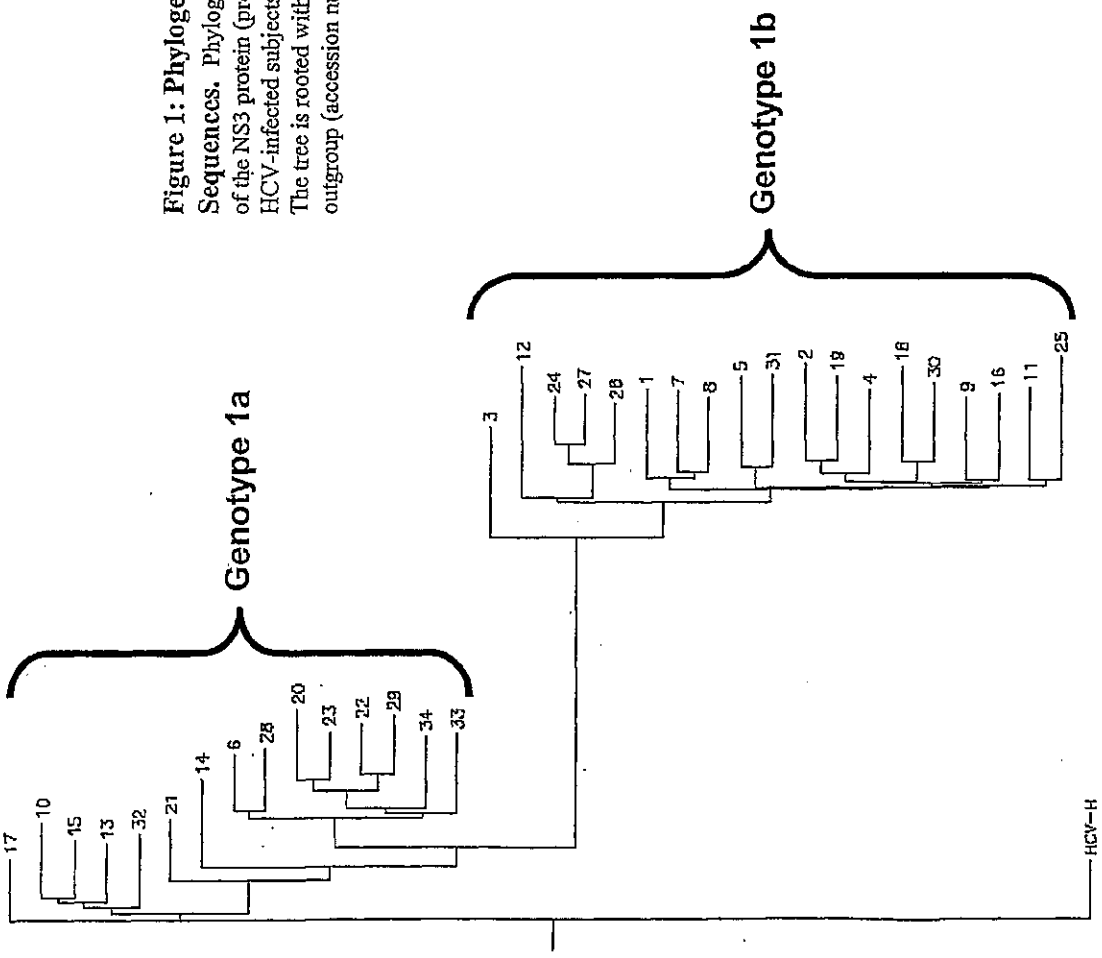


Figure 1: Phylogenetic Analysis of Baseline Sequences. Phylogenetic tree of the N-terminal 543 nucleotides of the NS3 protein (protease domain) from untreated genotype 1 HCV-infected subjects. Sequences cluster into subtypes 1a and 1b. The tree is rooted with the reference lab strain HCV-H as an outgroup (accession number M67463).

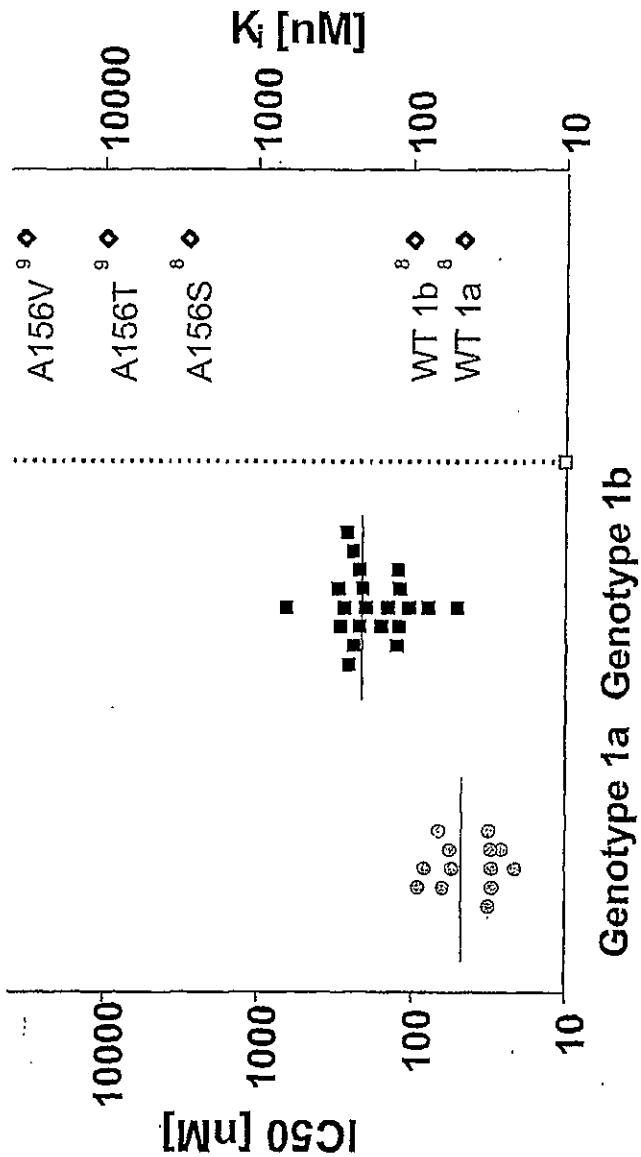
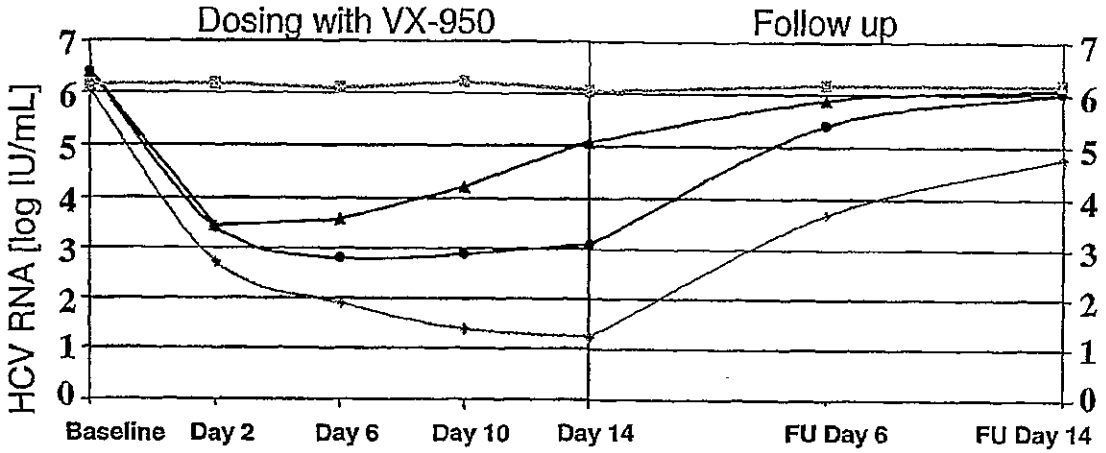


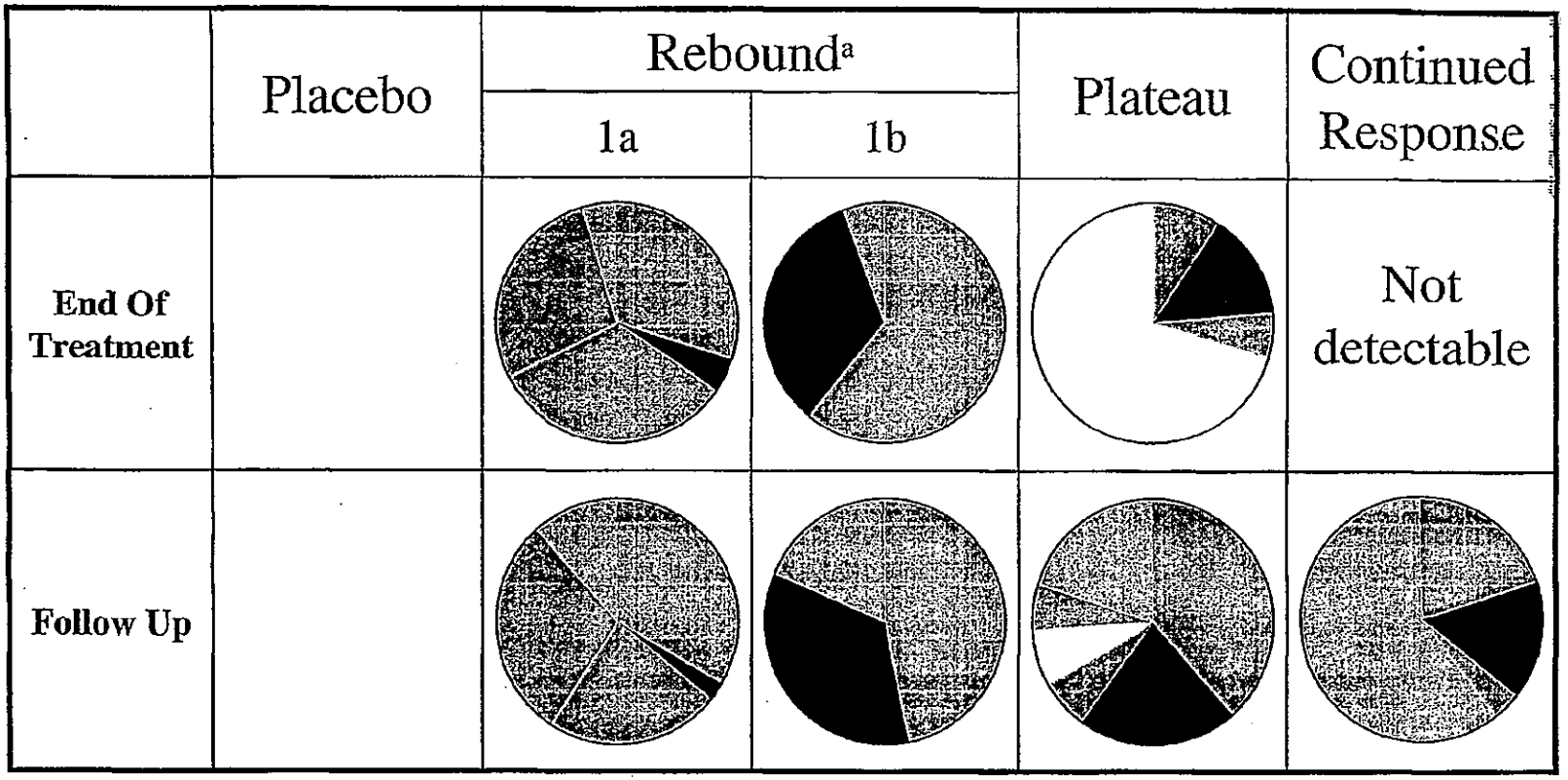
Figure 2: Baseline IC_{50}s for Genotype 1a and 1b. Protease variants from untreated subjects were expressed, purified, and tested for sensitivity to VX-950. The enzyme IC_{50} of VX-950 against each protease is shown (values are the mean of 2 independent experiments). Results are grouped by genotype 1a and genotype 1b. The line indicates the mean of all data points. Included for comparison are K_i values of previously published wild-type proteases and protease mutants known to confer resistance to VX-950 (A156V/T/S).



