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Suboptimal Etravirine Activity is Common During Failure of Nevirapine-Based Combination Antiretroviral Therapy in a Cohort Infected with Non-B Subtype HIV-1

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Abstract: *Objective*: The primary objective of this study was to estimate etravirine activity in a cohort of patients infected with non-B subtype HIV-1 and failing nevirapine-based therapy.

Materials and Methods: Genotypic resistance testing was performed if viral load was ≥ 1,000 copies/ml after receiving at least six months of therapy. Suboptimal response to etravirine was predicted by a score ≥ 2.5 on the Tibotec weighting schema, ≥ 4 in the Monogram schema, or classification as high to low-level resistant by a modification of the Stanford HIVdb algorithm (Version 5.1.2). Bivariate and multivariate analyses were conducted to determine the risk factors for suboptimal etravirine activity.

Results: The patients (n=91) were receiving nevirapine and lamivudine plus stavudine (57.1%) or zidovudine (42.9%). Median duration of nevirapine exposure was 53 weeks (IQR 46-101 weeks). The most common etravirine resistance associated mutations were Y181C (42.9%), G190A (25.3%), H221Y (19.8%), A98G (18.7%), K101E (16.5%), and V90I (12.1%). Suboptimal etravirine activity was predicted in 47.3 to 56.0%. There were disparities in mutations listed in Tibotec *vs* Monogram Schemas. Predicted suboptimal activity was not associated with nucleoside reverse transcriptase inhibitor (NRTI) used, gender, pretreatment or current CD4 cell count or viral load, subtype or NRTI mutations.

Conclusion: Etravirine has compromised activity in approximately half of the patients failing nevirapine-based first-line treatment in this cohort, which supports guidelines that caution against using it with NRTIs alone in such patients.

Keywords: Etravirine, non-B subtype, resistance, resource-limited setting, second-line.

INTRODUCTION

Etravirine is a diarylpyrimidine non-nucleoside reverse transcriptase inhibitor (NNRTI) with a higher genetic barrier to resistance than nevirapine or efavirenz [1]. Analyses of the DUET studies identified 17 etravirine resistance associated mutations (V90I, A98G, L100I, K101E/H/P, V106I, E138A, V179D/F/T, Y181C/I/V, G190A/S and M230L) with their effects being most significant with ≥ 3 of the mutations [2, 3]. These are the mutations in the December 2008 International AIDS Society-USA list of drug resistance mutations [4], but other mutations that may contribute to reduced virologic responses to etravirine include V106A, E138G/K/Q, V179E/L/M, Y181F, Y188L/I, V189I, G190E/Q/T, H221Y, P225H, K238N/T and E399D [5-7]. Schemas that account for differences in weight (impact) of each mutation and improve the correlation between genotype and phenotypic susceptibility have been proposed [3, 5]. The primary objective of this study was to estimate the proportion of the patients in our cohort for whom etravirine is likely to retain optimal activity in second-line antiretroviral therapy (ART).

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MATERIALS AND METHODS

Study Site

This study was conducted in Nigeria, a country with approximately 3 million HIV-infected persons [8]. All the patients were enrolled in the Harvard School of Public Health (HSPH) Track 1.0 United States President's Emergency Plan for AIDS Relief (PEPFAR) supported program following informed consent, which was subject to ethical approval by the Institutional Review Boards of the University of Ibadan/University College Hospital, National Institute for Medical Research, Lagos, Jos University Teaching Hospital, University of Maiduguri Teaching Hospital and the HSPH. First-line ART in the program primarily consisted of nevirapine plus two nucleos(t)ide reverse transcriptase inhibitors (NRTIs). Laboratory monitoring available to patients after treatment initiation included CD4 cell count every 3 months and plasma HIV RNA (viral load) every 6 months. Sixty percent of the patients in the program had virologic success, defined as viral load < 400 copies/mL after 48 weeks on nevirapine combined with lamivudine and zidovudine or stavudine. Referral-based genotyping was available to patients in virologic failure. Criteria for referral were plasma HIV RNA > 1,000 copies/ml after at least 6 months of uninterrupted ART pick-up from the pharmacy.

