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Usefulness of an HIV DNA Resistance Genotypic Test in Patients Who Are Candidates for a Switch to the rilpivirine/emtricitabine/tenofovir Combination


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Running title: Switch to the RPV/TDF/FTC and DNA genotype

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Abstract

BACKGROUND: In the context of a rilpivirine/emtricitabine/tenofovir switch in HIV-1 infected patients with at least one year of virologic success, we determined whether proviral DNA is an alternative to plasma HIV-RNA for resistance genotyping.

METHODS: Resistance associated mutations (RAM) in DNA after at least one year of virologic success (viral load (VL) <50 copies/mL) were compared with those identified in the last plasma RNA genotype available. Pilpivirine/emtricitabine/tenofovir RAM studied were: K65R, L100I, K101E/P, E138A/G/K/R/Q, V179L, Y181C/I/V, M184V/I, Y188L, H221Y, F227C and M230I/L in reverse transcriptase. We studied patients without virologic failure (VF) and with ≥1 VF (two consecutive VL>50 copies/mL). Kappa’s coefficient was used to measure agreement between the DNA and RNA genotypes.
RESULTS: In patients without (n=130) and with VF (n=114), RNA and DNA showed resistance to at least one drug of the rilpivirine/emtricitabine/tenofovir combination in 8% and 9% and in 60% and 45%, respectively. For rilpivirine RAM, correlation between RNA and DNA was higher in patients without VF than in patients with VF (kappa= 0.60 versus 0.19, p=0.026). Overall, prevalence of RAM was lower in DNA than in RNA.

CONCLUSION: The incomplete information provided by DNA genotypic test is more notable in patients with VF, suggesting that all resistance mutations associated with prior VF have not been archived in the proviral DNA or decreased to a level below threshold of detection. In the case where no historical plasma genotypic test is available, DNA testing might be useful to rule out switching to rilpivirine/emtricitabine/tenofovir.

INTRODUCTION
The second generation NNRTI rilpivirine (RPV) formulated in a single tablet regimen (STR) with tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) was approved by the European Medicines Agency and the US Food and Drug Administration as a once-daily oral treatment for adults infected with HIV-1 without mutations associated with resistance to TDF, FTC, or the NNRTI class, and harboring a viral load (VL) ≤100 000 HIV-1 RNA copies/mL. Current antiretroviral treatment guidelines recommend switching therapy in virologically suppressed patients to improve adherence or tolerability or to allow for treatment simplification\textsuperscript{1-3}. The SPIRIT study showed maintenance of virologic suppression at W48 for 89.3% of patients switching to rilpivirine/emtricitabine/tenofovir (RPV/FTC/TDF) from a ritonavir-boosted protease inhibitor (PI/r)-based regimen, compared with those who continued treatment with a PI/r regimen with a low risk of virologic failure (VF)\textsuperscript{4}. A study demonstrated switching from efavirenz (EFV/FTC/TDF to RPV/FTC/TDF) was safe and effective for virologically suppressed HIV-infected patients with EFV intolerance\textsuperscript{5}. Cohort studies have also shown efficacy and tolerability of switching to RPV/FTC/TDF\textsuperscript{6}. Thus
RPV/FTC/TDF is considered as an appropriate therapy for switch for simplification in virologically suppressed HIV infected patient. In this context, the use of previous plasma resistance genotypes was recommended to determine the susceptibility to this combination because of the possible presence of pre-existing drug resistance mutations leading to VF. Studies have shown that resistance testing performed on HIV DNA lacks sensitivity compared with accumulated drug resistances from previous plasma genotypes. However in patients fully virologically suppressed, the previous plasma genotypic test could be unavailable.

In the perspective of a switch to the combination RPV/TDF/FTC, the aim of this study was to determine whether proviral DNA is a potential relevant alternative to HIV-RNA for resistance genotyping in HIV-1 infected treated patients with at least one year of virologic suppression.