Subject Group	# of Subj	Definition
Placebo ■	6	No VL change
Rebound ◆	13	> 0.75 log VL change from nadir to day 14
Plateau ●	8	< 0.75 log VL change from nadir to day 14
Continued Response ▲	7	Continued decline throughout 14 days of VX-950 dosing

Figure 3: Subject Groups by Viral Response. *Subject groups are divided by HCV RNA response to 14 days of dosing with VX-950 based on mutational patterns. HCV RNA values shown are the mean values for each group of subjects.*

Figure 4: Summary of Viral Response and Mutation Patterns. Pie charts show the average percent of mutations at a given amino acid position for all subjects within a given response group.



[a] This rebound group is split by subtype to depict the fact that mutations at position 155 are only seen in 1a patients. All mutations at position 155 in plateau and continued response groups are found only in the genotype 1a patients of that group.

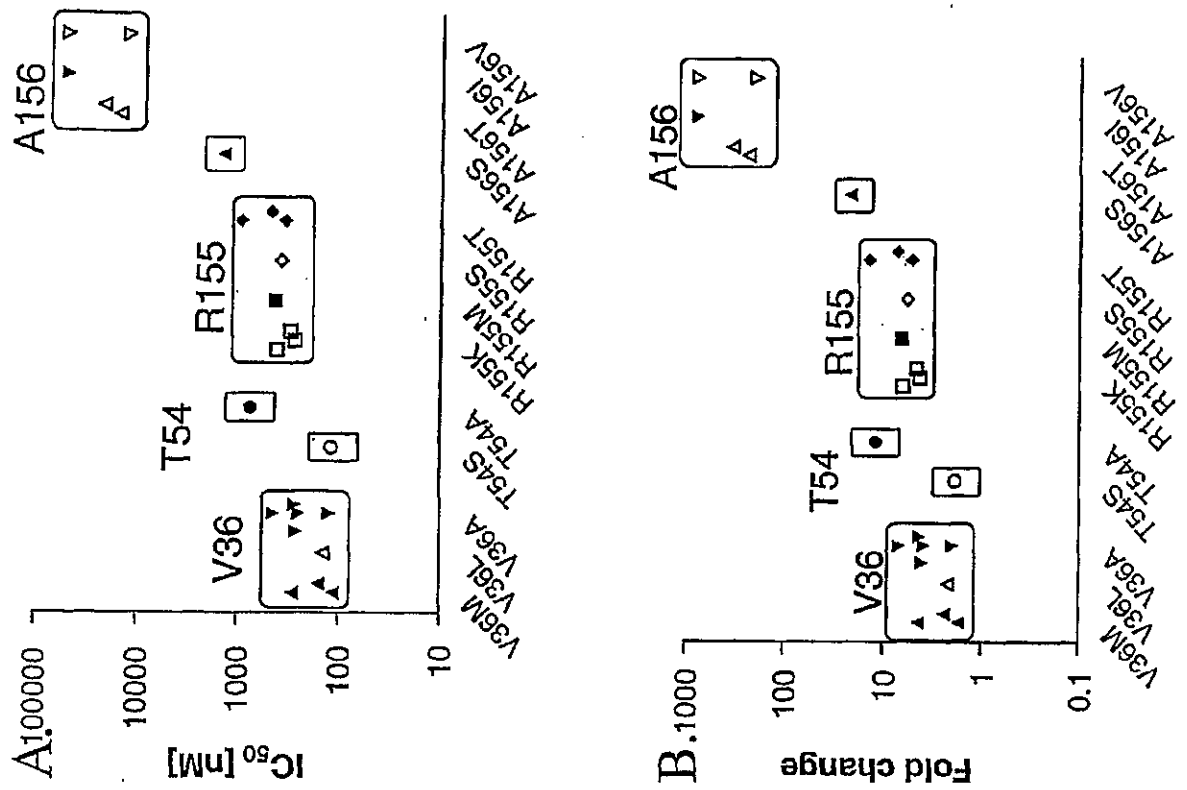


Figure 5: Enzymatic IC50 and Fold Change from Reference 1a Strain HCV-H of Protease Single Resistance Mutants to VX-950. Protease mutants selected in subjects dosed with VX-950 were expressed, purified and tested for sensitivity to VX-950. Values are the mean of 2 independent experiments for a given protease mutant sequence. The IC50 of VX-950 against each protease is shown (A) and compared to the reference strain HCV-H (B).

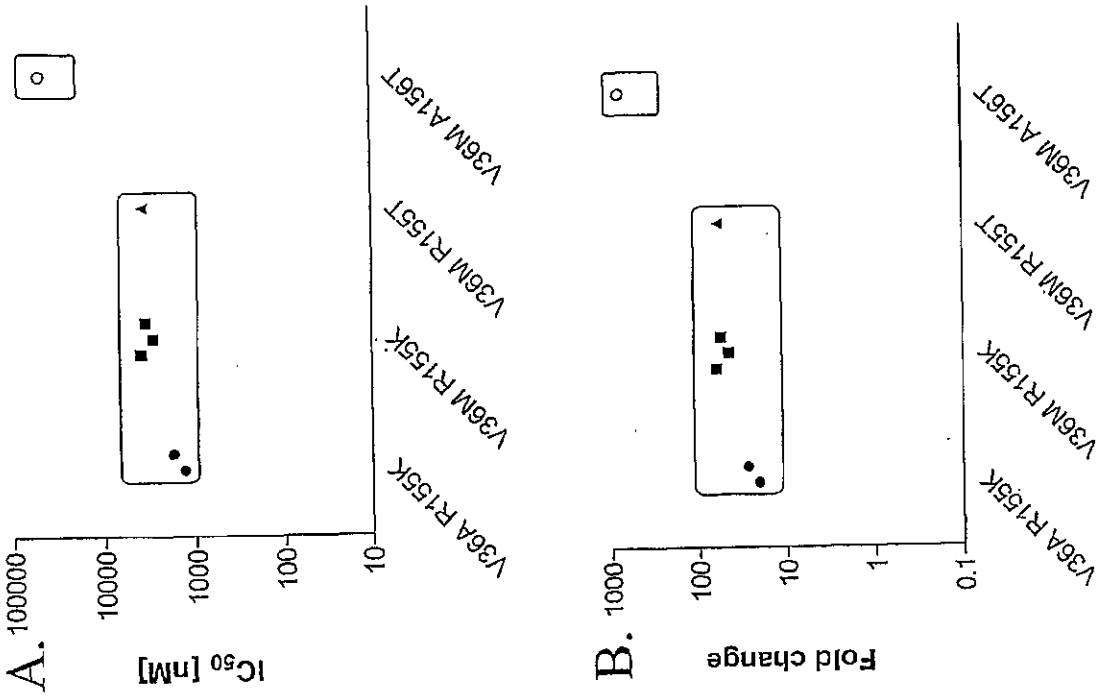
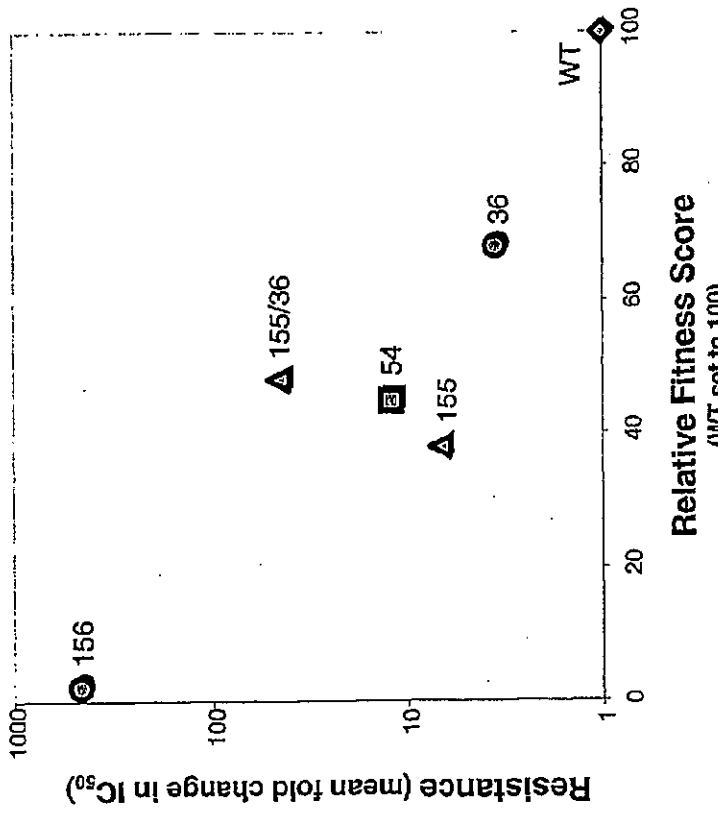
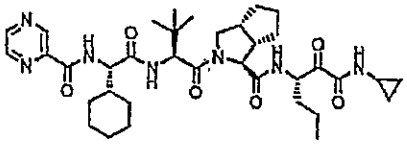


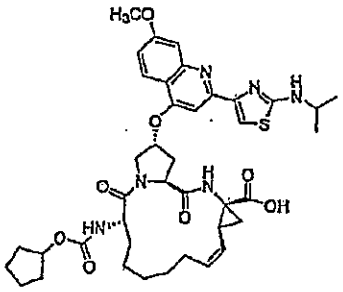
Figure 6: Enzymatic IC50 and Fold Change from Reference 1a Strain HCV-H of Protease Double Resistant Mutants to VX-950. Protease mutants selected in subjects dosed with VX-950 were expressed, purified and tested for sensitivity to VX-950. Values are the mean of 2 independent experiments for a given protease mutant sequence. The IC50 of VX-950 against each protease is shown (A) and compared to the reference strain HCV-H (B).

