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Rapid Selection of High-Level Resistance to Enfuvirtide (Fuzeon)

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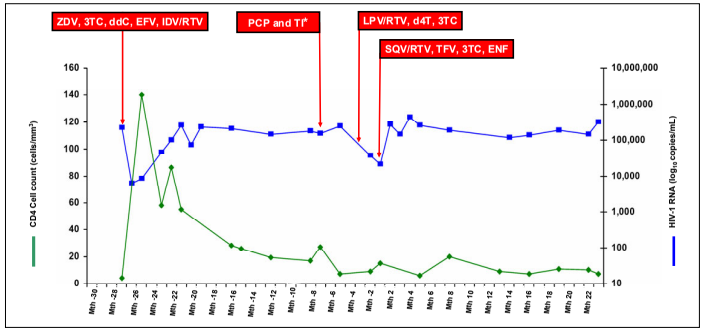
Background

- Reductions in enfuvirtide (ENF) susceptibility are conferred by various amino acid substitutions at positions 36-45 located within the ENF binding site (heptad repeat 1) of gp41.
- We describe the rapid selection of a virus variant bearing a rare single mutation conferring high level resistance to ENF.

Methods

- The patient was highly treatment experienced and had an HIV-1 RNA level of 147,864 copies/mL and a CD4 cell count of 27/mm^3.
- In the setting of an episode of pneumocystis carinii pneumonia he underwent a treatment interruption after which he began d4T, 3TC and LPV/RTV (Figure 1).
- Prior to a new salvage regimen a combination genotype-phenotype test (PhenoSense GT) was obtained (Figure 2).
- The salvage regimen comprised TDF, 3TC, SQV/RTV and ENF.
- Despite reported antiretroviral adherence the response to this therapy was poor with a paradoxical rise in HIV-1 RNA levels and a lack of CD4 T-cell count increase.
- ENF phenotyping was obtained on sequential samples from Day-1 using a single cycle HIV-1 *env* pseudovirion infectivity assay.
- GeneSeq HIV gp160 *env* sequences were determined at multiple time points by population and clonal methods using chain terminator reaction chemistry.
- The relatedness of baseline and follow-up sequences was investigated by the construction of a tree showing the relative distance between the patient strains and three reference sequences by amino acid comparison

Figure 1: Antiviral Therapies, HIV-1 RNA and CD4 T-cell Marker Data Over Time



*PCP: Pneumocystis carinii pneumonia, TI= Treatment interruption

Figure 2: Combination Phenotype-Genotype, PhenoSenseGT Prior to the ENF-Inclusive Regimen

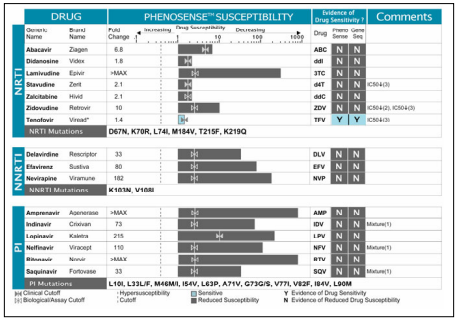
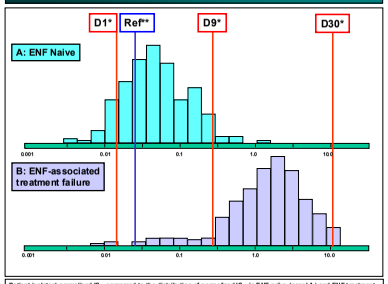


Table 1: Sequential ENF Susceptibility, Co-receptor Tropism, and HR1 Mutation Data

Time	T-20 FC	HR1 Mutations	Co-receptor Tropism
	1.0	Reference*	R5
Day 1	0.5	G36, V38	R5
Day 9	7.6	G36G/D, V38V/E/A	R5
Day 30	401.3	V38E	R5
Day 40	393.8	V38E	R5
Day 58	305.8	V38E	R5
Day 70	370.3	V38E	R5
Day 84	327.7	V38E	R5
Day 580	208.7	V38E	R5

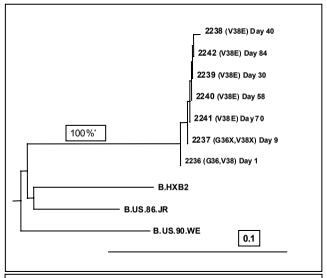
*Reference= reference isolate JRCSF

Figure 3: ENF Susceptibilities over Time Compared to the Reference Isolate JRCSF and to Populations that are ENF Naïve and Those with ENF-associated Treatment Failure



Patient isolates' normalized IC_{50} compared to the distribution of normalized IC_{50} in ENF naïve (panel A) and ENF treatment-failure (Panel B) populations.
*Normalized IC_{50} (mg/mL) = IC_{50} of the patient sample x [Average IC_{50} Ref ÷ Measured IC_{50} Ref].
The calculation of normalized IC_{50} mitigates changes in IC_{50} due to assay variation.
D1, D9 and D30 refer to ENF treatment days 1, 9 and 30, respectively
**Ref= reference isolate JRCSF

Figure 4: Tree Showing the Relative Distance Between Patient *env* Sequences



*Represents the bootstrap value; the frequency that the branch was present in exactly the same topology in 1000 resampled trees

Results Summary

- The Day-1 sample was fully susceptible to ENF (FC= 0.5) (Table 1, Figure 3)
- On Day 9 the ENF FC was 7.6. concurrent with the presence of G36G/D and V38V/E/A changes in HR1.
- Subsequent samples demonstrated high-level ENF resistance with V38E only.
- Clonal analysis of the gp160 *env* sequences from the Day 7 sample demonstrated that the G36D change was present on distinct strains from those with V38A or V38E.
- A distance tree of HR-1 sequences confirmed the relatedness of the fully susceptible Day 1 sample and the follow-up samples (Figure 4).
- Although rare the V38E mutation (in combination with the N42S change) has been associated with high level ENF resistance (395-fold) and reduced fitness (J. Lu et al., J Virol. 78:4628, 2004).

Conclusions

- This case demonstrates that rapid selection of high-level ENF resistance may occur in clinical practice, albeit uncommonly.
- High-level phenotypic resistance was associated with the emergence of an uncommon mutation V38E in the ENF binding site of the HR1 domain.
- The rapid outgrowth and maintenance of the V38E mutant on ENF therapy is consistent with a high selective advantage of this mutation.

Acknowledgements

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