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Genotypic Algorithm for Predicting Elvitegravir Susceptibility: Clinical Validation and Correlation with Phenotype

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BACKGROUND

- Elvitegravir (EVG) is a new integrase strand transfer inhibitor (INSTI) that has shown potent activity against HIV-1.
- A list of mutations associated with EVG failure in phase 2/3 clinical trials has been described [Ref 1] (Figure 1).
- We applied data mining and statistical analyses to a database comprised of matched EVG phenotypic susceptibility measurements and integrase (IN) sequences derived from specimens submitted for routine raltegravir (RAL) susceptibility testing to evaluate the impact of IN mutations on EVG susceptibility.

METHODS

- The biological cutoff for EVG, defined as the 99th-percentile of fold-change in IC50 (FC) for wild-type HIV, was established at FC=2.5 and was used to define reduced susceptibility.
- We identified and examined EVG resistance associated mutations (RAMs) by performing correlation analysis among 3,662 samples from a commercial RAL resistance testing database with matched genotype and EVG phenotype (referred to here as MGRM-EVG-DB). We derived a genotypic algorithm for predicting reduced susceptibility to EVG.
- The performance of this genotypic algorithm was assessed in 917 clinical specimens involved in EVG phase 2/3 trials, as well as within MGRM-EVG-DB.
- To evaluate the individual impact of each RAM on the phenotypic drug susceptibility within the MGRM-EVG-DB, the FC distribution of samples with and without each mutation in the absence of any other primary EVG RAM was investigated, and the difference was examined using the Mann-Whitney test.
- In order to analyze the frequency and impact of reported and newly identified EVG mutations in isolation as well as in combination with other RAMs, we measured the level of co-occurrence between mutations within the MGRM-EVG-DB.

Figure 1: Primary EVG INSTI (integrase strand-transfer inhibitor) RAMs from EVG clinical studies

	T	E	T		S	Q	N
elvitegravir	66	92	97		147	148	155
	I	Q	A*		G	R	H
	A	G				H	
	K					K	

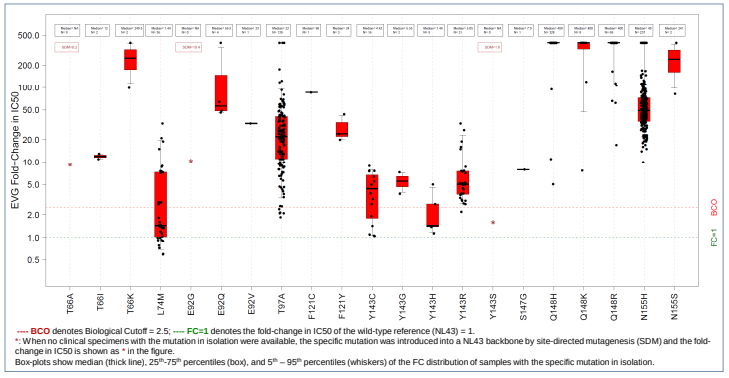
* May require additional mutations for reduced susceptibility to EVG

Table 1: Median EVG FC, N, and % frequency (N/Total) for each mutation in isolation

Mutation	Median EVG FC	p-value by MW test	N	Frequency (%)
T66A	9.2*	NA	SDM*	NA
T66I	12	0.25	2	0.06
T66K	249.5	0.06	2	0.06
L74M	1.44	0.01	36	1.02
E92G	10.4*	NA	SDM*	NA
E92Q	56.5	0.02	4	0.11
E92V	33	0.09	1	0.03
T97A	22	<0.001	139	3.92
F121C	86	0.09	1	0.03
F121Y	24	<0.001	3	0.08
Y143C	4.42	<0.001	16	0.45
Y143G	5.55	0.02	2	0.06
Y143H	1.44	0.08	5	0.14
Y143R	5.05	<0.001	31	0.87
Y143S	1.6*	NA	SDM*	NA
S147G	7.9	0.43	1	0.03
Q148H	400	<0.001	328	9.26
Q148K	400	<0.001	8	0.23
Q148R	400	<0.001	69	1.95
N155H	49	<0.001	237	6.69
N155S	241	0.06	2	0.06

*: When no clinical specimens with the mutation in isolation were available, the specific mutation was introduced into a NL43 backbone by site-directed mutagenesis (SDM) and the fold-change in IC50 (FC) is shown.
P-values are derived from Mann-Whitney (MW) test, comparing FC distributions of samples with and without each mutation in isolation.