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HIV-1 Resistance Genotyping and Subtype Determination

For genotyping, plasma samples from EDTA-separated blood were aliquotted and shipped in a dry liquid nitrogen container to the HSPH where testing was performed using Abbott's ViroSeq HIV-1 Genotyping System 2.0 assay. RNA was isolated from plasma, reverse-transcribed and amplified, and sequenced on an ABI 3100 capillary system. The sequence chromatograms were then edited using Abbott's ViroSeq software. To determine HIV-1 subtype, nucleotide sequences covering 297 bases in the protease gene and 1005 in reverse transcriptase were aligned in ClustalW along with reference sequences from Los Alamos repository. Neighbor-joining trees were used to classify the sequences by subtype.

Prediction of Etravirine Activity

To account for differences in the impact of NNRTI mutations on the antiviral activity of etravirine, we applied three independent schemas that incorporate the weight of specific NNRTI mutations and the total number of mutations.

1) Tibotec® Weighted 2008 etravirine RAM Score (Schema T) derived from analysis of the DUET studies and includes the 17 etravirine RAMs. In this schema, weighted mutation score of 0-2, 2.5-3.5 and ≥ 4 corresponded to response rates of 74% (highest response), 52% (intermediate response) and 38% (reduced response). We selected a score < 2.5 as the cut-off for optimal etravirine activity [3].

2) Monogram® Algorithm for Predicting Etravirine Susceptibility (Schema M) which was derived from analysis of etravirine fold-change in clinical samples submitted to Monogram for routine testing. In this schema, a score ≥ 4 was predictive of a fold-change of at least 2.9, the lower clinical cutoff for etravirine [7]. We selected a score < 4 as corresponding to optimal etravirine activity.

3) HIVdb (Version 5.1.2, Stanford University, available at http: //hivdb.stanford.edu/pages/algs/HIVdb.html) for which we dichotomized those classified as 'High-level resistance','Intermediate resistance' and 'Low-level Resistance' as predictive of lower clinical activity, and those classified as 'Susceptible' and 'Potential low-level resistance' predictive of optimal activity. Although the HIVdb algorithm assigns a small weight to K103N, this mutation was excluded from the genotype input used to interprete the algorithm because there is consensus that K103N does not significantly influence etravirine activity by itself [2-6].

Thus, lack of optimal etravirine activity was defined as a score ≥ 2.5 on the Tibotec weighting schema, ≥ 4 in the Monogram schema, or classification as high to low-level resistant by modified HIVdb.

Statistical Methods

All analyses were retrospective and conducted on de-identified data. The time that elapsed between the first ART pick-up from the pharmacy and the date plasma was collected for genotyping was defined as the duration of ART. Outcomes were defined based on the respective schemas and were dichotomized accordingly: (1) Schema T – score ≥2.5 *vs* score <2.5, (2) Schema M-score ≥4 *vs* score <4, and (3) High to low-level resistance *vs* susceptible to potential low-level resistance as classified by the modified HIVdb algorithm (K103N mutation was not included in the genotype input).

Continuous variables were assessed for normality by visual inspection of box-plots, normal quantile plots and the Shapiro-Wilk W test for normality. Overall, normality was assumed based on the Central Limit Theorem unless the distribution was significantly skewed (CD4+ cell count) or multimodal (length of treatment). All continuous variables were compared between groups with the non-parametric Mann-Whitney test and/or categorized into dummy-variable for appropriate comparisons. Medians were computed and presented in the tables.

Categorical variables were assessed with Fisher's Exact Test or X^2-test when appropriate. All non-subtype G samples (CRF02, CRF06, subtype A, other) were grouped together, effectively dichotomizing the variable. For analysis, CD4 counts were stratified as ≤200, 201-349, and ≥350. Logistic regression with a Bonferroni adjustment was used for multiple comparisons. Discordance between weighting schemas was the sum of the two mismatched cells. Gender and age, and CD4 count and viral-load were well correlated. For all analyses we report findings at significance level alpha = 0.05; performed with Stata 10.1 (StataCorp, College Station, TX).

