring new therapeutic options. We therefore conducted a systematic nationwide survey of HIV-1 resistance in patients with virological failure in France, and compared the results with those obtained in 2004.[4]

## Patients and methods

### Study population

HIV-1-infected patients were included in this study if they had been receiving cART for at least 6 months and if they had a confirmed plasma HIV-1 RNA value >50 copies/mL between April and September 2009. Up to 30 consecutive patients were enrolled in each participating virology laboratory. Participating laboratories belonged to the Agence Nationale de Recherches sur le SIDA et les Hépatites Virales (ANRS) AC11 network and participated in the ANRS quality control assessment of HIV-1 drug resistance sequencing.[5] The study was approved by the Comité Consultatif de Traitement de l'Information dans la Recherche Scientifique et Médicale (CCTIRS) and the Commission Nationale Informatique et Libertés (CNIL), in keeping with French law. The patients received full information concerning their participation in the study.

### Genotypic resistance analysis

The sequences of the protease, reverse transcriptase (RT), gp41 and integrase genes were determined in each laboratory, using the ANRS consensus technique (http://www.hivfrenchresistance.org), the Bayer TrueGene kit (Siemens Medical Solutions Diagnostics, Tarrytown, NY), the Abbott ViroSeq kit (Abbott Laboratories, Abbott Park, IL) or an in-house method. The gp41 and integrase genes were only sequenced if the patient was receiving enfuvirtide or raltegravir, respectively. Primary and secondary protease, RT, integrase and gp41 gene mutations were identified from the International AIDS Society resistance testing USA panel (http://www.iasusa.org/resistance_mutations/mutations_figures.pdf; update November 2011).

### Data collection

Sociodemographic data, clinical data, treatment histories and the treatment regimen at the time of virological failure were routinely collected. Respect for the inclusion criteria and all recorded data were checked by study monitors.

The deduced amino acid sequences of the different genes were collected in text or FASTA format and integrated in a specifically developed Access® database application.

### Statistical analysis

Weighted analyses were used to derive representative estimates of the percentages of patients harbouring viruses with mutations conferring resistance to drugs belonging to the nucleoside reverse transcriptase inhibitor (NRTI), protease inhibitor (PI) and non-NRTI (NNRTI) classes, weighting being based on the number of patients followed in each centre. The estimates of the number of patients followed in each centre were derived from the French Hospital Database on HIV (FHDH ANRS CO4) and from the ANRS Aquitaine CO3 Cohort. Crude analysis (without weighting) was used to estimate the percentages of patients harbouring viruses with mutations conferring resistance to an integrase or gp41 class drug, as the integrase and gp41 genes were only sequenced in patients receiving raltegravir or enfuvirtide. The data for enfuvirtide resistance mutations are not shown, because of the small sample size ($n=4$). Results are expressed as medians and IQRs or percentages.

The genotyping results were interpreted for each drug by using the ANRS algorithm (http://www.hivfrenchresistance.org; update October 2011, version 21). We also used the Stanford algorithm (http://hivdb.stanford.edu; update November 2011) to interpret resistance to tipranavir in patients with non-B viruses, as the ANRS algorithm for tipranavir is only valid for subtype B. The ANRS algorithm designates viruses as resistant (R), possibly resistant (I) or susceptible (S). In the Stanford system, viruses are ranked as follows: susceptible, potential low-level resistance, low-level resistance, intermediate resistance or high-level resistance. To condense these categories into three levels of resistance, we grouped the first two levels in category S, the third and fourth in category I, and the fifth in category R. We considered that an isolate was completely resistant to a class of drugs when it was ranked as resistant or possibly resistant to all the drugs considered for the class.

The rates of successful sequencing and of resistance to each drug and each class of drugs were analysed according to the plasma HIV-RNA level (51–500, 501–1000 and >1000 copies/mL). The $\chi^2$ test or Fisher's exact test was used to compare categorical variables and the Mann–Whitney *U*-test was used to compare continuous variables. Multivariable logistic models were used to identify factors associated with the risk of RT or protease gene amplification failure. Variables with univariate *P* values <0.15 were included in multivariable logistic models.

