# SENSITIVITY OF DRUG RESISTANT HIV-1 ISOLATES TO 2ND-GENERATION NNRTI'S

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## Introduction

South Africa has an estimated 5.7 million people infected with HIV-1 of whom ~1.8 million were receiving antiretroviral treatment by 2011.[1] The first-line regimen for adults is non-nucleoside reverse transcriptase inhibitor (NNRTI)-based, comprising of either efavirenz (EFV) or nevirapine (NVP), in combination with two nucleos(t)ide reverse transcriptase inhibitors. Both these first-generation NNRTIs have a low genetic barrier to the development of resistance and share substantial cross-resistance. Recent studies have shown that approximately 80% of first-line failures contain NNRTI resistance mutations.[2-4] As a consequence, failure on either drug necessitates a switch to a protease-based regimen. Although protease inhibitors are effective in controlling HIV-1 viremia, they are less tolerable and more expensive than the NNRTI-based regimen. Due to the extensive use of first-generation NNRTIs in South Africa and other developing countries, accessibility to alternative NNRTIs with an unrelated resistance profile is needed in order to delay regimen switch to a protease-based regimen.

Etravirine (ETR) and rilpivirine (RPV) are second generation NNRTIs with a high genetic barrier to the development of resistance and a resistance profile that only partially overlaps that of EFV or NVP.[5] In the DUET trials, ETR was demonstrated to be safe, tolerable and active in treatment-experienced patients with NNRTI resistant strains.[6] In the ECHO and THRIVE trials, rilpivirine had a non-inferior efficacy in treatment-naive patients compared to EFV, with a favorable safety profile.[7,8] Both ETR and RPV retain activity against viruses containing the K103N mutation in HIV-1 reverse transcriptase, the most prevalent NNRTI resistance mutation in current first-line failures. Although an accumulation of NNRTI mutations are required for resistance to ETR or RPV, a single mutation at codon Y181, frequently selected for by NVP, could be sufficient to cause resistance to both.[9] Indeed, an increased risk in ETR failure has been associated with the prior use of NVP.[10]

The aim of this study was to assess whether South African patients failing on an EFV/NVP-based first-line regimen would harbor viruses sensitive to second generation NNRTIs.

## Methods

Phenotypic susceptibility testing was performed on 33 patients using an in-house single-cycle HIV-1 phenotypic assay to assess their susceptibility to both first- and second-generation NNRTIs. Standard population-based Sanger-sequencing was performed to identify NNRTI resistance mutations present in the reverse transcriptase portion of the *pol* gene of HIV-1 in the samples.

## Results

As expected, most samples (n=31/33, 94%) were resistant to both EFV and NVP while only 36% (n=12/33) and 21% (7/33) were resistant to ETR and RPV respectively (table 1). Apart from sample DR64, with a single Y181C mutation, all samples resistant to ETR and/or RPV contained ≥2 NNRTI resistance mutations. In contrast, a single NNRTI resistance mutation was sufficient to cause resistance to EFV and/or NVP. The majority of samples with high-level resistance to ETR contained the Y181C mutation (58%, n=7/12). This mutation had a lower impact on RPV resistance as only 3 highly resistant samples contained Y181C.

Table 1. Genotype and phenotypic susceptibility of patient samples to first- (EFV and NVP) and second-generation (ETR and RPV) NNRTIs.

