Utility of HIV-1 DNA genotype in determining antiretroviral resistance in patients with low or undetectable HIV RNA viral loads

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SYNOPSIS

Objectives. To investigate the extent to which drug resistance can be evaluated on proviral HIV-1 DNA genotype compared to RNA genotype at different time-points.

Patients and methods. In HIV-1-infected patients routinely seen at a university hospital, who need to substitute their current ART, antiretroviral drug resistance was determined from DNA genotype and was compared to past RNA genotype (group 1) or same-day RNA genotype (group 2). A "resistance-sum" was defined as the sum of agents where resistance was present and was calculated across NRTI, NNRTI, and PI. We defined "loss-of-information" when a lower resistance sum was observed on DNA than RNA samples.

Results. Of the 74 and 26 patients included in group 1 and 2, respectively, most had a long median duration of known HIV-1 infection (17.4 and 14.2 years) and ART (15.3 years and 13.5 years). For group 1, the median (range) resistance sums between DNA/RNA were 0 (0-6)/1 (0-6) for NRTIs, 0 (0-4)/0 (0-4) for NNRTIs and 0 (0-7)/0 (0-8) for PIs, which were comparable for group 2. Loss-of-information on DNA was substantial for group 1 (37.8%) and less so for group 2 (11.1%). In multivariable analysis, only longer ART-duration was significantly associated with loss-of-information. Results were similar in patients harboring resistance to ≥1 agent.

Conclusions. In a real-life setting, genotyping DNA on PBMC has some degree of concordance compared to RNA. Loss-of-information on DNA would appear to coincide with longer periods of ART.

INTRODUCTION

With potent and well-tolerated ART, plasma viral loads (VL) of HIV can be easily suppressed to undetectable levels for the majority of HIV-infected patients. For example, data from the French Hospital Database on HIV have recently described 90.3% of treated patients from 2009-2011 exhibiting virological success.[1] Consequently, the evolution towards severe HIV-related disease has been drastically curtailed in infected individuals undergoing ART.[2]

Despite these successes, some individuals need to modify their ART regimen due virological failure (VF) at low levels of VL. Meanwhile, other patients with undetectable HIV plasma RNA might change treatment combinations as a result of toxicity to certain agents or to simplify their ART regimen.[3] In order to guide the choice of appropriate treatment, resistance testing would then be recommended. Plasma RNA genotype is the current standard to assess resistance to antiretroviral drugs (ARV);[4] however, when VL is low or undetectable, viral sequences from plasma RNA could be difficult to obtain and results on drug resistance mutations (DRM) could be unavailable. For instance, only 60% of samples with VL between 20 and 200 copies/ mL were successfully sequenced in a nation-wide study of on-treatment virological failures conducted in France.[5] Other ways to determine antiretroviral resistance are needed, specifically in the context of low or undetectable VL.

HIV-1 DNA from samples of PBMC can be sequenced and could provide some information to assess antiretroviral resistance. However, previous studies have shown that DNA genotype does not identify all DRMs compared to RNA genotype obtained after ART-initiation[6,7] while others have found good concordance and that mutations on DNA were predictive of virological failure.[8,9]

In this study, we aimed to investigate whether HIV-1 DNA genotype could provide reliable information to guide ARV choice. DRM and ARV resistance determined from DNA samples

were compared to (i) past RNA genotype spanning several years or (ii) same-day RNA genotype in treated patients substituting their current ART regimen. In addition, the net gain or loss in DRM information was examined within classes of antiretroviral agents. We also assessed the determinants for which information on antiretroviral resistance is lost when genotyping DNA.

PATIENTS AND METHODS

Study participants

HIV-1-infected individuals seeking care at the Infectious Diseases Department of Saint-Antoine Hospital (Paris, France) were consecutively included between 2010 and 2014 provided that ≥1 DNA genotype and ≥1 RNA genotype were available during clinical follow-up. DNA and RNA genotypes were strictly performed at the request of the treating physician and no selection strategy based on sample availability was used. Two groups were then constructed: patients with undetectable VL or experiencing low-level plasma viraemia, defined as VL <500 copies/mL, whose RNA genotype had been performed prior to DNA genotype (group 1) and patients with VL <1000 copies/ml whose DNA and RNA genotypes were performed on the same day (group 2).

Ethics

Written informed consent was obtained from patients for the use of stored samples and personal data when conducting non-interventional research.

Assessing clinical and virological characteristics

The following patient characteristics were obtained from a computerized medical-record database (DIAMM v8.6r0, Villers-lès-Nancy, France): age, sex, mode of transmission, date of HIV-1 diagnosis, AIDS-defining illness, and concomitant and nadir CD4+ T cell count. Complete history of ART regimens from treatment initiation until DNA genotyping was collected and verified by the treating physician. VLs were obtained from records at the Virology Department of Saint-Antoine Hospital.

HIV-1 RNA was quantified using Cobas AmpliPrep (Cobas Taqman HIV-1 assay, version 2.0; Roche Diagnostic, France). Total HIV-1 proviral DNA was retrospectively quantified using a real-time PCR assay (LTR HIV-1 DNA assay, Biocentrics, Bandol, France) from the same PBMC samples used to obtain DNA genotypes.[10]

Sanger sequencing and assessing DRM on plasma and PBMC samples

HIV-1 resistance testing was done on routine samples at the Virology Department of Saint-Antoine Hospital. Sanger sequencing was performed on both RNA and DNA for reverse transcriptase (RT) and protease sequences with nested-PCRs using primer sequences established by the Agence Nationale de Recherche sur le SIDA (ANRS) consensus method (described in www.hivfrenchresistance.org). RNA was used as template for reverse transcription before amplification. Sequence products were analyzed with the GENETIC ANALYZER 3500xl Dx (Applied Biosystem, Villebon Sur Yvette, France) and sequence alignment was performed using Seqscape software (Applied Biosystem). Sequencing on RT is expected to be successful in 60%, 78%, and 78% of patients with VLs of 51-200, 201-500, 501-1000 copies/mL[5] and 297 of 3430 (8.6%) requests for genotyping at our Virology Department from 2010-2014 were able to be sequenced. Mutations for NRTI, NNRTI and PI described in the ANRS 2014 v24 algorithm considered as resistance-associated mutations were recorded (Table S1).[11] Resistance and susceptibility to each agent pertaining to the three classes of ARV were determined on RNA and DNA sequences.

Statistical analysis

All analysis was stratified on study group. Characteristics of each group were summarized using number and percent for categorical variables and medians and interquartile ranges (IQR) for continuous variables.