MATERIAL and METHODS

In 244 HIV-1 infected patients treated in 2 centers (Nantes University Hospital and Pitié-Salpêtrière Hospital) with a prior available RNA resistance test, we retrospectively analyzed HIV DNA resistance genotype generated in PBMC after at least one year of virologic success (VL <50 copies/mL). Bulk sequences of the reverse transcriptase (RT) on RNA and DNA were determined using the ANRS consensus technique primer sequences described at http://www.hivfrenchresistance.org. We compared prevalence of HIV resistance mutations in the DNA and RNA genotype generated from the last detectable VL. We studied 2 groups of patients: 130 patients without previous VF (pre-therapeutic plasma genotype) and 114 patients with at least one previous VF (genotype on the more recent detectable VL).
regardless of the treatment they received (VF was defined as two consecutive VL>50 copies/mL).

RPV/TDF/FTC combination resistance mutations studied were defined according to the IAS list (K65R, K101E/P, E138A/G/K/R/Q, V179L, Y181C/I/V, M184V/I, Y188L, H221Y, F227C and M230I/L) in RT\textsuperscript{9}. Resistance to RPV was defined according the ANRS http://www.hivfrenchresistance.org/. Kappa’s coefficient was used to measure agreement between the DNA and RNA genotypes with values <0, 0-0.2, 0.21-0.4, 0.41-0.6, 0.61-0.8, and 0.81-1.00 indicating no, very low, low, middle, high, and very high concordance, respectively.

We studied factors associated to the correlation between DNA and RNA mutations: VL, CD4 number at the time of both genotypes, number of treatment lines, number of previous VF and mean time between last plasma RNA genotype and proviral DNA genotype.

RESULTS

We collected 244 pairs of DNA and RNA sequences for the RT gene (130 for patients without VF and 114 for patients with at least one VF). Characteristics of patients are presented in table 1. The mean time between last plasma RNA genotype and proviral DNA genotype was 46 and 37 months for patients without VF and with ≥1 VF, respectively. There are significant differences between the 2 groups for VL RNA (p≤0.0001), numbers of lines of treatment (p≤0.0001) and time between RNA and DNA genotypes (p=0.0025).
In patients without VF, the prevalence of studied resistance associated mutation (RAM) was very low in both RNA and DNA RT sequences (Figure 1a), with resistance to at least one drug of the RPV/FTC/FTC combination in 8.0% and 9.0%, respectively.

In patients with at least one prior VF, the prevalence of at least one RPV RAM was 24.6% and 18.4 % in RNA and DNA genotype, respectively. The most prevalent RPV RAMs were Y181C/I/V (12.3%) and E138A/G/K/Q/R (7.8%) in RNA genotype; and in DNA, E138A/G/K/Q/R (7.9%) and M230I/L (6.1%) (Figure 1b). Resistance to FTC and TDF was detected in 52.6% (M184V/I) and 0.9% (K65R) in RNA genotype and 37.7% (M184V/I) and 0.9% (K65R) in DNA genotype. Resistance to at least one drug of the RPV/FTC/FTC combination was 60.0% in RNA and 45.0% in DNA.

In patients without VF, concordance between resistance in RNA and DNA was not significantly higher than in patients with VF (kappa= 0.57 versus 0.43 respectively, p=0.36).

For RPV RAM, correlation between RNA and DNA was significantly higher in patients without VF than in patients with at least one VF (kappa= 0.60 versus 0.19 respectively, p=0.026).

Overall, prevalence of rilpivirine associated mutations was lower in DNA than in RNA genotypic test, except for mutations at positions E138 and M230 that are APOBEC driven mutations (G to A) (Figure 1b). The factors associated with a good correlation between resistance according RNA and DNA genotype were a higher VL at RNA genotype (p=0.0124), a shorter mean time between last plasma RNA genotype and proviral DNA genotype (p=0.0468) and a higher number of treatment lines (p=0.0006). Number of VF, subtypes, CD4 at the time of RNA genotype or DNA genotype were not associated with correlation of resistance in RNA and DNA.
In the context of switch to RPV/TDF/FTC therapy in HIV-1 infected treated patients, this study shows a good concordance between DNA and RNA genotypes in patients without prior VF and who are successfully suppressed for at least one year. However, DNA genotype is less informative than RNA genotype in patients with at least one prior VF. The good concordance in patients with no prior VF is in accordance with results of studies on naive patients showing that DNA genotype could be useful and even more informative than standard RNA genotyping. 

