

# Selection and Analysis of Human Immunodeficiency Virus Type 1 Variants with Increased Resistance to ABT-538, a Novel Protease Inhibitor

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**Inhibitors of the human immunodeficiency virus protease represent a promising new class of antiretroviral drugs for the treatment of AIDS. We now report the in vitro selection of viral variants with decreased sensitivity to a symmetry-based protease inhibitor, ABT-538, currently being tested in clinical trials. Molecular characterization of the variants shows that an isoleucine-to-valine substitution at position 84 results in a substantial decrease in sensitivity to the drug. Moreover, an additional mutation at position 82, valine to phenylalanine, further decreases viral susceptibility to ABT-538. Three-dimensional analysis of the protease-drug complex provides a structural explanation for the relative drug resistance induced by these two mutations. These findings emphasize the importance of closely monitoring patients receiving ABT-538 for the emergence of viral resistance and provide information that may prove useful in designing the next generation of protease inhibitors.**

Efforts to treat human immunodeficiency virus type 1 (HIV-1) infection, the cause of AIDS, have focused on the development of inhibitors of virally encoded enzymes necessary for successful completion of the viral life cycle (11). Among the agents in development, protease inhibitors represent a promising new class of antiretroviral drugs (7).

During HIV-1 replication, the *gag* and *gag-pol* gene products are synthesized as precursor polyproteins, the latter as a result of a ribosomal frame shift (14). As the nascent virus particle buds from a productively infected cell, these polyproteins are processed within the maturing virion by the viral protease to yield structural proteins, p17, p24, p7, and p6, as well as the essential enzymes, including reverse transcriptase, integrase, and protease (27). Inhibition of the HIV-1 protease results in the budding of immature noninfectious particles, thus interrupting completion of the viral life cycle (17, 19, 31). Unlike reverse transcriptase inhibitors, which are only effective in blocking viral replication in acutely infected cells, these anti-protease drugs can inhibit infectious virus production in both acutely and chronically infected cells (21).

The HIV-1 protease is an aspartyl protease composed of two identical 99-amino-acid monomers, combining to form a C<sub>2</sub>-symmetrical homodimer whose single active site contains the signature sequence Asp-Thr-Gly for aspartyl proteases at amino acids 25 to 27 and 125 to 127 (26). Amino acid substitution for the aspartic acid residue at position 25 results in inactivation of the HIV-1 protease and loss of viral infectivity (19). The tertiary structure of the enzyme was solved by several groups, and this information has allowed computer-

based design of inhibitors of HIV-1 protease (10, 20, 29). Generally, these inhibitors have been transition state peptidomimetic compounds, structurally similar to the *gag* and *gag-pol* polyprotein substrates (35). They bind to the active site and competitively inhibit enzymatic function. In contrast, ABT-538 (Fig. 1) was derived by optimization of the pharmacokinetic properties of a series of C<sub>2</sub> symmetry-based protease inhibitors (18) and has been selected, among a variety of protease inhibitors, for clinical development. It is a potent antiretroviral agent in vitro with demonstrated activity against a variety of laboratory strains and field isolates of HIV-1, with 50 and 90% inhibitory concentrations and (IC<sub>50</sub> and IC<sub>90</sub>, respectively) of 10 to 40 and 70 to 200 nM, respectively (18). The drug has a favorable pharmacokinetic profile, with an oral bioavailability of about 80%. In phase I-II clinical trials, ABT-538 has exhibited potent antiviral activity in vivo (our unpublished results).

In vitro HIV-1 resistance to a number of protease inhibitors, including A-77003 (12, 16), A-80897 (34), P9941 (25), and RPI 312 (9), has been reported. Similar to the observed resistance to the reverse transcriptase inhibitors, where specific amino acid substitutions in the enzyme are responsible for decreased drug sensitivity (23, 32, 36), HIV-1 resistance to protease inhibitors in vitro has been linked to a few specific amino acid substitutions within the viral protease. For example, an arginine-to-glutamine change at residue 8 (R8Q) conferred significant resistance to A-77003 (12) whereas a valine-to-alanine substitution at residue 82 (V82A) was responsible for substantial resistance to P9941 (25).

