Secondary resistance mutations in the R263K integrase inhibitor resistance pathway

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Background: HIV-1 resistance has been observed for all antiretrovirals tested so far, raising concerns about the long-term efficacy of these drugs. However, no resistance mutation against the strand-transfer inhibitor dolutegravir has been observed in treatment-naïve patients. In vitro selection studies performed in our laboratory demonstrated that, in the presence of dolutegravir the R263K mutation commonly emerges in integrase and is often associated with the secondary mutation H51Y. We have shown that R263K confers resistance to dolutegravir while the addition of H51Y to R263K further decreases HIV susceptibility to this drug. However, resistance correlated with a pronounced decrease in integration and viral replication. Although less common than H51Y, other secondary mutations such as M50I and E138K were selected in the presence of the R263K primary mutation. We further characterize the R263K resistance pathway by studying the effect of the M50I and E138K secondary mutations on HIV resistance to dolutegravir, as well as on the catalytic activity of purified recombinant integrase and on viral fitness.

Methods: The various relevant integrases were cloned into a vector for bacterial expression and mutated by site-directed mutagenesis. Recombinant integrases were purified and tested for strand-transfer activity in cell free assays, and Michaelis-Menten constants and maximal activities were calculated. The same mutations have been introduced into pNL43 HIV clones by site directed-mutagenesis and the resulting viruses were tested for resistance to dolutegravir and viral replication capacity in cell culture.

Results: Similar to the H51Y mutation, M50I and E138K fail to restore the catalytic activity of the R263K integrase, as well as the defect in viral replication associated with this latter mutation. However, the M50I and E138K secondary mutations have different effects on R263K than H51Y, with M50I influencing integrase interaction with viral DNA and E138K impacting the conformation of the catalytic site. Additionally, M50I confers a higher resistance level to dolutegravir than E138K.

Conclusion: None of the secondary mutations associated with R263K restores HIV integrase activity, integration, or viral replication capacity, suggesting that the R263K resistance pathway may be beneficial to patients receiving dolutegravir-containing regimens.