To assess the similarity of information between samples, we used an indicator of overall resistance defined herein as the “resistance sum.” Resistance sum was defined as the number of drugs where resistance was present and was calculated within classes of ARV (NRTI, NNRTI, and PI) and sequence source (RNA or DNA genotype). Only resistance to ARV was considered in this score, regardless of whether multiple DRM to the same agent was present. Distribution of resistance sums were truncated to >2 agents in analysis due to the sparse distribution of sums above this level. DNA and RNA genotypes were compared using percent agreement and intraclass correlation (ICC). In group 1, data from the most recent RNA genotype was used in analysis for patients with more than one genotype (*n*=19). We also conducted a sensitivity analysis among individuals with >1 prior RNA genotype whereby mutation presence was defined whenever it was observed on past RNA genotyping results.

To quantify the amount of information lost or gained with DNA genotype, we calculated a net difference in resistance sum-score comparing the number of agents where resistance was observed from DNA to RNA genotypes. Each agent was assigned the following: -1=resistance identified on RNA but not DNA, 0=resistance identified (or not identified) on both RNA and DNA, and 1=resistance identified on DNA but not RNA. The sum within individuals was calculated to create a score, which was provided in the overall group and within classes of ARV. Any values <0 and >0 were interpreted, respectively, as loss of and

gain in information from DNA genotype compared to RNA. Score distributions were assessed using frequency histograms.

Finally, to understand risk-factors associated with net difference in resistance information, we modeled mean differences in resistance sum-score between levels of determinates (Δ) and its 95% CI using linear regression. In a separate analysis, we defined a resistance sum-score <0, representing loss in information, as an end-point. Odds ratios and 95% CIs for various demographic, virological, and treatment correlates were estimated using logistic regression. For both end-points, multivariable analysis was performed in which all covariates with $p<0.1$ were placed in a full model and removed in backwards-stepwise fashion if the p-value was no longer above this threshold.

Statistical analysis was carried out using STATA statistical software (v12.1, College Station, TX) and significance was determined using a p-value <0.05.

RESULTS

Study population

A total of 100 patients met study inclusion criteria for one of the two cohorts: 74 with previous RNA genotypic results prior to DNA genotype (group 1) and 26 with concomitant RNA and DNA genotypes (group 2).

For group 1, 52 patients (71.6%) had undetectable VL (<50 copies/mL). The remaining 22 patients had low level viraemia with a median VL at 1.88 $\log_{10}$ copies/mL. RNA genotypic results were obtained a median 5.8 years prior to DNA genotype in this group, whose distribution was fairly uniform from 0.4 to 10.4 years. Fifteen (20.3%) patients from this group

had their RNA genotype performed before initiating ART. In addition, 19 patients had >1 RNA genotype prior DNA genotype with the number of retrospective RNA genotypes distributed as follows: two (*n*=10), three (*n*=7), four (*n*=1) and five (*n*=1). For group 2, median VL was at 2.51 $\log_{10}$ copies/mL for the 24 of 26 patients (92.3%) with detectable VL. Two (7.7%) patients from this group had RNA and DNA genotypes prior ART-initiation.

Patient characteristics for each group are given in Table 1. Patients in group 1 and 2, respectively, were predominately male (75.7% and 65.4%), harbored HIV-1 subtype B virus (75.3 and 69.2%), and had a long duration of known HIV-1 infection (median 17.4 and 14.2 years). Patients underwent ART for a median of 15.3 years (group 1) and 13.5 years (group 2), while receiving a median 9 (IQR=6-12) and 6 (IQR=4-11) ARV respectively, from ART initiation until DNA genotype. At the time of DNA genotype, ART was mostly prescribed in 3-drug-regimen (n=53, 52.5%): 2 NRTIs+1 NNRTI (n=17), 2 NRTIs+1 PI (n=20), 2 NRTIs+1 INSTI (n=6), or other combinations (n=10; 7 of which contained an INSTI). Non-3-drug-regimen consisted of monotherapy with ritonavir-boosted darunavir (n=2), dual therapy (n=9), or >3 agents from at least three different classes (n=27; 21 of which contained an INSTI).

Distribution of DRM in DNA and RNA genotypes

Wild-type genotype on both RNA and DNA was found in 23 patients (31.1%) from group 1 and 9 (34.6%) from group 2. Of those with RNA genotype prior to ART-initiation, 11/15 (73.3%) and 1/2 (50.0%) patients from groups 1 and 2, respectively, harbored strains without any antiretroviral resistance.

For patients with DRM, in group 1, the percentage of DRMs was consistently higher on the most recently performed RNA than on DNA genotype, which was observed for each ARV-class (Figure S1A). In contrast, this proportion was similar between sequence sources for all three ARV-classes in group 2 (Figure S1B).

Of note, mutations E138K, M184I and M230I on DNA, associated with APOBEC3G/F editing, were found in 4 patients for group 1 and 6 for group 2. Among them, one patient from each group harboring M184I on DNA had the M184V mutation detected on RNA, while another from group 2 had the mutation E138K on both DNA and RNA. Defective viruses with in-frame-stop codons, which are considered unable to replicate, were detected in DNA sequences in 9 patients for group 1 and 5 for group 2.

Distribution of ARV resistance

Prevalence of resistance to individual ARV is summarized in Figure 1A for group 1 and Figure 1B for group 2. When examining ARV-classes, resistance to at least one of the following was observed in group 1 from RNA and DNA sequences, respectively: NRTI, 38 (51.4%) and 30 (40.5%), NNRTI, 24 (32.4%) and 16 (21.6%), PI, 23 (31.1%) and 15 (20.3%). In the 19 patients with ≥ 2 RNA genotypic results prior to DNA genotype, a slightly lower proportion of drug resistance was observed using results from DNA compared to all previous RNA, respectively : NRTI, 8 (42.1%) and 15 (79.0%); NNRTI, 7 (36.8%) and 13 (68.4%); and PI, 8 (42.1%) and 10 (52.6%). In group 2, resistance to at least one of the following was observed from respectively RNA and DNA sequences: NRTI, 9 (34.6%) and 10 (38.5%); NNRTI, 9 (34.6%) and 10 (38.5%); PI, 5 (19.2%) and 6 (23.1%).

Similarity in resistance information between DNA and RNA genotyping

In Table 2, the concordance in number of agents for which resistance was observed, within each class of antiretroviral, was compared between DNA and RNA genotypes. Percent agreement was similar between groups 1 and 2 for NNRTI and PI classes, whereas group 1 had a roughly 20% lower percent agreement than group 1 for NRTIs. Nevertheless, ICC was much higher in group 2 compared to group 1 across all classes, with the smallest difference

observed for PIs. There were no remarkable differences in percent agreement and ICC when patients harboring defective viruses on DNA genotype were excluded from analysis (data not shown).

Net change in resistance information between DNA and RNA genotypes

The majority of patients did not have a net difference in overall number of ARV where resistance was observed: 46 (62.2%) in group 1 and 24 (88.9%) in group 2.
In group 1, more patients appeared to have more loss than gain in resistance information with respect to NRTI (31.1% versus 10.6%, Figure 2A left panel), NNRTI (17.6% versus 10.6%, Figure 2B left panel) and PI classes (18.9% versus 2.7%, Figure 2C left panel). In group 2, few patients exhibited loss of information in NRTI (3.7%, Figure 2A right panel) and NNRTI (7.4%, Figure 2B right panel) classes and no net change in resistance information was observed for PIs (Figure 2C right panel).

