



# Pre-HAART Drug Resistance Mutations in Subtype C HIV-1 in Zimbabwe

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## I. Background and Objectives

• Non-nucleoside reverse transcriptase (NNRTI) based combination antiretroviral therapy (ART), mostly using nevirapine (NVP), is the current standard first line regimen in resource limited settings.

• Prevention of mother to child transmission (pMTCT) in these regions often includes use of single dose (SD) NVP with or without short course AZT.

• Transmitted or acquired resistance prior to combination antiretroviral therapy (ART) may compromise the effectiveness of NVP-based HAART regimens.

## II. Methods

• Eighty-seven adults with AIDS initiated NVP-based ART in July 2003 in Chitungwiza, Zimbabwe.

• 54 women

• 32 had received short course (SC) AZT between 1999 and 2001,

• 24 single dose (SD) NVP between 2000 and 2003

• 3 had received SC AZT and SD NVP:

- 2 individuals – combination treatment during the same pregnancy
- 1 individual had SC AZT monotherapy during an initial pregnancy, and SD NVP for a subsequent pregnancy

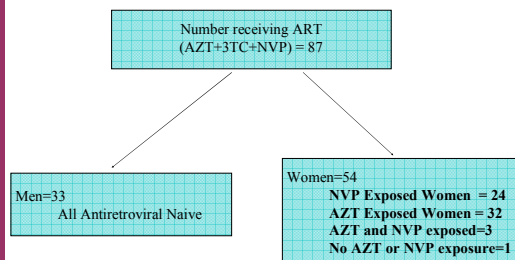
• 33 Men were the partners of exposed women, but reported no prior ARV exposure.

• Baseline (week 0) plasma samples were assessed from 25/87 (29%) with HIV RNA > 400 copies/mL at 16, 24, and/or 48 weeks on treatment.

• Drug resistance mutations in consensus plasma HIV RNA were detected using two methods:

- Population genotypic sequence assay
- Allele specific (AS) PCR for minority variant populations of K103N

## Patient Population



**Table 1**  
Baseline cohort characteristics by subsequent response to treatment

	Overall	*Viremic at 16, 24, or 48 weeks (n=28) *VL>400 copies/mL	Suppressed at 16, 24, and 48 weeks (n=59) *VL<400 copies/mL	*P Value
Median Age (Range; IQR)	34 (20-52; 6)	34 (24-42; 4.5)	33 (20-52; 7)	0.67
Median baseline CD4 per cu mm (Range; IQR)	123 (5-306; 105)	119 (5-224; 95)	124 (14-306 ; 110)	0.77
Median Baseline VL log <sub>10</sub> copies/ ml (Range; IQR)	5.02 (3-7.64; 0.92)	5.26 (3.9-6.4; 0.74)	4.9 (3-7.6; 0.93)	<b>0.017</b>
Median Baseline absolute lymphocyte count (Range; IQR)	966 (128-2972; 644)	976 (128-2972; 604)	996 (239-2372; 686)	0.98
Median Weight in kilograms (Range; IQR)	60 (42-80; 13)	58.9 (45-75; 14.5)	60 (42-80; 13)	0.89
Number NVP Exposed	24	9	15	0.85
Number AZT Exposed	32	12	20	0.8
Median time between SD NVP/ SC AZT exposure and ART initiation in months (Range; IQR)	28	33 (6-58; 26)	27 (2-52; 19)	0.28

\*P-values are calculated using chi square for categorical, and Wilcoxon rank-sum for continuous variables

## III. Results:

- Average reported adherence to ART was > 98%.
- In baseline samples prior to ART initiation, 5 of 25 subjects with virologic failure (20%) had detectable mutations in population sequence at codons associated with antiretroviral drug resistance:
  - K103N in 2 women
  - K238N in one woman
  - G190A in one man
  - T215/INST mixture in one man
- All 3 women with NNRTI associated drug resistance mutations reported SD NVP exposure 17 to 39 months prior to ART initiation.
- A polymorphism associated with NVP resistance, V179D, was identified in one woman.
- The ASPCR for K103N minority variants detected drug resistant mutants in five women:
  - Two women had high levels of AAC/T (>20% AAC) and K103N in consensus sequence
  - Three additional women had 0.35% - 3% AAC by ASPCR, above the lower limit of detection (0.2%), but wild-type virus by population sequence
  - Two of the women with low levels of K103N detected by ASPCR had been exposed to SD NVP 10 and 19 months before ART initiation, and one reported only AZT exposure

**Table 2**  
Drug resistance by population sequence and allele specific PCR for K103N

Subject	Baseline VL	*Viremic at 16,24, or 48 weeks *VL> 400 copies/ mL	Allele-specific PCR for K103N (% AAC/AAT)	Population Sequence RT Mutations
DF003	4.61	Yes	K103N (0.78% AAC)	WT
DF173	5.2	Yes	K103N (2.9% AAC)	WT
DF194	5.6	Yes	K103N (0.35% AAC)	WT
DF024	5.27	Yes	K103N (72.3% AAC/ 2.36% AAT)	K103N
DF089	4	Yes	K103N (71% AAC)	K103N
DP016	4.48	Yes	WT	G190A
DP182	5.06	Yes	WT	T215INST
DF069	5.08	Yes	WT	K238KN
DF020	6	Yes	WT	V179D
DF008	5.71	Yes	WT	WT
DF036	5.26	Yes	WT	WT
DF120	5.49	Yes	WT	WT
DF139	6.02	Yes	WT	WT
DF142	5.11	Yes	WT	WT
DF148	5.28	Yes	WT	WT
DF177	5.25	Yes	WT	WT
DF151	5	Yes	WT	WT
DP039	4.8	Yes	WT	WT
DP081	4.8	Yes	WT	WT
DF029	5.97	Yes	Fail	WT
DF050	6.36	Yes	WT	WT
DF103	5.4	Yes	WT	WT
DF111	unavailable	unavailable	WT	WT
DF162	4.92	Yes	WT	WT
DP047	5.13	No	WT	WT

## IV. Summary and Conclusions

- Population sequencing identified drug resistance mutations in 5 individuals:
  - 2 with NNRTI-associated drug resistance mutations at amino acid position 103 in the reverse transcriptase (RT) region of the *pol* gene
  - 2 (including 1 man without a personal history of NNRTI exposure) had NNRTI-associated drug resistance mutations at positions other than amino acid 103 in the reverse transcriptase region of the *pol* gene
  - 1 man without documented ART exposure with a T215F/Y revertant associated with AZT resistance
- A K103N allele specific PCR assay identified 5 individuals with drug resistance.
- The combination of ASPCR for minority variants at K103N and genotypic population sequence identified a evidence for drug resistance in baseline samples in a total of 8/25 (32%) individuals with viremia after 16 weeks or more of treatment with a NVP-based regimen.
- Surveillance for drug resistance mutations in AIDS patients with subtype C HIV may be warranted in settings where SD NVP and SC AZT are used for pMTCT. Screening for K103N by allele specific PCR may identify risk for virologic failure.