

DEVELOPMENT OF VIRCO®TYPE HIV-1 RESISTANCE ANALYSIS, INCLUDING CLINICAL CUTOFFS FOR TMC125 (ETRAVIRINE, ETR), A NEW NNRTI

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BACKGROUND

Phenotypic susceptibility of first generation NNRTIs (EFV, NVP, and DLV) is significantly affected by single amino acid substitutions in the viral genome resulting in elevated FC values and loss of clinical activity. Previous attempts to identify an intermediate range of activity and clinical response for these agents have been unsuccessful. Currently, quantitative resistance methods are not frequently used to guide the use of NNRTIs in clinical practice.

Etravirine, a next generation NNRTI, demonstrates a different resistance pathway from the first generation NNRTIs. No single mutation has been identified which results in high levels of resistance and loss of clinical activity. The accumulation of multiple mutations with different weighting factors is required before significant loss of activity has been observed.

Quantitative phenotypic resistance information interpreted via clinical cut-offs (CCOs) are described below which identify for the first time a clinically meaningful intermediate range of activity for an NNRTI.

METHODS

Based on clinical isolates with both drug susceptibility phenotypes (Antivirogram[®]) and viral genotypes, linear regression models were developed to predict ETR fold change in IC₅₀ (FC) from the viral genotype (*VirtualPhenotype*[™]-LM, vPT v 4.2.0.1).

Using treatment response data from the two phase III DUET trials and four Phase IIb trials, a separate linear regression model was developed to predict 8-week change in viral load for regimens including ETR as a function of

- Log₁₀ baseline viral load
- ETR FC as calculated by *VirtualPhenotype*[™]-LM from the baseline viral genotype
- Activity of the background regimen summarized as a continuous Phenotypic Susceptibility Score (cPSS)
- Use of T20 for the first time

Two CCOs were defined, corresponding to vPT calculated FC values associated with a 20% or 80% loss of the ETR response predicted for subjects infected with wild type strains.

ACKNOWLEDGEMENTS

- Virco
- Diagnostic Lab Operations
- Theresa Pattery
- Gaetan Muyldermans
- Tibotec R&D
- xLeo
- Brian Wasikowski
- Clinical study participants

SECTION 1

PREDICTING ETRAVIRINE FC FROM THE VIRAL GENOTYPE

- vPT calculations of ETR FC weigh the contributions of 284 mutations and mutation pairs in RT, and provide an accurate prediction of the measured FC (N=23191, R=0.85, MSE (cross-validated)=0.112)

FIGURE 1. REVERSE TRANSCRIPTASE GENE MUTATIONS AND MUTATION PAIRS IDENTIFIED BY VIRTUALPHENOTYPE™-LM AS SIGNIFICANT IN PREDICTING ETR FC