Figure 7: Inverse Correlation Between Resistance and Fitness





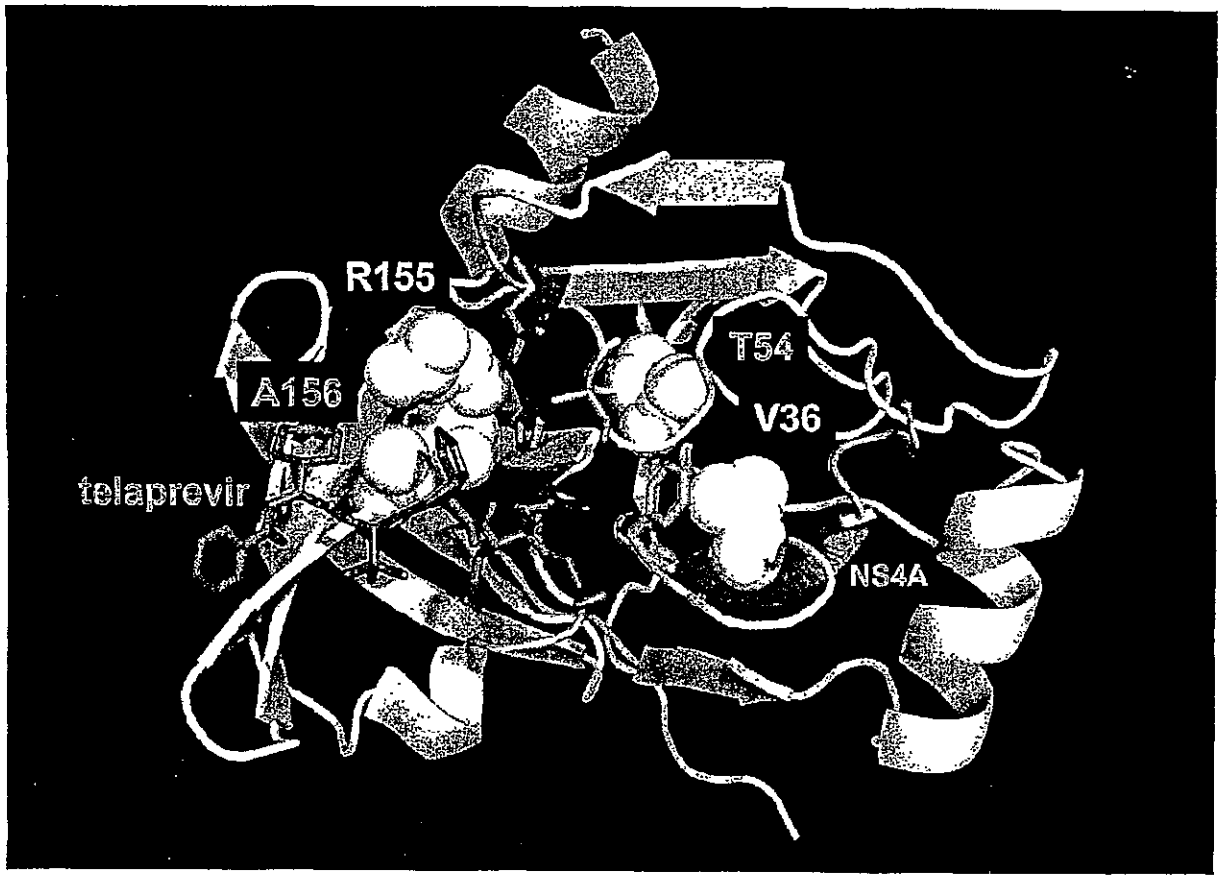
VX-950 (MW. 680)



BILN 2061 (MW. 775)

FIGURE 4 g

Location of Telaprevir-resistant Mutations in the HCV Protease



VERTEX

Figure 9

Methods for Phenotypic Analyses of Variants

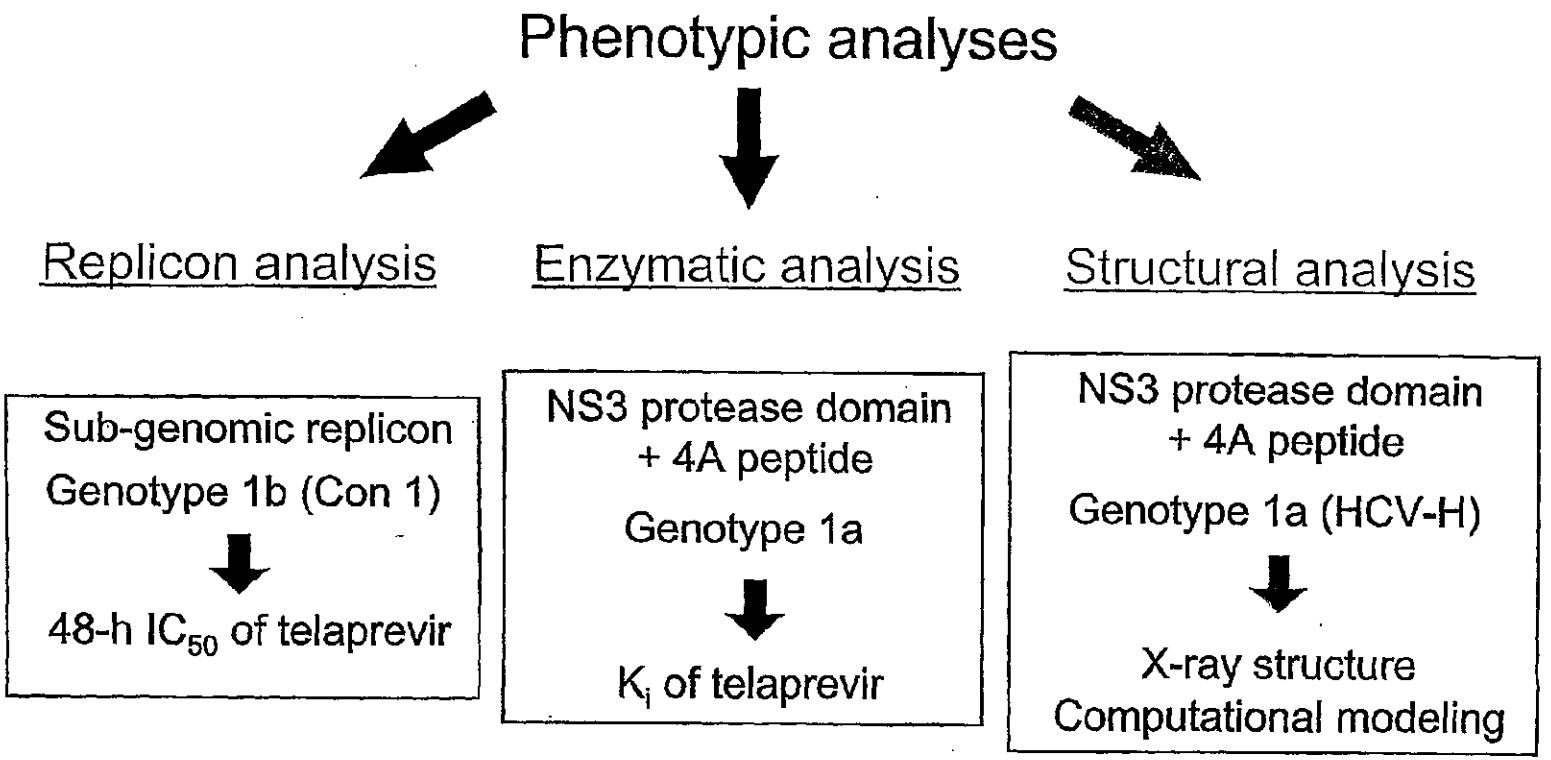


Figure 10

VERTE

V36 Substitutions Confer Low-level Resistance to Telaprevir

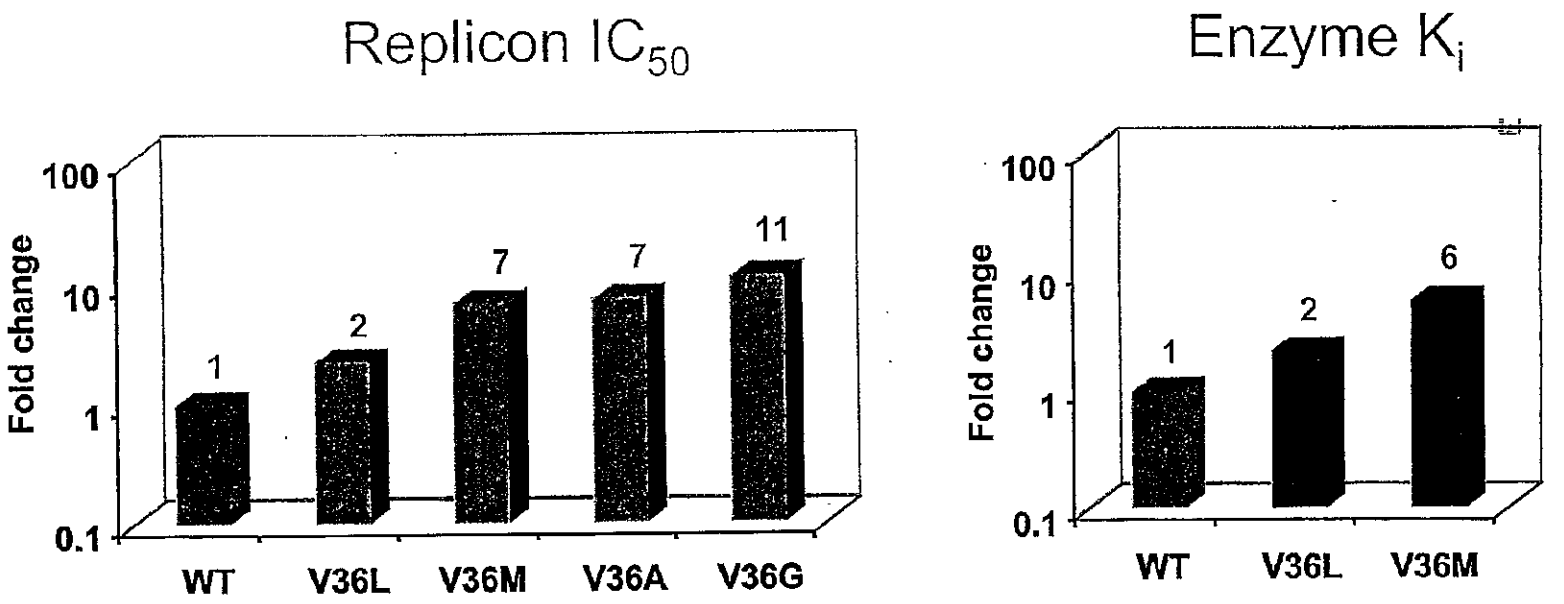
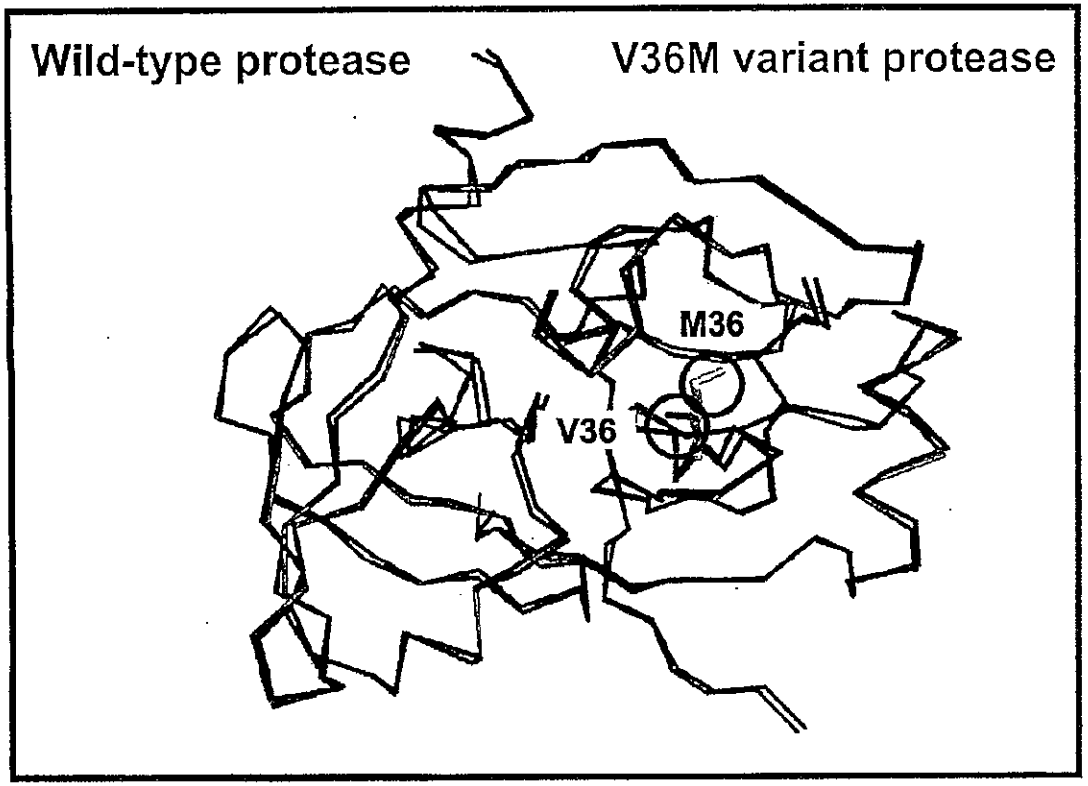


