

In Vitro HIV-1 Resistance Selection to GS-8374,
a Novel Phosphonate Protease Inhibitor:
Comparison with Lopinavir, Atazanavir, and
Darunavir

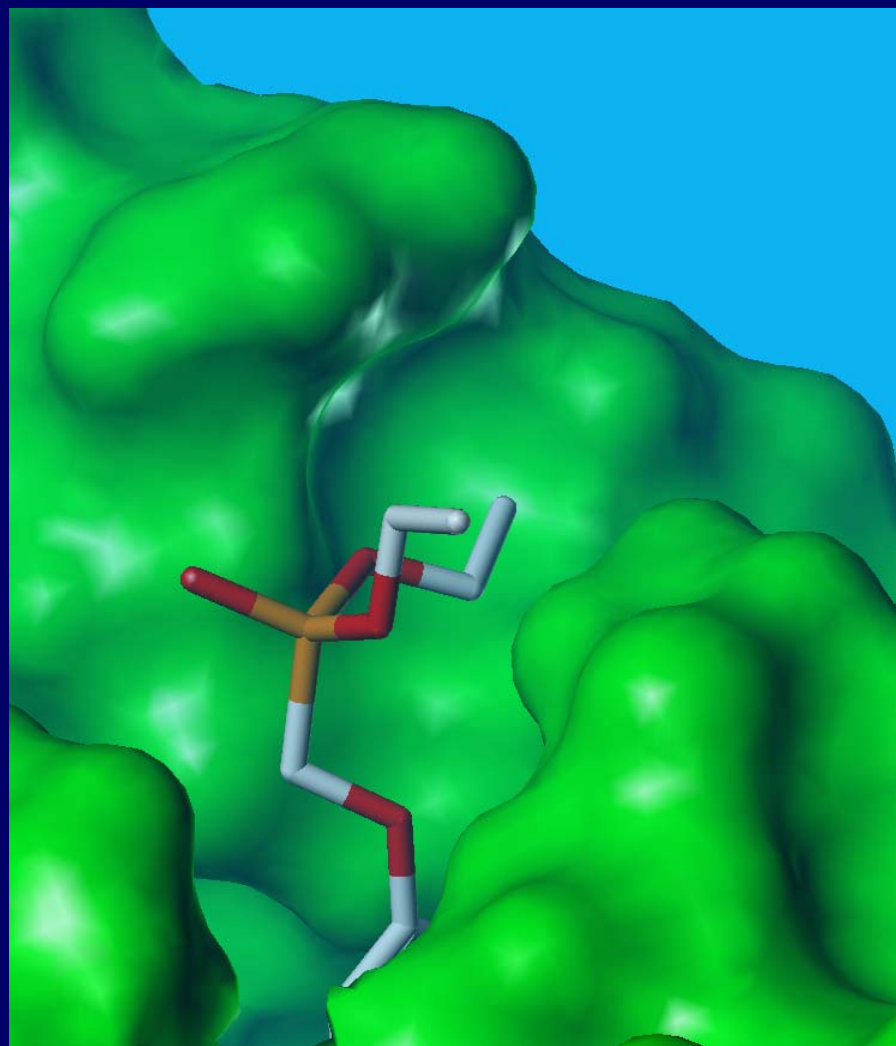
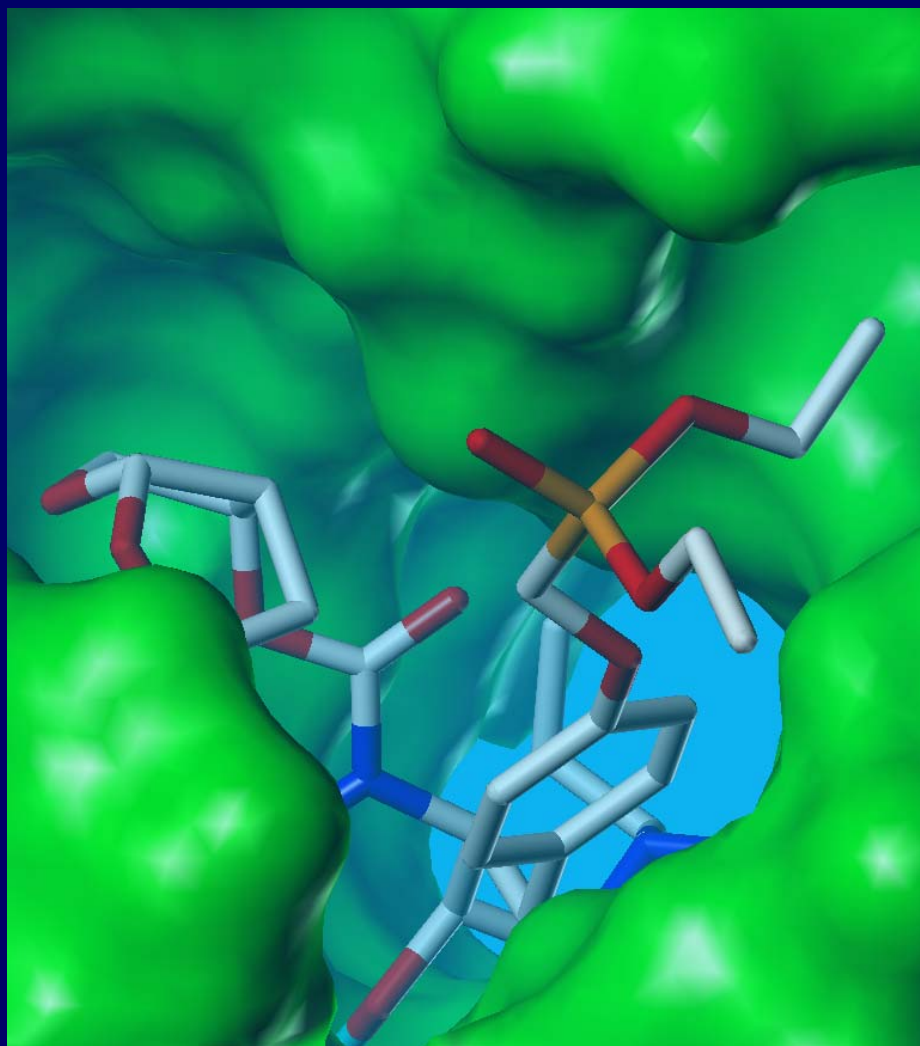
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GS-8374: Phosphonate Moiety Does Not Interact with the Protease Active Site