In this report, we describe the in vitro selection of HIV-1 variants with relative resistance to ABT-538. In addition, we have defined the genotypic basis for the observed resistance phenotype. Finally, by using site-directed mutagenesis, we have determined the critical amino acid substitutions in the HIV-1 protease which result in resistance to this antiretroviral agent.

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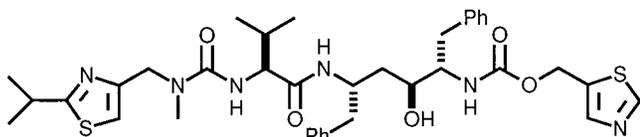


FIG. 1. Chemical structure of ABT-538. Ph, phenyl.

## MATERIALS AND METHODS

### Generation of resistant HIV-1 variants with reduced sensitivity to ABT-538.

HIV-1<sub>NL4-3</sub>, a virus derived from an infectious molecular clone (1), was incubated with  $2 \times 10^6$  MT-4 cells for 1 h, washed, and cultured in the presence of a subtherapeutic concentration of ABT-538 (0.002  $\mu$ M) (18). Viral replication was measured by p24 antigen determination in the supernatant with a commercial assay (Abbott Laboratories). When the p24 antigen concentration exceeded 10,000 pg/ml, the culture supernatant was collected, filtered, and stored at  $-80^\circ\text{C}$  for future use. Infected cells were washed and stored at  $-80^\circ\text{C}$  for subsequent analysis. One aliquot of the culture supernatant was used in the subsequent passage to infect fresh MT-4 cells in the presence of a higher concentration of ABT-538. As summarized in Table 1, this selection method was carried out for 22 passages while gradually increasing the drug concentration.

**Drug inhibition assay to determine the sensitivity of selected viral variants to ABT-538.** The 50% tissue culture-infective dose of each selected virus was determined as previously described (12) and calculated by the method of Reed and Muench (8). A dose of 500 50% tissue culture-infective doses of each virus was used to infect  $10^6$  MT-4 cells in a volume of 1 ml of RPMI 1640 medium supplemented with 10% fetal calf serum. After incubation for 1 h, infected cells were washed, resuspended in medium to a density of  $2 \times 10^5$ /ml, and plated onto 96-well plates in the presence of fivefold dilutions of ABT-538 (0.016 to 10.0  $\mu$ M). Each assay was performed in duplicate, and the p24 antigen produced was measured on day 4 in each culture supernatant. Percent inhibition was calculated by comparison with the supernatant p24 antigen level of a drug-free control.

**Sequence analysis of the protease coding region of the selected variants of HIV-1.** Infected cells from passages 19 and 22 were selected for sequence analysis. DNA was extracted and subjected to PCR to amplify the protease coding region. The primers used have already been described (12), and they contain *Eco*RI and *Sac*I recognition sites. The PCR solution used contained 10 mM Tris-HCl; 50 mM KCl; dATP, dCTP, dGTP, and dTTP each at 200  $\mu$ M; 0.2  $\mu$ M primers; 2.5 mM MgCl<sub>2</sub>; 2.5 U of *Taq* polymerase; and 1  $\mu$ g of DNA in a total volume of 100  $\mu$ l. PCR was performed for a total of 30 cycles in a Perkin-Elmer 9600 Thermocycler under the following conditions: melting at  $94^\circ\text{C}$  for 60 s, annealing at  $55^\circ\text{C}$  for 60 s, and extension at  $72^\circ\text{C}$  for 105 s. The amplified products were purified, digested with *Eco*RI and *Sac*I, and cloned into M13 as previously described (12). Single-stranded DNA was precipitated, purified, and sequenced by the dideoxy-chain termination method.

