Poster No. 55

Subgroup analysis of baseline (BL) susceptibility and early virological response to Enfuvirtide in the combined TORO studies

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Background and Rationale

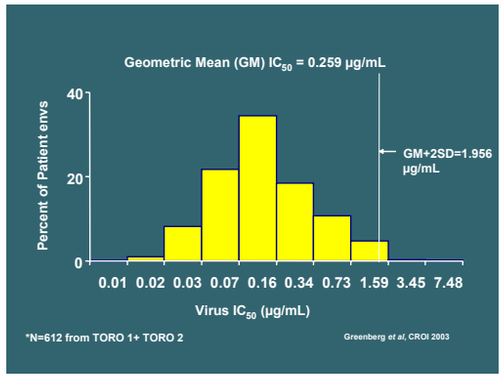
Enfuvirtide is the first Fusion Inhibitor that is approved by the USFDA, EMEA and the Swiss Health Authority. Previous studies described the characterization of resistance to ENF both *in vitro* [1] and *in vivo* [2; 3]. Taken together, these *in-vivo* studies suggested that: (1) the incidence of substitutions in the gp41 aa 36-45 region at baseline was low; (2) substitutions in gp41 aa 36-45 were observed in plasma virus from the majority of patients meeting protocol defined virological failure (VF) during ENF treatment; (3) virus isolates from patient blood cells exhibiting reduced susceptibility to ENF also displayed substitutions in this gp41 region. These results have led us to consider the region of gp41 aa 36-45 as a principal determinant of ENF resistance.

We extended our previous findings by analyzing in the TORO 1 & 2 Phase III clinical studies [4], plasma viral envelopes (Envs) from 661 patients taking ENF in combination with an optimized background (OB) regimen. Patients in the ENF+OB arm were analyzed for the following: (1) BL plasma virus Env susceptibility to ENF; (2) change in viral Env susceptibility to ENF from BL to VF; (3) a potential relationship between genotypic changes in the gp41 ectodomain and changes in ENF susceptibility between BL and VF. It is important to note that genotypic and phenotypic data were obtained for Envs from all evaluable patients at BL and for those meeting VF through 24 weeks.

The results showed that:

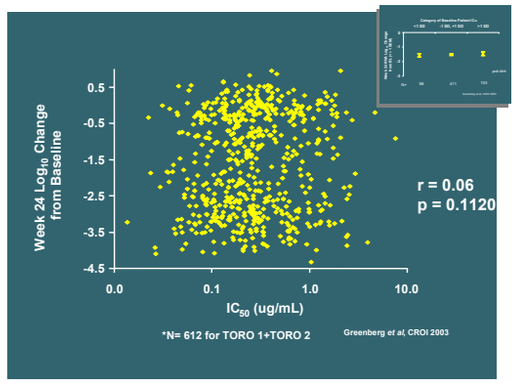
- The patient viral Envs BL susceptibilities to ENF were log normally distributed (Fig 1).

Figure 1: Histogram of Baseline IC_{50} for ENF



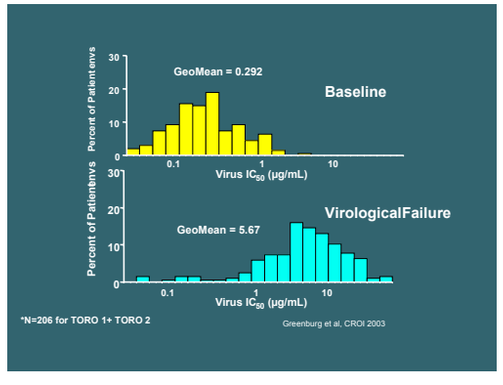
- There was no evidence of a correlation between BL IC_{50} to ENF and Virological RNA response at Week 24 using last observation carried forward (LOCF) (Fig.2).

Figure 2: Baseline ENF IC_{50} vs. Log_{10} Plasma HIV-1 RNA response* through Week 24



- The Week 24 RNA response was not diminished for patients whose virus exhibited BL IC_{50} that was either less or greater than 1 (shown in inset) or 2 SD from the GM (not shown).
- Viral Envs from patients who met VF had a 21-fold increase in the GM ENF IC_{50} from BL to VF (Fig 3). The most common concomitant genetic changes in gp41 amino acids (aa) 36-45 and the varying degrees of ENF susceptibility are shown in Table 1.