In patients from group 1, net difference in resistance sum-score was lower in those with longer duration of HIV infection (p=0.006), longer ART-duration (p=0.002), and number of ARV (p=0.01). Only longer ART-duration was significantly associated with net difference in resistance sum-score during multivariable analysis (Table 3). Loss of information was associated with older age (p=0.01), longer time since nadir CD4+ T cell count (p=0.004), longer known duration of HIV-infection (p=0.002), longer ART-duration (p<0.001), and number of ARV (p=0.003). Longer ART-duration was the only determinant significantly associated with loss of information in multivariable analysis (Table 3). These results did not substantially differ when examining only patients harboring drug-resistant strains (Table S2).

In group 2, any risk-factor analysis on the differences in resistance information between RNA and DNA genotypes was precluded by the lack of distribution in the resistance sum-score.

DISCUSSION

In a population of HIV-1 infected patients of which roughly two-thirds had viral strains with drug resistance to at least one ARV, there was some degree of information loss of antiretroviral resistance on current DNA compared to retrospective RNA genotypes when VL was low or undetectable. However, for some patients, DNA genotype does appear to give similar numbers of agents to which the strain is resistant compared to RNA. Higher concordance in DNA resistance information was also observed with RNA sequences from the same day versus several years in the past.

In the absence of RNA genotype due to low-levels of replication, resistance testing on DNA is sometimes the only means by which clinicians are able to select appropriate ART.[12] DNA genotype has been retrospectively compared to RNA genotype in other studies, demonstrating somewhat good concordance between past-RNA and DNA genotype in patients without viral failure[9] and adequate comparability with respect to number of DRM between past-RNA and non-defective DNA genotype.[13] We build on these works by demonstrating only marginally good comparability in the degree of antiretroviral resistance and whether information on resistance is lost with DNA genotyping, giving clearer guidance on the utility of this approach in identifying resistant drugs.

In our analysis, we considered a statistic by which the extent of information loss from DNA to RNA genotype could be assessed. This approach is somewhat analogous to comparing genotypic susceptibility scores between sample sources, as employed in other studies.[14–16] A score of “zero” is the optimal goal and suggests that DNA genotype would provide the same type of information without considering specific ARV. A negative score would indicate loss of information on the DNA sample compared to RNA, with lower values representing a greater degree of loss of information.

Our univariable analysis identified a number of risk-factors associated with loss-of-information from DNA genotype, which included longer time since nadir CD4+ T cell count, known duration of HIV-infection, known duration of ART and number of cumulative ARV since ART initiation. As these determinants are closely intertwined with ART-duration, it is unsurprising that longer ART-duration was the only factor associated with loss-of-information in multivariable analysis. ART-duration could be a simple proxy for extensive HIV-infection and potentially more complicated therapeutic history, lending to exposure to many ARV and selective pressures to develop DRM.[17] Agents will be switched as treatment progresses and the selective pressures of acquiring certain mutations might no longer present. In turn, previous DRM could be naturally purged from the reservoir during longer periods of treatment.[18] For instance, M184I/V on DNA is known to disappear in the absence of NRTIs pressure, which could explain the effectiveness of lamivudine-containing dual therapies in patients with a history of M184I/V mutation.[19]

Nevertheless, there are other explanations for the information loss on DNA genotype observed in our study. Our study population with longer HIV-infection and ART-exposure could have had a higher frequency of virological failure, which has also been associated with poor concordance between RNA and DNA genotypes in other studies.[6,7] Higher person-years of viral replication, representing in part less effective ART combinations when levels are higher, did not correlate with loss-of-information. However, it is difficult to differentiate virological failure than poor treatment adherence as an explanation for replicating HIV. Furthermore, the detection threshold from Sanger sequencing only allows detection of mutant variants as majority quasi species and could miss DRMs representing less than 15-25% of the viral population.[20] It could be that DRM found on previous RNA samples could have been archived as minority variants, creating further loss of information. Ultra-deep sequencing detecting low-frequency variants harboring DRMs has been found to aid prediction of antiviral resistance, but was not performed in our study.[21,22]

Mutation profiles in archived DNA are also known to evolve despite long periods of sustained virological control[23,24] and hence could affect interpretation and comparability of sequences determined on DNA versus RNA genotypes. As the loss of resistance in DNA was associated with longer duration of HIV-infection and longer history of being on ART, our results indirectly indicate that increased diversity of proviral DNA could be the reason for loss of resistance information. Clearance of archived DRM in proviral DNA after long periods of virological control, lacking therapeutic selective pressures, have been recently reported with reverse transcriptase, which could imply residual replication of more fit viruses within some reservoirs.25

For some patients, resistance to ARV was able to be detected on DNA but not on RNA genotype. APOBEC3 cytidine deaminases are known to induce G to A hypermutations in GG or GA dinucleotides on DNA, which could explain some of these cases.[26] Seven patients harbored APOBEC mutations E138K, M184I and M230I on HIV DNA without DRM on RNA, consistent with other studies.[8,19] The potential significance of these mutations is still unknown; nevertheless, others have shown that recombination could occur between hypermutated sequences and circulating viral populations, eventually leading to the emergence of resistant viral strains.[27] Others have also found that concordance is affected in DRM between DNA and RNA genotypes in case of defective proviruses.[13] We were unable to corroborate these results, yet only a small proportion of patients in our study harbored stop-codon mutations on DNA sequences and the study population was different.
For other cases in group 1, DRM might have developed due to a switch to another treatment after the most recent RNA genotype and thus the resistance observed on DNA might have captured selective pressures incongruent with those from past RNA genotypes.[28]

Importantly, the intraclass correlation comparing resistance information from DNA to RNA genotype was much lower for NRTIs than NNRTIs or PIs, as demonstrated in another study

evaluating detected resistance.[28] These results would imply that any susceptibility of losing DRM might occur more frequently with NRTI agents. NRTI is historically an older class of ARV and were likely given during early infection among those with longer ART-duration. The risk of observing archived NRTI resistance mutations that disappear over time could then be considered higher for these individuals.

Certain limitations of our study need to be addressed. First, the large heterogeneity of the studied population does make it difficult to generalize to other populations, such as those with less exposure to ART or HIV-infection duration, or to more specific treatment regimens. This heterogeneity could have also increased variation, which likely affected some of our analyses. Second, the low sample size could have limited the power to detect certain risk-factors associated with differences in genotype information between RNA and DNA. Third, genotypic results were obtained retrospectively and hence selection of patients was based on sample availability. Fourth, unlike many genotypic resistance scores, we considered intermediate resistance test results as resistance, which could be considered extreme for certain ARV. Finally, resistance testing of INSTI was not available at the time of study and was not included in our analysis. Other studies would be needed to confirm similar observations with this treatment class.