**Site-directed mutagenesis of specific sites within the protease coding region of HIV-1<sub>NL4-3</sub>.** The 4,300-bp *Sph*I-*Eco*RI fragment of HIV-1<sub>NL4-3</sub> was cloned into pALTER (Promega), and single-stranded DNA was used as the template for site-directed mutagenesis in accordance with the supplier's instructions. After confirmation of the desired mutation by DNA sequencing, the 4.3-kb fragment was isolated by agarose gel electrophoresis, purified, and reinserted into pNL4-3. After transformation into JM109 cells, recombinant colonies were confirmed by

direct sequencing. Mutant pNL4-3 DNA was purified by Wizard maxiprep (Promega) and used to transfect COS cells by the calcium phosphate precipitation method (4). At 48 h after transfection, cell-free supernatants were assayed for p24 antigen expression and, when positive, used to infect MT-4 cells. Viral supernatants were propagated in short-term cultures, titers were determined, and the supernatants were used for subsequent drug susceptibility studies as detailed above.

**Modeling of the wild-type HIV protease-ABT-538 complex.** Modeling was done with QUANTA and the coordinates of the A-78791-HIV protease complex (1HVJ) (3). The crystal structure of A-78791, the deshydroxy diol analog of A-77003, was used as a template for modification to produce the ABT-538 structure, since the P1-P2' core portions of these two inhibitors are chemically identical. The P3' pyridine ring of A-77003 was replaced by the dimethylthiazole group, and the P2-P3 portion was replaced by the P2 terminal thiazole attached via a methyl ester linkage. The two thiazole substituents of the inhibitor were rotated into conformations that were free of steric conflicts by visual inspection.

**Modeling of the V82F/I84V protease-ABT-538 complex.** The V82F/I84V mutant protease was generated by using the modeled wild-type protease-ABT-538 complex. The side chains at residues 82 and 182 and residues 84 and 184 were replaced with phenylalanine and valine, respectively, by using preferred side chain conformations for these residues. In the I84V mutant, the  $\Delta$  methyl group was simply removed. For the V82F mutant, the two methyl groups were substituted by an aromatic ring.

## RESULTS

**Resistant virus is generated by in vitro passages.** As shown in Table 1, we serially passaged HIV-1 while slowly increasing the ABT-538 concentration. Drug concentration selection was guided by the degree of viral replication of the previous passage. If the p24 antigen concentration in the culture supernatant from the previous passage exceeded 100,000 pg/ml, the drug concentration in the ensuing passage was increased to a greater extent than if the p24 value was between 10,000 and 100,000. It was not until passage 19 (P19) that clearly resistant variants emerged, with approximately fivefold increases in the drug IC<sub>50</sub>s and IC<sub>90</sub>s. It is worth noting that once this low level of viral resistance was established, it became possible to increase the drug concentration more rapidly and select for more resistant variants within three more passages. The P22 viral stock was approximately 10- to 25-fold less sensitive to ABT-538 than was parental HIV-1<sub>NL4-3</sub> (Table 1). This marked increase in the IC<sub>90</sub> suggests the presence of a highly resistant population in the viral swarm.

**The appearance of phenotypic resistance to ABT-538 is associated with specific genotypic changes in the protease.** Proviral DNAs from infected cells from P1, P19, and P22 were subjected to PCR to amplify the protease coding region. The deduced amino acid sequences of the P19 and P22 proteases are shown in Fig. 2. The P1 sequences were identical to those of the parental strain (data not shown), which has an arginine residue at position 57 instead of a glycine (24). In P19, an I84V substitution was seen in all 13 of the clones sequenced, suggesting that this is the critical mutation accounting for the observed reduction in drug sensitivity. In addition, 12 of 13 clones contained an M46I mutation. This substitution on the flap of the protease was previously reported by our group for an HIV-1 variant that was resistant to a related symmetry-based protease inhibitor (A-77003) (12). However, in that setting, the M46I mutation per se did not influence drug sensitivity; instead, it apparently compensated for the low replication kinetics of an R8Q mutant. One of 13 clones from P19 contained a V82F change in addition to the changes at residues 46 and 84. In the P22 sequences, this V82F mutation not only persisted but was observed in all of the clones sequenced. In addition to this change at residue 82 and the persistence of M46I and I84V, L63P appeared in 7 of 10 clones from P22. This substitution is a naturally occurring variation of HIV-1, as discussed below. An A71V substitution appeared in 7 of 10 clones as well. An alanine at protease residue 71 is seen in all