Figure 3: Viral Envs from VF patients exhibited a 21-fold decrease from baseline in susceptibility to ENF



Background and Rationale

Table 1: Change in ENF susceptibility for most common substitutions in gp41 aa 36-45

Substitutions in gp41 aa 36-45	Fold change from BL In ENF Susceptibility		
	N	Geo Mean	Range
V38A	27	42	(10 - 185)
V38V/A	8	36	(8 - 324)
G36D	6	32	(15 - 60)
N43D	19	26	(8 - 401)
V38M	6	15	(10 - 26)
N43N/D	5	9	(5 - 17)

- Substitutions confer varying decreases in ENF susceptibility

Greenberg *et al*, CROI 2003

Objective

The specific objectives of the current presentation are to further examine the relationship between:

I. Viral Env BL susceptibility to ENF and early virological HIV-1 RNA response for patientshaving a BL Genotypic Sensitivity Score (GSS) of 0 or 1;
II. To evaluate BL factors associated with changes in ENF IC_{50} from BL to VF;
III. To describe the association between BL GSS and the magnitude of decrease in susceptibility to ENF from BL to VF.

Methods

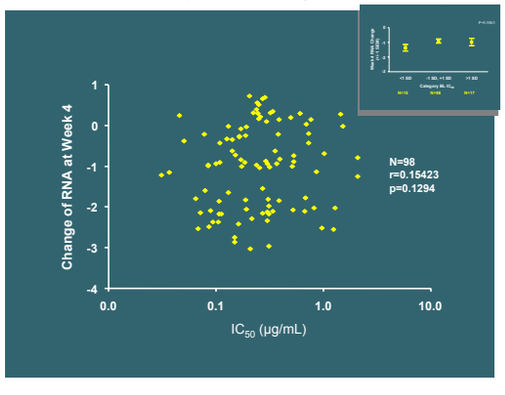
TORO 1 & 2 are randomized, open-label, controlled, multi-center, Phase III studies of patients receiving 90 mg BID of enfuvirtide by subcutaneous injection in combination with an optimized background (OB) regimen [5]. The ITT population included 661 patients randomized to ENF+OB. Resistance data were generated using the ViroLogic GeneSeq™ for GSS and the experimental PhenoSense™ Entry Assay for ENF susceptibility [6]. For viruses exhibiting dual tropism, the IC_{50} value is the higher of the IC_{50} values obtained from cell lines expressing CD4 and either CCR5 or CXCR4. Correlation between BL ENF susceptibility and virological response was assessed using nonparametric univariate linear correlation and multiple linear regression analyses. GSS at BL was calculated by assigning a score of 1 for each ARV in the OB regimen for which resistance mutations were absent at the screening visit. Also of note, no score was assigned for the use of ENF in the treatment regimen.

Results

I. Baseline Susceptibility to ENF and early HIV-1 RNA response in Patients with BL GSS of 0 or 1

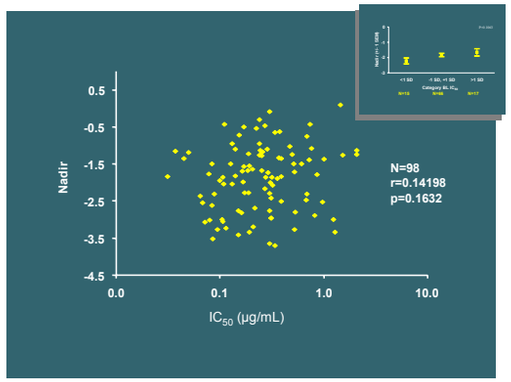
- Of the 612 patients with BL viral phenotype and complete virological response data, 98 patients had BL GSS=0 and 167 patients had a BL GSS =1.
- There was no correlation with BL ENF IC50 and log10 plasma HIV-1 RNA change from BL to week 4 in the GSS=0 or GSS=1 subgroups (r=0.15, p=0.13; and r=0.06, p=0.47, respectively). Similar results were obtained for both subgroups for virological response at Week 2 and nadir response through Week 24 and also after adjustment for prognostic factors (See Figs 4 and 5 for patients with BL GSS=0; results for patients with BL GSS=1 not shown)

Figure 4: Scatter plot of BL IC_{50} and RNA response at Week 4 (inset shows virological response for patients with GSS=0 and ENF susceptibilities less than, within and greater than 1 SD from the GM for patients with BL GSS=0. (For patients with BL GSS=1: N=167; r= 0.14; and p= 0.4717; Figure not shown)



Results

Figure 5: Scatter plot of BL IC_{50} and nadir RNA response through Week 24 (inset shows virological response for patients with GSS=0 and ENF susceptibilities less than, within and greater than 1 SD from the GM for patients with BLGSS=0. (For patients with BL GSS=1: N=167; r=0.09; and p=0.2672; Figure not shown)



II. Factors associated with change in IC_{50} from BL to VF

- Several factors that might be associated with changes in IC_{50} from BL to VF were evaluated by multiple regression analysis (See Table 2).