RESULTS

Participant Characteristics

Participants were treatment-naïve patients (n=91) who underwent resistance testing while failing nevirapine and lamivudine combined with stavudine (57%) or zidovudine (43%). Table **1** shows that they were predominantly female (60.4%), and had a median age of 36 years (IQR 32-42 years). The median pretreatment viral load and CD4 cell count were 5.17 $\log_{10}$ copies/ml and 77 cells/mm^3, respectively. The median viral load and CD4 cell count at the time of genotyping were 4.45 $\log_{10}$ copies/ml and 194 cells/mm^3, respectively. The median duration of ART at the time of genotyping was 53 weeks (IQR 46-101 weeks).

Prevalence of Specific NNRTI RAMs

The prevalence of NNRTI mutations associated with etravirine resistance in the patients and their distribution by thymidine analog used and HIV-1 subtype is shown in Table **2**. Seventy-one (71.4%) percent of the patients had at least one of the 17 mutations in Schema T and 13.2% had ≥ 3 of these mutations. Four mutations listed in Schema T were not present in our cohort. Schema M includes 18 mutations not listed in schema T and they ranged in prevalence from 0 to 7.7% with the exception of H221Y that was present in 19.8%. Overall, the most common mutations were Y181C (42.9%), G190A (25.3%), A98G (18.7%), H221Y (19.8%), K101E (16.5%), and V90I (12.1%). Only three of these high prevalence mutations (Y181C, K101E and V90I) are

Table 1. Characteristics of Study Participants, by Treatment

	Total Cohort	d4T + 3TC + NVP	ZDV+3TC+NVP
General Characteristics:			
Total number – N (%)	91 (100%)	52 (57.1%)	39 (42.9%)
Female gender – (%)	60.4	55.7	66.7
Age – median (years)	36.0	37.5	34.0
*Viral load – median (log_{10} c/ml)	4.45	4.36	4.54
Viral load (pretreatment) – median	5.17	5.23	5.13
*CD4 – median (cells/mm^3)	194	198	176
CD4 (pretreatment) – median	77	64	88
Duration of ART – median (weeks)	53	53	53
Subtypes:			
Subtype G – (%)	35.2	34.6	35.9
Non-subtype G – (%)	64.8	65.4	64.1
CRF02 – (%)	47.3	48.1	46.2
CRF06 – (%)	8.8	9.6	7.7
Subtype A – (%)	2.2	3.9	0
Other – (%)	6.6	3.9	10.3

ZDV = zidovudine; d4T = stavudine; 3TC = lamivudine; NVP = nevirapine.
*Value at the time of virologic failure.

represented in both T and M schemas. G190A and A98G are listed in Schema T while H221Y is listed in Schema M only. A98G was more frequent in patients infected with the G subtype.

Predicted Maximum Etravirine Activity in Second-Line ART

The proportions of patients predicted to have suboptimal response to ETV using Schema T, Schema M and modified HIVdb algorithm were 52.8%, 47.3% and 56%, respectively. Concordance between the Tibotec and Monogram schemas was 94.5%, but was lower between modified HIVdb algorithm and Tibotec or Monogram Schemas (92.3% and 89.0%, respectively). A98G, K101E, Y181C and G190A are some of the mutations driving the discordance, however given the small numbers and imbalanced comparator groups no clear conclusions can be made regarding relative weight of each mutation. In univariate and logistic regression (data not shown), predicted suboptimal activity was not associated with NRTI used (zidovudine *vs* stavudine); gender; pretreatment, current or change in CD4 cell count or viral load; subtype; or presence of NRTI mutations (M41L, K65R, D67N, 69 ins, K70R, L210W, T215Y/F, K219Q/E, M184V/I, K65R, L74V, Q151M)