TABLE 1. Drug sensitivity results for HIV-1 variants obtained by serial passages in the presence of increasing concentrations of ABT-538

Virus	Concn of ABT-538 used in selection ( $\mu$ M)	Sensitivity to ABT-538 ( $\mu$ M)	
		IC <sub>50</sub>	IC <sub>90</sub>
NL4-3	NA <sup>a</sup>	0.03	0.2
P1	0.002	0.03	0.2
P4	0.02	ND <sup>b</sup>	ND
P7	0.05	ND	ND
P10	0.15	0.03	0.2
P13	0.30	0.03	0.2
P16	0.60	ND	ND
P19	0.80	0.18	1.0
P20	0.90	ND	ND
P21	1.00	ND	ND
P22	2.00	0.80	2.0

<sup>a</sup> NA, not applicable.

<sup>b</sup> ND, not done.

	20	40	60	80	# of clones	
NL4-3	PQITLWQRPLVTIKIGGQLK	EALLDTGADDTVLEEMNLPG	RWKPKMIGGIGGFIKVRQYD	QILIEICGHKAIGTVLVGPT	PVNIIGRNLLTQIGCTLNF	
P19	.....	.....	.....I.....	.....	.....V.....	10
P19	.....	.....Q.....	.....	.....	.....V.....	1
P19	.....	.....Q.....	.....I.....	.....	.....V.....	1
P19	.....	.....	.....I.....	.....	.....F.V.....	1

	20	40	60	80	# of clones	
NL4-3	PQITLWQRPLVTIKIGGQLK	EALLDTGADDTVLEEMNLPG	RWKPKMIGGIGGFIKVRQYD	QILIEICGHKAIGTVLVGPT	PVNIIGRNLLTQIGCTLNF	
P22	.....	.....	.....I.....	.....P.....V.....	.....F.V.....	4
P22	.....	.....	.....I.....	.....P.....	.....F.V.....	3
P22	.....	.....	.....I.....	.....V.....	.....F.V.....	3

FIG. 2. Deduced amino acid sequences of proteases obtained for P19 and P22 variant viruses.

known HIV-1 subtypes, except the subtype O viruses from Cameroon and Gabon, in which a valine is common at that position (24). Variants of the parental HIV-1<sub>NL4-3</sub> protease with all five amino acid substitutions were seen in 4 of 10 clones, and this was the dominant genotype associated with the P22 virus phenotype.

**Amino acid substitutions at residues 82 and 84 result in resistance to ABT-538, whereas the changes at 46, 63, and 71 are likely compensatory.** By site-directed mutagenesis, each individual mutation was introduced into the protease coding region of wild-type NL4-3. In addition, two double mutants (M46I-I84V and V82F-I84V) and one triple mutant (L63P-V82F-I84V) were constructed. Drug sensitivity data for the mutant viruses are shown in Table 2. It is evident that the I84V substitution is the major mutation associated with resistance, resulting in a 10-fold reduction in sensitivity to ABT-538. The V82F substitution alone confers a lower degree of resistance, but as noted in the P22 sequences, when V82F and I84V occur together, the combination may account for the 10- to 20-fold increase in resistance. Unfortunately, the slow growth kinetics of the V82F-I84V double mutant and the L63P-V82F-I84V triple mutant have made drug sensitivity testing impossible. Efforts to construct quadruple and quintuple protease mutants in the HIV-1<sub>NL4-3</sub> background are under way. The changes at residues 46, 63, and 71 did not appear to result in significant changes in drug sensitivity when introduced singly or when M46I was added to I84V. The ABT-538 sensitivity data for I84V, V82F, and I84V-M46I mutants from one representative experiment are also shown in Fig. 3.

