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IMPACT OF HIV-1 GP41 AMINO ACID SUBSTITUTIONS (POSITIONS 36–45) ON SUSCEPTIBILITY TO T-20 (ENFUVRTIDE) *IN VITRO*: ANALYSIS OF PRIMARY VIRUS ISOLATES RECOVERED FROM PATIENTS DURING CHRONIC ENFUVRTIDE TREATMENT AND SITE-DIRECTED MUTANTS IN NL4-3

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BACKGROUND: T-20 (enfuvirtide) is a synthetic peptide inhibitor of HIV-1 gp41 mediated fusion, currently in pivotal Phase III clinical trials. Resistance to enfuvirtide has been studied by *in vitro* selection of escape variants and by examining the effect of substitutions observed in viruses from patients in clinical studies on enfuvirtide susceptibility. We previously reported on the enfuvirtide EC₅₀ of six NL4-3 site-directed mutants containing substitutions in gp41 amino acids (aa) 36–45. We also reported a correlation between the *in vitro* binding affinity of mutant gp41 heptad repeat 1 regions with fluoresceinated T-20 and the enfuvirtide EC₅₀ for the mutant NL4-3 constructs. Here we report on changes in enfuvirtide susceptibility for 21 constructs harbouring substitutions or combinations of substitutions observed in plasma virus obtained from patients during chronic enfuvirtide treatment. We also report on changes in enfuvirtide susceptibility for patient peripheral blood mononuclear cell (PBMC)- derived viruses harbouring some of the same substitutions.

METHODS: Population sequencing was used to identify substitutions in gp41 aa 36–45 in plasma HIV-1 from patients enrolled in enfuvirtide Phase II clinical studies. Substitutions were then introduced into a modified pNL4-3 parental laboratory HIV-1 strain (harbouring D36G in gp41) by site-directed mutagenesis. The enfuvirtide susceptibility of all NL-3 viruses and patient PBMC-derived viruses was tested in the MAGI/cMAGI assay. In addition, the gp41 ectodomain of PBMC virus isolates with decreased enfuvirtide susceptibility was sequenced. Fold changes were calculated from patient baseline viruses or the parental NL4-3 virus.

RESULTS: The NL4-3 site directed mutants containing the single substitutions G36D, G36S, V38A, V38M, Q40H, N42S, N42T, N42D, N42E, N43D, N43K, N43S, L44M or L45M had a geometric mean fold change in EC₅₀ of 3.6 (range 0.5–21) in comparison to the parent virus. Mutant NL4-3 viruses with the double substitutions G36S/L44M, G36S/V38M, V38A/N42D, V38A/N42T, V38E/N42S, N42T/N43S and N42T/N43K had a geometric mean fold change of 73 (range of 15–513), generally greater than viruses with a single substitution. Patient PBMC-derived virus isolates containing G36D, G36S, G36E, V38A, Q40H, N43D or N43K had a geometric mean fold change of 33 (range of 3.1–450). Patient PBMC derived viruses with N42T/N43S, N42T/N43K, G36S/L44M, Q40H/L45M, and N42T/L45M had a geometric mean fold change of 221 (range of 30–632).

CONCLUSION: Specific substitutions in gp41 amino acids 36–45 observed in plasma virus or clinical isolates during enfuvirtide treatment conferred decreased susceptibility to enfuvirtide *in vitro* in the modified NL4-3 virus and/or in primary clinical isolates. These results support the premise that this region of gp41 is a primary target for enfuvirtide and a principal locus for emergence of enfuvirtide resistance. Further study will be needed to characterize possible contributions of HIV-1 gp41 residues outside of aa 36–45 on enfuvirtide susceptibility.

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22

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