2V	75T	103L	135T & 181C	178M & 181C	181C & 318F	202V & 215F	230L
312D	77L	103H	135T & 286M	179D	181C & 379C	203K	234I
317A	90E	103W & 118R	135T & 317A	179D & 181C	181C & 386A	200I	238T
318F & 379C	90I & 179F	106A & 135T	138A	179D & 379C	181C & 386I	208F	243I
33L	90I & 386I	106A & 214F	138G	179E	181F	209Y	284K
311 & 100T	98G	100E & 178L	138G & 181C	179E & 181C	181G	209Y & 221E	286A
20R	98G & 100I	100I & 181C	138G & 386A	179E & 388L	181I	213G	288S
20R & 179F	98G & 100I	100I & 388L	139K	179F	181I & 223Y	214F	329L
20R & 181I	100I	100I & 215Y	139K & 181C	179F & 219R	181I & 348E	214F & 223D	331E
20R & 225H	100I & 101E	106L	138K & 215Y	179F & 386I	181I & 376S	214F & 370G	335D
35I	100I & 139I	106L & 208Y	139D	179I	181G	215D	335S
40F	100I & 165I	106L & 357T	139D & 181C	179I & 181C	181H	215E	339F
41L	100I & 178H	100M	139D & 184V	179I & 181V	181V & 386I	215F	348I
49R	100I & 181C	106M & 118I	139D & 210W	179I & 188L	181V & 400A	235F & 238H	356A
49R & 189I	100I & 184V	106M & 215Y	139D & 384I	179I & 390A	184V	215Y	357G
49R & 208Y	100I & 188L	106M & 357T	139A	179I & 208Y	184V & 219D	215Y & 219E	357S
49R & 118F	100I & 219R	108E & 181C	139K	179I & 214F	184V & 221V	215V & 219R	357T
49R & 90I	100I & 219R	108E & 181I	139R	179I & 215Y	184V & 386A	215V & 220H	358K
50I	100I & 318F	108E & 190A	142V	179I & 221V	188I	215V & 220H	358K
60I	100I & 348E	108E & 219E	151M	179E & 179E	188L & 214F	219I & 379C	360V
67G	100I & 386A	111I	151M & 179E	179I	188L & 219E	219E	360A
67H	101E & 181I	115F	151M & 219E	179H	188I & 181E	219H	369V
67H	101E & 214F	115F & 181I	151M & 210W	179T & 184V	188I & 317A	219I & 379C	376S
69.1V & 215Y	101E & 221V	115F & 184V	151M & 384I	179T	188L & 357T	219I	376S & 379C
69C	101E & 223H	118E & 138Q	154S	181C	189I	219R	376V
69I	101H	118E & 179I	158T	181C & 184V	189I & 386I	219R & 317A	377I
69C	101H	118E & 179H	162A	181C & 190A	190A & 223D	219R & 400A	379G
69S	101P & 178H	118E & 189S	162C	181C & 190S	190Q	219R	386I
70I	101P & 179I	118I & 214F	162H	181C & 214F	398E	231I	388R
73D	101P & 202H	120L	162Y	181C & 221C	190E & 214F	223E	395R
74I & 135T	101P & 190A	135L & 181C	165I	181C & 225H	190Q	224D	399D
74I & 178H	101P & 215F	135L & 188L	165I & 348I	181C & 230C	195K	228H	399G
74I & 221V	101P & 219R	135L & 215Y	166B	181C & 238H	202T	228H	400A
74I & 225H	101P & 223Q	135T	178L	181C & 238T	202V	230I	400S

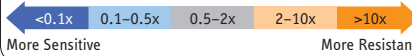
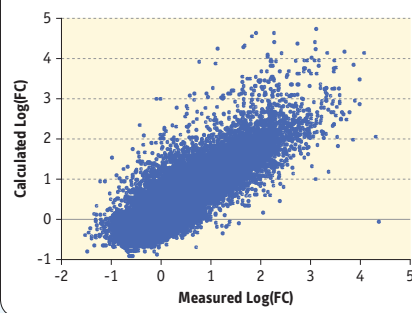


FIGURE 2. LRM PROVIDES AN ACCURATE ASSESSMENT OF ETR FC



SECTION 2

DEFINING ETRAVIRINE CLINICAL CUT-OFFS

- Data from the phase III DUET studies and four phase IIb studies (C203, C209, C223 and C227) were used to define and validate *virco*[®]TYPE HIV-1 Clinical Cut-Offs (CCOs) for ETR. Patients were randomly assigned to the dataset used for CCO development (N=563, 64% from phase III studies, 36% from phase II studies) or the CCO validation dataset (N=290, 70% from phase III studies, 30% from phase II studies)

- The linear regression model developed to predict 8 week viral load response considered four baseline characteristics

TABLE 1. BASELINE CHARACTERISTICS INCLUDED IN THE MODEL (DEVELOPMENT DATA SET)