In conclusion, genotyping DNA has some degree of concordance compared to previous RNA genotypes, specifically when determining the extent of drug resistance, in a real-life setting of patients with low or undetectable VL during ART. It is already recommended that DNA genotype could be carried out for patients who have no RNA genotype or for those patients without any knowledge of their ARV history.[4,15] Our results would contend that if neither ART-history nor RNA genotype is available, DNA genotype could provide some useful information on drug resistance to the major classes of antiretrovirals. Nevertheless, patients having extensive years of treatment with multiple lines of ARV regimens might have more loss-of-information on DNA genotype, making it more difficult to rule out ARV resistance.

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TRANSPARENCY DECLARATIONS

None to declare.

REFERENCES

1 Delaugerre C, Ghosn J, Lacombe J-M, Pialoux G, Cuzin L, Launay O, *et al.* Significant reduction in HIV virologic failure during a 15-year period in a setting with free healthcare access. *Clin Infect Dis* 2015; **60**:463–472.

2 Smith CJ, Ryom L, Weber R, Morlat P, Pradier C, Reiss P, *et al.* Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet* 2014; **384**:241–248.

3 Collins SE, Grant PM, Shafer RW. Modifying Antiretroviral Therapy in Virologically Suppressed HIV-1-Infected Patients. *Drugs* 2016; **76**:75–98.

4 Conseil national du sida et des hépatites virales, Agence Nationale de Recherche contre le SIDA et les Hépatites virales. Prise en charge médicale des personnes vivant avec le VIH. Prise en charge des situations d'échec virologique. https://cns.sante.fr/wp-content/uploads/2017/01/experts-vih_echec.pdf

5 Assoumou L, Charpentier C, Recordon-Pinson P, Grudé M, Pallier C, Morand-Joubert L, *et al.* Prevalence of HIV-1 drug resistance in treated patients with viral load >50 copies/mL: a 2014 French nationwide study. *J Antimicrob Chemother* 2017; **72**:1769–1773.

6 Delaugerre C, Braun J, Charreau I, Delarue S, Nere ML, de Castro N, *et al.* Comparison of resistance mutation patterns in historical plasma HIV RNA genotypes with those in current proviral HIV DNA genotypes among extensively treated patients with suppressed replication. *HIV Med* 2012; **13**:517–525.

7 Wirden M, Soulie C, Valantin M-A, Fourati S, Simon A, Lambert-Niclot S, *et al.* Historical HIV-RNA resistance test results are more informative than proviral DNA genotyping in cases of suppressed or residual viraemia. *J Antimicrob Chemother* 2011; **66**:709–712.

8 Derache A, Shin H-S, Balamane M, White E, Israelski D, Klausner JD, *et al.* HIV drug resistance mutations in proviral DNA from a community treatment program. *PloS One* 2015; **10**:e0117430.

9 Lambert-Niclot S, Allavena C, Grude M, Flandre P, Sayon S, Andre E, *et al.* Usefulness of an HIV DNA resistance genotypic test in patients who are candidates for a switch to the rilpivirine/emtricitabine/tenofovir disoproxil fumarate combination. *J Antimicrob Chemother* Published Online First: 26 May 2016. doi:10.1093/jac/dkw146

10 Avettand-Fènoël V, Chaix M-L, Blanche S, Burgard M, Floch C, Toure K, *et al.* LTR real-time PCR for HIV-1 DNA quantitation in blood cells for early diagnosis in infants born to seropositive mothers treated in HAART area (ANRS CO 01). *J Med Virol* 2009; **81**:217–223.

11 ANRS AC-11 Resistance Study Group. HIV-1 drug resistance genotypic interpretation's algorithm. 2014.http://www.hivfrenchresistance.org/tab2014.html

12 Conseil national du sida et des hépatites virales. Prise en charge médicale des personnes vivant avec le VIH. Optimisation d'un traitement antirétroviral en situation de succès virologique. https://cns.sante.fr/wp-content/uploads/2015/10/experts-vih_actualisation2015.pdf

13 Allavena C, Rodallec A, Leplat A, Hall N, Luco C, Le Guen L, *et al.* Interest of proviral HIV-1 DNA genotypic resistance testing in virologically suppressed patients candidate for maintenance therapy. *J Virol Methods* 2018; **251**:106–110.

14 Garcia F, Alvarez M, Fox Z, Garcia-Diaz A, Guillot V, Johnson M, *et al.* Predicting antiretroviral drug resistance from the latest or the cumulative genotype. *Antivir Ther* 2011; **16**:373–382.

15 Barth RE, Aitken SC, Tempelman H, Geelen SP, van Bussel EM, Hoepelman AIM, *et al.* Accumulation of drug resistance and loss of therapeutic options precede commonly used criteria for treatment failure in HIV-1 subtype-C-infected patients. *Antivir Ther* 2012; **17**:377–386.

16 Beerenwinkel N, Montazeri H, Schuhmacher H, Knupfer P, von Wyl V, Furrer H, *et al.* The individualized genetic barrier predicts treatment response in a large cohort of HIV-1 infected patients. *PLoS Comput Biol* 2013; **9**:e1003203.

17 Clutter DS, Jordan MR, Bertagnolio S, Shafer RW. HIV-1 drug resistance and resistance testing. *Infect Genet Evol* 2016; **46**:292–307.

18 Kabamba-Mukadi B, Duquenne A, Henrivaux P, Musuamba F, Ruelle J, Yombi J-C, *et al.* HIV-1 proviral resistance mutations: usefulness in clinical practice. *HIV Med* 2010; **11**:483–492.

19 Charpentier C, Montes B, Perrier M, Meftah N, Reynes J. HIV-1 DNA ultra-deep sequencing analysis at initiation of the dual therapy dolutegravir + lamivudine in the maintenance DOLULAM pilot study. *J Antimicrob Chemother* 2017; **72**:2831–2836.

20 Palmer S, Kearney M, Maldarelli F, Halvas EK, Bixby CJ, Bazmi H, *et al.* Multiple, linked human immunodeficiency virus type 1 drug resistance mutations in treatment-experienced patients are missed by standard genotype analysis. *J Clin Microbiol* 2005; **43**:406–413.

21 Mohamed S, Penaranda G, Gonzalez D, Camus C, Khiri H, Boulmé R, *et al.* Comparison of ultra-deep versus Sanger sequencing detection of minority mutations on the HIV-1 drug resistance interpretations after virological failure. *AIDS* 2014; **28**:1315–1324.

22 Vandenhende M-A, Bellecave P, Recordon-Pinson P, Reigadas S, Bidet Y, Bruyand M, *et al.* Prevalence and evolution of low frequency HIV drug resistance mutations detected by ultra deep sequencing in patients experiencing first line antiretroviral therapy failure. *PloS One* 2014; **9**:e86771.