Figure 11

VERTE

X-ray Structure of the V36M Variant Protease



V36M protease

no difference from wild-type protease

- except for residues 36 and 43

VERTE

Figure 12

V36 Does not Make Direct Contact with Telaprevir (VX-950)

- V36 does not make direct contact with telaprevir (VX-950)
- V36 is located next to the NS4A cofactor ß-strand
- V36 (and T54) make hydrophobic contacts with F43
- F43, in turn, interacts with the P1' group of telaprevir (VX-950)

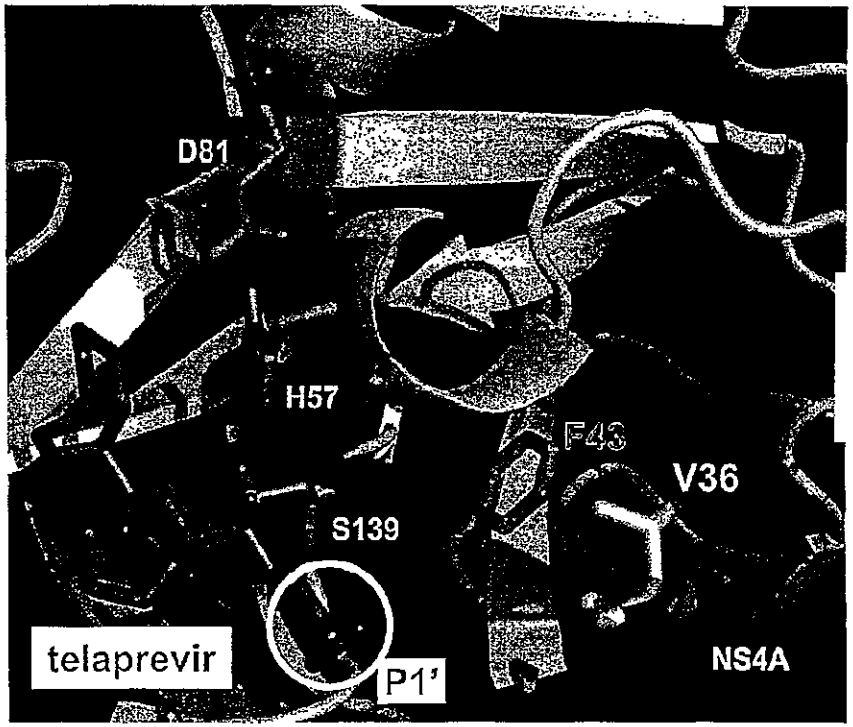
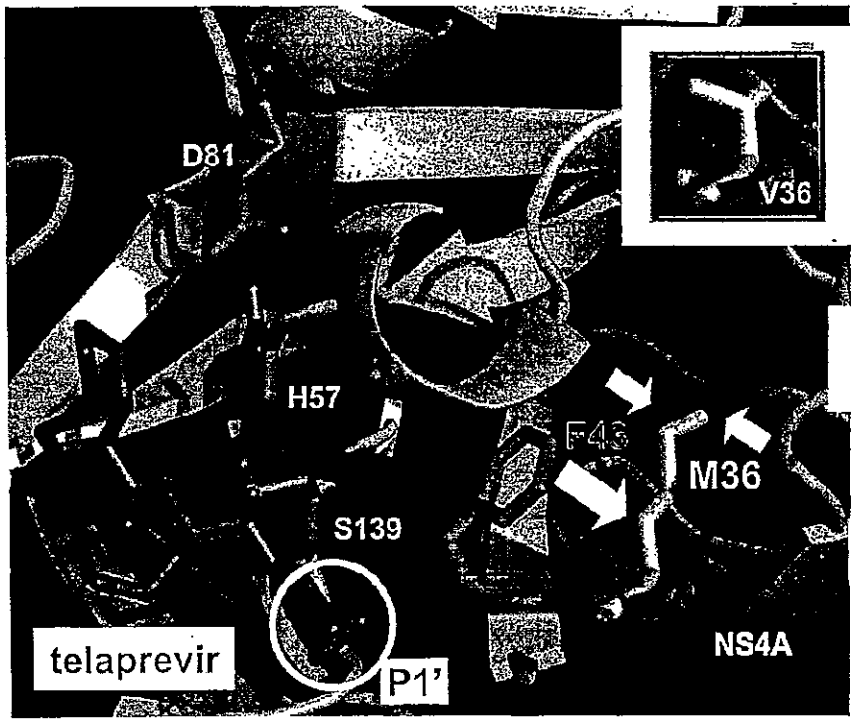


Figure 13

VERTE

V36M Variant in G1a: Low-level Resistance, More Fi

- Phenotype
  - 6-7 fold resistance to telaprevir
  - Average % of HCV RNA at day 14 in patients dosed with telaprevir alone
    - 43% in G1a (1-nt change)
    - 0% in G1b (presumably due to 2-nt change)
- Mechanism
  - Loss of hydrophobic interaction with F43 ➡ increased flexibility of F43 and P1' pocket
  - Loss of interaction with telaprevir P1' group
  - May gain additional interaction within the protease



VERTE

Figure 14

V36A Variant in G1a/b: Low-level Resistance, Less F

- Phenotype
  - Similar resistance as V36M
  - Average % of HCV RNA at day 14 in patients dosed with telaprevir alone
    - <10% in G1a (1-nt change)
    - 15% in G1b (1-nt change)
  - Less fit than V36M
- Mechanism
  - Loss of hydrophobic interaction with F43 ➡ increased flexibility of F43 and P1' pocket
  - Loss of interaction between F43 and telaprevir P1' group
  - Does NOT gain additional interaction with protease

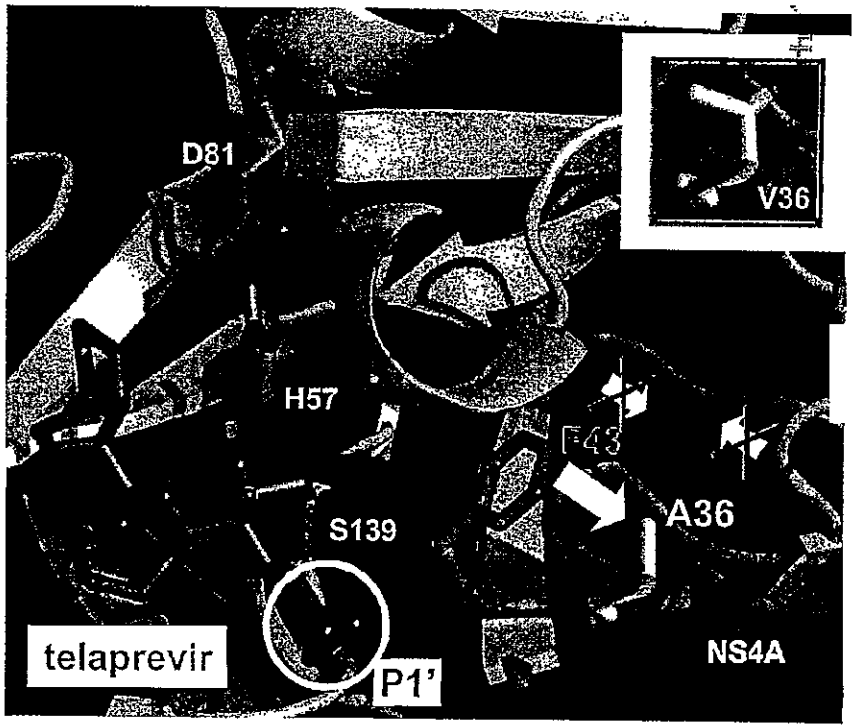


Figure 15

VERTE

V36G Variant in G1b: Low-level Resistance, Less Fit

- Phenotype
  - Similar resistance as V36M/A
  - Average % of HCV RNA at day 14 in patients dosed with telaprevir alone
    - 0% in G1a (1-nt change)
    - 14% in G1b (1-nt change)
  - Less fit than V36M
- Mechanism
  - Loss of hydrophobic interaction with F43 ➡ increased flexibility of F43 and P1' pocket
  - Loss of interaction between F43 and telaprevir P1' group
  - Does NOT gain additional interaction with protease