In Vitro Resistance Profile (PhenoSense™ assay)

- 24 viruses with high-level PI resistance (average of 10 mutations in protease)

Inhibitor	FC relative to WT		Viruses with FC>10 (n/24)	Mean EC ₅₀ [nM]
	Mean	Range		
TPV	5.9	0.5 - 27	4	624
GS-8374	6.2	0.6 - 26	3	7
DRV	29.8	1.0 - 157	16	20
SQV	62.8	1.5 - 127	19	252
APV	75.8	2.7 - 221	20	676
IDV	80.5	2.5 - 237	18	514
NFV	120	1.2 - 341	22	624
LPV	137	2.1 - 303	19	446
ATV	148	4.1 - 479	21	157
RTV	206	11 - 268	24	232

FC: fold change

(Callebaut et al, CROI 2007)

Phenotypic Susceptibility of Virus Selected by GS-8374

Virus	Mean EC ₅₀ nM, (Fold Change)							
	8374	DRV	ATV	LPV	APV	NFV	SQV	TPV
P62-ND (no drug)	6	2.8	4.5	14	12.5	6.5	3.8	85
P62-8374 (80 nM)	90 (15)	15 (5.5)	15 (3.3)	22 (1.6)	20 (1.6)	9.3 (1.4)	9.0 (2.4)	155 (1.8)

n=3

- P62-8374:**
- 15-fold reduced susceptibility to GS-8374
 - low-level cross-resistance to DRV and ATV
 - no cross-resistance to other PIs