23 Gantner P, Morand-Joubert L, Sueur C, Raffi F, Fagard C, Lascoux-Combe C, *et al.* Drug resistance and tropism as markers of the dynamics of HIV-1 DNA quasispecies in blood cells of heavily pretreated patients who achieved sustained virological suppression. *J Antimicrob Chemother* 2016; **71**:751–761.

24 Michelini Z, Galluzzo CM, Pirillo MF, Francisci D, Degli Antoni A, Vivarelli A, *et al.* HIV-1 DNA dynamics and variations in HIV-1 DNA protease and reverse transcriptase sequences in multidrug-resistant patients during successful raltegravir-based therapy. *J Med Virol* 2016; **88**:2115–2124.

25 Nouchi A, Nguyen T, Valantin MA, Simon A, Sayon S, Agher R, *et al.* Dynamics of drug resistance-associated mutations in HIV-1 DNA reverse transcriptase sequence during effective ART. *J Antimicrob Chemother* 2018; doi:10.1093/jac/dky130

26 Kieffer TL, Kwon P, Nettles RE, Han Y, Ray SC, Siliciano RF. G-->A hypermutation in protease and reverse transcriptase regions of human immunodeficiency virus type 1 residing in resting CD4+ T cells in vivo. *J Virol* 2005; **79**:1975–1980.

27 Kim E-Y, Bhattacharya T, Kunstman K, Swantek P, Koning FA, Malim MH, *et al.* Human APOBEC3G-mediated editing can promote HIV-1 sequence diversification and accelerate adaptation to selective pressure. *J Virol* 2010; **84**:10402–10405.

28 Zaccarelli M, Santoro MM, Armenia D, Borghi V, Gennari W, Gori C, *et al.* Genotypic resistance test in proviral DNA can identify resistance mutations never detected in historical genotypic test in patients with low level or undetectable HIV-RNA. *J Clin Virol* 2016; **82**:94–100.

TABLES

Table 1. Description of the study population

	Cohort	
	DNA genotype and past RNA genotype (group 1)	DNA and RNA genotype together (group 2)
	(*n*=74)	(*n*=26)
Male/Female (% male)	56/18 (75.7)	17/9 (65.4)
Age (years)*‡	48 (42-53)	47 (41-53)
Mode of transmission		
MSM	40 (54.0)	12 (48.0)
Heterosexual	20 (27.0)	11 (44.0)
IDU	5 (6.8)	-
Other	9 (12.2)	2 (8.0)
AIDS-defining disease	27 (36.5)	8 (33.3)
CD4 cell count (/mm^3)*‡	517 (363-772)	384 (190-558)
Nadir CD4 cell count (/mm^3)*	169 (48-246)	167 (79-226)
HIV-1 subtype		
B	55 (75.3)	18 (69.2)
Non-B	18 (24.3)	8 (30.8)
Years since ART-initiation*‡	15.3 (6.5-18.6)	13.5 (6.9-17.0)
Number of antiretroviral agents since ART-initiation*‡	9 (6-12)	6 (4-11)
Number of agents within class since ART-initiation*‡		
NRTI	5 (3-6)	2 (0-5)
NNRTI	1 (1-2)	1 (0-1)
PI	3 (2-4)	2 (2-3)
INSTI	0 (0-1)	0 (0-1)
Number of current antiretroviral agents*‡	3 (3-4)	3 (3-3)
HIV-1 RNA viral load >50 copies/mL‡	21 (28.4)	24 (92.3)
HIV-1 RNA viral load (log_{10} copies/mL)*†‡	1.88 (1.80-2.16)	2.51 (2.18-4.23)
Cumulative years <50 copies/mL*	7.3 (4.1-10.6)	9.6 (5.3-12.3)
Total known number of virological failures*¶	2 (0-5)	2 (0-4)
HIV-1 proviral DNA >40 copies/10^7 cells*‡	51/63 (81.0)	20/4 (83.3)
HIV-1 DNA viral load (log10 copies/10^7 cells)*‡	2.90 (2.18-3.11)	2.99 (2.37-3.69)
Years between HIV-1 RNA and DNA‡ genotypes*	5.8 (2.6-7.4)	-

Median (IQR) is given when indicated (*). Otherwise, all statistics are reported in *n* (%).

†Only among patients with detectable HIV-1 RNA.

‡Data obtained at the time of DNA genotype, for patients of group 1(DNA genotype with past RNA genotype).

¶Virological failure was defined as two consecutive visits with a pVL >50 copies/mL or one pVL>200 copies/mL.

Table 2. Comparing number of patients harboring drug resistance mutations to antiretroviral classes between DNA and RNA genotypes

	NRTI								NNRTI								PI							
DNA genotype	Past RNA genotype* (group 1)				Current RNA genotype** (group 2)				Past RNA genotype* (group 1)				Current RNA genotype** (group 2)				Past RNA genotype* (group 1)				Current RNA genotype** (group 2)			
	0	1	2	>2	0	1	2	>2	0	1	2	>2	0	1	2	>2	0	1	2	>2	0	1	2	>2
0	**32**	3	3	6	**16**	1	0	0	**46**	1	6	5	**14**	1	1	0	**50**	2	0	7	**19**	1	0	0
1	2	**1**	1	4	1	**1**	0	0	1	**0**	0	0	2	**0**	0	1	0	**2**	0	2	1	**1**	0	1
2	1	0	**2**	3	0	0	**1**	1	2	0	**4**	1	0	0	**1**	1	1	0	**0**	0	1	0	**0**	0
>2	1	1	0	**14**	0	1	0	**4**	1	0	1	**6**	1	0	0	**4**	0	0	0	**10**	0	0	0	**2**
% agree.	66.2				84.6				75.7				73.1				83.8				84.6			
ICC	0.592				0.910				0.529				0.747				0.636				0.784			

Numbers represent the number of agents within each class where drug resistance was observed. HIV-1 DNA sequences was compared to past HIV-1 RNA (*) or concomitant HIV-1 RNA sequences (**) in two separate cohorts. Numbers in bold represent concordant information.

