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Ultradeep sequencing detection of the R263K integrase inhibitor drug resistance mutation

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Sir,

HIV-1 transmitted drug resistance (TDR) can impair the first-line antiretroviral therapy response. Moreover, minority resistant variants (MRVs) can be a source of virological failure if they are present before antiretroviral treatment. Nevertheless, few data are available for integrase strand transfer inhibitor (ISTI) TDR and studies about the impact of integrase MRVs on virological failure are contradictory. Furthermore, universal genotypic analysis of integrase gene is not recommended before treatment initiation, whereas the use of ISTI in clinical practice is largely increasing. In this context, we have evaluated integrase majority resistant variants and MRVs in HAART-naive patients by Sanger sequencing and ultradeep sequencing (UDS).

The population studied was composed of 92 treatment-naive patients recently diagnosed with HIV-1 (2013–15): 70 MSM, 8 heterosexual patients (including one intravenous drug user), and 14 patients with unknown sexual orientation. Patients were followed by the Department of Infectious Diseases and Internal Medicine of Pitié-Salpêtrière Hospital (Paris, France) and at the University Hospital of Rome Tor Vergata (Rome, Italy). Information was obtained from the existing electronic database and/or medical record.

Integrase genotypic resistance analysis was performed in all subjects from amino acids 53 to 288. Integrase Sanger sequencing was conducted according to the Agence Nationale de Recherche sur le SIDA et les hépatites virales (ANRS) consensus method as previously described.[1] The subtype determination was performed using the Module HIV from SmartGene® (Zug, Switzerland) whose methodology is based on BLAST. UDS was performed by using GS-Junior platform according to manufacturer recommendations in both clinical centres as previously described.[2,3]

Table 1. Characteristics of patients and global prevalence of ISTI mutations; patients, $n = 92$

Age (years), median (IQR)	36.1 (29.8–45.0)
Viral subtype B, %	58.2
HIV-1 RNA (log_{10} copies/mL), median (IQR)	4.9 (4.4–5.4)
CD4 cell count (cells/mm^3), median (IQR)	461 (320–577)
Time since diagnosis (days), median (IQR)	12.0 (2.3–31.5)
Prevalence of ISTI mutations with Sanger (95% CI)	$n = 7$; 7.6% (2.2%–13.0%)
Prevalence of ISTI mutations with UDS (95% CI)	$n = 10$; 10.9% (4.5%–17.2%)

An amplicon variant analyser was used to analyse the UDS data, with a detection threshold of MRV of 2% (forward and reverse).

Resistance was interpreted according to the last version of the ANRS algorithm (www.hivfrenchresistance.org). The mutational load was considered as the number of mutated copies/mL (product of percentage of a mutation by viral load).

An average of 3464 reads per nucleotide position was amplified. Characteristics of patients are presented in Table 1. Viruses of seven patients harboured majority resistant variants by Sanger sequencing (five L74I and two E157Q mutations). In three patients, viruses harboured MRVs detected by UDS only: two R263K mutations (at a rate of 9.7% the mutational load is 7099 copies/mL, and at 13.5% the mutational load is 8345 copies/mL, in the presence of a L74I mutation detected by both techniques) and one E138K mutation (at 4.8%, 111 copies/mL). All these mutations were retrieved/detected among MSM patients infected by a subtype B virus.

None of the three classical ISTI signature resistance mutations (at positions 143, 148 and 155) was retrieved. However, among MSM treatment-naive patients, the prevalence of ISTI-associated resistance mutations seems to be relatively high [10.0% by Sanger (95% CI: 3.0%–17.0%) and 14.3% by UDS (95% CI: 6.1%–22.5%)]. Moreover, our results are consistent with what has been previously shown by Sanger sequencing in the study on French patients diagnosed at the time of primary HIV-1 infection in which the E157Q first generation ISTI mutation, was detected in 5 of 331 cases (1.5%).[4] Indeed, mutations detected by Sanger sequencing (E157Q, L74I) are known to be mainly related to polymorphism in databases, whereas mutations detected by UDS only (E138K, R263K) are not.[5,6] By querying a large Italian anonymous database including 1307 integrase plasma Sanger sequences from HIV-1-infected drug-naive patients, the R263K mutation was detected only in five samples (0.4%), often as a mixture with the WT virus R263R (D. Armenia, M. M. Santoro, D. Di Carlo, C. Gori, A. Bertoli, V. Borghi, F. Ceccherini-Silberstein, C. Mussini, M. Andreoni, A. Antinori and C. F. Perno, University of Rome Tor Vergata, unpublished results). *In vivo*, the R263K mutation had been described in experienced but also in ISTI-naive patients failing a dolutegravir once daily regimen.[7,8] This mutation had been described as a dolutegravir resistance-associated mutation selected in cell culture with a negative impact on viral fitness.[9] This unfavourable impact might explain why the R263K mutation was detected only by UDS as a minority species in two patients in our study, whose viruses are not phylogenetically related (genetic distance >8% between sequences). Indeed, we can suppose that the low viral fitness related to this mutation avoids the spread of these variants among intra-patient quasispecies.

