FREQUENT DISCORDANCE BETWEEN ETRAVIRINE PHENOTYPE & GENOTYPE IN SUBTYPE C ART FAILURE

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BACKGROUND/OBJECTIVE

- Etravirine (ETR) is a second-generation NNRTI that is used as a component of combination antiretroviral therapy (ART) for treatment-experienced persons.
- The extent of cross-resistance between nevirapine (NVP) and efavirenz (EFV) and ETR is not well defined especially in low- and middle-income countries (LMIC) where switches from first-line ART may be delayed.
- To address this gap, the susceptibility to ETR in individuals infected with HIV-1 subtype C experiencing virologic failure while on a first-line NNRTI-containing regimen was investigated.

METHODS

Sample Acquisition

- Residual plasma from samples sent for routine HIV drug resistance testing at Lancet Laboratories, South Africa
- Targeted criteria:
  - Subtype C infection
  - Failing first-line therapy after >6 months of ART
  - Viral load of >10,000 RNA copies/ml
  - Contains ≥ 1 NNRTI mutation in reverse transcriptase (Stanford HIVdb)
  - Included N=12 ART-naïve controls for calculating composite IC_{50} for Fold Change (FC) values

Sample Size and Description

135 Subtype C Samples

- 6 Extraction Failures
- 14 PCR Failures
- 3 Cloning Failures

112 Samples with Drug Susceptibility Results

Treatment History
12/112: ART-naïve controls
88/112: 88% on EFV-based ART
12/112: 12% on NVP-based ART

HIV-1 RNA Levels
Median: 116,772 c/ml
Range: 9,355 – 2,555,245 c/ml

Cloning and Phenotyping

Extract HIV-1 RNA from donor plasma
↓
Generate cDNA & PCR amplify RT full-length sequence (aa 1-560)
↓
Clone donor full-length HIV-1 RT into xxLAI viral vector
↓
Transfect cells with donor-derived viral vector and prepare viral stocks
↓
Determine viral susceptibility to ETR† using TZM-bl cells*

*Fold-Change (FC) values were calculated using a composite IC_{50} from 12 treatment-naïve HIV-1 subtype C isolates from the same region[1].
†The phenotyping Clinical Cut-off of 2.9 was previously established using Phenosense® by Coakley et al.[3] based on viral susceptibility to ETR of virus cloned from participants in the DUET trials[4,5].
‡This analysis was completed before HIVdb v8.7 was available that included the score of 15 for A98G+F227C and 10 for V106I. No changes were added for ETR in HIVdb v8.8 (latest).

Genotype Scoring

HIVdb v8.4‡ weight factor for ETR RAMs

Etravirine RAM	Final weight factor
K101E+Y181C	5
K101E+Y188L	5
K101E+G190A	5
K101E+G190S	5
A98G+Y181C	5
A98G	10
L100IV	10
K101H	10
E138A/G/K/Q/R	10
V179D/E/L	10
Y188L	10
G190A/C/S/T/V	10
H221Y	10
V179T+Y181C	10
Y181C+G190A/C/S/T/V	10
K101E	15
V179F	15
Y181F/G/S	15
M230I	15
V179F+Y181C	15
L100I	30
Y181C	30
F227C	30
M230L	30
G190E/Q	45
K101P	60
Y181I/V	60

HIVdb v8.4 weighted genotype score#

0 – 9	Susceptible
10 – 14	Potential Low-Level Resistance
15 – 29	Low-Level Resistance
30 – 59	Intermediate Resistance
≥ 60	High-Level Resistance

#For comparison to phenotype, the genotype score categories "potential low-level" and "low-level" were grouped with "susceptible" and "intermediate" respectively[2].

Results

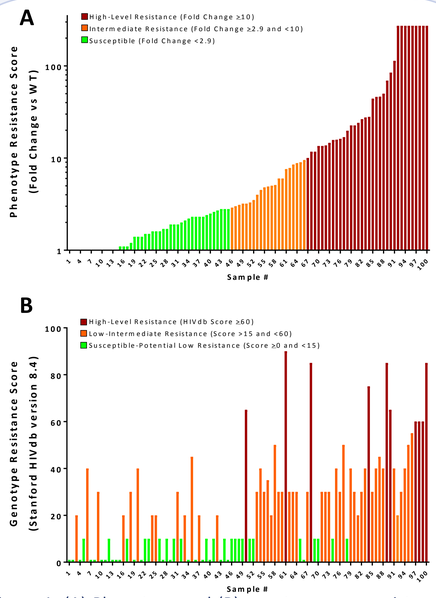


Figure 1: (A) Phenotype and (B) genotype cross-resistance to etravirine of plasma-derived HIV-1 subtype C viruses from 100 individuals on failing first-line antiretroviral therapy.

