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## ***IN VITRO* SELECTION OF THE T215Y AND K65R MUTATIONS BY STAVUDINE AND DEMONSTRATION OF HIGH-LEVEL RESISTANCE TO STAVUDINE**

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**BACKGROUND:** Stavudine is a nucleoside reverse transcriptase (RT) inhibitor widely used in the treatment of HIV-1-infected persons. However, the genotypic and phenotypic correlates of stavudine resistance and treatment failure are poorly understood. Zidovudine-associated mutations, including 215Y, are selected in some stavudine-experienced patients. However, the effect of these mutations on phenotypic resistance to stavudine is not evident in current assays. To better understand resistance to stavudine, we monitored genotypic changes in nine recombinant viruses during culture with stavudine and assessed changes in stavudine susceptibility by different phenotypic assays.

**METHODS:** Recombinant viruses were generated using cloned RT sequences from either HXB2 or treatment-naïve HIV-1-infected persons. Three had wildtype (WT) RT, and six had 215D or 215C mutations, which differ from 215Y by one nucleotide.

**RESULTS:** Of the six viruses that had 215C or 215D, four acquired the 215Y mutation after a mean of 57 days (range=32–89) in culture with stavudine, compared to only 23 days (range=18–27) with zidovudine. None of the three WT viruses selected 215Y in 104 days (range=94–110) of culture with stavudine. Interestingly, seven of the nine viruses selected the K65R mutation; four acquired K65R only, and three acquired K65R before acquiring either 215Y (two viruses) or V75A (one virus). HIV-1K65R/T215Y replicated less efficiently than HIV-1K65R or HIV-1T215Y, suggesting that co-existence of K65R and 215Y has a negative impact on viral fitness. Reverse transcriptases carrying K65R with or without 215Y or V75A showed high-level resistance to stavudine-TP (median=18-fold, range=5–48) in a commercial RT assay that uses polyA/oligo dT and dUTP/dTTP. In contrast, resistance to stavudine was low or undetectable in two phenotypic assays. The median change in IC<sub>50</sub> values for stavudine was 1.6-fold

(range=1.1–1.8) in the PhenoSense assay (recombinant single-cycle replication) and 1.1-fold (range=0.9–2.4) in the MT-4/MTT assay (whole-virus multiple replication cycle). The inability of culture-based assays to detect resistance was restricted to stavudine and was confirmed in site-directed mutants carrying K65R or 215Y/K65R.

**CONCLUSION:** We show that 215Y is selected by stavudine *in vitro*, confirming a selective advantage for this mutation in the presence of this drug. We also show that K65R is frequently selected by stavudine, supporting a primary role for this mutation in stavudine resistance. The high level of stavudine resistance associated with K65R suggests that K65R may compromise the treatment efficacy of this drug. Similarly, the ability of HIV-1215D and HIV-1215C to rapidly select 215Y *in vitro* implies that patients infected with these viruses and treated with stavudine may be at increased risk for acquiring 215Y. The detection of stavudine resistance by an enzymatic assay and not by culture-based assays has important implications for phenotypic resistance testing for stavudine.

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