Furthermore, in our current dataset, one patient with R263K harboured the TDR mutation K103N in the reverse transcriptase gene. This patient was the only one who has initiated an integrase inhibitor-based regimen (tenofovir, emtricitabine and raltegravir) among patients with mutated viruses. Nevertheless, the virological outcome was a success with a viral load <50 copies/mL after 3, 6 and 12 months under this treatment. Indeed, *in vitro*, R263K mutants obtained by side-directed mutagenesis were hypersensitive to raltegravir (fold-change from 0.5 to 0.8).[10]

In conclusion, with the increased use of ISTI in clinical practice, the presence of ISTI-associated resistance mutations in naive patients should be considered in the future, and our results suggest that integrase pre-treatment genotypic analysis might be useful. However, the impact of integrase MRVs should be further studied.

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Transparency declarations

None to declare.

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APOL1 variants may induce HIV-associated nephropathy during HIV primary infection

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Sir,

HIV-associated nephropathy (HIVAN) is still associated with high mortality,[1] and is usually a late complication of HIV chronic infection.[2] Since 2010, a link has been strongly established between APOL1 genetic variants and HIVAN, especially in the African population.[3]

A middle-aged man from Ivory Coast was admitted to hospital for a flu-like illness lasting for 1 month. He had already received several antibiotic regimens and previous malaria and HIV tests were negative. He had been on amlodipine/valsartan for many years for hypertension and his plasma creatinine level 1 month before was 1.25 mg/dL (110 μmol/L).

His weight had increased from 101 to 113 kg with pitting oedemas in the legs and frothy urine for 1 month. Diffuse small peripheral lymphadenopathies were present. Clinical examination was otherwise normal.

Biological assessment showed an acute kidney injury with indication for dialysis [serum creatinine 20.3 mg/dL (1781 μmol/L), blood urea nitrogen 40.7 mmol/L, potassium 5 mmol/L and carbon dioxide 15 mmol/L] and a nephrotic syndrome with low serum albumin (1.3 g/dL), high-level proteinuria (7 g per day) and a urinary protein/creatinine ratio of 1.72. Ultrasound found normal-sized hyperechogenic kidneys (12 cm each).

A renal biopsy showed typical HIVAN: one-third of the glomeruli were sclerotic with all but one displaying a collapsing focal segmental glomerulosclerosis (FSGS) with hypertrophic podocytes (Figure 1a). The tubulointerstitial area revealed many cystic tubular lesions associated with a moderate inflammatory infiltrate (Figure 1b). Acute tubular necrosis lesions and a discrete patchy fibrosis were also present. The immunofluorescence study was negative for IgA, IgG, IgM, C3, C1q and albumin.

The HIV test (ELISA) confirmed with a western blot (New Lav Blot, Bio-Rad) returned positive. The p24 antigen was negative. The initial CD4 count was 594 cells/mm^3 (17%) and the plasma HIV-1 RNA was 1.2 million (6.04 log) copies/mL. The patient tested negative for syphilis, hepatitis B and C and opportunistic infections except for a cytomegalovirus (CMV) replication (3 log DNA copies/mL). The HIV-1 strain was a CRF02_AG subtype with a CCR5 tropism, and the genotyping assay displayed no resistance-associated mutation.

ART was quickly initiated with raltegravir and etravirine with addition of abacavir to the regimen when HLA B5701 returned negative. Dialysis was continued for 2 weeks. Considering the severity of renal failure and the nephrotic syndrome, the patient received both angiotensin-converting enzyme (ACE) inhibitors and corticosteroid therapy. Ten days after initiating treatment, plasma creatinine decreased and stabilized at about 2.3 mg/dL (200 μmol/L); the protein/creatinine ratio also decreased to 0.46.

After 6 weeks of treatment, the HIV-1 load decreased to 449 copies/mL. The CD4 count increased to 1063 cells/mm^3 (45%) with a CD4/CD8 ratio of 1.18 and lymphadenopathy disappeared.

To confirm a recent HIV infection, we used both a supplemental western-blot assay (HIV Blot 2.2, MP Biomedicals,