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Minority resistant HIV-1 variants and the response to first-line NNRTI therapy

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ABSTRACT

Background: The presence of low-frequency HIV-1 variants with mutations making them resistant to non-nucleoside reverse-transcriptase inhibitors (NNRTI) could influence the virological response to first-line NNRTI therapy.
Objectives: This study was designed to describe the proportions and quantities of NRTI and NNRTI-resistant variants in patients with successful first-line NNRTI therapy.
Study design: We evaluated the presence of drug-resistance mutations (DRMs) prior to treatment initiation in 131 naive chronically HIV-1-infected patients initiating NNRTI-based first-line therapy. DRMs were detected by ultradeep pyrosequencing (UDPS) on a GS Junior instrument (Roche).
Results: The mean HIV RNA concentration was 4.78 ± 0.74 log copies/mL and the mean CD4 cell count was 368 ± 184 CD4 cells/mm^3. Patients were mainly infected with subtype B (68%) and 96% were treated with efavirenz. The sensitivity threshold for each mutation was 0.13–1.05% for 2000 reads. Major NRTI-resistant or NNRTI-resistant mutations were detected in 40 patients (33.6%). The median frequency of major NRTI-resistant mutations was 1.37% [IQR: 0.39–84.1], i.e.: a median of 556 copies/mL [IQR: 123–37,553]. The median frequency of major NNRTI-resistant DRMs was 0.78% [IQR: 0.67–7.06], i.e.: a median of 715 copies/mL [IQR: 391–3452]. The genotypic susceptibility score (GSS) of 9 (7.3%) patients with mutations to given treatment detected by UDPS was 1.5 or 2.
Conclusions: First-line NNRTI-based treatment can produce virological success in naïve HIV-1-infected patients harboring low-frequency DRMs representing <1% of the viral quasispecies. Further studies are needed to determine the clinical cut-off of low-frequency resistant variants associated to virological failure.

1. Background

HIV-1 drug resistance genotyping is recommended to optimize first-line antiretroviral therapy and thus prevent virological failure [1]. Current tests for drug resistance fail to detect low-frequency drug-resistance mutations (DRMs) present in less than 15–25% of the total quasispecies because they are based on bulk population sequencing [2,3]. Several studies have used recently developed ultra-sensitive methods, including allele-specific PCR [4,5] and ultra-deep sequencing [6–8], to show that low percentages of viruses with DRMs could contribute to subsequent treatment failure. There is no consensus as to whether more sensitive techniques should be used routinely to detect and quantify minor populations of drug resistant HIV-1 variants that are not detected by bulk population sequencing.

Low-frequency variants of HIV-1 with altered drug sensitivity could be clinically relevant in two main settings. First, when detecting minority variants that are resistant to non-nucleoside reverse transcriptase inhibitors (NNRTI) before starting first-line NNRTI-based treatment [9,10] and, second, when detecting low frequencies of CXCR4-using variants before using CCR5 antagonists [11].

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There is still confusion about which HIV-1 minority variants are clinically significant and how their presence affects clinical practice. Most studies published to date used techniques focusing on limited predefined DRMs [9]. Few have used ultra-deep sequencing methods which give access to all known DRMs and allow haplotyping [12–15]. The enhanced sensitivity of these assays facilitates the detection of minority variants and any resistance artifacts generated by the method [8,16]. However, appropriate clinical cut-offs and the proportion of low-frequency DRMs associated with virological success or failure are missing.

2. Objectives

NNRTI-based treatment is the recommended first-line highly active antiretroviral therapy (HAART); it is the most commonly prescribed treatment for naïve patients [17,18]. The presence of low-frequency NNRTI DRMs could influence the virological response. This study was designed to describe the proportion and quantity of minority resistant variants observed in patients successfully treated with first-line NNRTI therapy.

3. Study design

3.1. Population

A total of 131 naïve patients chronically infected with HIV-1, given first-line NNRTI therapy between 2008 and 2011 and who experienced virological success (HIV-1 RNA <50 copies/mL) 6 and 12 months after treatment initiation, were studied. All were followed at Toulouse University Hospital (France). The concentrations of HIV-1 RNA in plasma samples were measured by real-time RT-PCR (COBAS^{TM} Ampliprep/COBAS^{TM} Taqman HIV test; Roche Diagnostics; sensitivity: 20 copies/mL). All patients gave their informed consent for these virological studies.