We used FHDH ANRS CO4 to estimate the percentage of HIV-1-infected patients receiving cART in 2009 and the percentage of those with viral load >50 copies/mL. This allowed us to estimate the percentage of treated patients who could potentially transmit resistant viruses and the percentage of treated patients harbouring viruses with complete resistance to at least three classes of drugs.

Amino acid sequences ($n=498$) obtained during our previous survey (Multivir 2004)[4] were re-analysed with the algorithms used in the present survey (Multivir 2009) in order to compare the frequencies of drug resistance between the two surveys in patients with plasma HIV-RNA >1000 copies/mL, as this was an inclusion criterion in the Multivir 2004 study.

The SPSS software package version 19.0 for Windows (SPSS Inc., Chicago, IL, USA) and SAS® statistical software version 9.3 (SAS Institute, Inc., Cary, NC, USA) were used for all analyses.

## Results

### Characteristics of the patients

Nine hundred treated HIV-1-infected patients with viral load >50 copies/mL were screened between April and September 2009 in 33 French laboratories and 1 Swiss laboratory for inclusion in this study. One hundred and forty-four patients were ineligible [inclusion criteria not met ($n=62$) or viral load <50 copies/mL at the last visit ($n=82$)]. The protease and RT genes were successfully sequenced in 506 of the remaining 756 patients. Amplification was attempted in 60 of the 97 patients receiving raltegravir and was successful in 35 cases.

Two-thirds of the patients were men, and the median age was 46 years (IQR 39–51 years). Median plasma viral load was 2.57 $\log_{10}$ copies/mL (IQR 2.05–3.49). The percentage of patients with plasma viral load >1000 copies/mL was 35.6. The median CD4 cell count was 390 cells/mm$^3$ (IQR 225–581). The patients had been exposed to a median of 8 antiretroviral drugs overall (IQR 6–10) and to 5 NRTIs (IQR 3–6), 2 PIs (IQR 1–3) and 1 NNRTI (IQR 0–1). The median total duration of ART was 10.4 years (IQR 4.6–13.4). Fifty-eight percent of patients had received three different drug classes. The antiretroviral treatments at the time of virological failure were tenofovir (58.6%), emtricitabine (48.4%), lamivudine (34.9%), abacavir (29.7%), zidovudine (12.1%), didanosine (5.9%), stavudine (1.4%), efavirenz (9.1%), etravirine (6.5%), nevirapine (5.5%), lopinavir/ritonavir (27.4%), atazanavir (26.5%) including atazanavir/ritonavir (21.9%), darunavir/ritonavir (14.7%), saquinavir/ritonavir (3.9%), fosamprenavir/ritonavir (2.8%), tipranavir/ritonavir (2.1%), indinavir (0.6%) including indinavir/ritonavir (0.3%), raltegravir (13.3%), maraviroc (2.2%) and enfuvirtide (1.5%).

### *Factors predictive of amplification failure*

Exposure to the different drugs at the time of virological failure was well balanced between the groups in which RT and protease gene amplification was successful and unsuccessful, with the exception of nevirapine, which was associated with amplification failure ($P=0.008$). Amplification of both the RT and protease genes was successful in 21%, 46%, 64%, 69%, 77%, 80% and 96% of samples from patients with plasma viral loads of 51–100, 101–150, 151–200, 201–300, 301–500, 501–1000 and >1000 copies/mL ($P<0.001$), respectively. The univariate analyses showed that age at genotyping, transmission group, CD4 count and plasma viral load at genotyping, and duration on cART were significantly associated with unsuccessful resistance genotyping. Multivariable analysis showed that only plasma viral load independently predicted amplification failure: the risk of failure was 34.6 times higher (95% CI 17.3–69.1) and 7.1 times higher (95% CI 3.0–16.7) when plasma viral load was 51–500 and 501–1000 copies/mL, respectively, than when it was >1000 copies/mL.