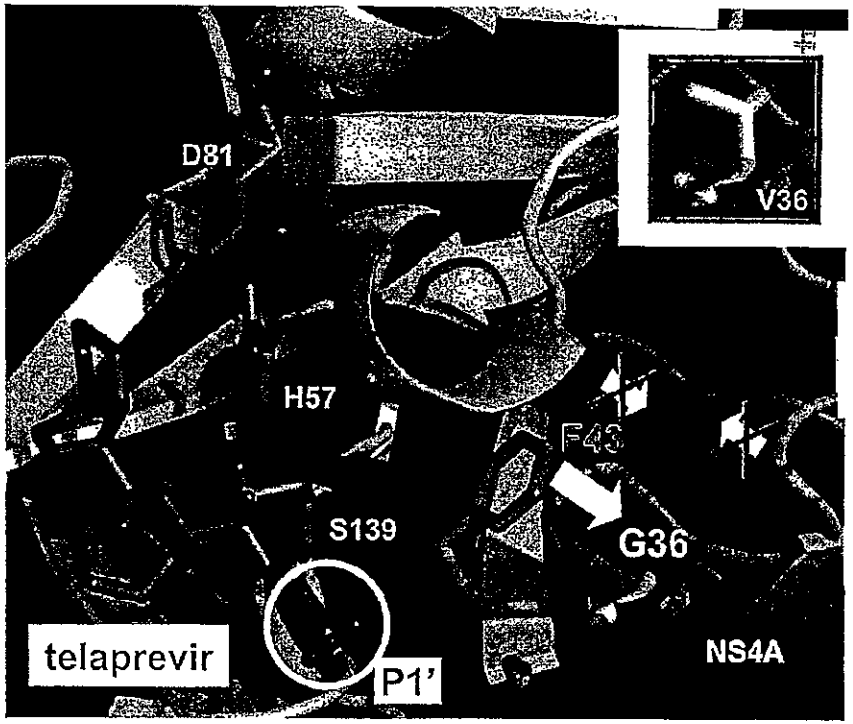
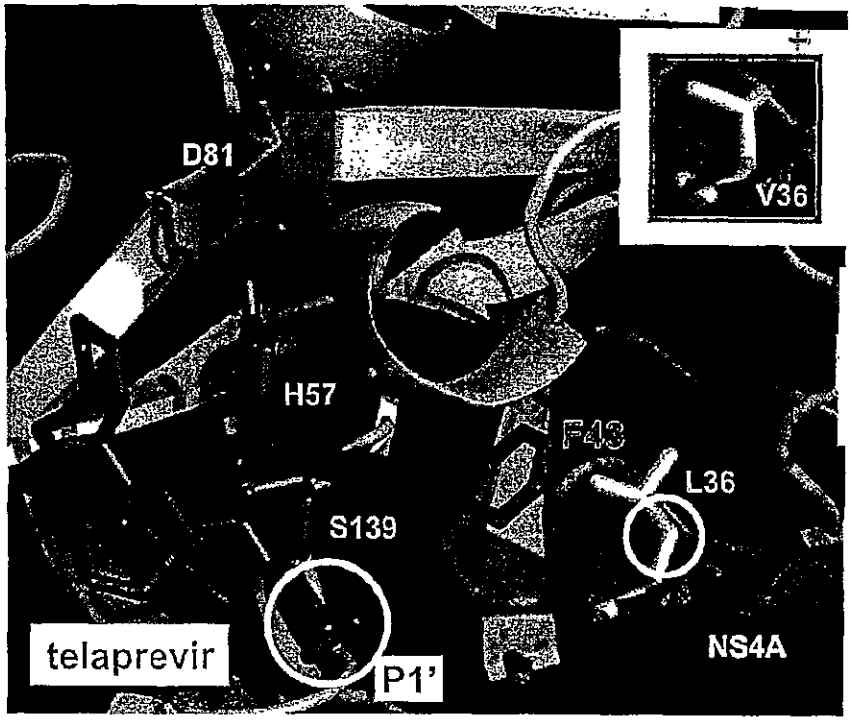


Figure 16

VERTE

V36L Variant: No Resistance, Rare in G1 (Polymorphism)

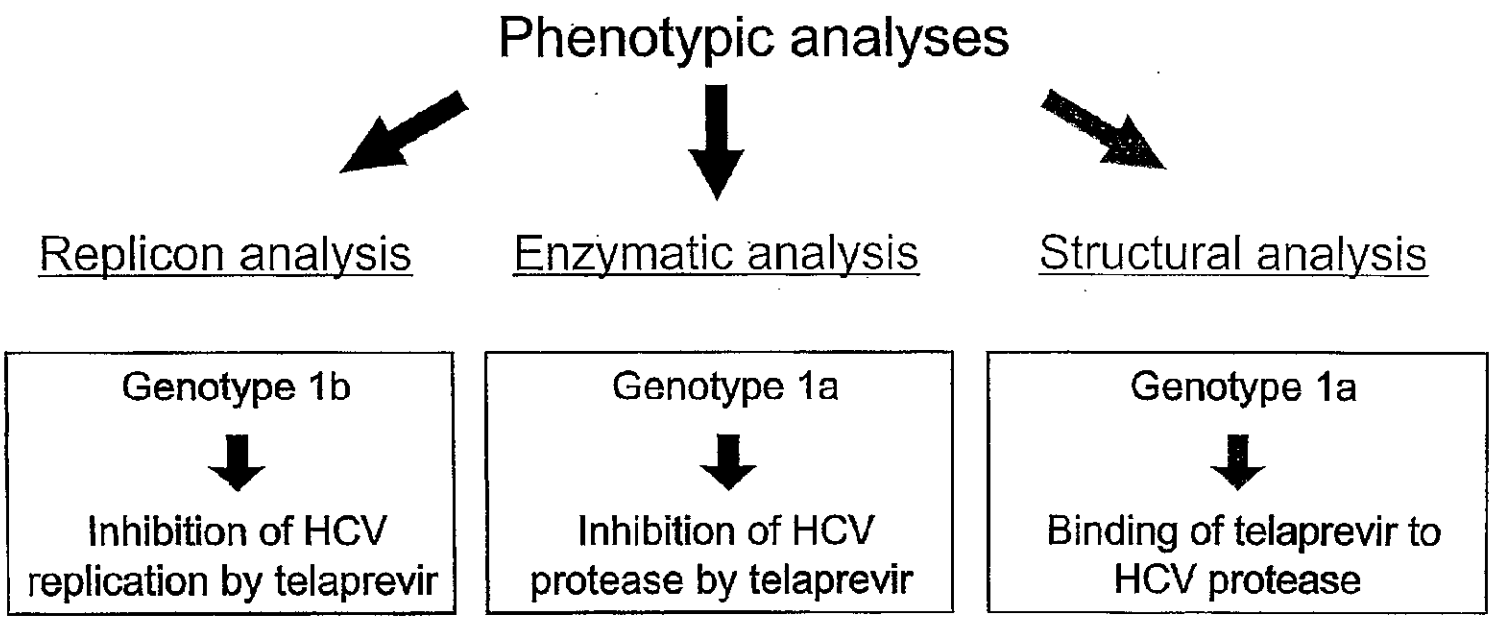
- Phenotype
  - No resistance
  - Average % of HCV RNA at day 14 in patients dosed with telaprevir alone
    - 0.7% in G1a (polymorphism)
    - 0% in G1b
  - L36 exists in G2, G3, or G4 HCV NS3 protease
- Mechanism
  - Little loss of hydrophobic interaction with F43
  - Little loss of interaction between F43 and telaprevir P1' group



VERTE

Figure 17

Methods for Phenotypic Analyses of Variants



Definition of resistance (in fold change):	
< 3-fold	(no resistance)
3- to 25-fold	(low-level resistance)
25- to 50-fold	(intermediate-level resistance)
>50-fold	(high-level resistance)

Figure 18

VERTEX

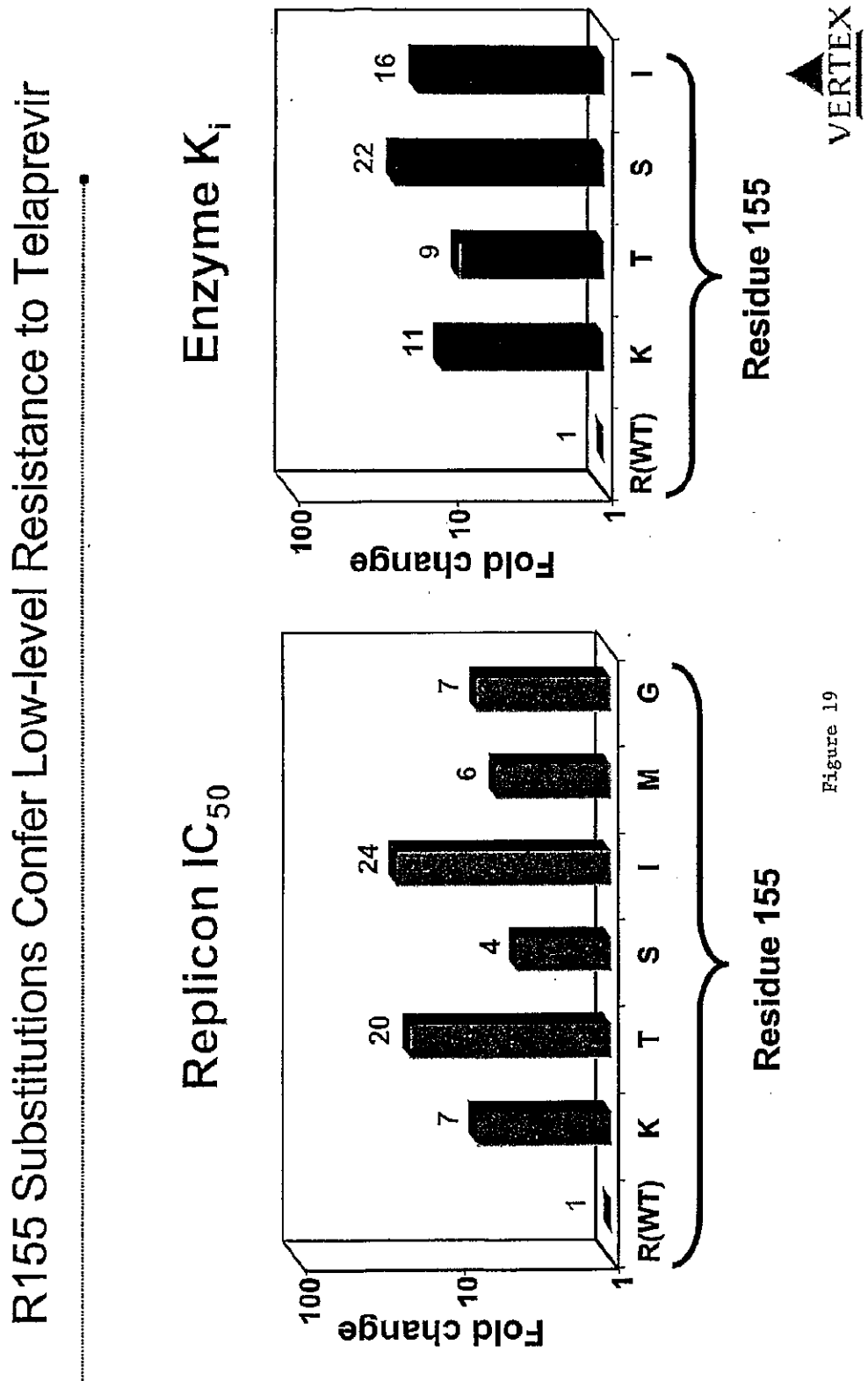
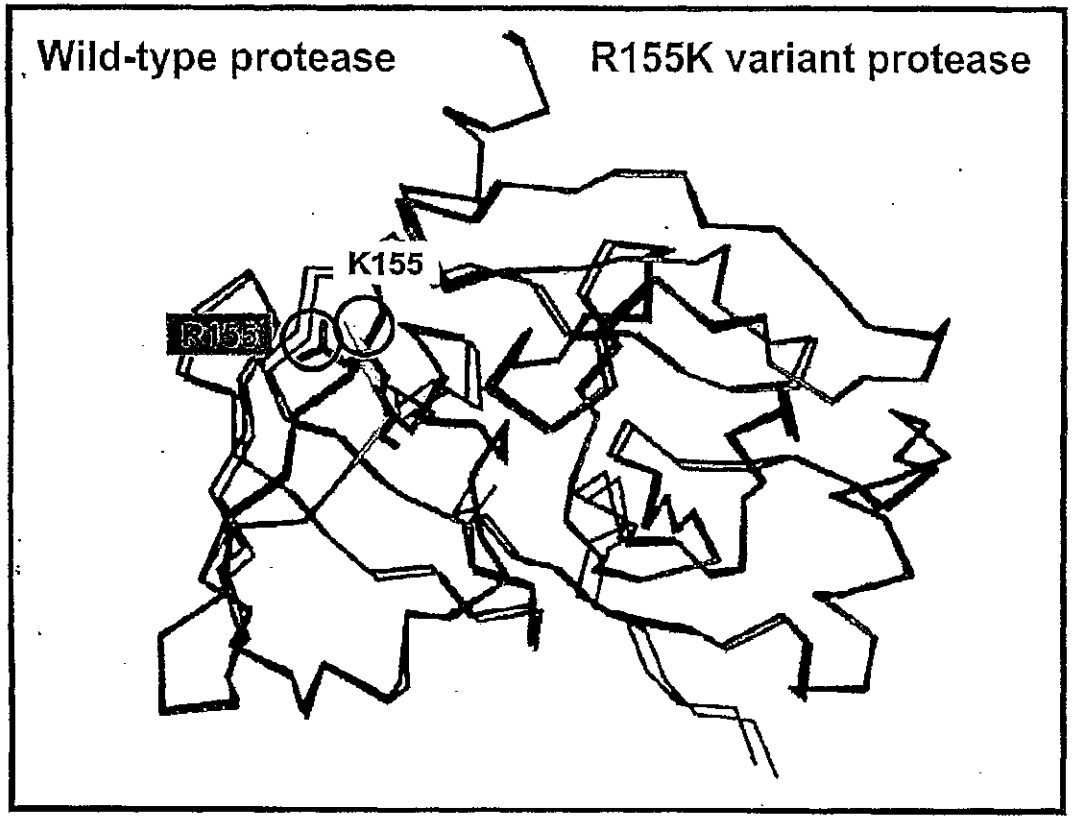