Figure 2: Distribution of EVG FC within MGRM-EVG-DB, with each mutation, when no other primary EVG INSTI RAM is present



---- BCO denotes Biological Cutoff = 2.5; ---- FC=1 denotes the fold-change in IC50 of the wild-type reference (NL43) = 1.
*: When no clinical specimens with the mutation in isolation were available, the specific mutation was introduced into a NL43 backbone by site-directed mutagenesis (SDM) and the fold-change in IC50 is shown as * in the figure.
Box-plots show median (thick line), 25th-75th percentiles (box), and 5th – 95th percentiles (whiskers) of the FC distribution of samples with the specific mutation in isolation.

Table 2: EVG phenotype-genotype concordance among samples from the commercial integrase resistance testing database (MGRM-EVG-DB)

N=3,662 from MGRM-EVG-DB	EVG FC ≤ 2.5	EVG FC > 2.5
EVG RAM not Detected	2,384	29¥
EVG RAM Detected	83‡	1,166
Sensitivity = 98%		
Specificity = 97%		
Overall Concordance = 97%		

‡:Virus populations with mixtures that may exhibit lower level of reduced susceptibility compared to unmixed populations are included in this set.
¥: 90% (26/29) of these samples had a FC between 2.5 and 3.5.

Table 3: EVG phenotype-genotype concordance among samples from EVG clinical trials (*GS-US-236-0102, GS-US-236-0103, GS-US-236-0104, GS-US-183-0105, GS-US-183-0145*)

N=917 samples from EVG clinical trials	EVG FC ≤ 2.5	EVG FC > 2.5
EVG RAM not Detected	656	11¥
EVG RAM Detected	34‡	216
Sensitivity = 95%		
Specificity = 95%		
Overall Concordance = 95%		

‡: Virus populations with mixtures that may exhibit lower level of reduced susceptibility compared to unmixed populations are included in this set.
¥: 64% (7/11) of these were baseline samples and had a FC between 2.5 and 3.

RESULTS

- IN substitutions **T66A/I/K, E92G/Q/V, T97A, F121C/Y, S147G, Q148H/K/R**, and **N155H/S** were associated with reduced susceptibility to EVG. Most of these substitutions have been observed in EVG clinical studies.
- The entire FC distribution of specimens with each of these individual mutations as a single INSTI RAM was above the cutoff, except for T97A with 5% of samples below 2.5 (Figure 2 & Table 1). Notably, >80% of the samples with T97A showed co-occurrence with other mutations, particularly with L74M or Y143X.
- In addition, **L74M** and **Y143C/G/H/R/S,** which often occur in combination with other RAMs, showed moderate increase in FC when no other primary EVG RAMs were present and are considered possibly resistant to EVG (Figure 2 & Table 1).
- Using a genotypic algorithm that includes all of these mutations, sensitivity to detect reduced susceptibility to EVG within MGRM-EVG-DB is 98%, and specificity is 97% (Table 2).
- This genotypic algorithm was validated using a clinical trial dataset. High sensitivity, specificity, and overall accuracy (95%) for detecting EVG resistance was achieved (Table 3).
- We observed considerable co-occurrence rates (>30%) among pairs of mutations at G140+Q148, V151I+N155H, E138K+Q148R, L74M+T97A, and T97A+Y143X.

CONCLUSIONS

- The genotypic algorithm developed based on correlative analyses performed on a database of matched phenotype and genotypes accurately predicts EVG resistance in viruses derived from patients who participated in EVG clinical trials as well as in the setting of RAL cross-resistance.
- Periodic surveys of emerging IN mutation patterns and correlation with phenotypic drug susceptibility may be necessary to sustain the current accuracy of the EVG genotypic algorithm, particularly following more wide-spread use of the drug.

REFERENCES
1. ME Abram, RM Hluhanich, DD Goodman, et al; Effect of Primary Elvitegravir Resistance Mutations in HIV-1 Integrase on Drug Susceptibility and Viral Replication Fitness; DRW 2012

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