### *Genotypic resistance patterns*

The prevalence of resistance mutations to NRTIs, NNRTIs and PIs is shown in Figure 1. The M184V and/or I mutations, selected by lamivudine and emtricitabine, were present in 36% of patients. NRTI resistance mutations selected by thymidine analogues (M41L, D67N, K70R, L210W, T215Y/F and K219Q/E) were the most frequent other mutations (10.3%–23.3%). Multiple NRTI resistance mutations, such as the Q151M complex and the 69 insertion complex, were present in 1% and 0% of samples, respectively. The K65R and L74V mutations were found in 2.1% and 6.0% of patients, respectively (Figure 1a). The most frequent NNRTI resistance mutations were K103N and Y181C/I/V, which were detected in 12.2% and 10.1% of patients, respectively (Figure 1b). The prevalence of major protease resistance mutations ranged from 0.3% (N83D) to 18.8% (I54L/M/V/A/T/S) (Figure 1c). Raltegravir resistance mutations were frequent (66%) in patients with failing raltegravir regimens ($n=35$): E92Q ($n=3$), Y143R/H/C ($n=5$), Q148H/R/K ($n=9$) and N155H ($n=9$).

### *Drug resistance interpretation*

The prevalence of resistance and possible resistance to drugs belonging to the NRTI, PI and NNRTI classes ranged from 17% (didanosine) to 37% (emtricitabine and lamivudine), from 8% (darunavir) to 25% (indinavir) and from 11% (etravirine) to 20% (efavirenz and rilpivirine), respectively. Resistance or possible resistance to at least one antiretroviral drug was observed in 59% of samples (at least one NRTI in 49%, one PI in 29% and one NNRTI in 23%). The percentages of patients whose viruses were not susceptible to any drugs in the NRTI, PI or NNRTI classes were 8.8%, 4.5% and 8.6%, respectively.

The frequency of resistance or possible resistance to all members of at least one drug class (NRTI, PI or NNRTI) was 16.5%. There was complete resistance to one class in 12.1% of cases, to two classes in 3.5% of cases [NRTI+NNRTI (1.8%) and NRTI+PI (1.1%), NNRTI+PI (0.6%)] and to all three classes in 0.9% of cases.

### *Correlation between viral load and resistance*

The frequency of resistance or possible resistance to at least one antiretroviral drug increased significantly with plasma viral load: 49% in the group with plasma HIV RNA of 51–500 copies/mL, 65% in the group with plasma HIV RNA of 501–1000 copies/mL and 67% in the group with plasma HIV RNA >1000 copies/mL. The proportion of patients with viruses resistant to at least one drug in the NRTI or NNRTI class was significantly higher when plasma viral load was >500 copies/mL than when it was 51–500 copies/mL. For resistance to PIs, the proportion was higher when the plasma viral load was >1000 copies/mL. The percentages of patients with viral loads of 51–500, 501–1000 and >1000 copies/mL whose viruses were resistant to all NRTIs were 6.7%, 16.5% and 13.9%, respectively ($P=0.029$); the corresponding proportions for PIs were 1.7%, 0% and 9.9% ($P<0.001$) and those for NNRTIs were 4.3%, 10.2% and 11.5% ($P=0.029$).

### *Comparison between 2004 and 2009*

In the Multivir 2004 and 2009 studies, 498 and 259 patients, respectively, had plasma HIV-RNA >1000 copies/mL, with median plasma HIV-RNA levels of 4.0 $\log_{10}$ copies/mL (IQR 3.5–4.7) in 2004 and 3.9 $\log_{10}$ copies/mL (3.3–4.6) in 2009, of whom 85% and 67%, respectively, had viruses with resistance or possible resistance to at least one antiretroviral drug ($P<0.001$), as follows: at least one NRTI in 81% and 55% of patients ($P<0.001$), at least one NNRTI in 44% and 30% ($P<0.001$) and at least one PI in 51% and 39% ($P=0.002$). The prevalence of resistance to most drugs fell between 2004 and 2009. No difference in the prevalence of etravirine, saquinavir or atazanavir resistance was found between 2009 and 2004. By contrast, resistance to darunavir was significantly more frequent in 2009 than in 2004. Complete or possible resistance to all members of at least one drug class was more frequent in 2004 than in 2009 (35% versus 24%, $P=0.001$).