Table 3. Determinants of change in resistance information when using DNA compared to past RNA genotype

	Net difference in resistance sum-score**				Loss in information##			
	Univariable		Multivariable		Univariable		Multivariable	
	Δ (95% CI)	*p*	Δ (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Age*	-0.1 (-0.1, 0)	0.12			1.08 (1.02-1.14)	0.01		
CD4+ cell count#	0.1 (-0.2, 0.3)	0.5			0.97 (0.83-1.13)	0.7		
Nadir CD4+ cell count #	0.3 (-0.2, 0.7)	0.3			0.79 (0.56-1.11)	0.17		
Time since nadir CD4+ cell count*	-0.1 (-0.2, 0)	0.06			1.14 (1.04-1.24)	0.004		
B versus non-B HIV-1 subtype	0.1 (-1.6, 1.7)	0.9			1.36 (0.46-4.02)	0.6		
HIV-1 RNA viral load†	0.0 (-2.2, 2.2)	0.9			0.45 (0.10-1.99)	0.3		
Time since first HIV-1 VL<50 cps/mL*	-0.1 (-0.2, 0.1)	0.4			1.06 (0.96-1.18)	0.3		
Cumulative average HIV-1 RNA VL†	0.3 (-0.5, 1.1)	0.5			0.64 (0.36-1.12)	0.12		
Cumulative years <50 copies/mL	-0.1 (-0.3, 0)	0.12			1.10 (0.99-1.22)	0.07		
Undetectable HIV-1 RNA VL†	-0.2 (-1.8, 1.4)	0.8			1.93 (0.67-5.53)	0.2		
Known HIV-infection duration*	-0.1 (-0.2, 0)	0.006			1.12 (1.05-1.21)	0.002		
ART duration*	-0.2 (-0.3, -0.1)	0.002	-0.2 (-0.3, -0.1)	0.002	1.19 (1.09-1.31)	<0.001	1.19 (1.09-1.31)	<0.001
Number of antiretroviral agents	-0.2 (-0.4, 0)	0.01			1.21 (1.07-1.38)	0.003		
Known number of virological failures¶	-0.2 (-0.4, 0.1)	0.18			1.09 (0.93-1.27)	0.3		
Time between measures*	-0.1 (-0.4, 0.1)	0.3			1.03 (0.88-1.21)	0.7		
Virological failure versus treatment switch	-0.6 (-2.0, 0.8)	0.4			1.29 (0.51-3.25)	0.6		

Analysis conducted on patients from group 1 only. Units represented in: *per year; #per 100/mm^3; †per $\log_{10}$ copies/mL; ¶Virological failure was defined as two consecutive visits with a pVL >50 copies/mL or one pVL>200 copies/mL.

**End-point is absolute difference in number of agents where drug resistance was observed on DNA compared to past RNA genotyping results.

In the multivariable model, the following variables were excluded as their *p*-value was no longer below the pre-specified threshold: time since

nadir CD4+ cell count (p=0.883), duration of HIV infection (p=0.672), number of antiretroviral agents (p=0.609), and cumulative years <50 copies/mL (p=0.453).

[##]End-point is whether HIV-1 DNA provides less resistance information than past HIV-1 RNA genotyping results. In the multivariable model, known HIV-infection duration was not included due to collinearity with ART duration. The following variables were excluded as their p-value was no longer below the pre-specified threshold: time since nadir CD4+ cell count (p=0.791), age (p=0.514), number of antiretroviral agents (p=0.431), and duration of HIV-infection (p=0.149).

FIGURE LEGENDS

Figure 1. Comparing percentage of resistance to individual antiretroviral agents between HIV-1 RNA versus HIV-1 DNA genotypes

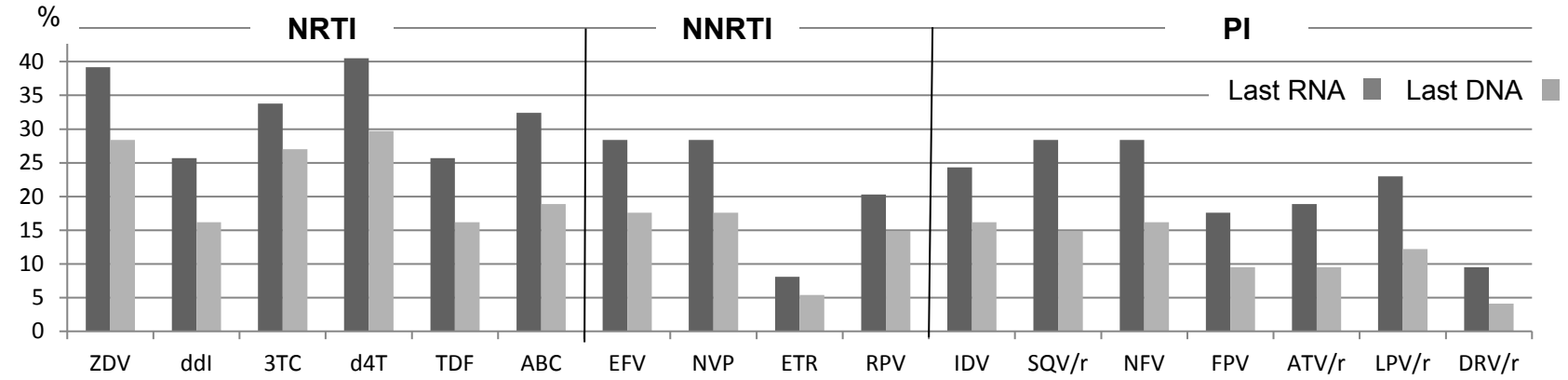
Proportion of resistance to individual antiretroviral agents identified from HIV-1 DNA genotype is compared to previous HIV-1 RNA genotype (**A**) or HIV-1 RNA obtained at the same time (**B**).

Figure 2. Histogram of net change in resistance information between HIV-1 RNA and DNA genotypes.

Frequency of net difference in number of agents where drug resistance was observed on HIV-1 DNA compared to past HIV-1 RNA genotype (group 1; left panels) or compared to same-day HIV-1 RNA genotype (Group 2, right panels). Histograms are provided within nucleoside/nucleotide reverse transcriptase inhibitors (NRTI, **A**), non-NRTIs (NNRTI, **B**), and protease inhibitor (PI, **C**) antiretroviral classes. Drugs are shown on the *x*-axis: ZDV, zidovudine; ddI, didanosine; 3TC/FTC lamivudine/emtricitabine ; TDF, tenofovir ; ABC, abacavir ; EFV, efavirenz ; NVP, nevirapine ; ETR, etravirine; RPV, rilpivirine; IDV, indinavir; SQV, saquinavir; NFV, nelfinavir; FPV, fosamprenavir; ATV, atazanavir; LPV, lopinavir; DRV, darunavir; /r when PI are boosted with ritonavir.

Figure 1.

