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INTRODUCTION

Little is known about the impact of the viral genetic background on the nature and the pattern of protease mutations emerging in the course of resistance.

There is substantial sequence variation in gag, the main substrate of the protease, which affects principally the p17 matrix (MA) and p6 proteins.

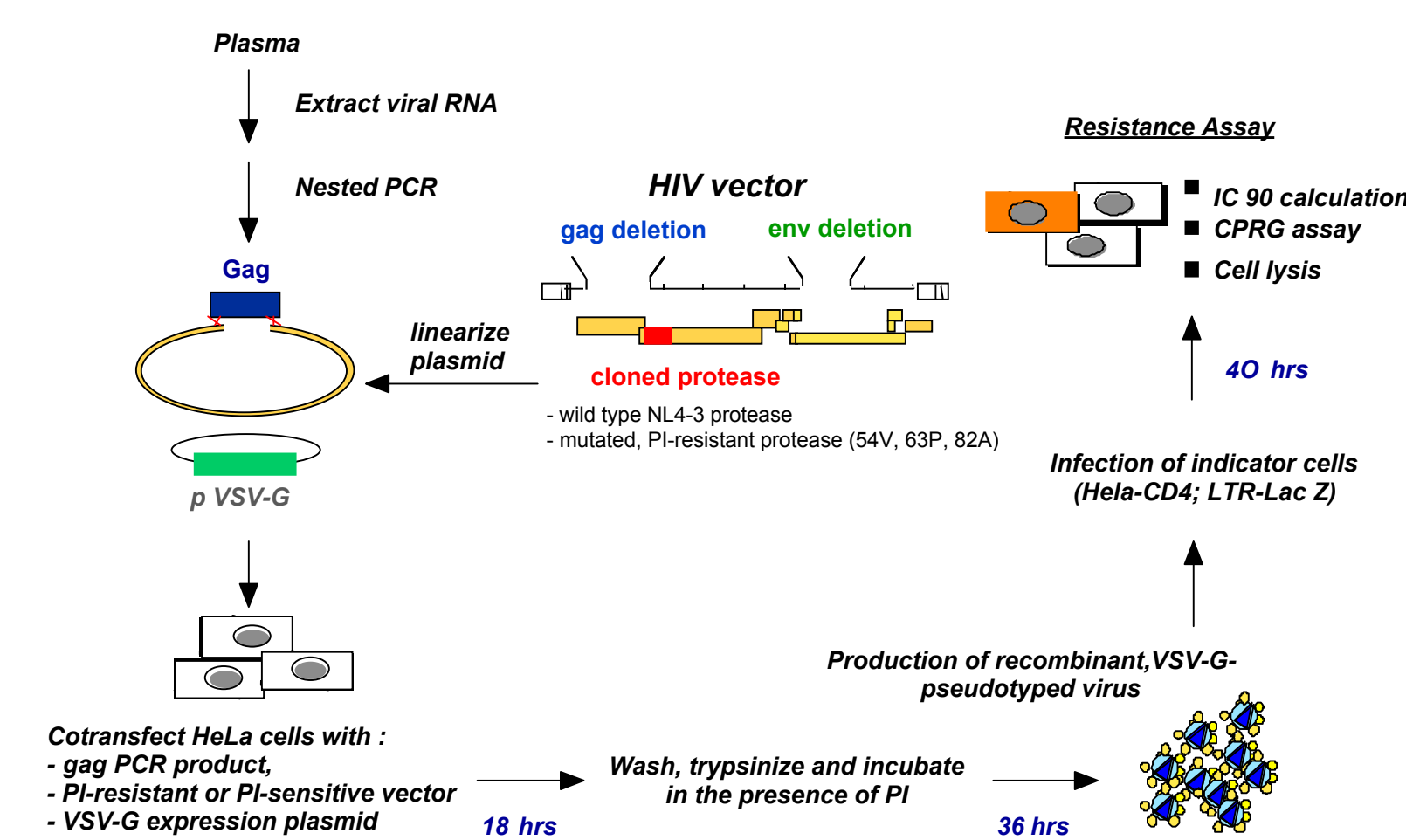
Although a few gag aminoacid changes in cleavage sites have been clearly associated with resistance to protease inhibitors, the impact of gag polymorphisms on the development of resistance to protease inhibitors is not known.