## Discussion

We determined the prevalence of HIV-1 resistance to antiretroviral drugs in treated patients with viral loads >50 copies/mL in 2009 in France. Viral sequences were successfully obtained in 67% of 756 patients. Plasma viral load was the only factor independently associated with the risk of amplification failure (increasing from 21% for viral load 51–100 copies/mL to 96% for >1000 copies/mL). This association is already known, but very few studies have quantified and clearly demonstrated such a relationship.

In 2009, 86.0% of patients included in the FHDH ANRS CO4 were receiving cART, of whom 15.0% had viral loads >50 copies/mL. Using the ANRS algorithm and extrapolation on the whole French database, 8.9% of all treated patients could contribute to the spread of resistance and 0.08% had complete resistance to three antiretroviral drug classes.

The frequency of resistance mutations decreased significantly over time. For example, thymidine analogue mutations (TAMs), known to reduce viral susceptibility to most approved NRTIs,[6] significantly decreased from 16%–51% in 2004 to 10%–23% in the 2009 survey. This decline may be explained by lesser use of zidovudine and stavudine, which tend to select TAMs. TAMs are

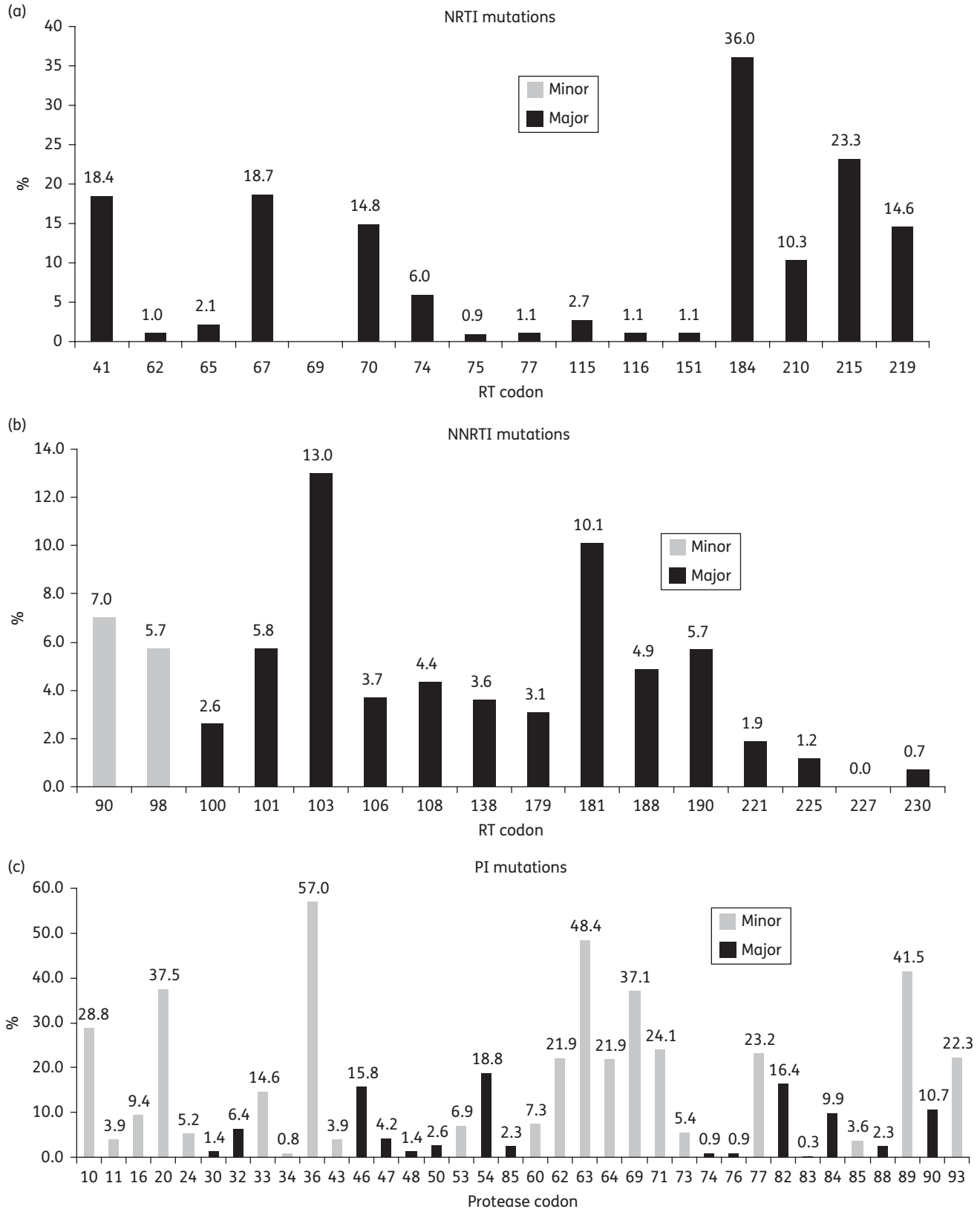


**Figure 1.** Prevalence of resistance mutations. Resistance mutations are those listed on the web site www.iasusa.org (updated autumn 2011). Major and minor resistance mutations are indicated.

relatively infrequent at the time of virological failure of a triple-drug regimen. Mutation K103N, the most common mutation seen in patients with failing regimens based on efavirenz and nevirapine,[7] was present in 10.3% of cases, a proportion consistent with the failure rate of these regimens in this study. The prevalence of major PI mutations ranged from 0.3% (N83D) to 18.8% (I54L/M/V/A/T/S), in keeping with the results of a previous study.[8]

The prevalence of resistance to at least one antiretroviral drug was 59%, and resistance to NRTIs was more frequent than resistance to PIs and NNRTIs (49%, 29% and 23%, respectively). These prevalence rates are close to those estimated in Europe in 2008.[9] The proportions of patients with viruses resistant to all drugs of the NRTI, PI and NNRTI classes were 8.8%, 4.5% and 8.6%, respectively. Regarding second-generation NNRTIs, the prevalence of resistance ranged from 11% (etravirine) to 20% (rilpivirine). More than 80% of viruses resistant to efavirenz or nevirapine were also resistant to rilpivirine. This suggests that care should be taken when using rilpivirine to treat HIV-1 infection, even in treatment-naive patients,[10] because of the high prevalence (2.8%) of transmitted NNRTI-resistant viruses.[11] Resistance to raltegravir was only analysed in patients taking raltegravir at the time of sampling, and was found in 66% of cases, in keeping with data from the ANRS Aquitaine CO3 Cohort.[12]

