



Detection of Drug Resistance by Population Sequencing and Allele Specific PCR for K103N after Nevirapine-based HAART in Subtype C HIV-1 in Zimbabwe

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Background and Objectives

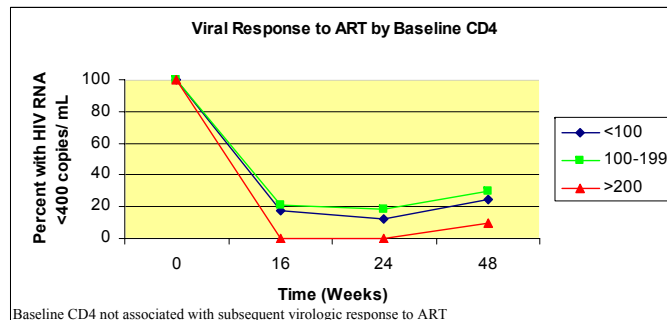
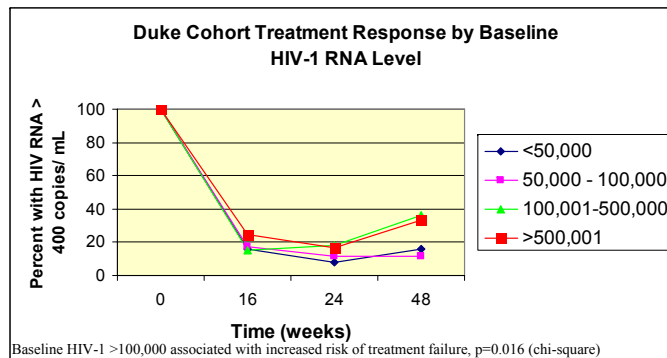
• Nevirapine (NVP) based combination antiretroviral therapy is the most common first line regimen in resource limited settings.

• Patterns of drug resistance mutations selected by combination NVP-3TC-AZT may identify potential second-line treatment strategies.

• Lower cost, sensitive assays such as allele specific amplification for specific resistance associated mutations are emerging as adjunctive tools to more costly genotypic sequence based methods to evaluate for emergence of drug resistance.

Methods

- Eighty-seven adults with AIDS initiated NVP-based ART in July 2003 in Chitungwiza, Zimbabwe.
- 54 women had previously been exposed to ARV for prevention of mother-to-child transmission
 - 31 had received short course (SC) AZT
 - 24 had single dose (SD) NVP
 - 3 had received SC AZT and SD NVP
- 33 Men were the partners of exposed women, but reported no prior ARV exposure.
- On treatment plasma samples with VL > 400 copies/mL were extracted, reverse transcribed and amplified by PCR.
- The second round amplicon was tested with a quantitative ASPCR for K103N. A positive K103N ASPCR was defined as > 0.2% prevalence of asparagine (AAC or AAT).
- Di-deoxy consensus sequencing was performed by homebrew nested sequencing with primers optimized for subtype C *pol*.



Results

- Average reported adherence to ART was > 98%.
- 28/87 (32%) subjects on treatment had VL > 400 copies/mL at 16, 24, and/or 48 weeks.
- 22 were viremic at 48 weeks (median VL = 5.26; range: 4-6.02 log₁₀ copies/mL)
- 29 sequences were obtained from 20 subjects:
 - Of the 20 subjects with sequences, 4 achieved viral suppression by 48 weeks
 - 11/20 had an NNRTI associated mutation
 - 10 an M184V
 - 1 D67DN
 - All 11 had concurrent NNRTI resistance mutations
 - 15/20 subjects had NNRTI resistance mutations
 - 8 had the K103N mutation alone
 - 7 had other NNRTI resistance mutations
 - A98G, K101E, K103S, V108I, Y181C, Y188C, G190A, and/or K238N, in various combinations
 - One had only a Y181C mutation
- ASPCR was performed on amplicon from the 29 sequenced samples:
 - 12 subjects had K103N detected by ASPCR (15 samples positive for K103N by ASPCR)

Table 2

Drug resistance by population sequence and allele specific PCR for K103N

ID	NVP	Baseline Sequence	Sequence - Week 16	Sequence - Week 24	Sequence - Week 48
DF024	1	K103N	K103N, M184V		K103S, G190A, M184V
DF089	1	K103N			M184V K103N V108I
DF069	1	K238KN	K103N		
DF194	1	WT	K103N	K103N	WT
DF162	1	WT	WT		
DF173	1	WT			WT
DF142	1	WT			M184V K103N
DF020	0	V179D		M184V V179D Y181C	M184V V179D Y181C
DF003	0	WT	M184V K101EK G190A	M184V K101E G190A	M184V K101E G190A
DF139	0	WT	M184V K103N	M184V K103N	
DF151	0	WT	WT	Y181C	Lost to follow up
DF050	0	WT	WT		
DF029	0	WT		M184V K103N	
DF120	0	WT			M184V G190S
DF148	0	WT			M184V K101E V108IV Y181CY G190AG
DP016	N.A.	G190A		M184V A98G Y181C	
DP182	N.A.	T215INST			WT
DP039	N.A.	WT	Y181CY G190A	D67DN K101EK K103N G190A	
DP081	N.A.	WT		Y181CY Y188CY	G190A, K238N
DP047	N.A.	WT		WT	

DP = Male
DF = Female

Detection of drug resistance by ASPCR vs. population sequence

ASPCR for K103N

	ASPCR Positive +	ASPCR Negative -
Drug Resistance +	14	9
Drug Resistance -	1	5

- 14 pairs - drug resistance detected by K103N specific ASPCR and genotype
- 9 samples - WT by ASPCR but sequence showed other NNRTI resistance: Y181C or G190A/S
- 1 sample - K103N detected by ASPCR but was WT by genotype sequence
- 5 pairs - Wild type by both ASPCR and sequence

Summary and Conclusions

- In subtype C HIV infection, NVP+3TC+AZT predominantly selects the NNRTI associated drug resistance mutation, K103N, and lamivudine associated M184V.
- Thymidine analog mutations are rare within the first year of treatment.
- NVP resistance includes K103N in 53% and G190A/S and/or Y181C in the other 47%.
- In 75% of subtype C samples with virologic failure and consensus mutation(s) associated with NNRTI resistance, ASPCR for the K103N mutation identifies > 0.2% AAC or AAT.
- ASPCR for K103N may be negative in early failure with G190A/S or Y181C by population sequence.

Patient Population

