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***IN VITRO* RESISTANCE DEVELOPMENT FOR A SECOND-GENERATION NNRTI: TMC125**

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BACKGROUND: [TMC125](#) is a NNRTI under clinical development with potent *in vitro* antiviral activity against many NNRTI resistance mutations. We describe the development of resistance mutations under selective pressure of TMC125.

METHODS: Wild-type (HXB2) and K103N viruses were grown in the presence of increasing drug pressure. Emerging viral supernatants were amplified in MT4 cells, proviral DNA was extracted, amplified and sequenced. Viral RNA was isolated for clonal analysis. Site-directed mutants were generated with the QuickChange kit (Stratagene) and tested in a pseudotyped single-cycle assay.

RESULTS: As a control, HXB2 was passaged with increasing efavirenz (EFV) concentrations. EFV selected for L100I, K103N, L214F and P225H. A combination of L100I, K103N and L214F conferred reduced susceptibility with a >1000-fold increase in IC₅₀. Other amino acid changes included T39A, I94L, H96L, S156P, D192N, G196R, E203K, Q207R, T216A and S268G. TMC 125 selected for T39A, E138K, V179F, Y181C, L214F, F227L, M230I/L; with polymorphisms at: E40K, K70R, Q91L, L109M, R125G, A158T, Q174P, G196R, N265T, D256G and E291K. Clonal analysis from virus selected at 360 nM passage concentration revealed 9/24 clones containing the triple mutant E138K+V179F+Y181C. The virus pool from this passage step showed an IC₅₀ of 514 nM (>700-fold change). At 10 µM, TMC125 selected for mutations at E138K, V179F, Y181C, L214F and M230L. The quadruple mutant V179F+Y181C+L214F+M230L showed decreased susceptibility to EFV, CPV, GSW678248 and TMC125 by 133-fold, >600-, >600- and >850-fold, respectively. TMC125 selected mutants from K103N included: K102Q, E122K, Y146S, Y181C, while the K103N reverted to wild-type. Sitedirected mutagenesis studies to introduce identified positions for single-cycle assay testing are in progress.

CONCLUSIONS: TMC125 lost susceptibility with mutants: V179F, Y181C, L214F and M230L. In combination, these mutations conferred a >800 shift in susceptibility. The mutation patterns selected conferred cross-resistance to EFV, CPV and GSW678248.

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