#692 - Subtype-dependent Resistance to Protease Inhibitors by the Human Immunodeficiency Virus Type 1 (HIV-1) Protease L89M Polymorphism.

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Background : to evaluate the possible influence of HIV-1 subtype non-B polymorphism L89M on the protease for resistance to each of the six FDA-approved protease inhibitors (PIs). This Leu to Met polymorphism is the same found on the neighbor amino acid (aa.), L90M, that confers resistance to Saquinavir (SQV) and Nelfinavir (NFV).

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CONSENSUSB  PQITLWQRPL VTIKIGGQLK EALLDTGADD TVLEEMNLPG RWKPKMIGGI GGFIKVRQYD QILIEICGHK AIGTVLVGPT PVNIIGRNLL TQIGCTLNF 99
PNL43 (Bwt) .........I .......... .......... ...G....R .......... .......... ..T.G..... .......... .......... ......... 99
TVGG13(Fwt) .......... .....N..V... ..R..... .......... ....DX.... K..... ..... .....K... N.C..... .......... ...S..... .....M.. ......... 99
CONSENSUSF  .......... ....V...R. .......... ....DI.... K..... ..... .....K... ..P....Q. .......... .....M.. ......... 99
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Figure 1.

Methods : *Molecular Virology* - site-directed mutageneses were performed over plasmid-cloned drug susceptible protease genes of subtype B and F (Bwt and Fwt), to respectively create and reverse the M polymorphism of aa. 89; as well for the creation of L90M mutation. The same panel of 89 and/or 90 mutants was created using cloned protease genes of the two subtypes carrying complex resistance mutation profiles for Ritonavir and Indinavir (named Bmdr and Fmdr). All the constructs were phenotyped in quadruplicates for PI susceptibility by MT-4 cell–MTT based cell viability assay. Competitive assays (fitness assays) were realized for the mutants, using the Bwt 89L90L clone as the reference virus. The viral relative concentrations in each point collected were evaluated by Snapshot multiplex kit assay (Applied Biosystems, USA). *Molecular modelling* - the crystallographic structures of unbound protease and the nelfinavir-protease complex were retrieved from Protein Data Bank (PDB) under codes 3PHV and 1OHR, respectively. The nelfinavir topology was built from the crystallographic coordinates using the PRODRG server. Atomic charges for the protonated ligand were obtained from *ab initio* calculations. This structure was submitted to full geometry optimization using *ab initio* quantum-mechanical computations at the 3-21G level with GAMESS and the minimum energy conformation submitted to single-point calculation at the 6-31G** level. Each protease structure was mutated, giving the HIV-PrB-L89M and HIV-PrF (I15V, E35D, M36I, R41K, R57K, Q61N, L63S, and L89M) structures (Figure 1). These protease structures were solvated in a bath of SPC water molecules with chloride ions. The Lincs and Settle methods were applied with Particle-Mesh Ewald method. The simulation lengths were 3.2 ns for each protease structure. The first 500ps were considered equilibration. Restraints were not used.

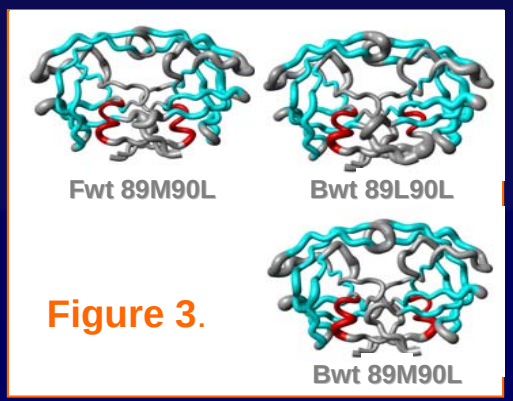

Figure 3.

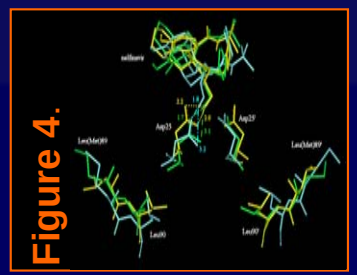

Figure 4.

Results : L89M mutation conferred 2-, 4.5-, 6-, 4.7-, 4.5- and 3.4-fold increases in EC50 for SQV, IDV, NFV, RTV, APV and LPV respectively, on Fwt 89M90L virus; comparable to the fold resistance values obtained with Bwt 89L90M virus. Surprisingly, Bwt 89M90L clone behaved phenotypically similar to the susceptible Bwt 89L90L virus, excepted for IDV. While Fwt 89L90M clone presented 3-, 2-, 4-, 2-, 1.8- and 2-fold resistance increase higher than its counterpart Bwt 89L90M for SQV, IDV, NFV, RTV, APV and LPV, respectively (Table 1). The Bwt 89M90L and Bwt 89M90M replicate with a lesser extent than the reference virus, while Bwt 89L90M had minimally increase replicative fitness compared to Bwt 89L90L (Figure 2). The Fwt 89M90L had a replication capacity similar to that reference virus, while Fwt 89L90M replicates better than Bwt 89L90L. Fwt 89L90L and Fwt 89M90M possess a decrease in their replicative capacity compared to the Bwt reference. As it can be seen in figure 3, the three complexes shared similar features but, in general, HIV-PrF (C) showed to be more rigid (lower rsmd values). Most of the hydrogen-bond interactions between NFV and HIV-Pr were maintained during the MD simulations. This interaction was not noticed in the corresponding complexes of NFV with HIV-PrF, HIV-PrB-L89M complexes or in the crystallographic structure and must be taken into account for the recognition of this inhibitor. To confirm these results, MD simulations of free (not complexed with NFV) HIV-PrB and HIV-PrB-L89M showed that the Leu89→Met89 mutation induced an increase of almost 0.5 Å in the distance Asp25-Asp25' (Figure 4).