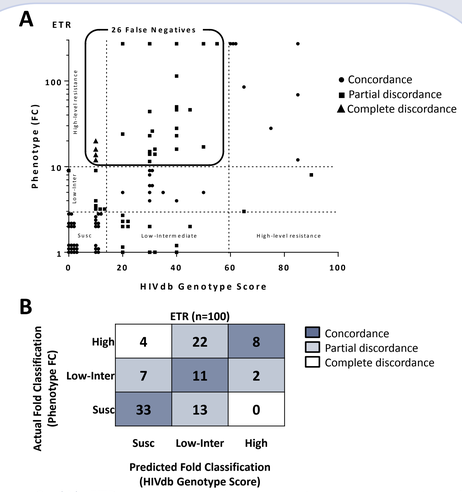
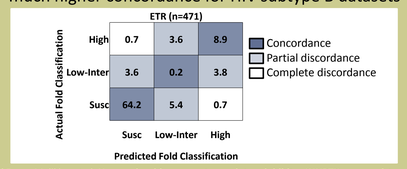


Figure 2. (A) ETR phenotype does not strongly correlate with genotypic score (r=0.47) for HIV-1 subtype C isolates with (B) 44% of actual fold classifications partially discordant and 4% completely discordant to the predicted fold classification.

ETR genotypic resistance prediction algorithms were shown to have much higher concordance for HIV subtype B datasets[2]



Modified From: Melikian et al. **Non-nucleoside reverse transcriptase inhibitor (NNRTI) cross-resistance: implications for preclinical evaluation of novel NNRTIs and clinical genotypic resistance testing**. J Antimicrob Chemother 2014 Jan;69(1):12-20. doi: 10.1093/jac/dkt316. Epub 2013 Aug 9

Results

Table 1. The NNRTI mutations L100I, Y181C, M230L and the NRTI mutation K65R are associated with ETR Cross-Resistance Phenotype Score.

Mutation	Resistant (>2.9-fold) N = 54	Susceptible (<2.9-fold) N = 58	Odds	P-value	Adjusted‡
NNRTI-Associated Resistance Mutations					
V90I	5 (9%)	0 (0%)	Inf	0.024	0.567
A98G	10 (19%)	4 (7%)	3.068	0.087	0.775
L100I	**12 (23%)**	**1 (2%)**	**16.286**	**0.001**	**0.049**
K101H	0 (0%)	3 (5%)	0.000	0.244	0.952
K101E	5 (9%)	5 (8%)	1.082	1.000	1.000
K101P	0 (0%)	0 (0%)	na	na	na
K103N	31 (58%)	24 (41%)	1.909	0.130	0.812
K103S	3 (6%)	3 (5%)	1.078	1.000	1.000
V106M	18 (34%)	26 (44%)	0.615	0.248	0.952
V106I	0 (0%)	0 (0%)	na	na	na
V108I	8 (15%)	5 (8%)	1.843	0.382	1.000
E138A	4 (8%)	5 (8%)	0.848	1.000	1.000
E138K	2 (4%)	1 (2%)	2.192	0.608	1.000
E138G	0 (0%)	0 (0%)	na	na	na
V179D	11 (21%)	2 (3%)	7.163	0.007	0.233
V179F	0 (0%)	0 (0%)	na	na	na
V179T	0 (0%)	0 (0%)	na	na	na
V179L	0 (0%)	0 (0%)	na	na	na
Y181C	**16 (30%)**	**0 (0%)**	**Inf**	**<0.001**	**0.001**
Y181I	0 (0%)	0 (0%)	na	na	na
Y181V	0 (0%)	0 (0%)	na	na	na
Y188L	5 (9%)	1 (2%)	5.816	0.104	0.775
G190E	0 (0%)	0 (0%)	na	na	na
G190A	9 (17%)	17 (29%)	0.597	0.273	0.970
G190S	0 (0%)	0 (0%)	na	na	na
H221Y	6 (11%)	2 (3%)	3.500	0.152	0.901
P225H	7 (13%)	6 (10%)	1.344	0.769	1.000
F227C	0 (0%)	0 (0%)	na	na	na
M230L	**10 (19%)**	**1 (2%)**	**12.955**	**0.003**	**0.174**
NRTI-Associated Resistance Mutations					
M41L	7 (13%)	10 (17%)	0.746	0.610	1.000
K65R	**27 (51%)**	**8 (14%)**	**6.620**	**<0.001**	**0.006**
D67N	6 (11%)	11 (19%)	0.557	0.306	0.957
K70R	7 (13%)	6 (10%)	1.344	0.769	1.000
Y115F	7 (13%)	7 (12%)	1.130	1.000	1.000
M184V	41 (77%)	41 (69%)	1.500	0.397	1.000
M184I	5 (9%)	0 (0%)	Inf	0.021	0.605

‡ An adjusted p-value <0.2 is statically significant.

