574 Improved Genotypic Algorithm for Predicting Etravirine Susceptibility: Comprehensive List of Mutations Identified Through Correlation with Matched Phenotype

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BACKGROUND

• Etravirine (ETR) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) which has shown activity against many HIV-1 strains with multiple NNRTI resistance mutations.

• Recent studies (Benhamida 2008 and Vingerhoets 2008) have shown that a weighted factor applied to more extensive list of mutations minimizes discordance rate of ETR susceptibility by phenotype versus genotype.

• We evaluated a new weighting algorithm applied to the combined list of mutations within Monogram's matched phenotype and genotype database from the most recent year of commercial patient testing.

METHODS

• We studied phenotype and genotype results of 4,923 samples containing at least one NNRTI mutation from the following lists:

- ❖ Expanded list of ETR muations derived from combining two studies [1, 2]: V90I, A98G, L100I, K101E/H/P, K103R, V106A/I/M, E138A/G/K/Q, V179D/E/F/I/L/M/T, Y181C/F/I/V, Y188L, V189I, G190A/E/Q/S/T, H221Y, P225H, M230L, K238N/T.
- ❖ NNRTI resistance associated mutations: A98G, L100I, K101E/P, K103N/S, V106A/M, Y181x, Y188x, G190x, P225x, F227x, M230L, P236L, where x represents any amino acid substitution.

• ETR reduced susceptibility as measured by fold-change of IC50 (FC) ≥ lower clinical cutoff (2.9) was compared to a weighted score applied to the expanded list of mutations.

• Weights for individual mutations and threshold for reduced susceptibility on overall score were optimized to minimize discordance rate between phenotype and genotype.

1. Purpose of the Study

• To develop an enhanced genotypic algorithm for detecting resistance to ETR, by applying a combination of two weighted score systems, and other resistance associated mutations identified thru correlation analysis with phenotypic response.

• The two independent studies that reported extended lists of ETR resistance associated mutations and their weight factors are:

- ❖ MGRM: developed by Monogram Biosciences thru minimizing discordance to phenotypic susceptibility [1].
- ❖TBTC: developed by Tibotec thru correlation with virologic outcome [2].

2. Optimizing Genotypic Weighted Score by Minimizing Discordance to Phenotype

• Samples reported after 2009 that included at least one NNRTI resistance associated mutation, or one of the mutations in the expanded list, were included in the test set.

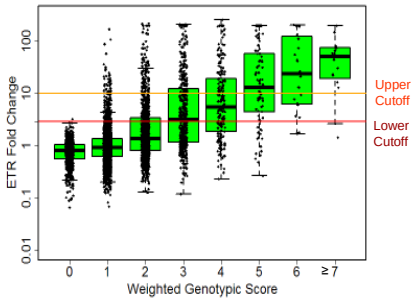
• Weights in range of 1 – 4 were assigned to mutations based on the rank order of both score systems.

• Weights for individual mutations and genotypic score cutoff (GCO) were optimized by performing iterations of testing of the total weighted factor (TWF) in the dataset, and minimizing the discordance rate as in FC ≥ 2.9 and TWF < GCO (false negative rate), and FC < 2.9 and TWF ≥ GCO (false positive rate).

3. Results: Mutations and Weight Factors

Mutation	MGRM Weight	TBTC Weight	Enhanced Weight
V90I	1	1	1
A98G		1	1
L100I	4	2.5	4
K101E	2	1	2
K101H	1	1	1
K101P	4	2.5	4
K103R			1
V106A	2		2
V106I		1.5	2
V106M	1		1
E138A	3	1.5	3
E138G	3		3
E138K	2		2
E138Q	1		1
V179D	1	1	1
V179E	3		3
V179F	1	1.5	1
V179I			1
V179L	2		2
V179M	1		1
V179T		1	1
Y181C	4	2.5	4
Y181F	1		1
Y181I	4	3	4
Y181V	4	3	4
Y188L	2		2
V189I	1		1
G190A		1	1
G190E	1		1
G190Q	3		3
G190S		1.5	2
G190T	1		1
H221Y	1		1
P225H	1		1
M230L	3	2.5	3
K238N	3		3
K238T	1		1

4. Distribution of ETR FC by Enhanced Genotypic Score



5. Performance of the Enhanced Genotypic Score

N = 4,923 with ≥ 1 ETR Mutation	ETR FC < 2.9	ETR FC ≥ 2.9
Total Score < 4	69.5%	1.8%
Total Score ≥ 4	11.8%	16.9%

False Negative Rate = 9.9% (Sensitivity = 90.1%)

False Positive Rate = 14.5% (Specificity = 85.5%)

Overall Discordance = 13.6%

6 . Performance of the Original MGRM Score in the New Test Set

N = 4,923 with ≥ 1 ETR Mutation	ETR FC < 2.9	ETR FC ≥ 2.9
MGRM Score < 4	72.4%	3%
MGRM Score ≥ 4	9%	15.6%

False Negative Rate = 16.3% (Sensitivity = 83.7%)

False Positive Rate = 11% (Specificity = 89%)

Overall Discordance = 12%

RESULTS

• Sensitivity to detect ETR FC ≥ 2.9 was 90.1% and discordance rate for all samples was 13.6% compared to 83.7% and 12% for the original MGRM weighted score, respectively. The improved sensitivity was accompanied by modest increase in number of samples with FC < 2.9 but a weighted score ≥ 4, from 11 by the original score to 14.5 by the enhanced algorithm.

CONCLUSIONS

• Using this optimized genotypic score, sensitivity for detecting resistant viruses was improved by 6.4% thru inclusion of more mutations associated with phenotypic reduced susceptibility to ETR. Adversely, the rate of identifying ETR FC < 2.9 decreased by 3.5%.

• Discordant cases with high genotypic score but which are phenotypically susceptible to ETR may be caused by increased sensitivity due to NRTI mutations.

• Phenotype remains the reference methodology to optimally determine ETR susceptibility.

ACKNOWLEDGEMENTS

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REFERENCES

1. J Benhamida, C Chappey, E Coakley, NT Parkin. HIV-1 Genotype Algorithms for Prediction of Etravirine Susceptibility: Novel Mutations and Weighting Factors Identified Through Correlations to Phenotype. DRW 2008.
2. J Vingerhoets, M Peeters, H Azijn, L Tambuyzer, A Hoogstoel, S Nijs, MP de Béthune, G Picchio. An update of the list of NNRTI mutations associated with decreased virologic response to etravirine (ETR): multivariate analyses on the pooled DUET-1 and DUET-2 clinical trial data. DRW 2008.