Genotype Resistance Profiles in Patients Failing an NNRTI-Containing Regimen, and Modifications After Stopping NNRTI Therapy

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Resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) develops quickly and independently if they are used in combination with NRTIs or protease inhibitors (PIs) as rescue therapy, mainly due to the low genetic barrier of this class of drugs. In this study we examined clinical, therapeutic, and virologic characteristics in 88 patients with mutations conferring resistance to NNRTIs, and in 11 patients 1

year after stopping NNRTI therapy. Between patients administered Nevirapine (NVP) and those taking Efavirenz (EFV), no statistical differences were found in CD4 cell count, HIV viral load, time on NNRTI therapy, or number of PIs administered previously. A slow decline in the detectability of mutations encoding NNRTI resistance was found. *J. Clin. Lab. Anal.* 16:76–78, 2002. © 2002 Wiley-Liss, Inc.

Key words: HIV; resistance; salvage therapy; NNRTI

INTRODUCTION

The virologic efficacy of antiretroviral therapy (ARVT) does not necessarily depend on the inclusion of protease inhibitors (PIs) in therapeutic regimens. Drug combinations containing non-nucleoside reverse transcriptase inhibitors (NNRTIs) represent a valid alternative to PI-containing regimens, as recently demonstrated in patients with intermediate or advanced HIV disease (1–3).

Patients who experience virological failure with ARVT usually harbor HIV-1-mutated variants that are resistant to several antiretroviral drugs, thus limiting the success of subsequent therapies (4–6).

No information is currently available on the decay rate of resistant variants after NNRTI pressure is removed. To gain further insight into this topic, we studied the genotypic characteristics of the viral population of patients failing an NNRTI-based ARVT both at the time of failure and after changing to an NNRTI-free regimen.

MATERIAL AND METHODS

Study Design

The study included a retrospective selection of patients in a large clinical setting in northern Italy, where more than 1,300 HIV-infected patients are followed.

Study Population

The study population comprised 88 patients in the HIV cohort followed by the Institute of Infectious Diseases of the Uni-

versity of Brescia (1,300 HIV-positive patients), who were retrospectively selected according to their genotypic resistance to NNRTIs. If the patient was already on NNRTI therapy, it was completely interrupted and replaced by a new, NNRTI-free regimen. If a new failure was observed within 12 months (as it was in 11 patients), a genotypic resistance assay was performed again. Virologic failure was considered when a <1-log reduction of HIV viral load by 8 weeks, failure to suppress plasma HIV RNA to undetectable levels (<200 copies/ml) within 6 months of initiating therapy, repeat detection of viremia after initial suppression to undetectable levels, or any reproducible significant increase from a minimum of plasma HIV RNA were observed.

Genotypic Resistance Assay

Plasma samples were direct-sequenced after HIV RNA extraction by a kit from Visible Genetics, Inc. (Paris, France). Briefly, a 1.3K sequence was generated with a single-tube reverse transcriptase-polymerase chain reaction (RT-PCR) technique. The protease and transcriptase genes were then bidirectionally sequenced using a sensitive single-tube technique (CLIP™ (Visible Genetics, Inc.)) that generates

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fluorescently-labeled terminated copies of DNA. The method uses two different dye-labeled primers for each of the three sequencing reactions: protease (codons ≤ 1 to ≥ 99), RT beginning (codons ≤ 39 to ≥ 143), and RT middle (codons ≤ 138 to ≥ 244). Next, electrophoresis was performed. Resistance mutations considered as coding NNRTI resistance included codons G100I, K101E, K103N, V106A, V108I, Y181C, Y188L/C, G190A, and P236L of the RT gene.

RESULTS

Eighty-eight patients were eligible for the study because they had genotypic resistance to NNRTIs registered in their clinical charts at the time they failed their therapeutic regimen. They were retrospectively selected to enter the study.

NNRTI resistance-associated mutations were found in 13 patients completely new to this class of drugs; 58 patients were on therapy with a regimen including Nevirapine (NVP), and in 17 patients Efavirenz (EFV) was part of the treatment regimen. A regimen including NVP was the first ARVT in 23 of 58 patients. Their viro-immunologic profile and time on therapy is reported in Tables 1 and 2.

No significant differences were noted in newly NNRTI-treated or NVP- or EFV-treated patients as far as the time on treatment was concerned. However, the latter group tended to have lower CD4 counts and higher viral loads, and was heavily pretreated. Baseline characteristics (time on therapy and viro-immunological status) of patients treated with NVP-based ARVT at the time of failure did not differ regardless of whether the treatment was their first-line therapy.

Several different NNRTI-resistant mutations were identified in patients on NVP or EFV therapy, and in newly NNRTI-treated patients (Table 3). Finally, modification of NNRTI resistance profiles in patients on NVP-containing regimens were analyzed when a new virological failure was identified within 12 months after a genotypic-driven, NNRTI-free regimen was prescribed. In 11 patients a new virologic failure was observed and a second resistance test was made. The observed mutations are reported in Table 4.

Only four of 11 patients who failed again within 12 months despite the genotypic-driven, NNRTI-free regimen lost all NNRTI-encoding mutants. Mutation K103N was still present in four of six patients who presented this mutation at the time of the switch. Of four patients who carried the Y181C mutation, only one had the mutation when the second failure occurred.