The replication kinetics of each virus were determined and compared to that of parental virus NL4-3. As shown in Fig. 4, the growth curves of the L63P and I84V mutant viruses are nearly superimposable on that of the wild-type virus. The kinetics of A71V and V82F mutant viruses are slightly slower; however, the double mutant (V82F-I84V) and the triple mu-

tant (L63P-V82F-I84V) have significantly impaired growth properties. From these results, it does appear that the L63P mutation may have partially restored the replication capacity of the V82F-I84V mutant. Judging from the sequence data and the observation that the P22 variant grows as well as the parental virus, it is likely that the changes at residues 46, 63, and 71 together somehow allow the virus to retain its replicative capacity despite the detrimental consequences of the combined V82F and I84V mutations.

**Viruses resistant to ABT-538 exhibit various degrees of cross resistance to other protease inhibitors.** The P22 variant and the V82F and I84V mutant viruses were screened for sensitivity to two other protease inhibitors currently being tested in clinical trials, Ro 31-8959, also known as saquinivir (6), and L-735,524 (33). Relative changes in sensitivity to these compounds based on the calculated IC<sub>90</sub>s are shown in Table 3. The three mutant viruses appeared to have comparable resistance to L-735,524 and ABT-538. In contrast, less cross resistance to Ro 31-8959 was observed.

**Computer modeling of ABT-538 bound to the HIV-1 protease confirms the importance of the I84V and V82F mutations in conferring resistance to ABT-538.** To understand the structural basis for the reduced sensitivity of I84V and V82F mutant

TABLE 2. Drug sensitivity results for HIV-1 mutants created by site-directed mutagenesis

Virus	Sensitivity to ABT-538 (μM)		Drug sensitivity relative to parental virus (fold)
	IC <sub>50</sub>	IC <sub>90</sub>	
NL4-3	0.03	0.1	1
I84V	0.2	1.0	8-10
V82F	0.15	0.4	4-5
I84V/M46I	0.2	0.8	8-9
L63P	0.03	0.1	1
M46I	0.02	0.07	1
A71V	0.02	0.07	1

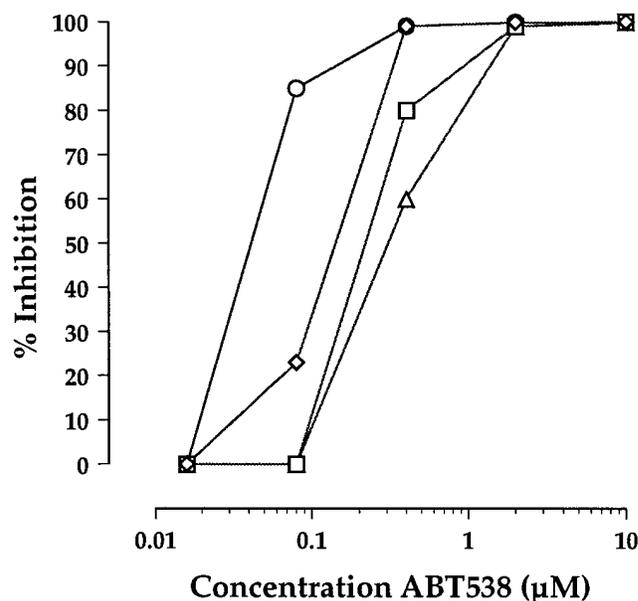


FIG. 3. Drug sensitivity of HIV-1 mutants generated by site-directed mutagenesis. Symbols: ○, NL4-3; ◇, V82F; □, I84V; △, M46I/I84V.