A


%
NRTI
NNRTI
PI
Last RNA
Last DNA
40
35
30
25
20
15
10
5
0
ZDV
ddI
3TC
d4T
TDF
ABC
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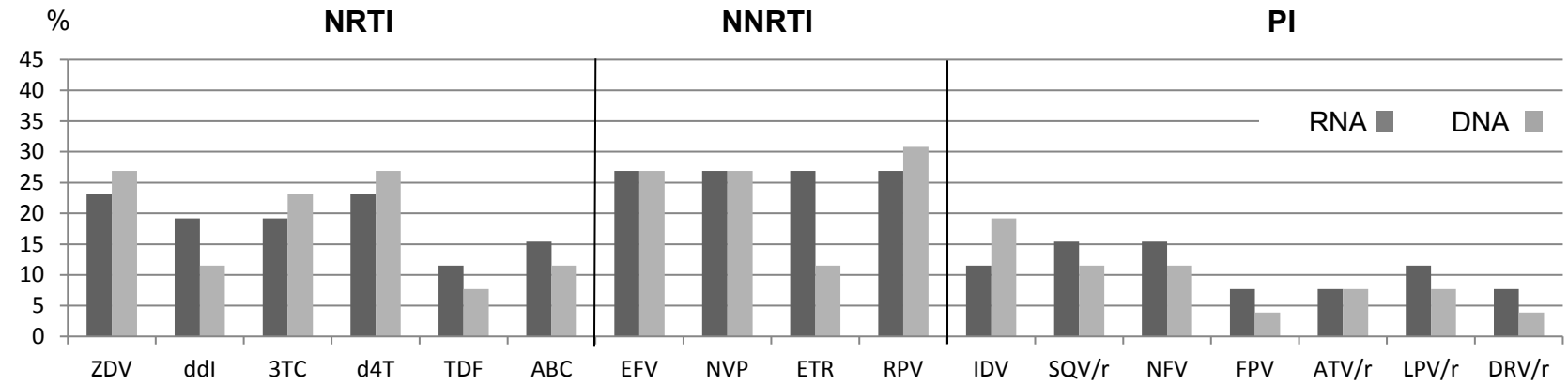
NVP
ETR
RPV
IDV
SQV/r
NFV
FPV
ATV/r
LPV/r
DRV/r

B


%
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3TC
d4T
TDF
ABC
EFV
NVP
ETR
RPV
IDV
SQV/r
NFV
FPV
ATV/r
LPV/r
DRV/r

Figure 2.

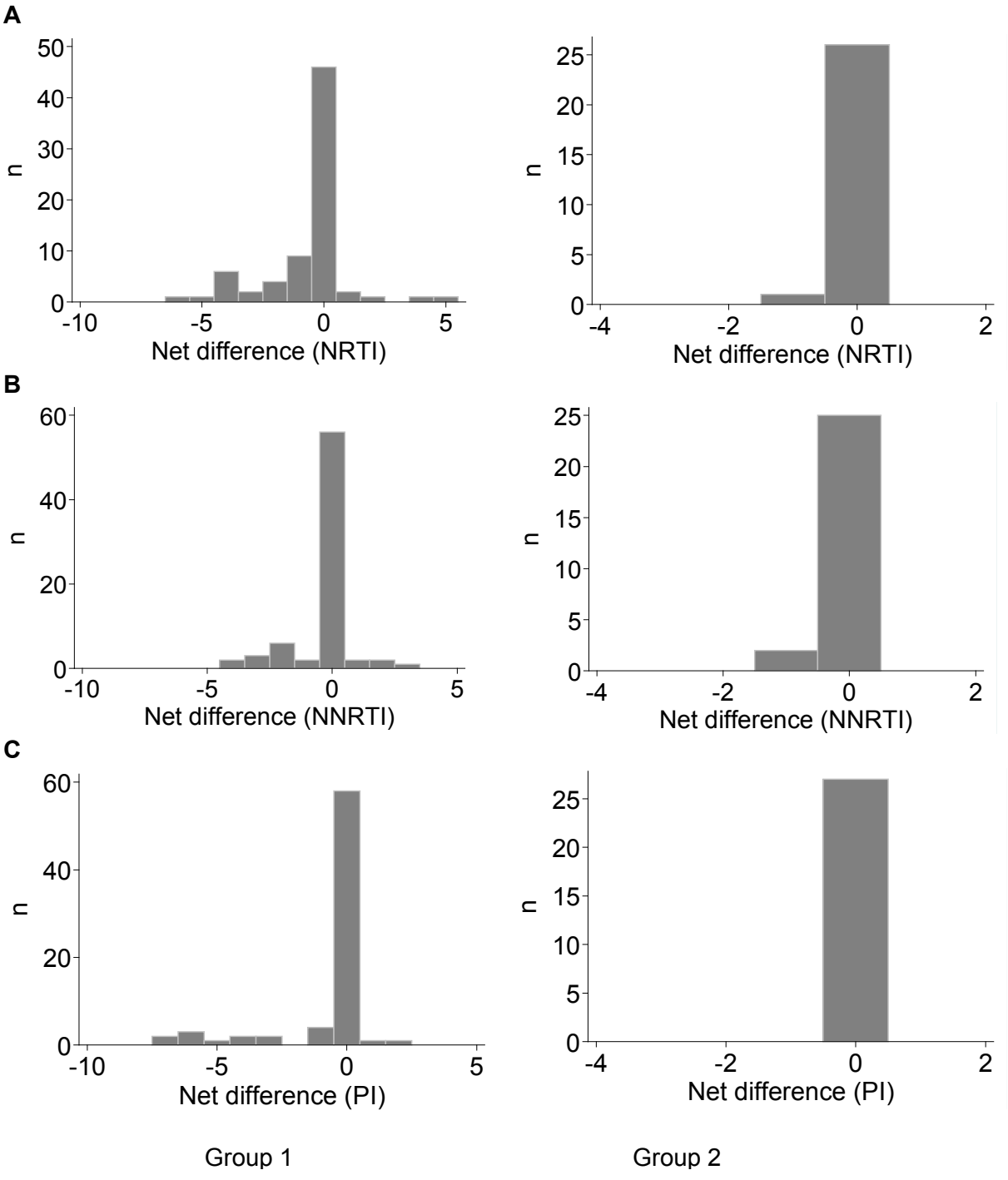

A
n
Net difference (NRTI)
B
n
Net difference (NNRTI)
C
n
Net difference (PI)
Group 1
Group 2

Table S1. List of Drug Resistant Mutations analyzed in the ANRS 2014 consensus Method

	NRTI	**NNRTIs**
Mutations on reverse transcriptase	M41L, E44D, K65E/N/R, D67N, T69D/N/S, K70E/R, L74V/I V75A/M/S/T Y115F Q151M, M184V/I, L210W, T215A/C/D/E/F/G/H/I/L/N/S/V/Y, K219Q/E Insertion at codon 69	V90I A98G; A98S (for HIV-1 subtype C only) L100I K101E/H/I/P/R K103H/N/S/T V106A/I/M E138A/G/K/Q/R/S V179D/F/I/L/M/T, Y181C/I/V Y188C/H/L G190A/C/E/Q/S/T/V H221Y P225H M230I/L/V

	PI	
Mutations on protease for PI	L10F/I/M/R/V V11I I15A/V G16E K20I/M/R/T L24I D30N V32I L33F M36I/L/V M46I/L, I47A/V G48V I50V F53L/W/Y I54A/L/M/S/T/V	Q58E D60E I62V L63P H69I/K/N/Q/R/Y A71I/L/T/V G73A/S/T T74P L76V V77I V82A/C/F/G/M/S/T I84A/V I85V N88S/D L89I/M/R/T/V L90M