Inhibition of Gag Processing by GS-8374

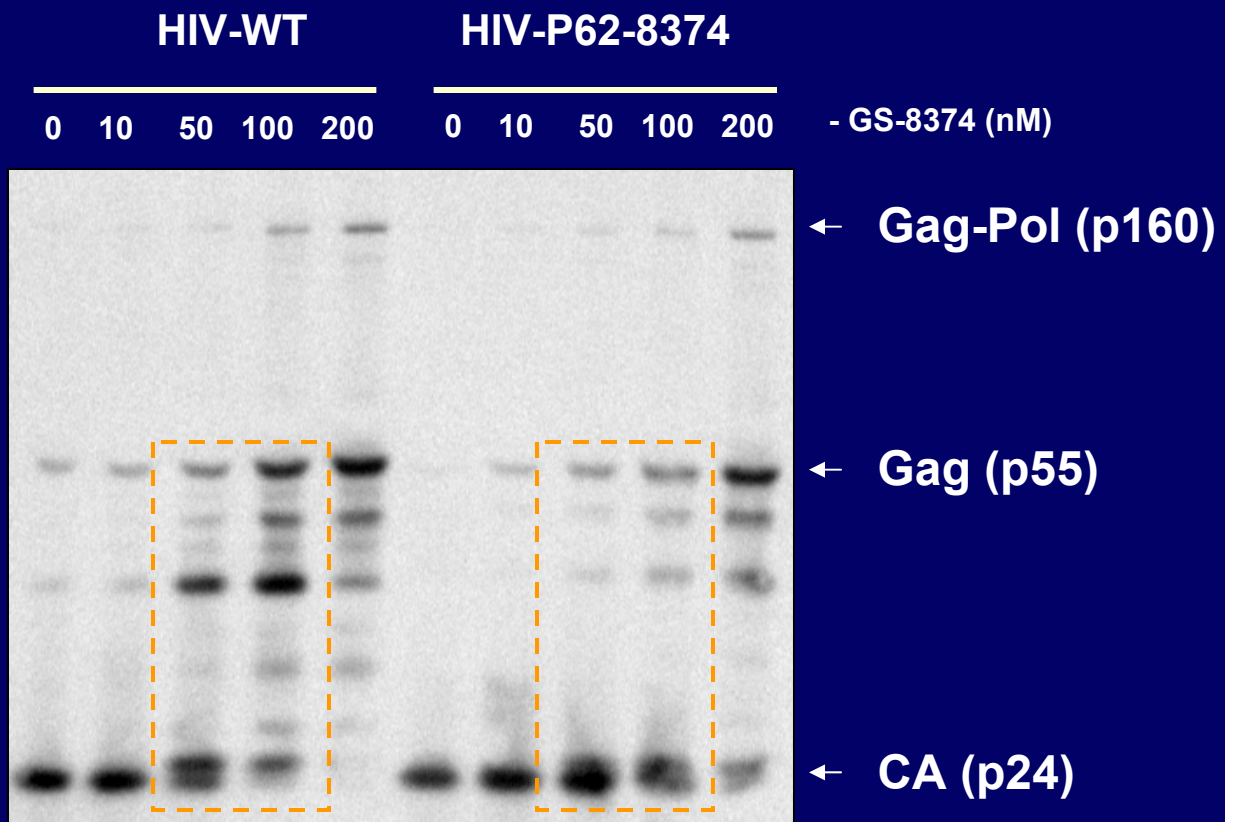
Cloning of P62-8374 gag-pol

Transfection of 293T cells

Virions harvested at 48 h

PAGE-SDS

Western-Blot anti-p24



Virus Supernatant

Single Genome Sequencing of Virus P62-8374



Gene	mutation	n/22	%
Protease	R41K	22	100
Gag	V128I, M200I, Δ 382NFR384, I401T, R409K, Y441H	1	4.5
	K112E, K113, V128I, M200I, H219P, Δ 382NFR384, I401T, R409K, Y441H	14	63.5
	K112E, K113, V128I, K162R, M200I, H219P, Δ 382NFR384, H400R, I401T, R409K, Y441H	7	32
TFP-P6* frameshift	D437N, L441P g2097a, t2110c	22	100