We systematically investigated all treated patients with plasma viral loads >50 copies/mL at enrolment, and found that the prevalence of resistance increased significantly with viral load. Interestingly, the prevalence of resistance to at least one antiretroviral drug was 49% among patients with viral loads between 51 and 500 copies/mL. This supports guidelines recommending resistance monitoring for all patients with viral load >50 copies/mL,[13–15] even if genotypic resistance tests are less efficient at low viral loads, as shown here.

We compared the prevalence of ARV resistance observed here with that observed in a 2004 survey, in patients with plasma HIV-RNA >1000 copies/mL (an inclusion criterion in the 2004 study). We found that drug resistance was less prevalent in 2009 than in 2004, except for darunavir. This is in keeping with previous results.[8] The decline was not statistically significant for saquinavir and atazanavir. In contrast, the prevalence of resistance to darunavir was higher in 2009 than in 2004 (19% versus 12%, $P=0.008$), probably because darunavir was made available through an expanded access programme in January 2006 in France. Thus, darunavir resistance observed in 2004 was mainly due to cross-resistance. In 2009, in addition to cross-resistance mutations, specific darunavir resistance mutations (V11I and T74P), frequently selected during darunavir failure,[16] contributed to the increase in resistance observed in 2009.

Thus, we found that, in France, resistance to antiretroviral drugs among patients with virological failure on cART was less frequent in 2009 than in 2004. This decline is supported by the fact that the median plasma HIV RNA level was similar in the two sets of samples (4.0 in 2004 versus 3.9 $\log_{10}$ copies/mL in 2009, $P=0.152$). In addition, patients in the 2009 survey had been exposed to much more antiretroviral treatment and for a longer period than those in the 2004 survey.

Although this frequency may decline in future and despite the good performance of new drugs, special attention should be paid to patients harbouring viruses resistant to at least three ARV classes.[17]

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## Transparency declarations

None to declare.

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