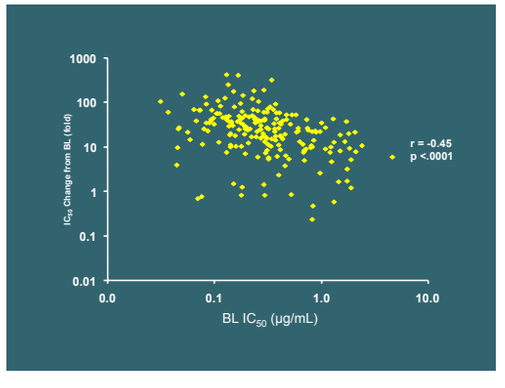
Table 2: Summary of multiple regression analysis of factors associated with change in IC_{50} from BL to VF

Factor	Single Tropic	Dual Tropic	All Patients
Protocol	NS	-	NS
Baseline CD4+ Cell Count	NS	NS	NS
Baseline Log RNA	NS	NS	NS
PSS	-	NS	-
GSS	NS	NS	NS
Lopinavir/r Experience	NS	NS	NS
Adherence to enfuvirtide	NS	NS	NS
Adherence to enfuvirtide+OB	+	NS	+
Log enfuvirtide AUC(0-12h)	NS	NS	NS
Log enfuvirtide Trough	NS	NS	NS
VF Study Week	NS	NS	+
Log RNA Change from Baseline	NS	NS	NS
Log Baseline IC_{50}	-	-	-
X4 Tropism	NS	N/A	NS
R5 Tropism	N/A	N/A	-
Non-B Viral Clade	NS	NS	NS
N42S or N42N/S at Baseline	NS	+	NS
Any Change in gp41 aa 36-45	+	+	+
Viral Co-receptor switch from Baseline	NS	NS	NS

*Factor associated with greater IC_{50}, $p<0.05$; - factor associated with less IC_{50}, $p<0.05$; NSNot significant at $p<0.05$; N/ANon-applicable

- A negative correlation (r=0.45; p=<0.0001) was seen between BL IC_{50} and Change in ENF susceptibility at VF (See Fig. 6)

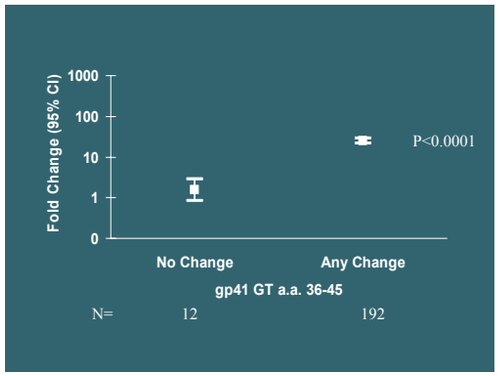
Figure. 6: Scatter plot of BL IC_{50} vs. IC_{50} change through Week 24



- A positive correlation (p=<0.0001) was seen between the emergence of substitutions in gp41 aa 36-45 and decrease in ENF susceptibility at VF (See Fig. 7)Z

Results

Figure. 7: ENF IC_{50} Fold Change from BL at VF by any Substitution in gp41 aa 36-45 at VF



III. Baseline GSS and decrease in susceptibility to ENF at VF

- An analysis of the relationship between BL GSS and GM decrease in susceptibility to ENF at VF revealed that patients with lower BL GSS had significantly greater decreases in susceptibility to ENF than those with higher GSS (r=–0.21, p=0.003) (See Fig. 8).

Figure. 8: Relationship between fold change in IC_{50} from BL and the BL GSS/PSS category

Discussion

- The observation that the BL susceptibility to ENF was not correlated with the virological response measurements examined thus far (Week 2, Week 4, nadir or LOCF through Week 24) may suggest that the in vitro IC_{50} measurements are shifted from the IC_{50} values operational in vivo. One possible explanation may be that the *in vivo* ENF concentrations which are applicable were sufficiently above the threshold concentrations to effect the virological responses observed, however, other explanations cannot be excluded at this time and will be the subject of further analyses.

Conclusions

- A significant correlation was not observed between BL *in vitro* susceptibility to ENF and virological response in patients with the lowest GSS, however, other unknown factors may be influencing virological response.
- Multiple regression analysis of the several factors that might be associated with change in susceptibility to ENF from BL to VF revealed that: (1) Log BL IC_{50} was negatively associated and (2) substitutions in gp41 aa 36-45 were positively associated with the change in ENF susceptibility at VF.
- The inverse correlation observed between BL GSS and reduced susceptibility to ENF at VF suggests a relative susceptibility to ENF is preserved when combined with additional active agents in the optimized background

References

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