R155 Substitutions Confer Low-level Resistance to Telaprevir
Replicon IC_{50}
Fold change
100
10
1
R(WT)
K
T
S
I
M
G
1
7
20
4
24
6
7
Residue 155
Enzyme K_i
Fold change
100
10
1
R(WT)
K
T
S
I
1
11
9
22
16
Residue 155
VERTEX

Figure 19

X-ray Structure of the R155K Variant Protease



R155K protease

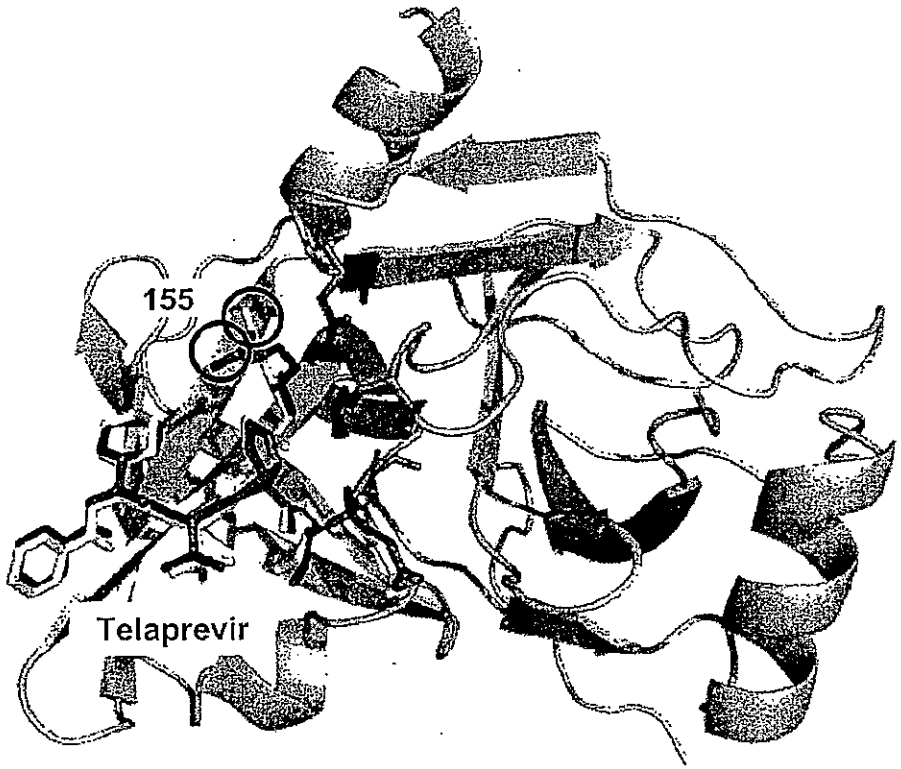
no difference from wild-type protease

- except for residue 155 itself

VERTEX

Figure 20

Computer Model of Telaprevir Binding to the R155K Variant Protease



R155K protease

- Conformation of bound telaprevir remains virtually unchanged
- Loss of some interactions with telaprevir P2 group
- Similar models for other R155 variant proteases

VERTEX

Figure 21

V36 or T54 Substitutions Confer Low-level Resistance to Telaprevir

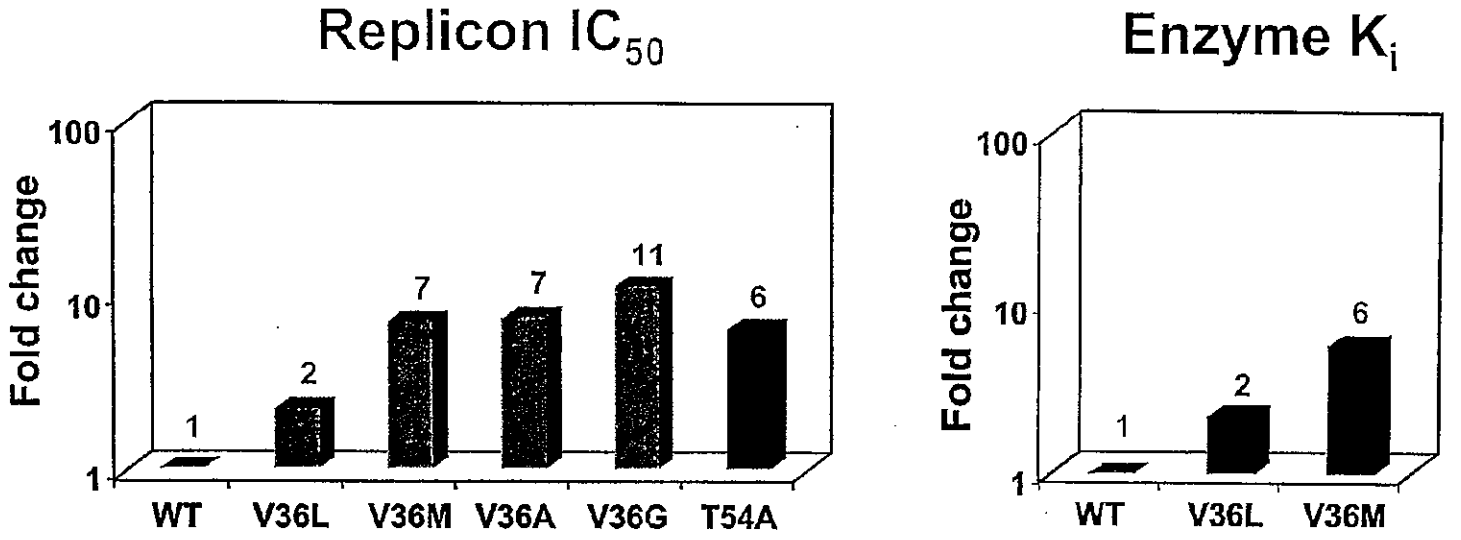


Figure 22

VERTEX

Computer Model of Telaprevir Binding to the V36M Variant Protease

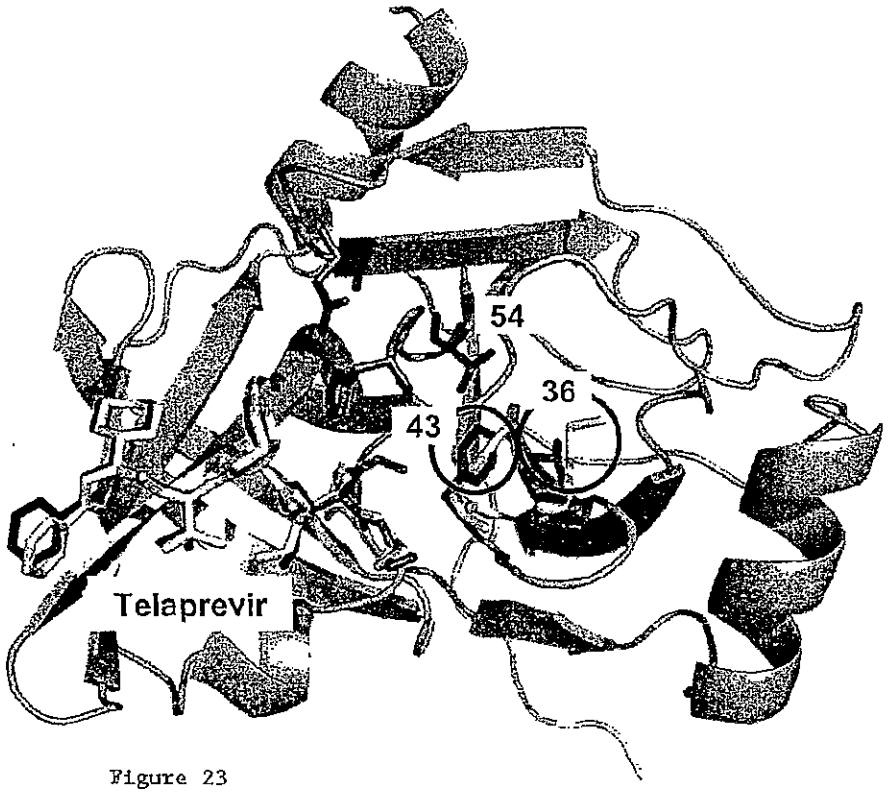


Figure 23

V36M protease

- Conformation of bound telaprevir remains virtually unchanged
- Loss of interactions with telaprevir P1' group
- Similar models for other V36 variant proteases

