



# The V165I and T206S/S230N Mutations in Human Immunodeficiency virus-1 Integrase Confer Resistance

## to the Pyranodipirimidine V-165 and Reduce Replication Capacity

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### Abstract

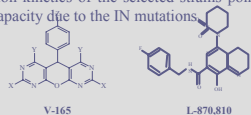
**Background:** Next to the diketo acids (DKAs), selective inhibitors of the strand transfer step of the HIV-1 integration process (integrase strand transfer inhibitors, INSTIs), the pyranodipirimidines (PDPs) were identified as a second class of authentic integrase (IN) inhibitors. V-165 is the most potent congener. PDPs interact with the binding of IN to the DNA and are thus referred to as integrase binding inhibitors or INBIs.

**Methods:** We have now studied the development of antiviral resistance to V-165 by growing wild type HIV-1 strains in the presence of increasing concentrations of this compound. The selected strains were analysed genotypically and phenotypically. Mutant integrase enzymes were generated and evaluated in an enzymatic oligonucleotide based assay and by fluorescence correlation spectroscopy for their activity, affinity for DNA and susceptibility to the different integrase inhibitors.

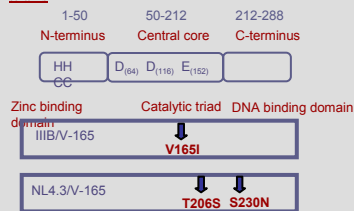
**Results:** The IN mutation V165I was identified in a selected strain starting from HIV-1(III<sub>B</sub>) whereas the double mutation T206S/S230N emerged in a selected strain starting from HIV-1(NL4.3). The mutant viruses showed a more than 9-fold reduction in susceptibility to V-165. Interestingly, a minor phenotypic cross-resistance to DKAs was observed. The specific enzymatic activity determined in the oligonucleotide assay of the V165I and T206S/S230N mutants was reduced by 2-fold as compared to wild type IN activity. Although the mutant viruses were resistant to inhibition by V-165, the corresponding enzyme displayed only a 2 to 3-fold reduction in sensitivity to V-165.

#### Figure 1: HIV-1 integrase inhibitors

The representative structures of the selected strains pointed to a hampered replication capacity due to the IN mutations.



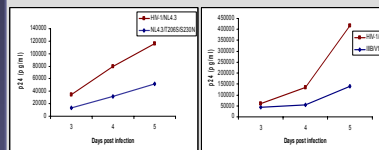
**Figure 2: Genotypic analysis of the V-165 resistant strains**



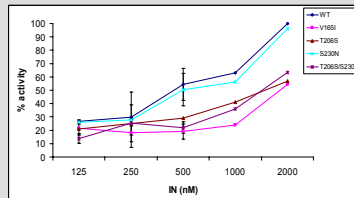
**Table 1: Phenotypic analysis of the V-165 resistant strains**

Resistance		IC <sub>50</sub> (µg/ml)	Fold
<b>V-165</b>	HIV-1(III <sub>B</sub> )	5.4 ± 1.7	
	III <sub>B</sub> /V165I	> 50	> 9.2 x
	HIV-1(NL4.3)	4.9 ± 1.8	
	NL4.3/T206S/S230N	32.1 ± 15.6	6.7 x
<b>L-870,810</b>	HIV-1(III <sub>B</sub> )	0.036 ± 0.041	
	III <sub>B</sub> /V165I	0.155 ± 0.207	4.3 x
	HIV-1(NL4.3)	0.007 ± 0.002	
	NL4.3/T206S/S230N	0.008 ± 0.001	1.2 x
<b>Ritonavir</b>	HIV-1(III <sub>B</sub> )	0.078 ± 0.026	
	III <sub>B</sub> /V165I	0.067 ± 0.008	1.2 x
	HIV-1(NL4.3)	0.081 ± 0.053	
	NL4.3/T206S/S230N	0.057 ± 0.036	0.7 x

**Figure 3: Replication capacity**



**Figure 4: Specific enzymatic activity determined in an oligonucleotide based assay**



**Table 2: Susceptibility of the different enzymes towards V-165 determined in the oligonucleotide based assay**

	IC <sub>50</sub> (ng/ml)	Fold increase
WT	1.8 ± 0.9	
V165I	3.3 ± 1.5	2.7 x
S230N	3.0 ± 1.8	2.1 x
T206S/S230N	3.7 ± 1.6	2.2 x

**Table 3: Affinity of the mutated IN enzymes for DNA and susceptibility to different integrase inhibitors as determined by fluorescence correlation spectroscopy (FCS)**

#### A. Affinity for DNA

	K <sub>a</sub> (µM <sup>-1</sup> )	Fold decreased affinity for DNA
WT	17.47 ± 1.46	
V165I	10.77 ± 0.28	1.6 x
T206S	ND	ND
S230N	3.72 ± 0.46	4.7 x

#### B. Susceptibility to V-165

	K <sub>i</sub> (nM)	Fold resistant
WT	0.037 ± 0.006	
V165I	0.131 ± 0.026	3.5 x
T206S	0.062 ± 0.0012	1.7 x
S230N	ND	ND
T206S/S230N	0.065 ± 0.001	1.8 x

#### C. Susceptibility to L-870,810

We measured by FCS the effect of the mutations on the binding of the IN enzyme to DNA. L-870,810 is an inhibitor of the strand transfer reaction of IN and therefore no inhibitory effect of L-870,810 could be determined at concentrations up to 1 mg/ml.

### General conclusion

Passaging of HIV-1 in the presence of V-165 resulted in V165I and T206S/S230N mutations in HIV IN. These mutations were associated with reduced replication capacities of the selected virus strains. We confirmed the importance of these IN mutations in an enzymatic assay and in a DNA binding assay using FCS.

Apparently, these mutations interfere with the enzymatic activity and DNA binding capacity of HIV-1 IN. This may explain the difficulty in selecting PDP-resistant strains in cell culture.