

POSTER 42**NEW MECHANISM OF HIV RESISTANCE: INVESTIGATING CLEAVAGE SITE MUTATIONS IN GAG**

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Inhibitors of HIV protease are important antiviral drugs. However, their effect is compromised by rapid resistance development. It has been shown that selection of mutations in the protease coding region is followed by mutations in the HIV polyprotein cleavage sites that compensate for the impaired proteolytic activity of the mutated protease.

In a recent paper (Nijhuis *et al.* 2007), mutations in the NC-p1 cleavage site of HIV Gag that can cause resistance to protease inhibitor drugs were described. Interestingly, these mutations were selected without prior mutations in the HIV-1 protease. In order to gain insight into the underlying mechanism that causes this resistance we designed 13-mer substrates (ERQAN-FLGKIWPS, wild type) mimicking the NC-p1 cleavage site of the wild-type and mutated virus strains from patients as well as from *in vitro* evolution experiments. The peptides were digested with wild type HIV-1 protease, with subsequent separation of substrates and products using HPLC. Integration of peak area was performed in order to quantify the concentrations. The peptide ERQAN-FLGETWPS showed a 2.4 fold higher activity than the wild type peptide, which correlates with the increased replicative capacity *in vitro* of the corresponding mutated virus. For this peptide a unique cleavage pattern was observed, yielding four products. The identities of these products were determined using LC-MS and preparative HPLC. In order to validate the use of peptide substrates for investigation of cleavage efficiency in the NC-p1 site, we cloned, expressed, and purified the full length double mutant and wild type NCp1 fusion proteins and analyzed the cleavage of these polypeptides by HIV PR. The consequences of these findings for resistance development in HIV will be discussed