DISCUSSION

Among patients failing nevirapine-based ART, we investigated the prevalence of NNRTI mutations associated with etravirine resistance and estimated the proportion for whom etravirine is likely to be optimally active and therefore contribute to the goal of having at least two fully active antiretroviral agents in second-line ART. The most common mutations were Y181C (42.9%), G190A (25.3%), A98G (18.7%), H221Y (19.8%), K101E (16.5%), and V90I (12.1%). The prevalence of Y181C is lower than the 59.5% reported in Thailand where Sungkanuparph *et al.* evaluated 158 patients who had failed initial NNRTI-based ART [9]. This difference probably relates to the longer median duration of NNRTI exposure in the Sungkanuparph study (88 weeks *vs* 53 weeks). As expected, Y181C was more common than the 4-36.9% prevalence reported in studies where efavirenz use was dominant [10-13]. G190A was the second most common mutation in our study as well as in the Thailand study (26.3% *vs* 33.5%). However, there are very striking differences in the prevalence of A98G (19.6% *vs* 0.8%), K101E (20.8% *vs* 0.8%), and V90I (12.4% *vs* 0.8%), perhaps due to differences in the predominant HIV-1 subtypes.

The definition of etravirine activity used by Sungkanuparph *et al.* (≤ any 2 of the initial 13 etravirine resistance associated mutations) significantly overestimates retention of full etravirine activity because it does not include more recently described etravirine resistance mutations and does not account for differences in the impact of each mutation. For example, that method classifies patients with Y181C alone or even Y181C plus another high impact mutation as retaining etravirine activity, whereas Y181C has sufficient impact to significantly compromise etravirine activity by itself, although etravirine has configurational flexibility and can bind to reverse transcriptase in the presence of some single NNRTI mutations [14]. Other high-impact mutations and their prevalence in our study are L100I (0%), K101P (1.1%), Y181I (0%), Y181V (1.1%) and M230L (2.2%). If had we used the same definition as Sungkanuparph *et al.* 89% of the patients in our study would have been classified as retaining

Table 2. NNRTI Mutations Present in the Cohort, Weight and Prevalence

Mutation	M	T	Overall Prevalence N (%) 91 (100)	Prevalence by Thymidine Analog*			Prevalence by Subtype*		
				ZDV N (%)	d4T N (%)	*p*-val	G N (%)	NON-G N (%)	*p*-val
V90I	1	1	11 (12.1)	6 (54.6)	5 (45.5)		2 (18.2)	9 (81.8)	
A98G	†	1	17 (18.7)	11 (64.7)	6 (35.3)		10 (58.8)	7 (41.2)	0.046
K101E	2	1	15 (16.5)	8 (53.3)	7 (46.7)		4 (26.7)	11 (73.3)	
K101H	1	1	1 (1.1)	1 (100)	0 (0)		0 (0)	1 (100)	
K101P	4	2.5	1 (1.1)	0 (0)	1 (100)		0 (0)	1 (100)	
V106A	2	†	5 (5.5)	3 (60.0)	2 (40.0)		3 (60.0)	2 (40.0)	
V106I	†	1.5	1 (1.1)	0 (0)	1 (100)		0 (0)	1 (100)	
E138A	3	1.5	1 (1.1)	1 (100)	0 (0)		0 (0)	1 (100)	
E138Q	1	†	7 (7.7)	5 (57.1)	3 (42.9)		3 (42.9)	4 (57.1)	
V179E	3	†	4 (4.4)	1 (25.0)	3 (75.0)		3 (75.0)	1 (25.0)	
Y181C	4	2.5	39 (42.9)	14 (35.9)	25 (64.1)		14 (35.9)	25 (64.1)	
Y181V	4	3	1 (1.1)	0 (0)	1 (100)		1 (100)	0 (0)	
Y188L	2	†	3 (3.3)	0 (0)	3 (100)		2 (66.7)	1 (33.3)	
G190A	†	1	23 (25.3)	12 (52.2)	11 (47.8)		9 (39.1)	14 (60.9)	
G190S	†	1.5	1 (1.1)	0 (0)	1 (100)		0 (0)	1 (100)	
H221Y	1	†	18 (19.8)	9 (50.0)	9 (50.0)		5 (27.8)	13 (72.2)	
M230L	3	2.5	2 (2.2)	1 (50.0)	1 (50.0)		0 (0)	2 (100)	
K238T	1	†	2 (2.2)	0 (0)	2 (100)		1 (50.0)	1 (50.0)	