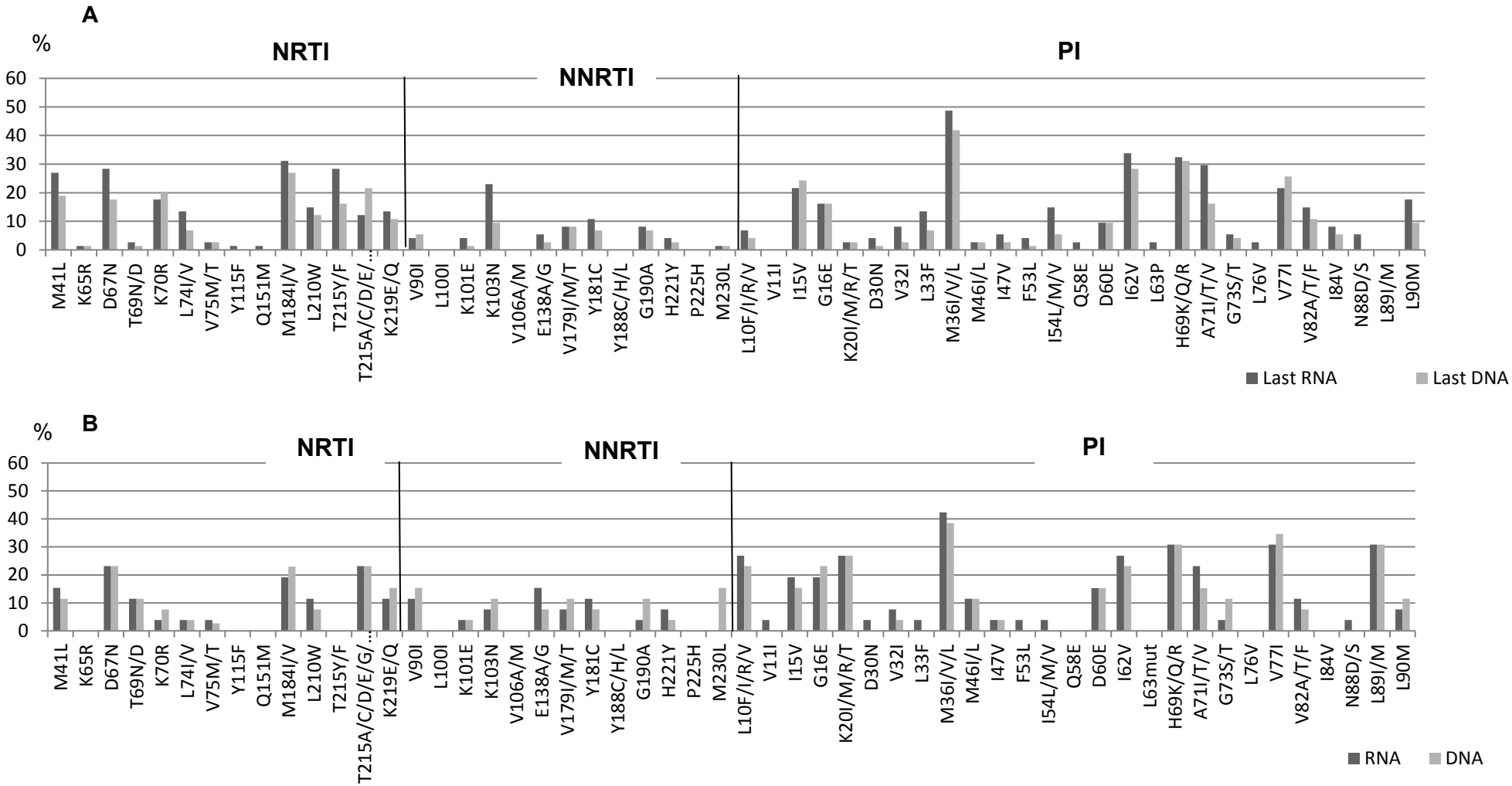
Table S2. Determinants of change in resistance information when using DNA compared to past RNA genotyping (in patients with drug resistance mutations)

	Net difference in number**				Loss in information##			
	Univariable		Multivariable		Univariable		Multivariable	
	Δ (95% CI)	*p*	Δ (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Age*	-0.1 (-0.2, 0.1)	0.3			1.12 (1.01-1.24)	0.03		
CD4+ cell count#	0.1 (-0.4, 0.6)	0.7			1.01 (0.77-1.32)	0.9		
Nadir CD4+ cell count #	0.4 (-0.5, 1.2)	0.4			0.92 (0.57-1.49)	0.7		
Time since nadir CD4+ cell count*	-0.2 (-0.4, 0.0)	0.12			1.25 (1.07-1.47)	0.005		
B versus non-B HIV-1 subtype	0.9 (-1.7, 3.6)	0.5			0.94 (0.22-4.09)	0.9		
HIV-1 RNA viral load†	1.4 (-3.1, 5.9)	0.5			0.21 (0.02-2.75)	0.2		
Time since first HIV-1 VL<50 cps/mL*	-0.1 (-0.4, 0.2)	0.7			1.05 (0.89-1.23)	0.6		
Cumulative average HIV-1 RNA VL†	0.7 (-0.6, 1.9)	0.3			0.48 (0.21-1.13)	0.10		
Cumulative years <50 copies/mL	-0.1 (-0.4, 0.1)	0.3			1.11 (0.95-1.30)	0.17		
Undetectable HIV-1 RNA VL†	-0.7 (-3.4, 2.0)	0.6			2.14 (0.48-9.77)	0.3		
Known HIV-infection duration*	-0.1 (-0.3, 0.1)	0.18			1.09 (0.98-1.21)	0.13		
ART duration*	-0.2 (-0.4, 0.0)	0.045	-0.2 (-0.4, 0.0)	0.045	1.23 (1.03-1.46)	0.02	1.23 (1.03-1.46)	0.02
Number of antiretroviral agents	-0.2 (-0.4, 0.1)	0.16			1.11 (0.96-1.29)	0.16		
Known number of virological failures¶	-0.3 (-0.7, 0.1)	0.11			1.15 (0.90-1.48)	0.3		
Time between measures*	-0.1 (-0.5, 0.3)	0.6			1.01 (0.81-1.26)	0.9		
Virological failure versus tx switch	-1.3 (-3.6, 0.9)	0.2			1.30 (0.36-4.68)	0.7		

Analysis conducted on patients from group 1 with identified drug resistance mutations from past HIV-1 RNA genotyping. Units represented in: *per year; #per 100/mm^3; †per $\log_{10}$ copies/mL. ¶Virological failure was defined as two consecutive visits with a pVL >50 copies/mL or one pVL >200 copies/mL.

The same multivariable models from Table 3 were applied *a priori*. **End-point is absolute difference in number of agents where drug resistance mutations were observed on DNA compared to past RNA genotyping results. ##End-point is whether DNA provides as much or more resistance information than past RNA genotyping results.

Figure S1. Comparing proportion of specific resistance mutations from RNA and DNA genotyping



Proportion of resistance mutations identified from HIV-1 DNA is compared to that from a previous RNA genotype (**A**) or RNA genotype obtained at the same time (**B**).