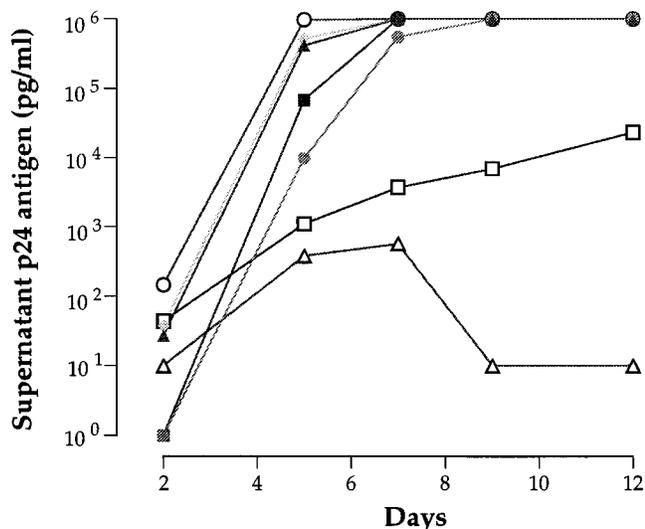


FIG. 4. Replication kinetics of HIV-1<sub>NL4-3</sub> and variants generated by site-directed mutagenesis. Symbols: ○, NL4-3; ◇, L63P; ■, A71V; ●, V82F; ▲, I84V; △, V82F/I84V; □, L63P/V82F/I84V. Each curve represents p24 antigen measurements of supernatants from MT4 cell cultures infected with a standardized inoculum of cell-free supernatants from COS cells transfected with either a wild-type or a mutant plasmid.

viruses, a model of ABT-538 bound to HIV protease was constructed on the basis of the crystal structure of the related inhibitor A-78791 (13). Figure 5 illustrates the effect of the I84V and V82F substitutions in HIV-1 protease on the interaction with ABT-538 at the S<sub>1</sub> and S<sub>1</sub>' subsites. At the S<sub>1</sub>' subsite, both Ile-184 and Val-182 contact the benzyl group of the inhibitor (Fig. 5A, left panel) but the modeled mutations, I184V and V182F, have different effects (Fig. 5A, right panel). I184V decreases the interaction with the C<sub>β</sub> group of the benzyl side chain of ABT-538, whereas V182F results in a very severe spatial overlap with the phenyl ring of ABT-538 at the P<sub>1</sub>' site. Avoidance of the spatial overlap requires a considerable conformational change in either the inhibitor or the enzyme that will likely result in further decreased interactions with the P<sub>1</sub> benzyl group of ABT-538. The effect on the S<sub>1</sub> subsite is similar to what is observed at the S<sub>1</sub>' subsite, as shown in Fig. 5B. However, Ile-84 makes an additional contact through its C<sub>γ</sub>2 methyl group with the C<sub>γ</sub> methyl group of the valine side chain (P<sub>2</sub>) of the inhibitor. The latter interaction may also be reduced in the V82F-I84V double mutant as a result of conformational readjustments due to the V82V mutation. Despite the more dramatic structural effect of the modeled V82F mutant, slightly greater resistance was observed with the I84V mutant (Table 3). This behavior may be due to structural compensation effects such as those that have been observed with the V82A mutant of HIV-1 protease complexed with A-77003 (2). In this example, main chain rearrangements

led to unexpected repacking of enzyme and inhibitor side chain atoms in the S<sub>1</sub> subsite. A more accurate understanding of the structural effects of the I84V-V82F mutations on drug binding must await crystallographic studies of the complexes.