In patients with at least one prior VF, the rate of selected resistance associated mutations to RPV in RNA was somewhat lower than in previous studies conducted in patients pre-exposed to an NNRTI-based regimen but naive to RPV. Indeed, in our study the 3 main RPV RAMs at codons 181, 101 and 138 had a frequency of 12.3, 2.6 and 5.3% versus 18 to 22.6%, 7 to 20.5% and 5.3 to 14% in these 3 studies, respectively. This lower frequency could be due to the fact that our studied population not exclusively contained NNRTI failing patients, nevertheless the 3 mains RPV RAM are similar to those observed in others studies. In the HIV DNA, we showed a lower prevalence of the RPV RAM: 101, 181 and 221 with 0.9%, 3.5% and 0.9% versus 7%, 18% and 4% in the study of Gallien et al. However, patients of this study were selected to have a prior VF especially to NVP or EFV and had a prior history of triple class failure.

In patients with prior VF, our study generally confirmed results of previous studies on the discordance between DNA and RNA genotypes. Indeed, in a large number of patients with undetectable or low VL under treatment, a study showed a concordance between DNA and RNA of 26.3% for NNRTI mutations. Another study demonstrated that mutations conferring
resistance to at least one antiretroviral drug were detected exclusively by RNA genotyping or exclusively by DNA genotyping in 47% and 1% of patients for NNRTIs, respectively.\textsuperscript{8}

Overall, prevalence of RAM was generally lower in DNA than in RNA genotypic tests, except for mutations at positions E138 and M230 that are APOBEC driven mutations. Indeed, APOBEC induces G to A viral mutation and this mechanism could explain the persistence of mutations in archived cellular proviral DNA.\textsuperscript{15}

The incomplete information provided by the DNA test is more notable in patients with at least one prior VF, suggesting that all resistance mutations associated with the prior VF may not have been archived in the proviral DNA or not detected with classical Sanger sequencing.

A good correlation between prior RNA genotype and current DNA genotype was significantly associated with a higher VL at RNA genotype and a shorter mean time between last plasma RNA and proviral DNA genotype. This suggest that DNA genotypic testing, with current techniques, might be suboptimal in case of low intracellular VL of resistant viruses and that archived resistant viruses might decrease over time. Further studies would be warranted to explore the interest of Ultra Deep Sequencing on DNA and the clinical relevance of minority variants.

From a clinical perspective, we recommend that before switching to RPV/TDF/FTC, one takes into consideration full treatment history and available past plasma genotypic testing, and in the absence of prior plasma genotype, avoid use of RPV/TDF/FTC if RAM to this combination are detected using a DNA genotype in a virologically suppressed patient.

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Transparency declarations

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REFERENCES


Table 1: Characteristics of patients according the 2 groups: without previous virologic failure (VF) and patients with ≥1 VF. *corresponding to pretreatment VL for patients without previous VF and VL at failure for patients with at least one VF

<table>
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<tr>
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<th>Without VF N=130</th>
<th>With at least one VF N=114</th>
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<tr>
<td></td>
<td>Median (standard deviation)</td>
<td>Median (standard deviation)</td>
</tr>
<tr>
<td>VL RNA log copies/mL*</td>
<td>4.5 (1.2)</td>
<td>3.0 (1.1)</td>
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<td>CD4 at time of RNA genotype</td>
<td>347 (230.2)</td>
<td>358 (251.6)</td>
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<tr>
<td>Number of cells/mm3</td>
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<tr>
<td>CD4 at time of DNA genotype</td>
<td>586 (311.6)</td>
<td>537 (338.2)</td>
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<tr>
<td>Number of cells/mm3</td>
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<tr>
<td>Time between RNA and DNA</td>
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<td>29.5 (36.8)</td>
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<tr>
<td>genotypes (month)</td>
<td></td>
<td></td>
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<tr>
<td>Number of lines of treatment</td>
<td>3 (3.3)</td>
<td>12 (7.5)</td>
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Figure 1: Prevalence of resistance mutations to RPV/TDF/FTC in RNA and in DNA: a) in patients without previous virologic failure. b) in patients with at least one virologic failure.* correspond to statistical significant difference of prevalence of resistance mutations between DNA and RNA (p<0.05)
b)