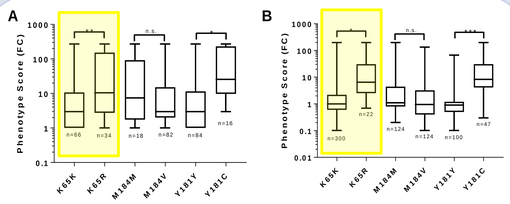


Figure 3. K65R is associated with high ETR phenotypic resistance in (A) HIV-1 subtype C samples and (B) HIV-1 subtype B ETR phenotyping data accessed through the Stanford HIVdb.

Table 2. K65R is associated with HIV sequences containing the NNRTI mutations V179DFT, Y181CIV and M230L.

NNRTI RAMs	K65R n=34 (%)	K65K n=66 (%)	P Value (Fisher's Exact)	P value summary
V90I	3 (9)	2 (3)	0.3335	n.s.
A98G	2 (6)	12 (18)	0.1304	n.s.
L100I	7 (21)	6 (9)	0.1242	n.s.
K101EHP	5 (15)	8 (12)	0.7586	n.s.
V106I	1 (3)	1 (2)	>0.999	n.s.
E138AGKQ	5 (15)	9 (14)	>0.999	n.s.
V179DFT	**9 (27)**	**5 (8)**	**0.0148**	**Significant**
Y181CIV	**9 (27)**	**7 (11)**	**0.0488**	**Significant**
G190SA	9 (27)	18 (27)	>0.999	n.s.
M230L	**8 (24)**	**3 (5)**	**0.0067**	**Significant**

Results

Table 3. There was no change in ETR susceptibility in recombinant HIV-1 virus clones containing 65K vs. 65R (site directed mutagenesis).

Sample	Sample Type	IC50 (nM)	Mutations NRTI	Mutations NNRTI	Other (bulk:clone discordant)
Wildtype	Bulk cloned	1.2	None	None	-
G100	Bulk cloned	17.5	M41LM,**K65R**,M184V	V106M,E138A,V179D	T39DE, K103KR, I135IL
G100.3	Single clone 1	2.0	M41L, **K65R**, M184V	V106M,E138A,V179D	T39D, K102R
G100.3.1	Single clone 1	1.9	M41L, M184V	V106M,E138A,V179D	T39D, K102R
G100.5	Single clone 2	74.8	M41M, **K65R**, M184V	V106M, E138A, V179D	T39E, K103R, I135L
G100.5.5	Single clone 2	76.0	M41M, M184V	V106M, E138A, V179D	T39E, K103R, I135L
G165	Bulk cloned	29.8	A62V,**K65R**,M184I	V106M,V179D,M230L	-
G165.4	Single clone	41.7	A62V, **K65R**, M184I	V106M, V179D,M230L	-
G165.4.1	Single clone	58.0	A62V, M184I	V106M, V179D,M230L	-

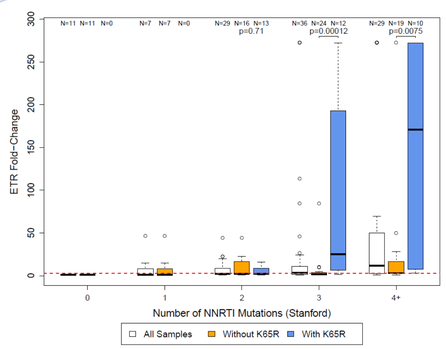


Figure 4.The correlation between K65R and ETR phenotypic resistance is related to the total number of NNRTI-resistance associated mutations.

Summary

- The HIVdb scores for A98G, K101H, E138A/K, V179D, Y188L, G190A, H221Y and P225H may overestimate & L100I, Y181C and M230L may underestimate ETR phenotypic resistance.
- The NRTI mutation K65R was associated with ETR resistance but reversion to 65K in two samples had no effect on ETR susceptibility, suggesting it may be a marker of resistance rather than a direct cause of resistance.

Conclusions

- **Phenotypic cross-resistance to ETR is common in first-line NNRTI-containing ART failure in HIV-1 subtype C from South Africa.**
- **Genotype-based algorithms differentially classify ETR susceptibility in HIV-1 subtype C.**
- **Updated weightings of combinations of ETR-associated mutations may be needed to improve genotype prediction of ETR phenotype in HIV subtype C.**

References

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