## DISCUSSION

Viral resistance to antiretroviral agents remains a critical obstacle in the path to the development of cogent strategies for treatment of HIV-1 infection. In this study, we demonstrated significant *in vitro* resistance to ABT-538 (Table 1 and Fig. 3), a novel inhibitor of HIV-1 protease now being tested in clinical trials in the United States and Europe. The degree of resistance observed is similar to the reported *in vitro* resistance to 2',3'-dideoxyinosine (32) but greater than the reported low-level resistance to 2',3'-dideoxycytidine (36). It is significantly less than the documented 100-fold resistance to zidovudine (22) and the 1,000-fold reduction in sensitivity to the non-nucleoside inhibitors of reverse transcriptase nevirapine (28) and L-697,661 (30) seen *in vivo* and *in vitro*.

The increased resistance to ABT-538 emerges from amino acid substitutions at positions 82 and 84 in the HIV-1 protease (Fig. 2). These substitutions are the result of single nucleotide changes in the protease coding region and map to the substrate binding cleft of the enzyme (Fig. 5). As demonstrated by site-directed mutagenesis, each mutation confers significant resistance to ABT-538 (Fig. 3 and Table 2). The I84V substitution was reported by El-Farrash et al. to confer resistance to RPI 312, a synthetic HIV-1 protease peptide inhibitor (9). As this compound and ABT-538 share the structural feature of a P<sub>1</sub> benzyl side chain, it may follow that the observed patterns of *in vitro* resistance are similar.

Substitutions at amino acid 82 have been reported by our group (12), as well as by others (16, 25). In this study, we detected a unique substitution, V82F. The amino acid at position 82 is an important contact residue that forms part of the S<sub>1</sub>-S<sub>1</sub>' binding pocket and extends into the S<sub>3</sub>-S<sub>3</sub>' pocket of the viral protease (35). As demonstrated by our modeling (Fig. 5), this amino acid substitution significantly alters the interaction between the enzyme and ABT-538. The recent crystal structure determination of the V82A mutant of HIV-1 protease complexed to A-77003 also reveals that the residue 82 and 182 regions of the enzyme are highly flexible (2).

Similar to the reverse transcriptase, HIV-1 protease appears to tolerate multiple amino acid substitutions which result in reduced drug susceptibility. We have documented the association of M46I, L63P, and A71V with the resistance-producing changes at residues 82 and 84. In our previous report, we found that M46I appeared to improve the replication kinetics of a severely deficient R8Q mutant virus (12). Our present data confirm our previous findings that the nature of the M46I substitution is probably not directly related to drug resistance. Molecular dynamics simulations indicate that the M46I substitution in the flap of the protease favors a closed conformation relative to the wild type when binding either a substrate or an inhibitor (5). Our data suggest that this mutation, along with L63P and A71V, serves to compensate for the changes at residues 82 and 84, as evidenced by the difference in viral kinetics between the P22 variant (similar to HIV-1<sub>NL4-3</sub>) and the double and triple mutants created by site-directed mutagenesis (Fig. 4). Amino acids 63 and 71 are not in the vicinity of the active site of the enzyme, nor are they found at important substrate-binding sites (35). They do, however, interact with each other and may stabilize the structure of the monomeric subunit of the HIV-1 protease.

The L63P variant is a naturally occurring variant of HIV-1

TABLE 3. Relative resistance of selected HIV-1 variants to other protease inhibitors

Virus	Fold increase in IC <sub>50</sub> of:		
	ABT-538	L-735,524	Ro 31-8959
HIV-1 <sub>NL4-3</sub>	1	1	1
P22	10	10	3
I84V	8	5	2
V82F	5	3	2