In this study we have examined the effects of different primary gag sequences on the level of susceptibility to protease inhibitors of viruses carrying either wild-type or mutant protease sequences.

METHODS

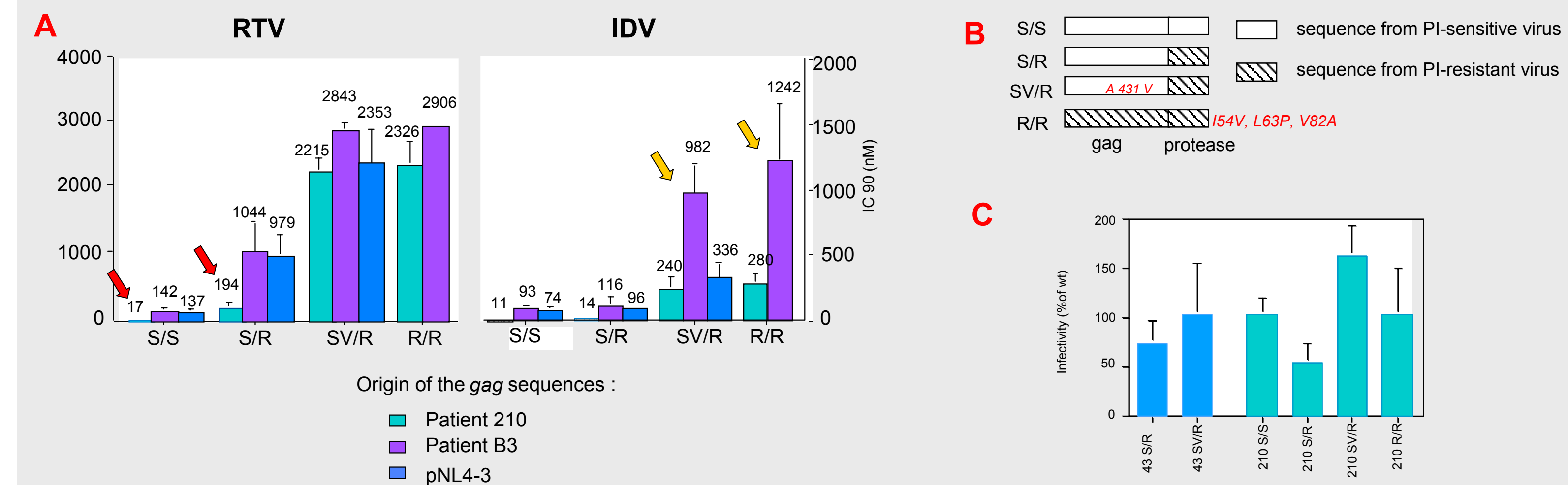
In the experiments depicted on fig.1 and fig.3, primary gag sequences were cloned next to either a WT pNL4-3 protease or to a resistant protease carrying mutations I54V, L63P and V82A, and the resulting recombinants were tested for PI susceptibility.

In the experiments depicted on fig.2, we used a specifically designed rapid gag recombinant virus assay based on cotransfection of PCR-amplified gag sequences with a gag-deleted clone carrying either a WT or mutant protease (see below).



