

Genotypic Analysis of Reverse Transcriptase in Treatment-naive HIV-1 Patients Treated with Lamivudine, Stavudine, and Nevirapine and/or Efavirenz

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Abstract

Introduction: The 2NN trial enrolled 1216 HIV-1+ treatment-naive patients from 6 continents in a 48-week investigation of antiretroviral regimens containing nevirapine (NVP) and/or efavirenz (EFV) on a background of lamivudine (3TC) and stavudine (d4T). To evaluate HIV-1 genotypic characteristics, including viral subtype and resistance-associated mutations, a random sample of 174 patients was selected and a group of 102 additional patients who were considered virologic failures was identified. Since 21 of the patients in the random sample were virologic failures, the study included 123 virologic failures. The trial is completed and the results are final.

Methods: Baseline specimens from all selected patients were sequenced for reverse transcriptase (RT) and protease (PR) genes, and analyzed for subtype and predicted resistance to RT inhibitors and PR inhibitors using the VIRCO Virtual Phenotype. On treatment specimens with viral load >1000 copies/ml from 123 virologic failures were sequenced and analyzed similarly. Comparisons included random sample versus virologic failure, and early versus late virologic failure specimens, overall, and between treatment groups. *P* values were based on chi-squared and rank tests, as appropriate.

Results: In the random sample 44% were subtype B, 34% subtype C and 22% other subtypes. In the virologic failures, 42% were B, 48% C and 10% other (*P*=0.011). At baseline 1 patient from the random sample was predicted resistant to NNRTIs while 12 patients in the virologic failure group were predicted resistant to NNRTIs and/or 3TC. At last on treatment observation 70 (57%) virologic failure patients had predicted resistance to one or more of the drugs in their regimen. These patients had significantly higher baseline viral load and lower CD4 count than those predicted susceptible (medians 5 vs 4.6, *P*=0.07; 95 vs 230, *P*=0.003, respectively). With NVP BID there were significantly more NNRTI-associated mutations after 24 weeks than at or before 24 weeks (*P*=0.003) while there was no difference with EFV (*P*=0.43).

Conclusions: Virologic failure was more common in subtype C. Baseline NNRTI resistance was associated with virologic failure. Among virologic failures, predicted resistance was associated with higher baseline viral load and lower CD4+ cell count.

Introduction

The 2NN trial compared d4T+3TC+EFV with d4T+3TC+NVP QD with d4T+3TC+NVP BID with d4T+3TC+NVP QD+EFV in antiretroviral-naive HIV-1-infected patients. All regimens effectively reduced viral loads to levels below detection with sustained response for 48 weeks.

Methods

A random sample of patients enrolled in an international randomized trial of NNRTI/NRTI HAART regimens for treatment-naive HIV-1-infected patients was selected to have baseline specimens sequenced for HIV-1 reverse transcriptase (RT) and protease. The random sample included 150 non-failure patients and was stratified based on region in order to insure representation of different HIV-1 subtypes.

Patients who failed to achieve plasma viral load (pVL) <50 copies/mL or failed to sustain pVL <50 were selected to have baseline and on-treatment specimens sequenced for HIV-1 RT and protease.

The VIRCO Virtual Phenotype™ system was used to amplify sequence, perform automated sequencing, subtype virus, and interpret results by predicting resistance. In addition, results were compared with those listed by VGI as resistance associated, with the addition of the V106M mutation seen in subtype C.

The random patients were compared with the virologic failure patients.

Sequences at baseline and on treatment were compared to identify the observed frequency of emergence of new mutations. Results were compared between subtypes and between treatment groups.

Results

Table 1. Baseline comparison between random sample and virologic failures

		Random sample	Virologic failure	
	Number	174	102	
Region	Thailand/Australia	32 (18%)	7 (7%)	
	Europe/North America	44 (25%)	22 (22%)	
	South Africa	62 (36%)	57 (56%)	
	South America	36 (21%)	16 (16%)	
	Other	0	0	
Subtype	B	76 (44%)	43 (42%)	
	C	60 (35%)	49 (48%)	
	Other	37 (21%)	9 (9%)	
	Predicted resistance	Total	1	12
		3TC only	0	4
NVP only		0	1	
NVP/EFV		1	4	
d4T/3TC/NVP		0	1	
	3TC/NVP/EFV	0	2	
Resistance-associated mutations	NNRTI	8 (4.6%)	11 (10.8%)	
	NRTI	12 (6.9%)	12 (11.8%)	

- More virologic failures than randomly selected patients were subtype C
- More of the virologic failures were from South Africa (56%) than the randomly selected patients (36%); the opposite was true for patients from Thailand and South America, which may account for the higher incidence of subtype C among failures
- 12 of 13 patients with predicted resistance at baseline were virologic failures
- There were more patients with resistance-associated mutations than with predicted resistance

Table 2. For specimens with predicted resistance, mean number of resistance associated mutations as a function of time on treatment