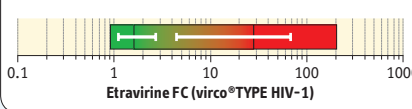
Log baseline viral load	Median: 4.62	Q1-Q2: 4.16–5.24
cPSS of background	<1	38%
	1–<2	49%
	2–<3	12%
	≥3	1%
DRV FC at baseline	≤CCO1	39%
	CCO1–CCO2	49%
	>CCO2	10%
Use of T20 for the first time		29%

- Two CCOs were defined as the baseline ETR FC associated with 20% and 80% loss of wild type response to ETR (FIGURE 3). The responses predicted for patients with different characteristics as a function of ETR FC at baseline can be normalized as percent loss of ETR response, which is independent of other variables in the model. Clinical cut-offs can then be defined as the baseline ETR FC associated with 20% and 80% loss of the ETR response

Validation

1. Assess variability of CCO through bootstrap analysis (1000 iterations). FIGURE 4 depicts the clinical cutoffs and the 95% confidence intervals

FIGURE 4. CLINICAL CUT-OFFS AND THE 95% CONFIDENCE INTERVALS



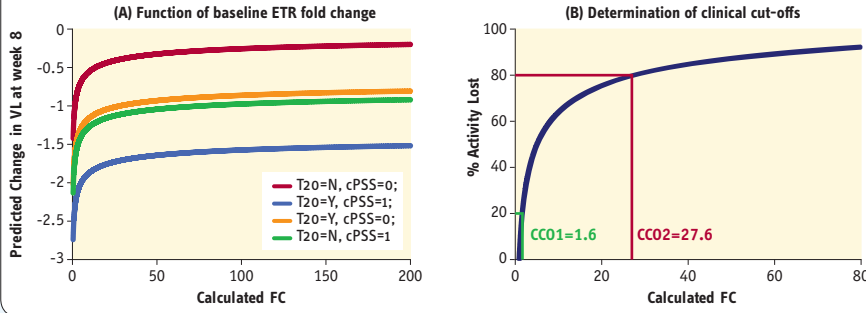
2. Test association of baseline ETR susceptibility with response. TABLE 2 shows a stronger association with clinical response (defined as 1 log drop in viral load) for clinical cut-offs as compared to biological cut-offs both in the development data set and in the unseen validation data set that was not used for clinical cut-off development
3. The ETR resistance continuum was explored by presenting the % responders (defined as <math><50</math> copies/mL at week 24) and the median log₁₀ viral load drop (FIGURES 5 AND 6)
 - Resistance categories were based on the clinical cut-offs defined in this poster (1.6 and 27.6) and the breakpoints in the package insert (3 and 13)

TABLE 2. ODDS RATIO AND 95% CONFIDENCE INTERVAL FOR RESPONSE (>1 LOG VIRAL LOAD REDUCTION AT 8 WEEKS)

	DEVELOPMENT DATASET (N=563)		UNSEEN VALIDATION DATASET (N= 290)	
	OR	(LL, UL)	OR	(LL, UL)
BCO	2.1	(1.4, 3.3)	3.5	(1.8, 6.7)
CCO	3.7	(2.0, 6.9)	10.0	(3.8, 25.6)

BCO: Biological Cut-Off, CCO: Clinical Cut-Off, OR: Odds Ratio, LL: Lower Limit of the 95% confidence interval, UL: Upper Limit of the 95% confidence interval

FIGURE 3. PREDICTED RESPONSE AS (A) FUNCTION OF BASELINE ETR FOLD CHANGE (B) DETERMINATION OF CLINICAL CUT-OFFS



RESULTS

FIGURE 5. VIROLOGIC RESPONSE TO ETRAVIRINE CONTAINING REGIMENS IN BASELINE SUSCEPTIBILITY GROUPS DEFINED BY VIRCO®TYPE HIV-1 CCOs (WEEK 8 AND WEEK 24—DROP OUT AS FAILURE (DOF))