| Patient ID | NNRTI exposure | Genotype NNRTI resistance mutations | Phenotype (Fold-change) ETR | RPV | EFV | NVP |
|---|---|---|---|---|---|---|
| SAVE 1186 | EFV | V106M,Y188C | 0.5 | 0.6 | 42.7 | 59.9 |
| SAVE 1302 | EFV | K101H,K103N,G190A | 0.7 | 1.1 | 42.7 | 59.9 |
| SAVE 2229 | EFV | V106M | 0.7 | 0.2 | 4.0 | 5.4 |
| SAVE 2041 | EFV | K103N | 0.8 | 0.6 | 41.6 | 59.9 |
| TOGA 134184 | NVP | A98G,K103N | 1.1 | 0.9 | 42.7 | 59.9 |
| SAVE 1412 | *NR | K103N | 1.3 | 1.3 | 42.7 | 59.9 |
| SAVE 1383 | EFV | K103N,V106A,G190A | 1.3 | 1.9 | 42.7 | 59.9 |
| TOGA 311368 | EFV | M230L | 1.4 | 0.9 | 1.3 | 1.9 |
| TOGA 605248 | NVP | K101H,G190A | 1.4 | 0.8 | 42.7 | 59.9 |
| SAVE 1379 | EFV | V106M,G190A | 1.5 | 0.8 | 42.7 | 59.9 |
| TOGA 357702 | EFV | K103N,V108I | 1.6 | 1.1 | 42.7 | 59.9 |
| TOGA 064124 | NVP | K103N | 1.7 | 1.3 | 40.2 | 59.9 |
| TOGA 56522 | NVP | K103N | 1.8 | 1.3 | 42.7 | 59.9 |
| SAVE 1434 | EFV | V90I,K103N,P225H | 2.3 | 1.7 | 42.7 | 59.9 |
| TOGA 063884 | NVP | K103N | 2.7 | 1.7 | 42.7 | 59.9 |
| TOGA 480117 | NVP | V106M,Y188C | 3.0 | 1.7 | 42.7 | 59.9 |
| TOGA 301226 | EFV | K103N,H221Y,P225H | 3.2 | 1.6 | 42.7 | 59.9 |
| SAVE 1500 | NVP | K101E,V106M,E138A,F227L | 3.2 | 1.8 | 42.7 | 59.9 |
| TOGA 437809 | EFV | A98G,K103N,V108I,P225H | 4.8 | 5.0 | 42.7 | 59.9 |
| SAVE 2452 | EFV | K103N,E138A,P225H | 5.3 | 7.4 | 42.7 | 59.9 |
| TOGA 102710 | EFV | K103N,Y181C | 8.6 | 5.2 | 28.1 | 59.9 |
| DR150 | NVP | V106M,Y181C | 10.9 | 1.2 | 42.7 | 59.9 |
| SAVE 1400 | EFV | K101E,Y188L | 11.2 | 75.8 | 42.7 | 59.9 |
| TOGA 62695 | EFV | V106M,V179D,Y188C | 12.6 | 2.4 | 42.7 | 59.9 |
| DR146 | EFV | K103N,V108I, Y181C,H221Y | 17.8 | 4.9 | 42.7 | 59.9 |
| SAVE 1252 | EFV | K101E,G190A,H221Y,M230L | 19.0 | 18.8 | 42.7 | 59.9 |
| TOGA 33184 | EFV | L100I,K103N | 24.8 | 12.4 | 42.7 | 59.9 |
| DR49 | NVP | V108I,Y181C | 25.1 | 3.3 | 40.6 | 59.9 |
| TOGA 135372 | NVP | Y181S,Y188H,H221Y | 42.5 | 8.2 | 42.7 | 59.9 |
| SAVE 1154 | EFV | V106M,M184V,Y188L,H221Y | 47.7 | 75.8 | 42.7 | 59.9 |
| DR64 | NVP | Y181C | 47.7 | 17.0 | 27.9 | 59.9 |
| DR41 | NVP | A98G,Y181C,M230L | 47.7 | 16.9 | 28.9 | 59.9 |
| DR122 | NVP | Y181C,H221Y | 47.7 | 23.3 | 30.7 | 59.9 |
| Total number susceptible | | | 18 | 20 | 1 | 1 |
| Total number with low-level resistance | | | 1 | 2 | 1 | 0 |
| Total number with intermediate resistance | | | 2 | 4 | 0 | 1 |
| Total number with high-level resistance | | | 12 | 7 | 31 | 31 |

Susceptible Low-level resistance Intermediate resistance High-level resistance

*NR: Not reported

NOTE: This table indicates the genotypic and phenotypic resistance profiles of patients (n=33) failing on an EFV- or NVP-based first-line regimen. The genotype indicates the various NNRTI resistance-associated mutations present in the reverse transcriptase (RT) gene of the HIV-1 strain obtained from the patient. The phenotype indicates the level of phenotypic resistance to ETR, RPV, EFV and NVP. Values represent the ratio, or fold-change (FC), of the inhibitory concentration-50 ($IC_{50}$) of the sample virus compared to that of a wild-type reference virus for a particular drug. A value of FC>1 indicates a decrease in drug susceptibility that infers some level of drug resistance. The level of phenotypic resistance is classified as “susceptible” (FC=1), “Low-level resistance” and “Intermediate resistance” (FC>1 but <10), and “High-level resistance” (FC≥10). The samples are ordered according to ETR FC.

## Discussion

These data suggest that HIV strains from patients failing on NVP or EFV would mostly show sensitivity to ETR and RPV. Currently there are no clear guidelines in South Africa for the use of 2nd generation NNRTIs and several factors could impact on their use. The prevalence of ETR/RPV-related NNRTI mutations in sub-Saharan Africa is low and the predicted resistance to ETR and RPV uncommon.[3] The use of these 2nd-generation NNRTI will mostly be influenced by high frequencies of Y181 mutants, as selected for by NVP, and the accumulation of NNRTI mutations. By prioritizing the use of EFV over NVP, and frequent viral load monitoring to prevent the accumulation of resistance associate mutations, 2nd-generation NNRTI might be a viable option for first-line failures.

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