	NVP QD		NVP BID		EFV		NVP/EFV	
	<36 weeks N=9	36–48 weeks N=6	<36 weeks N=26	36–48 weeks N=22	<36 weeks N=15	36–48 weeks N=16	<36 weeks N=9	36–48 weeks N=13
Resistance associated mutations (mean)	2.44	3.33	2.50	3.32	2.40	2.69	2.89	3.38
<i>P</i> value early vs late	-	0.169	-	0.009	-	0.387	-	0.811

- Selection of resistance-associated mutations occurred during the first 24 weeks of treatment in all treatment groups
- More resistance-associated mutations accumulated by weeks 36–48, especially with NVP BID

Table 3. Emergent changes from baseline sequence seen at least 4 times or known to be associated with NNRTI resistance

	Total	other	B	C	NVP	EFV	NVP/EFV
Number	119	9	51	59	62	37	20
M184V	39	2	17	20	25	9	5
M184I	7	1	2	4	5	1	1
K103N	35	2	11	22	8	17	10
Y181C	16	0	6	10	15	1	0
N348I	15	2	7	6	9	3	3
K101E	11	2	4	5	8	2	1
K101Q	3	1	2	0		2	1
V106M	11	0	0	11	1	6	4
V106A	5	0	3	2	4	1	0
V106I	4	1	2	1	3		1
G190A	11	1	5	5	8	1	2
K65R	8	0	3	5	6	1	1
H221Y	7	0	2	5	6	1	0
T369I	7	0	3	4	4	2	1
T369A	4	0	1	3	1	1	2
T369V	4	0	3	1	2	2	0
V108I	6	0	3	3	5	1	0
R277K	6	0	3	3	4	1	1
Q334N	5	0	0	5	3	1	1
V365I	5	0	3	2	1	2	2
E6K	4	0	1	3	3	1	0
K20R	4	1	2	1	0	3	1
V35I	4	0	1	3	3	1	0
T39K	4	1	0	3	2	2	0
M41L	4	1	2	1	2	1	1
V90I	4	0	3	1	3	0	1
E122K	4	1	2	1	2	2	0
I135T	4	1	2	1	2	1	1
A158T	4	0	2	2	4	0	0
T200A	4	0	2	2	1	1	2
E203K	4	2	0	2	1	1	2
Q207E	4	0	1	3	0	2	2
L228R	4	0	0	4	2	0	2
R284K	4	0	4	0	3	0	1
D324E	4	0	0	4	1	2	1
R358K	4	0	2	2	2	2	0
T386A	4	0	2	2	2	1	1
Y188C	3	0	1	2	2	1	0
Y188L	3	0	1	2	2	1	0

■ NNRTI associated ■ NRTI associated
■ Prevalent at baseline ■ Imbalanced between groups

- K103N and V106M were associated with EFV exposure, both more common with subtype C HIV-1
- Y181C, K101E, V106A/I, G190A, V108I and Y188 C/I were associated with NVP exposure. All were seen with both subtype B and subtype C
- NRTI-associated mutations were more common with NVP use than with EFV use

Table 4. Comparison of baseline characteristics for patients with virtual phenotype predicting susceptibility and predicting resistance at time of treatment failure

		Predicted Susceptible	Predicted Resistant
	N	53	70
pVL (log ₁₀)	Median	4.6	5.0
	(<i>P</i> value)		(<i>P</i> =0.009)
CD4 + cell count	Median	230	95
	(<i>P</i> value)		(<i>P</i> =0.003)
Number of RT mutations/polymorphisms	Median	25	26
	(<i>P</i> value)		(<i>P</i> =0.34)

- Patients who had virus predicted to be resistant after treatment failure had more advanced HIV-1 infection at treatment initiation, with significantly higher baseline pVL and lower baseline CD4+ cell counts

Discussion

There was very little pre-existing resistance to trial drugs in this patient population. Resistance was predicted for 0.6% (1/174) of the random sample. Predicted resistance for the full study population leads to an estimated 7 patients in the full 1216 patient trial population. Since 12 patients with pre-existing predicted resistance were found among the virologic failures, we can conclude that the overall rate of pre-existing predicted resistance is close to 1%.

The most common emergent mutations are those associated with resistance to 3TC, EFV, and NVP. Lamivudine mutations M184V and M184I are more prevalent after NVP than after EFV. K103N and V106M are more prevalent with subtype C and with EFV. Y181C, K101E, G190A, and V108I are more prevalent with NVP. H221Y and a mutation at N348 were observed in the INCAS trial in patients who had other resistance-associated mutations. Their role in resistance has not been investigated. K65R would be expected to be associated with resistance to 3TC and was seen primarily with NVP treatment.

Conclusions

- Virologic failure was more common in patients with subtype C HIV-1
- Predicted resistance at baseline was associated with virologic failure
- Among virologic failures, predicted resistance at time of treatment failure was associated with higher baseline viral load and lower CD4+ cell count
- Emergent resistance mutations were observed for 3TC and the NNRTIs, most commonly for the NNRTIs

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