

**POSTER 8****K70Q EXPANDS MULTI-DRUG RESISTANCE (MDR) OF HIV-1 CONTAINING “Q151M COMPLEX” REVERSE TRANSCRIPTASE MUTATIONS**

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Multi-drug-resistant (MDR) HIV-1 carrying the “Q151M complex” (Q151Mc) reverse transcriptase (RT) mutations (Q151M/F116Y/F77L/V75I/A62V) is resistant to many nucleoside RT inhibitors (NRTIs) including zidovudine, didanosine (ddI), zalcitabine, stavudine, abacavir, and lamivudine (3TC). However, Q151Mc is not significantly resistant to tenofovir (TDF). We have isolated from a TDF-treated HIV patient a Q151Mc-containing clinical isolate with significant phenotypic resistance to TDF. Genotypic analysis of this patient's isolates through various stages of therapy revealed that phenotypic susceptibility to TDF emerged upon appearance of the previously unreported K70Q mutation in the Q151Mc background. To confirm the potential effect of K70Q in TDF resistance, we used site-directed mutagenesis to prepare HIV-1 molecular clones with the K70Q, Q151Mc, or K70Q/Q151Mc mutations. Virological analysis of these viruses revealed that addition of only the K70Q RT mutation by itself in HIV-1 did not significantly affect resistance to TDF and resulted in marginal resistance to ddI and 3TC. However, addition of the same mutation in the Q151Mc background (Q151Mc/K70Q) resulted in an enhanced resistance to all FDA-approved NRTIs, including a 10-fold resistance to TDF. Steady-state kinetics biochemical analysis of the recombinant enzymes demonstrated that the K70Q mutation does not significantly affect incorporation of TDF-diphosphate into the nascent chain. However, the K70Q/Q151M RT showed an increase in TDF resistance in “rescue” assays (primer extension in the presence of ATP). Structural analysis suggests that the K70Q mutation may influence binding of the ATP substrate of the excision reaction.

In conclusion, addition of K70Q to Q151Mc enhances resistance to TDF and other NRTIs. Enhancement of TDF resistance is caused by increasing the efficiency of the excision mechanism. The novel pattern of TDF-resistance may have clinical implications for the design of therapeutic strategies for NRTI-experienced patients with MDR mutations.