P62-8374 Recombinant Viruses and Resistance Profile

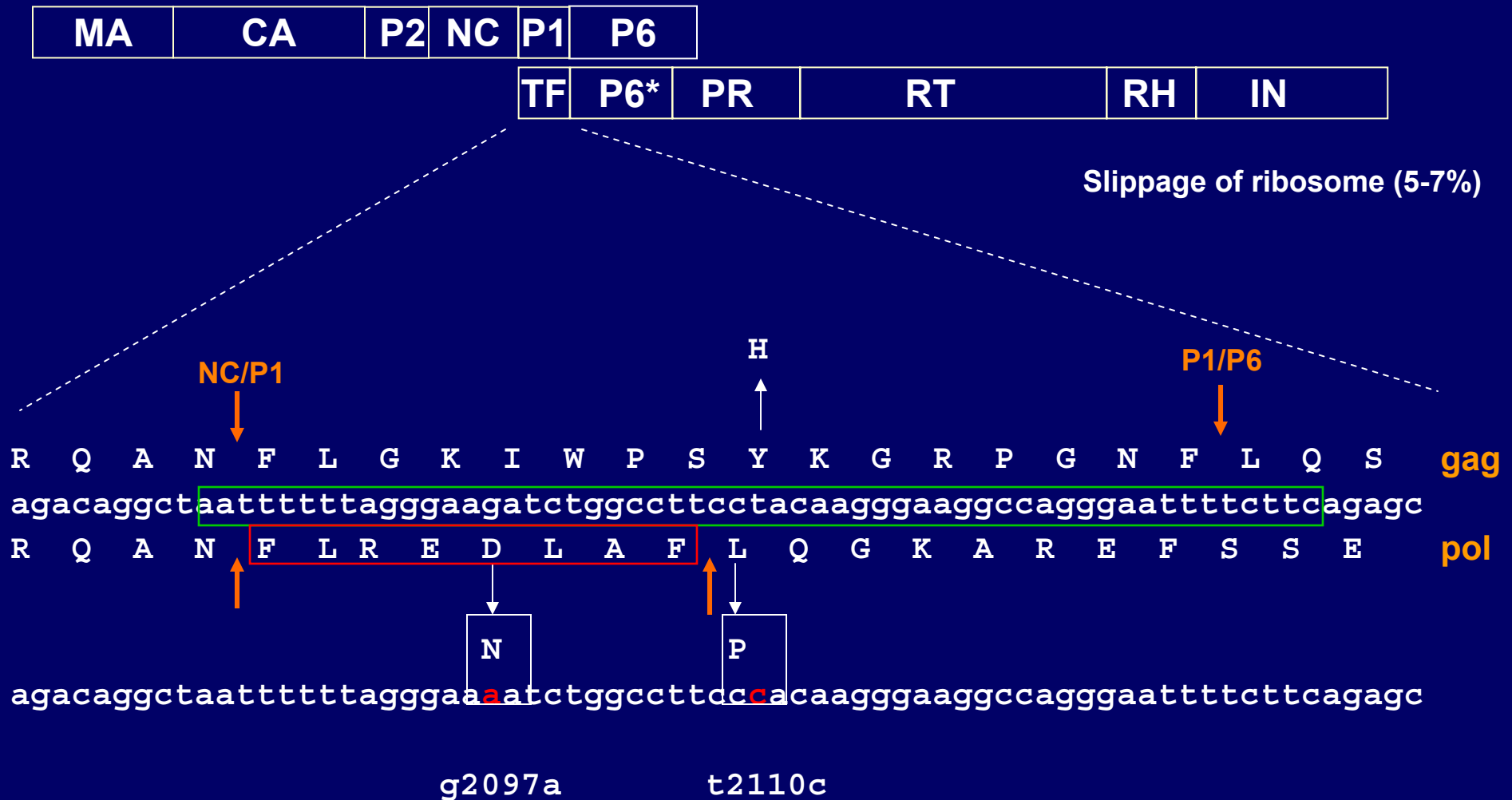


Virus	code	Mean EC ₅₀ nM, (Fold Change)				
		8374	DRV	ATV	LPV	APV
HIV - WT	-	4.0	2.5	2.7	6.8	10.3
HIV w/ Gag-Pro	A	66.3 (16.5)	19.0 (7.6)	14.7 (5.5)	25.3 (3.7)	14.5 (1.4)
HIV w/ Gag	B	60.0 (15)	13.8 (5.5)	13.3 (5.0)	29.5 (4.4)	14.0 (1.4)
HIV w/ Pro	C	5.4 (1.3)	2.8 (1.1)	3.8 (1.4)	8.5 (1.3)	10.8 (1.0)

Mutations in Gag are responsible for resistance to GS-8374

n=3

P1 Processing Sites in Gag and Gag-Pol



Possible Mechanisms of Resistance (1)

HIV-1 WT

P62-8374

```

    CA
    A  A
    UG
    CG
    CG
    UA
    UA
    CG
    CG
    GC
    GC
    UA
    CG G
    \  G
    UA A
    AU
    GU
    AU
    AU