VERTEX

V36M and R155K Substitutions Are Additive in Conferring Resistance to Telaprevir

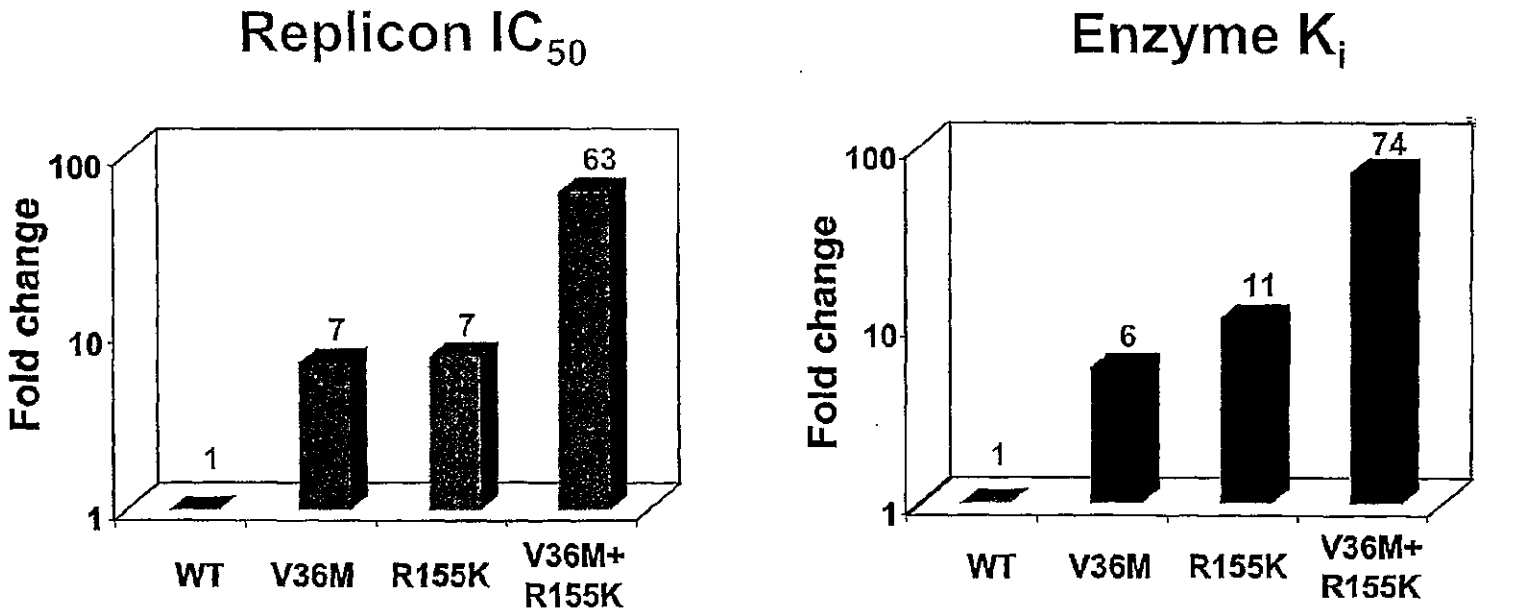


Figure 24

VERTEX

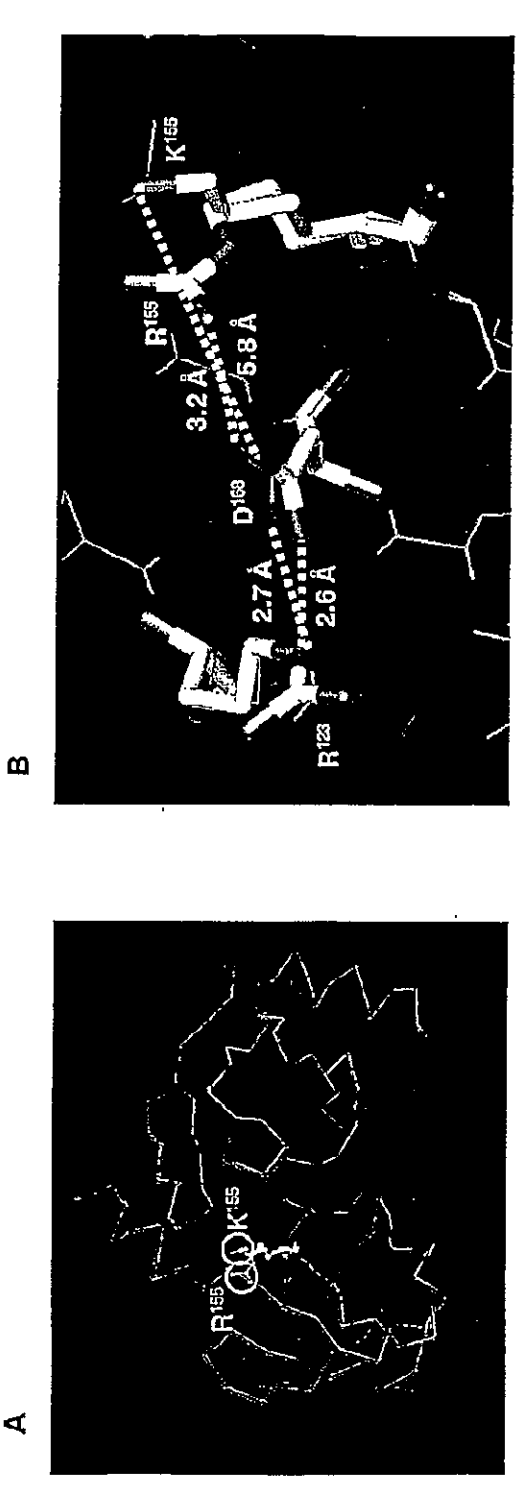


Figure 25

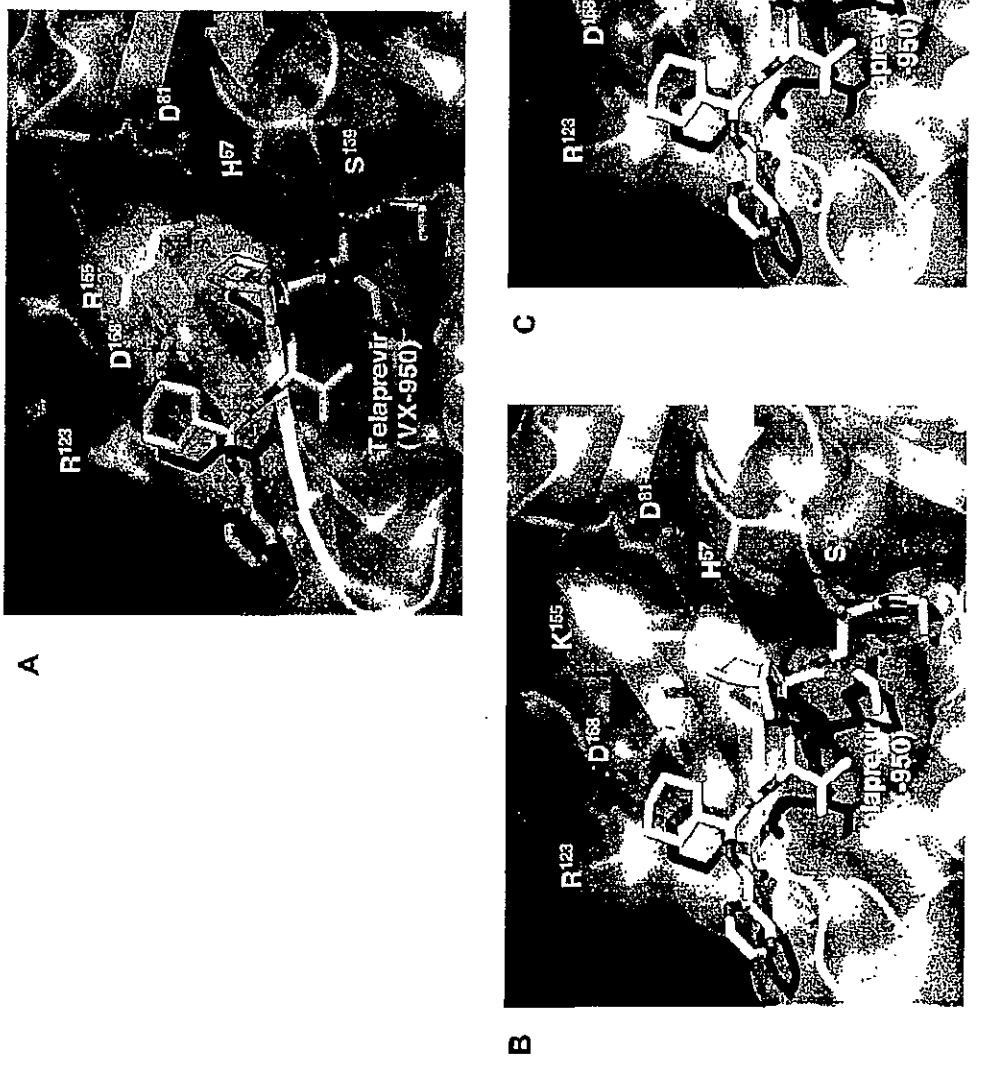


Figure 26

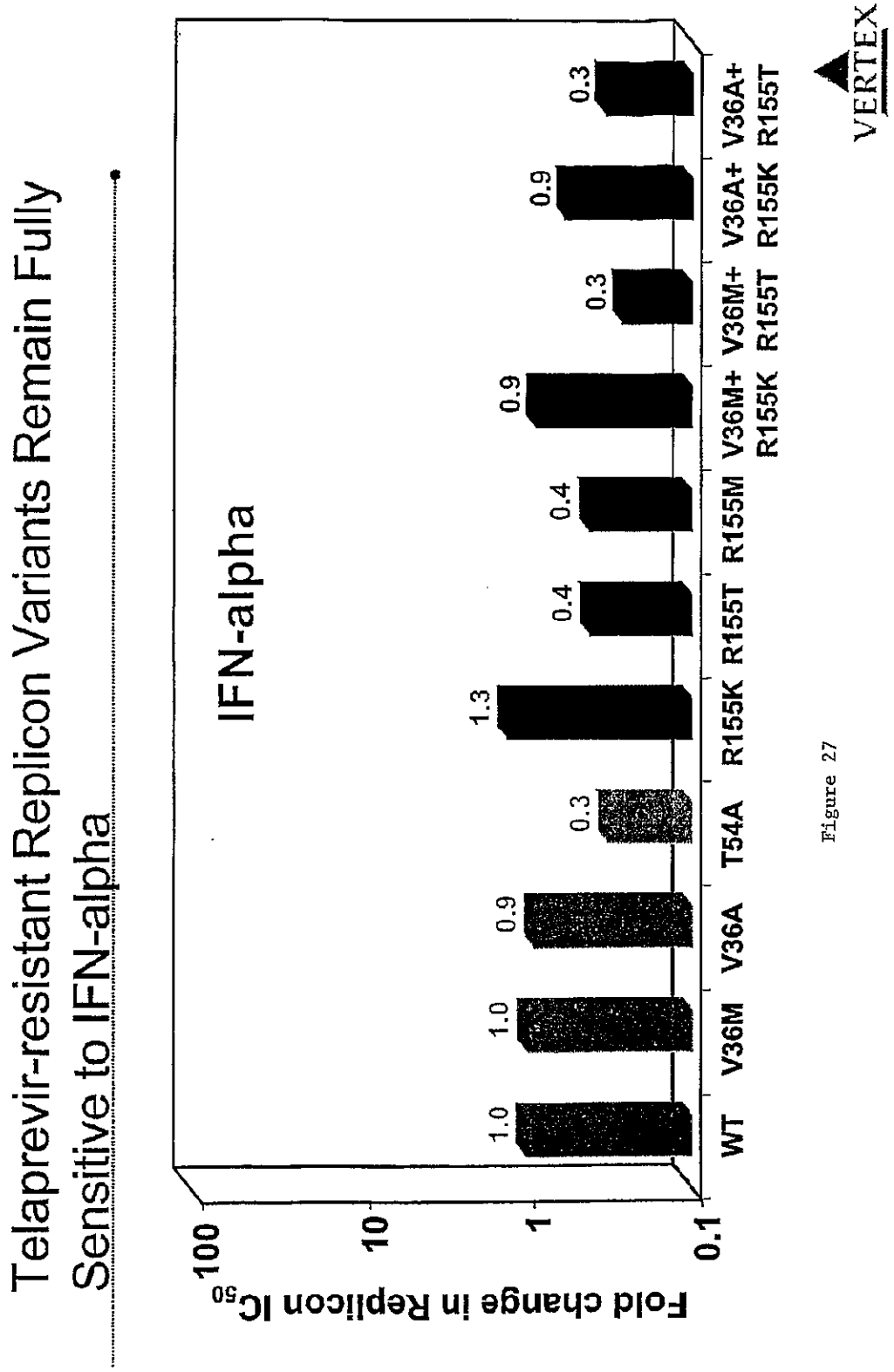

Telaprevir-resistant Replicon Variants Remain Fully Sensitive to IFN-alpha
IFN-alpha
Fold change in Replicon IC50
100
10
1
0.1
1.0
1.0
0.9
0.3
1.3
0.4
0.4
0.9
0.3
0.9
0.3
WT
V36M
V36A
T54A
R155K
R155T
R155M
V36M+ R155K
V36M+ R155T
V36A+ R155K
V36A+ R155T
VERTEX