M and T are independent weights assigned to mutation in Schema M and T, respectively
† = mutation is not listed in the Schema (T or M)
Mutations listed in Schema T or M but not present in any patient were L100I, V106M, E138G, E138K, V179D, V179F, V179L, V179M, V179T, Y181F, Y181I, V189I, G190E, G190Q, G190T, P225H, K238N
Only *p*-values <0.05 are shown in the table.
*Denominator is the total number with mutation as listed in Overall Prevalence.

etravirine susceptibility, which is expectedly higher than the 75% reported in the Sungkanuparph study because of the shorter duration of antiretroviral exposure in our patients (a median of 53 *vs* 88 weeks). That approach would have been erroneous, however, as etravirine cannot be considered to be fully active in many of those patients. The methods in the current study predicted suboptimal etravirine activity in 47.3 to 56.0% of the patients.

Some of the differences in the Schemas used in this study are noteworthy. Four of the NNRTI mutations in Schema T are not retained in Schema M, while Schema M includes several mutations not listed in Schema T, including H221Y that was present in 19.8%. H221Y was first reported in 2003 at which time it was proposed as an NRTI-associated mutation [15], but it is now known to be associated with NNRTI exposure [16, 17].

Concerns have been raised about etravirine if used without PIs in second-line therapy by previous investigators. TMC 125-C227 was a study of etravirine *vs* investigator-selected PI, each combined with two NRTIs in PI-naïve patients who had failed NNRTI-based initial ART. That study was terminated after a median 14.3 weeks because of poorer virologic responses in the etravirine arm, an outcome that was attributed to drug resistance [18]. Our study also shows modest rates of full etravirine activity after failing nevirapine-based ART.

Some limitations should be considered when interpreting this study. First, some mutations that may affect etravirine susceptibility are not listed in the schemas. One of such is E399D that was reported to confer a14.4-fold reduction in etravirine susceptibility [6]. In addition, we relied on bulk genotyping, which misses NNRTI resistant mutants that exist in minority drug-resistant populations [19]. Such minority variants have been shown to compromise response to efavirenz [20]. Further, genotyping was performed after a median of 53 weeks nevirapine exposure whereas such virologic monitoring or resistance testing is rarely available in resource-limited settings. As such, continuation of a failing regimen with accumulation of mutations is likely to be more common and problematic than shown in this study [21]. In conclusion, etravirine lacks full activity in approximately half of the patients failing nevirapine-based ART in our cohort, which supports guidelines that it should not be used with NRTIs alone in such patients [22, 23], especially when resistance testing is not available.

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REFERENCES

[1] Vingerhoets J, Azijn H, Fransen E, *et al.* TMC125 displays a high genetic barrier to the development of resistance: evidence from *in vitro* selection experiments. J Virol 2005; 79(20): 12773-82.

[2] Vingerhoets J, Buelens A, Peeters M, *et al.* Impact of baseline NNRTI mutations on the virological response to TMC-125 in the phase III clinical trials DUET-1 and DUET-2. Antivir Ther 2007; 12 : S34.

[3] Vingerhoets J, Peeters M, Azijn H, *et al.* An update of the list of NNRTI mutations associated with decreased virological response to etravirine: multivariate analyses on the pooled DUET-1 and DUET-2 clinical trial data. Antivir Ther 2008; 13(Suppl 3): A26.

[4] Jonhson VA, Brun-Vezinet F, Clotet B *et al.* Update on the drug resistance mutations in HIV-1. Topics HIV Med 2008; 16(5): 138-45.

[5] Benhamida J, Chappey C, Coakley E, Parkin NT. HIV-1 genotype algorithms for prediction of etravirine susceptibility: novel mutations and weghting factors identified through correlation to phenotype. Antivir Ther 2008; 13(Suppl 3): A142.

[6] Poveda E, de Mendoza C, Pattery T, González Mdel M, Villacian J, Soriano V. Phenotypic impact of resistance mutations on etravirine susceptibility in HIV patients with prior failure to nonnucleoside analogues. AIDS 2008; 22(17): 2395-8.

[7] Hirsch M, Günthard H, Schapiro J, *et al.* Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society-USA panel. Clin Infect Dis 2008; 47: 266-85.