    GC
    GU
    AAUUUUUUAG UC
  
```

$\Delta\Delta G = -2.10$
kcal/mol

More slippage ?

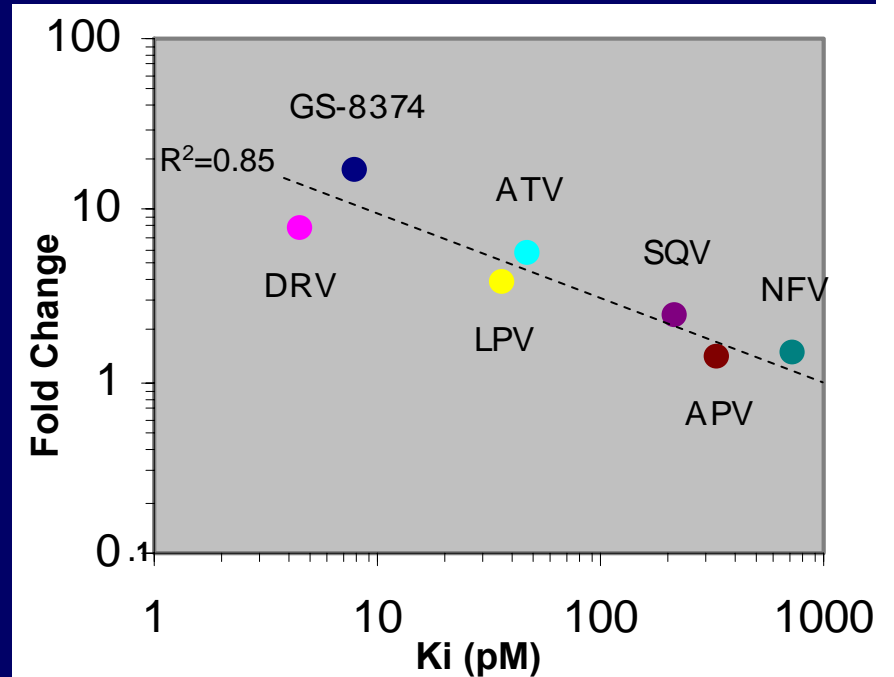
$\Delta G = -24.10$ kcal/mol

```

    CA
    A  A
    CG
    CG
    CG
    UA
    UA
    CG
    CG
    GC
    GC
    UA
    CG G
    \  G
    UA A
    AU
    AU
    AU
    AU
    GC
    GU
    AAUUUUUUAG UC
  
```

$\Delta G = -26.20$ kcal/mol

Correlation of Affinity for HIV Protease and Resistance to P62



Fold Change = $EC_{50} \text{ P62} / EC_{50} \text{ WT}$

Possible Mechanisms of Resistance (2)

Transframe Peptide (TFP)
FLREDLAF is a natural inhibitor
of HIV protease:

FLREDLAF $K_i = 98 \mu\text{M}$

FLREN**L**AF $K_i > 2000 \mu\text{M}$

Loss of inhibition ?