TABLE 1. Characteristics and antiretroviral treatment history at time of the NNRTI-regimen failure

Mean time on NNRTI therapy	6.16 $\pm$ 3.17 mo
Mean time on NVP therapy	6.34 $\pm$ 3.22 mo
Mean time on EFV therapy	5.52 $\pm$ 3.00 mo
Mean of CD4 cell count	228.97 $\pm$ 153.14 /ml
Mean of HIV viral load	63,421 $\pm$ 194,987 copies/ml
N° previous PIs (mean)	1.3 $\pm$ 1.1

TABLE 2. Characteristics of patients failing NVP and EFV regimens

	NVP-including regimen	EFV-including regimen
Mean of CD4 cell count	217.95 $\pm$ 157.77 /ml	178 $\pm$ 136.04 /ml
Mean of HIV viral load	58,591 $\pm$ 182,640 copies/ml	79,900 $\pm$ 237,975 copies/ml
N° previous PIs (mean)	1.05 $\pm$ 1.67	2.17 $\pm$ 0.9

DISCUSSION

Resistance to drugs in the NNRTI class develops quickly in the presence of even low-grade viral replication due to the low genetic barrier of this class of drugs. A single key mutation in the NNRTI-specific pocket site or in the surrounding domain can induce a high level of phenotypic resistance to the entire class of drugs (6,7).

In our study, 14.7% of patients harboring viruses with NNRTI-resistant mutations have never been administered NNRTIs. Transmission of K103N variants to newly infected persons has previously been described with a frequency of around 12.5% (8,9). None of our patients were newly HIV infected. Thus, whether these mutations had been received from source or whether they were selected during replication in an NNRTI-free environment remains unclear.

NVP and EFV were previously compared with a PI-containing regimen in randomized, controlled clinical trials, and they were found to be at least as effective as, and better tolerated than the PIs (2,3). They are therefore considered a valid alternative to PIs.

This study showed that the Y181C mutation was predominant in the patients on NVP, as previously described (10,11). It is interesting that the K103N mutation, which individually confers cross-resistance to all NNRTIs, was selected on 17.2% of our patients failing on the NVP regimen but on 70.5% of patients on the EFV regimen.

This suggests that some patients failing on NVP regimens

TABLE 3. Resistance profiles of patients on NNRTI therapy (mutated codons)

Mutated codon	Naive for NNRTI (n = 13)	On NVP (n = 58)	On EFV (n = 17)
103	8 (61.5%)	10 (17.2%)	12 (70.5%)
181	2 (15.3%)	15 (25.8%)	—
108	1 (7.6%)	—	1 (5.8%)
190	—	1 (1.7)	—
106	—	1 (1.7)	—
101	—	1 (1.7)	—
188	—	—	1 (5.8%)
103 + 181	—	5 (8.6%)	—
108 + 181	—	2 (3.4%)	—
181 + 190	1 (7.6%)	—	—
181 + 100	1 (7.6%)	—	—
> 3 mutations	—	23 (39.6%)	3 (17.6%)

TABLE 4. Modification of resistance profiles off NNRTI therapy (mutated codons)

Patient no.	On NNRT	Off NNRTI	Time interval (mo)
1	103	103	9
2	181/103	181/103	12
3	103	—	12
4	108	—	10
5	106	106	9
6	103	—	5
7	103	103	9
8	181/190/188	181	10
9	103	103	12
10	181	181	9
11	108/181	—	12

can benefit from EFV as a component of a salvage regimen. However, the presence of more than three mutations coding NNRTI resistance was more frequent in patients failing NVP therapy (39.6% vs. 17.6%). In our study, 29% of NVP genotypic-resistant patients remained susceptible to EFV, but none of the EFV genotypic-resistant patients were still susceptible to NVP. Therefore, sequencing NNRTIs is difficult and only after starting with NVP. Few patients are good candidates for successful EFV treatment (9,12).

Furthermore, very few studies have focused on the decline rate of NNRTI-resistant viruses after specific drug pressure has been removed. Devereux et al. (13) described a decline in primary mutations 4 months after stopping combination therapy with PIs. Our study shows that the clearance of NNRTI-resistant viruses is less rapid. Only in 36% (4/11) of patients all NNRTI conferring resistance mutations were completely lost within 12 months. Direct sequencing from a plasma RNA whole viral population presents some limitations, since the method will characterize the genotypic status of only the dominant part of the viral pool, and may mask a persisting mixed pool or an emerging or hidden shift in the population.

Our data confirm that NNRTI-resistant mutations emerge quickly, independently of the number of PIs used previously. They can remain for 1 year even in the absence of NNRTI pressure, but also can be present in patients new to this class of drugs.

Given the small number of salvage therapy cases, the presumably high heterogeneity of the cohort in terms of their past experience with salvage antiretroviral agent exposure,

and the variability of resampling times, no conclusions can be drawn from the present data about the “decline rate” of the resistance-mutation viral variants beyond the fact that in the circulating viruses these mutations predominantly persisted for 9–12 months.

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