[8] UNAIDS 2008 Report on the global AIDS epidemic. Available at http: //www.unaids.org/en/KnowledgeCentre/HIVData/GlobalReport/2008/. Accessed on January 19, 2009.

[9] Sungkanuparph S, Manosuthi W, Kiertiburanakul S, Piyavong B, Chantratita W. Evaluating the role of etravirine in the second-line antiretroviral therapy after failing an initial non-nucleoside reverse transcriptase inhibitor-based regimen in a resource-limited setting. Curr HIV Res 2008; 6(5): 474-6.

[10] Scott C, Grover D, Nelson M. Is there a role for etravirine in patients with nonnucleoside reverse transcriptase inhibitor resistance? AIDS 2008; 22: 989-92.

[11] Poveda E, Garrido C, de Mendoza C, *et al.* Prevalence of etravirine (TMC-125) resistance mutations in HIV-infected patients with prior experience of non-nucleoside reverse transcriptase inhibitors. J Antimicrob Chemother 2007; 60: 1411-3.

[12] Llibre J, Santos J, Puig T, *et al.* Prevalence of etravirine-associated mutations in clinical samples with resistance to nevirapine and efavirenz. J Antimicrob Chemother 2008; 62(5): 909-13.

[13] Picchio G, Vingerhoets J, Staes M, *et al.* Prevalence of TMC125 resistance-associated mutations in a large panel of clinical isolates. 15th Conference on Retroviruses and Opportunistic Infections. 3-6 February 2008; Boston, MA, USA. Abstract 866.

[14] Das K, Clark AD, Lewi PJ Jr, *et al.* Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. J Med Chem 2004; 47: 2550-60.

[15] Gonzales MJ, Wu TD, Taylor J, *et al.* Extended spectrum of HIV-1 reverse transcriptase mutations in patients receiving multiple nucleoside analog inhibitors. AIDS 2003; 17: 791Y 799.

[16] iang S, Xing H, Si X, Wang Y, Shao Y. Polymorphism of the protease and reverse transcriptase and drug resistance mutation patterns of HIV-1 subtype B prevailing in China. J Acquir Immune Defic Syndr 2006; 42(4): 512-4.

[17] Ceccherini-Silberstein F, Svicher V, Sing T, *et al.* Characterization and structural analysis of novel mutations in human immunodeficiency virus type 1 reverse transcriptase involved in the regulation of resistance to nonnucleoside inhibitors. J Virol 2007; 81(20): 11507-19.

[18] Ruxrungtham K, Pedro RJ, Latiff GH, *et al.* Impact of reverse transcriptase resistance on the efficacy of TMC125 (etravirine) with two nucleoside reverse transcriptase inhibitors in protease inhibitor-naïve, nonnucleoside reverse transcriptase inhibitor-experienced patients: study TMC125-C227. HIV Med 2008; 9(10): 883-96.

[19] Johnson JA, Li JF, Wei X, *et al.* Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naïve populations and associate with reduced treatment efficacy. PLoS Med 2008; 5(7): e158.

[20] Paredes R, Lalama C, Ribaudo H, *et al.* Presence of minor populations of Y181C mutants detected by allele-specific PCR (ASPCR) and risk of efavirenz failure in treatment-naive patients: results of an ACTG A5095 case-cohort study. Abstract 83. Program and abstracts of the 15th Conference on Retroviruses and Opportunistic Infections. February 3-6, 2008; Boston, Massachusetts.

[21] Lapadula G, Calabresi A, Castelnuovo F, *et al.* Prevalence and risk factors for etravirine resistance among patients failing on non-nucleoside reverse transcriptase inhibitors. Antivir Ther 2008; 13(4): 601-5.

[22] Hammer SM, Eron JJ Jr, Reiss P, *et al.* Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. JAMA 2008; 300(5): 555-70.

[23] WHO. Antiretroviral therapy for HIV infection in adults and adolescents recommendations for a public health approach (2006 revision). Available at http: //www.who.int/hiv/pub/guidelines/adult/en/index.html. Accessed July 21, 2009.

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