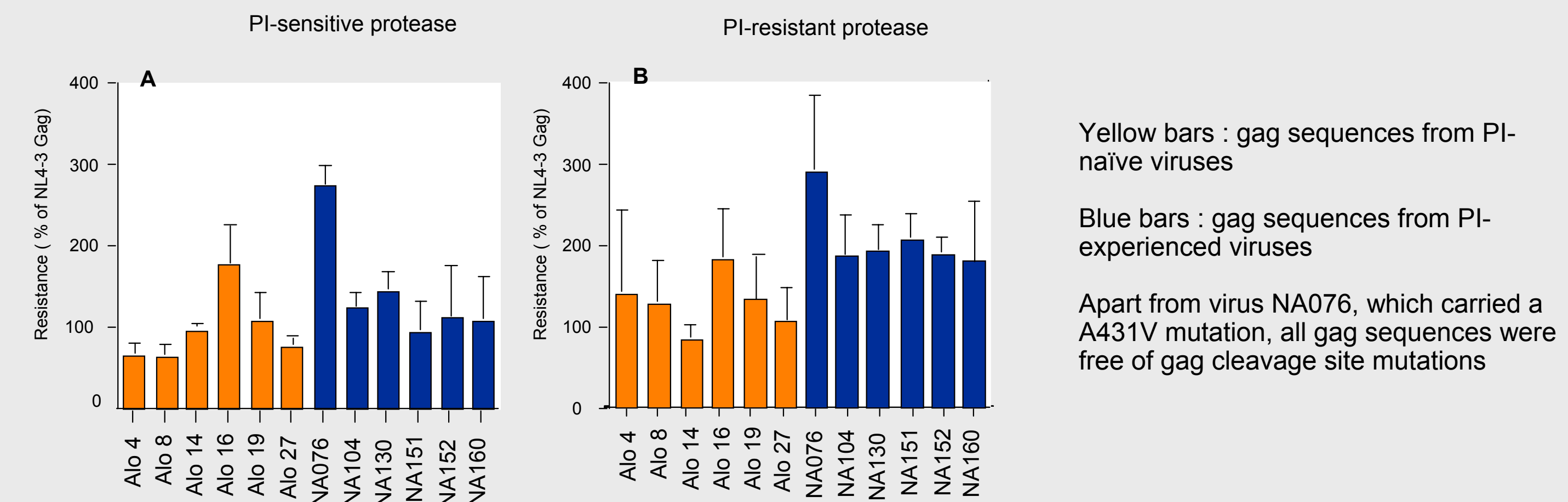
RESULTS

Figure 1 : effect of primary gag sequences on PI susceptibility



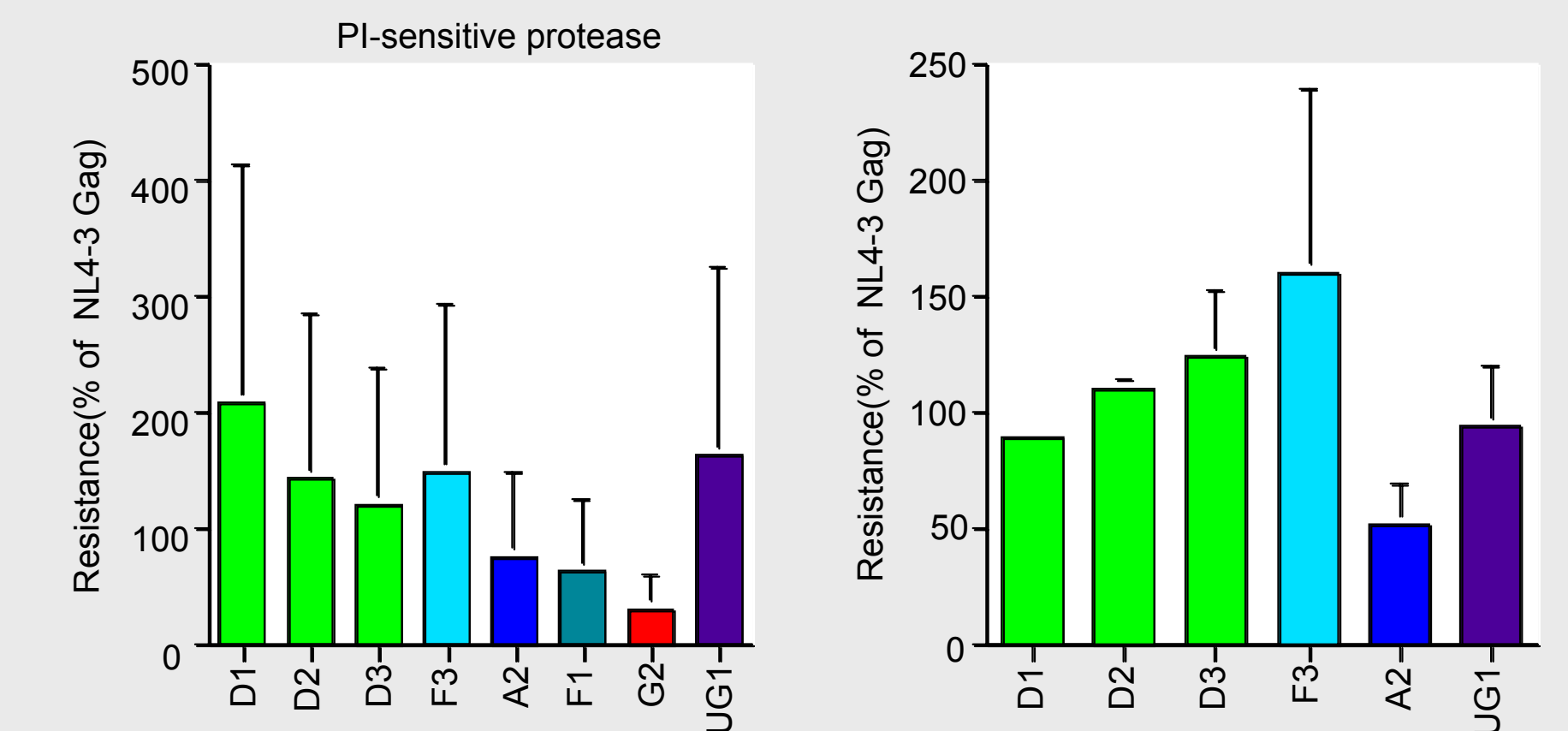
- Gag sequences from two viruses obtained before and after PI escape were tested for their effect on RTV and IDV susceptibility of recombinant virus carrying a sensitive or a resistant protease (panel B)
- Gag sequence from PI-sensitive virus 210 significantly increases RTV and IDV susceptibility of both the sensitive and the resistant protease (panel A, red arrows). In the context of primary gag sequence B3, mutation A431V in Gag increases IDV resistance of the mutated protease (panel A, yellow arrows).
- Increased susceptibility conferred by 210 gag cannot be explained by decreased replicative capacity (panel C)

Figure 2 : effect of primary gag sequences from PI-naïve or PI-exposed viruses



- The impact of primary gag sequences on RTV susceptibility was evaluated using a variant of the recombinant virus assay (see materials and methods).
- Notable disparities in the effect of different primary PI-naïve gag sequences were observed (panel A, yellow bars).
- Gag sequences from PI-exposed viruses appeared to induce a significant increase in the relative IC90 of RTV (panel B, blue bars), even in the absence of gag cleavage site mutations.

Figure 3 : effect of non-subtype B Gag sequences



Some gag sequences from non-B subtypes markedly affect PI susceptibility of WT or mutant protease, relative to control NL4-3 gag.

CONCLUSIONS

Variations in primary gag sequences can significantly affect susceptibility to protease inhibitors, whether paired to a wild-type or a resistant protease.

Increased susceptibility conferred by gag is not explained by decreased replicative capacity

Gag-related variations in PI susceptibility may be particularly important for viruses from non-B subtypes

Evolution of gag under pressure by protease inhibitors may affect susceptibility of the associated protease, due to changes that are distinct from well-characterized resistance-associated mutations in gag cleavage sites.