TFP/P6* cleavage site:

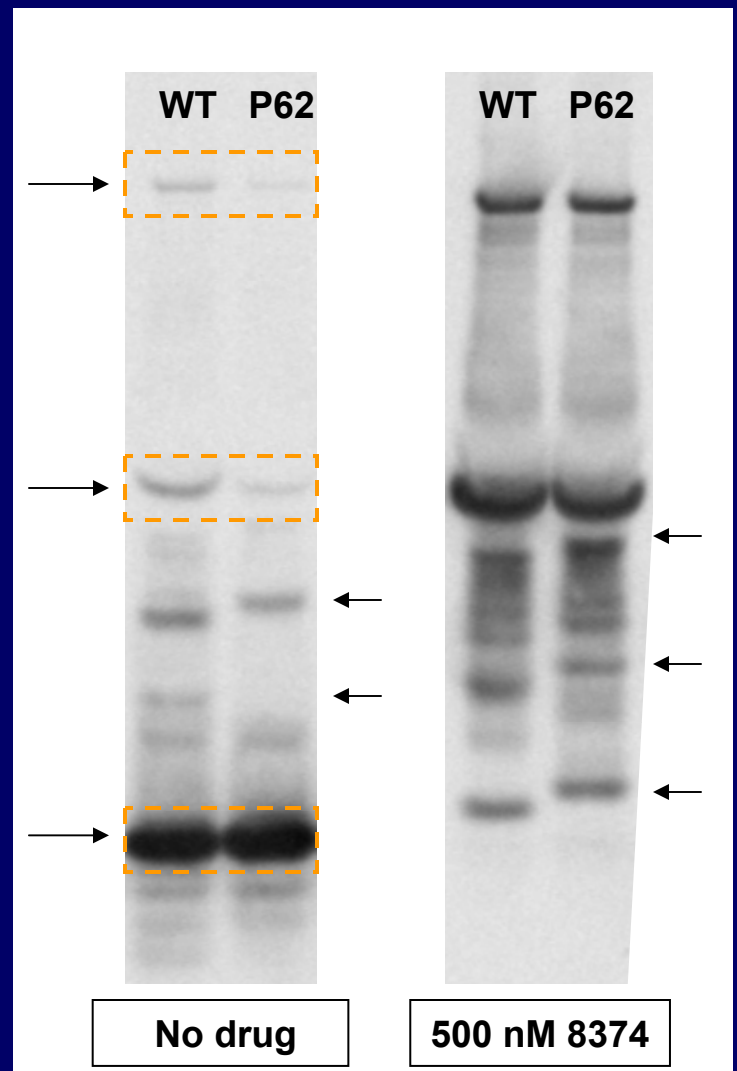
WT: DLAF LQGK
 ↓
P62: **N**LAF **P**QGK
 ↑

More efficient cleavage ?

Gag-Pol (p160)

Gag (p55)

CA (p24)



Conclusions

- **Development of *in vitro* resistance to GS-8374 is slow**
- **GS-8374-resistant selected virus shows:**
 - **Fifteen-fold reduced susceptibility to GS-8374**
 - **Low cross-resistance to atazanavir, darunavir**
 - **No cross-resistance to other tested PIs**
- **High genetic barrier for resistance with Protease mutation:**
 - **No primary mutation in Protease**
 - **Multiple mutations in Gag conferring resistance**
- **Potential mechanism of resistance:**
 - **Mutations in Gag generate more effective substrate**
 - **Mutations in frameshift lead to higher production of PR**
 - **Mutations in TFP could reduce its natural inhibition of PR**

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