Table 1

clones		Fold Resistance [a] ± SD[b] SAQUINAVIR (2,5)	INDINAVIR (3,0)	RITONAVIR (3,5)	NELFINAVIR (4,0)	AMPRENAVIR (2,5)	LOPINAVIR (4,0)
Bwt	89L90L	1,00 ± 0,127	1,00 ± 0,625	1,00 ± 0,124	1,00 ± 0,314	1,00 ± 0,192	1,00 ± 0,159
	89M90L	1,00 ± 0,329	2,96 ± 0,095	0,77 ± 0,135	1,10 ± 0,069	1,47 ± 0,136	0,77 ± 0,240
	89L90M	2,12 ± 0,480	2,80 ± 0,871	3,82 ± 0,819d	5,46 ± 0,956[d]	2,90 ± 0,173[d]	1,48 ± 0,239
	89M90M	1,67 ± 0,399	2,04 ± 0,020	2,71 ± 0,303	4,44 ± 0,410[d]	2,57 ± 0,195[d]	1,32 ± 0,150
Fwt	89L90L	0,51 ± 0,182	1,23 ± 0,331	0,33 ± 0,068	0,82 ± 0,154	0,74 ± 0,143	0,78 ± 0,104
	89M90L	2,06 ± 0,237	5,60 ± 0,150[d]	4,74 ± 1,387[d]	6,20 ± 0,556[d]	4,47 ± 0,337[d]	3,44 ± 0,607
	89L90M	6,42 ± 0,967[d]	6,51 ± 0,430[d]	7,45 ± 1,901[d]	23,39 ± 4,605[d]	5,13 ± 1,880[d]	3,87 ± 0,344
	89M90M	0,85 ± 0,256	2,42 ± 1,101	2,48 ± 0,212	2,80 ± 0,828	2,08 ± 0,528	0,90 ± 0,111

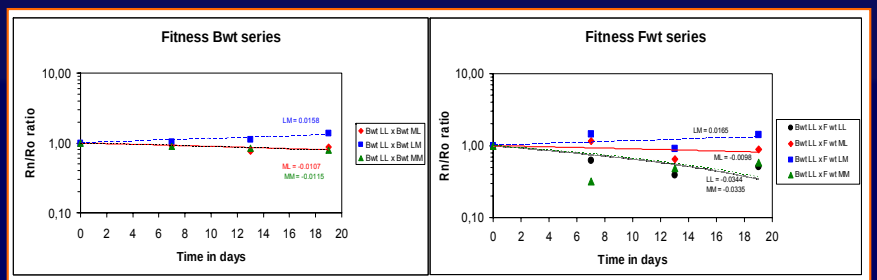

Figure 2

Conclusion: The L89M mutation impacts phenotypic resistance to PIs differently for F and B clade viruses. These results are more notable for NFV, IDV and RTV, where the 89M subtype non-B polymorphism of the Fwt virus promoved a similar or higher level of resistance than its counterpart Bwt 89L90M. The replicative fitness assay results explain the lack of L89M polymorphism on clinical samples from subtype B, as well as the rare subtype F isolates carrying L90M, due to the low fitness of F 89M90M and the needing for these viruses to accumulate the L89I mutation.

References:

Brindeiro P.A., *et al.* Testing genotypic and phenotypic resistance in Human Immunodeficiency Virus Type 1 isolates of clade B and others clades from children failing antiretroviral therapy. *J. Clin. Microbiol.* 2002, 40(12):4512-4519; **Caride, E., *et al.*** Genotypic and phenotypic evidence of different drug-resistance mutation patterns between B and non-B subtype isolates of Human Immunodeficiency Virus type I found in Brazilian patients failing HAART. *Virus Genes* 2001, 23(2):193-202; **Martinez-Picado, J., *et al.*** Replicative fitness of protease inhibitor-resistance mutants of human immunodeficiency virus type 1. *J. Virol.* 1999, 73(5):3744-3752; **Maschera, B., *et al.*** Analysis of resistance to human immunodeficiency virus type 1 protease inhibitors by using matched bacterial expression and proviral infection vectors. *J. Virol.* 1995, 69:5431-5436.

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