N348I in HIV-1 RT Confers Resistance to Etravirine and Rilpivirine but Restricts the Advent of E138K

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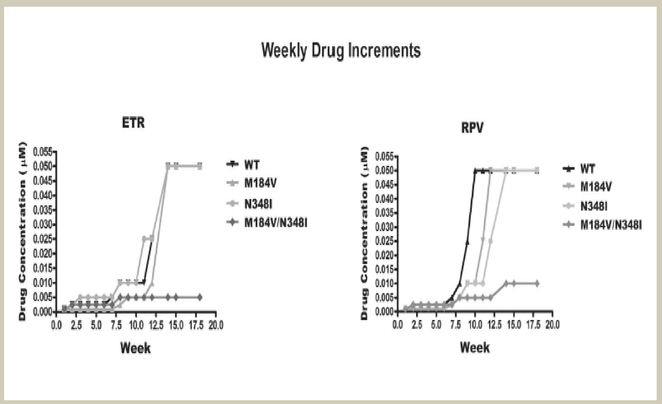
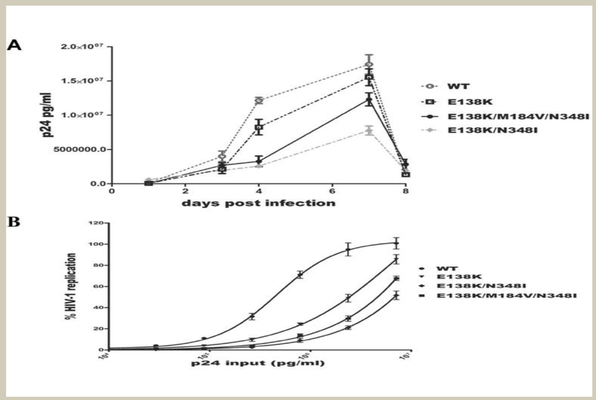
Background:

N348I is a Mutation in the Connection Domain of RT that Commonly Emerges in Treatment Experienced Patients and that is Strongly Associated with M184V. N348I Compensates for the Deficit in Enzyme Processivity Associated with the Latter Mutation.

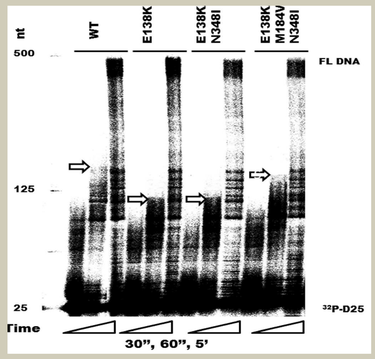
We Wished to Analyze this Mutation Alone and in Association with M184V to Assess if N348I can Prevent or Delay the Emergence of E138K Under Etravirine and Rilpivirine Pressure and what is the Impact of this Mutation on Viruses that Present E138K.



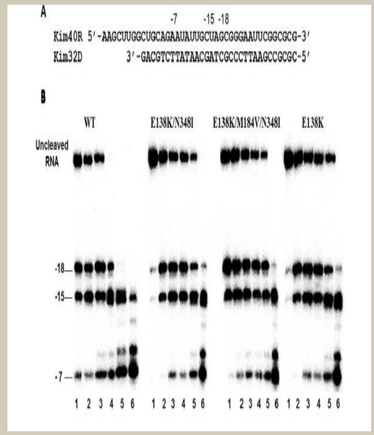
The Graph Shows the Weekly Increment of ETR or RPV Concentrations for Selection of Resistant Variants in CBMCs Using WT Virus and Viruses Containing the N348I and M184V/N348I Substitutions at Baseline. CBMC were Cultured in the Presence of Progressively Increasing Concentrations of Drugs.



A)In CBMCs the Replication Kinetics of Mutants and WT were Assessed on the Basis of p24 Levels in Culture Supernatants Sampled at Variable Time Points. B) In TZM-bl Cells the Luciferase Activity was Measured and the Viral Replication Level was Expressed as a Percentage of Relative Light Units (RLU) with Reference to WT.

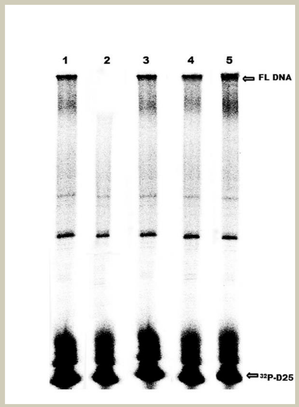


Time Course Experiments Showing the Effect of the E138K, E138K/N348I and E138K/M184V/N348I Mutations on the Efficiency of RT Processive DNA Polymerization. The Longest Extension Products Generated at 60 Seconds are Identified by Arrows and Indicate Differences in Polymerization Efficiencies.



RNase H Activity of WT and Mutant Recombinant RT Enzymes

A)Graphic Representation of the RNA/DNA Substrate Duplex Used to Monitor the Cleavage Efficiency of Mutant and WT RTs. B)RNase H Activity was Analyzed by Monitoring Substrate Cleavage in Time-Course Experiments. The Uncleaved Substrate and Cleaved Products Relative to the 3'-Terminus of the DNA Primer are Indicated on the Left.



Comparative Analysis of Enzyme Processivity of WT RT and RT Enzymes Containing Specific Mutations. Processivities Were Determined by the Size Distribution of DNA Products in Fixed-Time Experiments. The Figure Shows a Gel From a Representative Experiment.

Conclusions: In Summary, we Have Shown that the N348I Mutation alone or in Combination with M184V, can Prevent or Delay the Emergence of the E138K Substitution by Diminishing Viral Replication Capacity.

We Have also Provided Mechanistic Insights into the Reasons for This Decreased Replication Capacity at Enzyme Level.