3.2. Bulk population sequencing

Genotypic resistance tests were performed at treatment initiation. The HIV-1 reverse transcriptase gene was amplified by nested PCR and sequenced from bulk PCR products in both directions by the dideoxy chain 10 termination method (BigDye Terminator; Applied Biosystems) on an ABI 3130 DNA sequencer. The prevalence of resistant HIV-1 populations was analyzed using the latest International AIDS Society list of mutation (2013) [19] and the French resistance algorithm (2013, v23) (www.hivfrenchresistance.org).

3.3. Ultra-deep pyrosequencing (UDPS)

Plasma samples, collected for virological analyses at treatment initiation and stored frozen at −80 °C, were used to perform UDPS. HIV-1 RNA was extracted from 1 mL samples of plasma with virus loads of 708–3,090,295 copies/mL using QIAamp RNA extraction kits (Qiagen, Courtaboeuf, France). The next-generation ultradeep pyrosequencing (UDPS) HIV-1 assay currently being developed by Roche for the GS Junior system was used to amplify HIV-1 before UDPS. Briefly, first-strand cDNA was generated with two gene-specific oligonucleotides. This was used to produce four partly overlapping amplicons covering the HIV-1 pol gene (protease and reverse transcriptase gene codons 1–251). These were purified and quantified. Equimolar amounts of all four amplicons from each sample were pooled and subjected to clonal amplification on beads using reagents that enabled sequencing in both the forward and reverse directions. The beads were isolated and those bearing enriched DNA were counted. Ultra-deep pyrosequencing was carried out on 500,000 beads loaded onto the PicoTiter plate and sequenced on a Genome Sequencer Junior (Roche-454 Life Sciences). The amplicon nucleotide sequence reads were aligned with a consensus sequence HXB2. The capacities of two analytical programs, GS Amplicon Variant Analyzer (AVA) software and DeepCheck®-HIV, to analyze the ultra-deep pyrosequencing results were compared. GS Amplicon Variant Analyzer (AVA) software describe all the mutations whereas DeepCheck®-HIV describe only mutation with frequencies >1%. Data presented were based on AVA analysis. We used the latest International AIDS Society list of mutation (2013) [19] to identify resistance mutations and the French resistance algorithm (2013, v23) to interpret the involvement of each codon in resistance to each drug. Mutations conferring drug resistance on HIV alone are considered to be major mutations, while mutations that confer resistance only when associated with other mutations are defined as minor mutations. The genotypic sensitivity score (GSS) was interpreted using the ANRS algorithm: each antiretroviral was assigned a score 1 if susceptible, 0.5 for intermediate resistance and of 0 for high resistance.

Table 1
Description of mutations observed on each plasmid clones by sanger sequencing.

Clone	Subtype	Mutations (sanger sequencing)
1	B	V179I
2	B	V179I G190A T215D
3	B	WT[a]
4	B	WT
5	CRF06	WT
6	B	WT
7	CRF02	K103 N
8	B	WT
9	B	L100I, K103 N, E138 K, Y188L
10	B	M41L, A98G, G190A, V179I, L210 W, T215S

[a] WT: wild type.

3.4. UDPS technical error rate

We assessed the frequency of errors resulting from PCR amplification and pyrosequencing of the codon of interest by comparing the pyrosequencing data from a panel of 10 plasmid clones with their respective clonal sequence obtained by the Sanger method. Five plasmid clones had a wild type sequence and the others had one to six mutations on the polymerase gene (Table 1). The mean error rate and confidence interval were determined, and the upper confidence limit of the error rate was used to calculate the sensitivity of pyrosequencing for a given number of reads. Poisson distribution was used to distinguish authentic variants from artifactual sequences resulting from errors arising during PCR amplification and ultra-deep pyrosequencing. Only those variants yielding a P value of <0.001 according to the Poisson model were considered authentic. The detection threshold as a function of the read number was determined for each position. For 2000 reads, the thresholds of UDPS were as follow: M41 (0.15%), K65 (0.20%), D67 (1.05%), K70 (0.15%), L74 (0.25%), M184 (0.20%), L210 (0.18%), K219 (0.15%) for NRTI DRMs and L100 (0.50%), K101 (0.55%), V106 (0.15%), E138 (0.39%), Y181 (0.15%), Y188 (0.20%), G190 (0.15%), H221 (0.24%) for NNRTI DRM.

4. Results

4.1. Study population and drug resistance in bulk population sequencing

The demographics and other baseline characteristics of the patients are shown in Table 2. HIV subtype B was found in 89 (68%) patients and CRF02 in 22 (16.8%). Most patients were treated with a