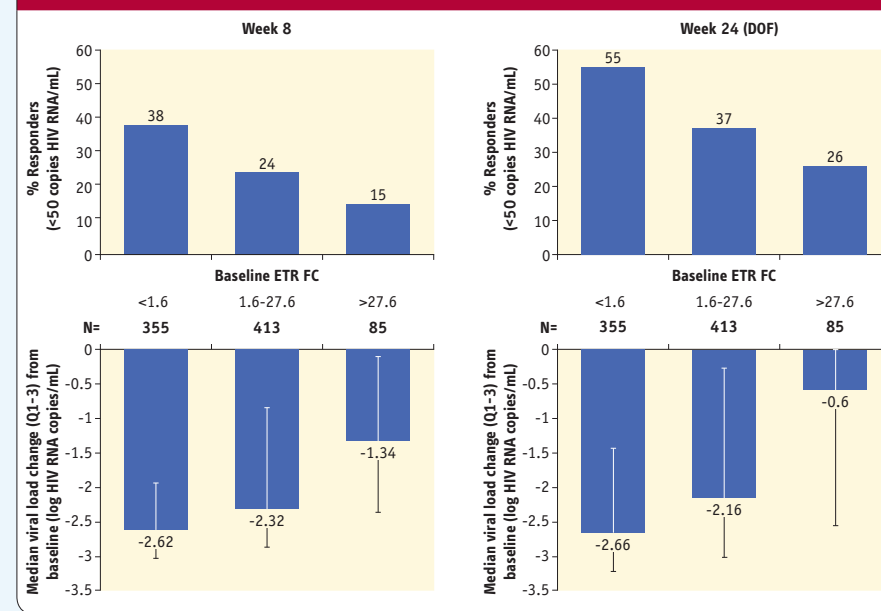
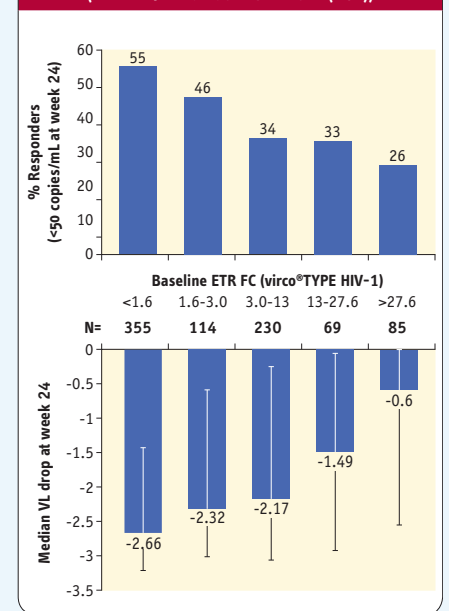


FIGURE 6. EXPLORATORY ANALYSIS OF REGIMEN RESPONSE ACROSS THE ETR RESISTANCE CONTINUUM (WEEK 24—DROP OUT AS FAILURE (DOF))



DISCUSSION

- The data set used for CCO development included the phase II data in addition to the phase III (DUET) data. Inclusion of the phase II data added to the diversity of etravirine containing treatment regimens (e.g. ±darunavir, ±T-20) considered in defining CCOs
- Although the CCOs were calculated based on 8-week virologic response, differences in response as a function of etravirine resistance at baseline persisted through week 24. This continuing association between etravirine susceptibility at baseline and treatment response suggests that it is indeed appropriate to define CCOs for this NNRTI

CONCLUSION

- We have integrated complex interactions among multiple mutations in RT to provide accurate quantitative predictions of etravirine susceptibility
- Clinical cut-offs for etravirine of 1.6 and 27.6 FC have been defined for the *virco*[®]TYPE HIV-1 report by the same approach used for all other drugs. The applicability of these CCOs to patients different from those in the Phase II/III studies has not yet been evaluated
- In the population studied, response to etravirine containing regimens declined gradually as resistance to etravirine at baseline increased. Responses among subjects with baseline ETR FC between *virco*[®]TYPE HIV-1 CCO1 and CCO2 remained substantial