Figure 27

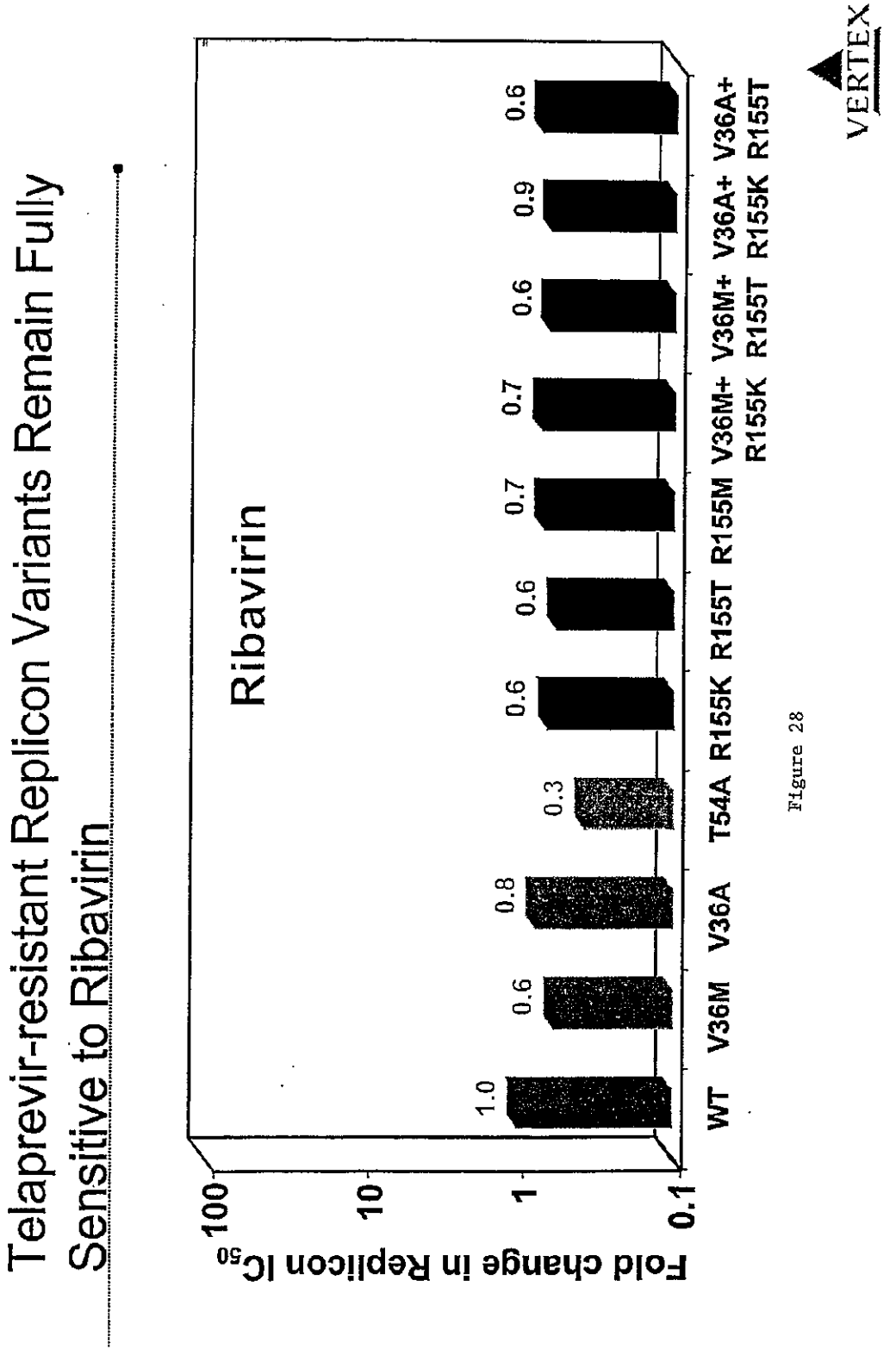
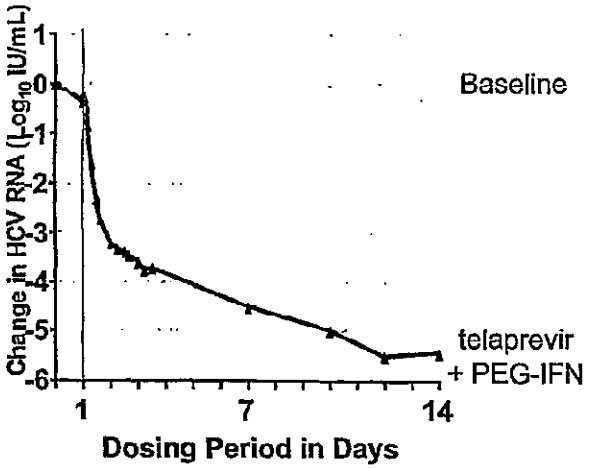

Telaprevir-resistant Replicon Variants Remain Fully Sensitive to Ribavirin
Ribavirin
Fold change in Replicon IC_{50}
100
10
1
0.1
1.0
0.6
0.8
0.3
0.6
0.6
0.7
0.7
0.6
0.9
0.6
WT
V36M
V36A
T54A
R155K
R155T
R155M
V36M+ R155K
V36M+ R155T
V36A+ R155K
V36A+ R155T
VERTEX

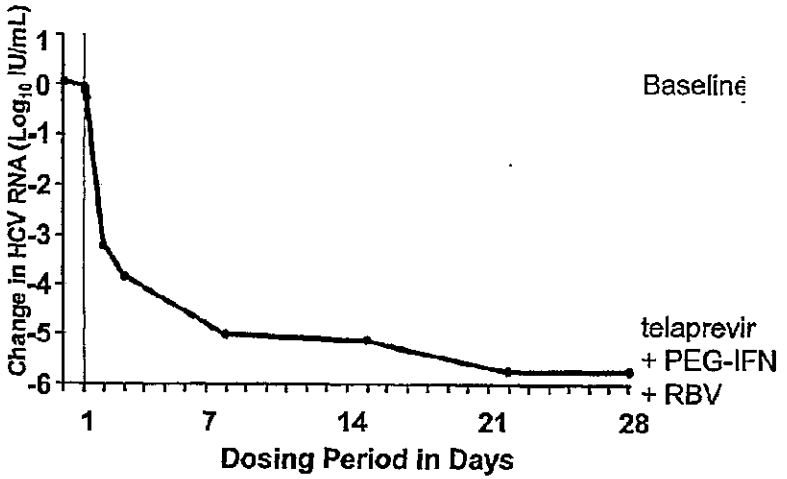
Figure 28

Telaprevir Combination Therapy Suppressed Emergence of Viral Resistance and Prevented Viral Break-through during Dosing

Phase 1b: Telaprevir + PEG-IFN



Phase 2a: Telaprevir + PEG-IFN/RBV



At Day 14 (telaprevir + PEG-IFN):

- 4/8 patients undetectable (<10 IU/mL)

At Day 28 (telaprevir + PEG-IFN/RBV):

- All 12 patients undetectable (<10 IU/mL)

No subjects had viral breakthrough during dosing of telaprevir in combination with PEG-IFN +/- RBV

VERTEX

Figure 29

HCV Sequence Diversity and Resistance Mutations

- Sequence changes (mutations) in the HCV genome occur spontaneously and frequently as a natural result of viral replication
  - HCV polymerase has a high error rate (~1 error per genome)
  - High rate of HCV replication (~10^{12} virions produced per day)
- Therefore, variants with mutations conferring resistance to a specifically targeted antiviral most likely exist at a low frequency prior to drug treatment
- Strong inhibition of wild-type virus with a specifically-targeted antiviral drug selects for drug-resistant virus
- NS3 protease mutations observed in Ph 1b trial of telaprevir alone
  - V36M/A/G
  - T54A
  - R155K/T/M/S/I/G
  - A156S/V/T (observed previously in replicon-based in vitro studies)
  - V36M/A+R155K/T

Figure 30

VERTEX

Mechanisms of Resistance to HCV Protease Inhibitors

- In the active site
  - A156S/V/T (Lin 2004, Lu 2004, & Lin 2005)
    - Steric hindrance (direct) to inhibitor binding
  - D168A/V (Trozzi 2003, Lin 2004 & Lu 2004)
    - Steric hindrance (indirect) to inhibitor binding
  - R155K/T/S/I/M/G (Lin AASLD 2006)
    - Loss of contact (direct) to inhibitor
- Outside the active site
  - **V36M/A/G ??**
  - T54A

Figure 31

VERTE

Conclusions

- Mechanism of resistance to HCV protease inhibitors
  - A156S/V/T: Steric hindrance (direct) to telaprevir binding
  - D168A/V: Steric hindrance (indirect) to BILN 2061 binding
  - R155K/T: Loss of contact (direct) to telaprevir
  - **V36M/A/G: Loss of contact (indirect) to telaprevir**
    - **V36M: Low-level resistance, more fit than V36A/G**
    - **V36A/G: Low-level resistance, less fit than V36M**
    - **V36L: No resistance (polymorphism)**

VERTE

Figure 32

Summary

- Telaprevir-resistant variants can be classified into two groups:
  - Low-level resistance (<25–fold): V36, T54, R155, A156S
  - High-level resistance (>50–fold): A156V/T or V36+R155
- Mechanism of resistance to telaprevir:
  - Loss of interaction to the P1' group (V36)
  - Loss of interaction to the P2 group (R155)
- Telaprevir-resistant variants remain sensitive to IFN-alpha and ribavirin in replicon cells
- No subjects had viral breakthrough during dosing of telaprevir in combination with PEG-IFN +/- ribavirin

Figure 33

VERTEX