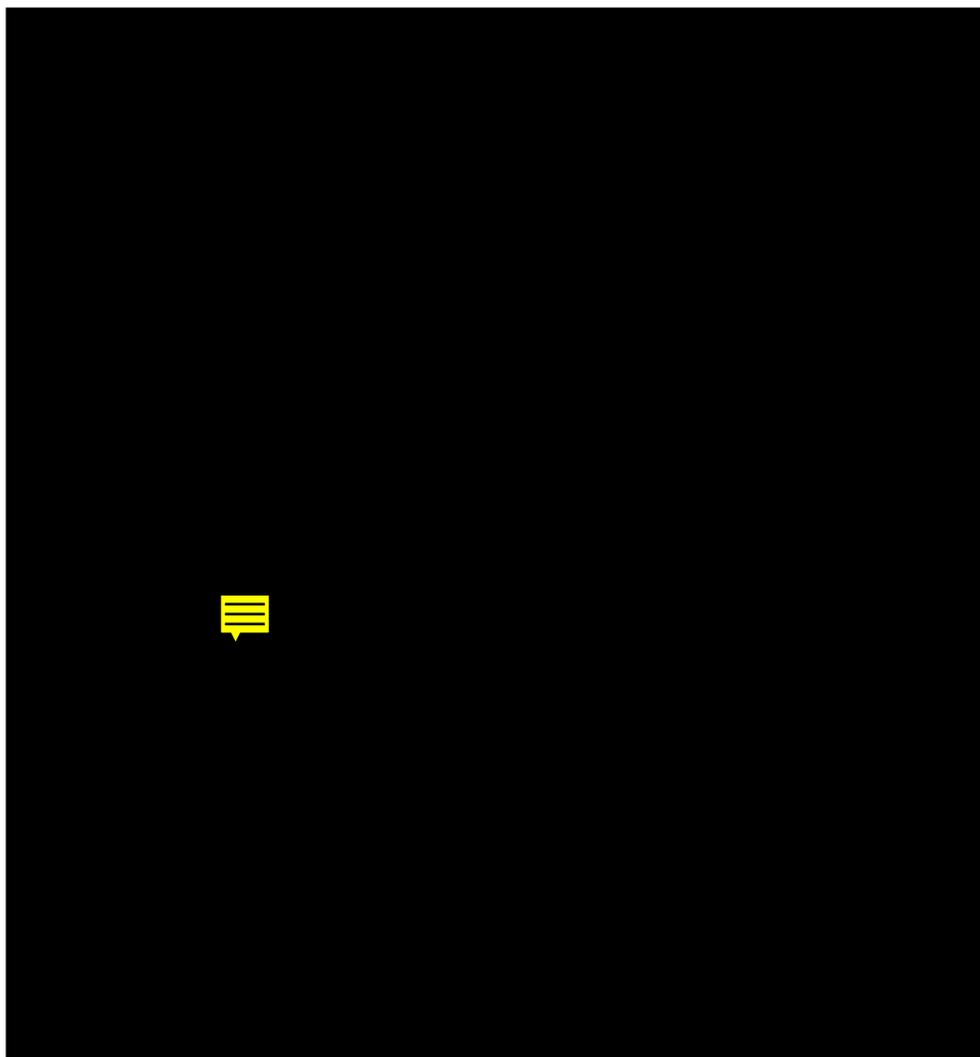


FIG. 5. Computer-generated models of HIV protease-ABT-538 complexes of wild-type HIV-1<sub>NL4-3</sub> and the V82F/I84V double mutant. (A) Interactions between ABT-538 and the protease at the S<sub>1</sub>' subsite. (B) The same interactions at the S<sub>1</sub> binding subsite. Note the effects of the double mutation on the van der Waals interactions.

and is seen in HIV-1 isolates JRCSF, SF-2, CAM1, and YU2 (24). The A71V substitution has been seen only in divergent Cameroon strains ANT70 and the MVP5180 (24). Although these two variants have been reported to occur naturally, 63P and 71V have not been found simultaneously. Our working hypothesis is that these mutations are selected for during passage because together they confer an advantage, probably as a compensation for the deleterious effects of mutations at residues 82 and 84. However, we cannot rule out the possibility that although when we introduced them singly into the NL4-3 background we could not detect a phenotypic change in viral sensitivity to ABT-538, these mutations in combination contribute to the significant resistance observed in the P22 variant.

We have also found that the pattern of *in vitro* resistance to ABT-538 is similar to that observed for another protease inhibitor, L-735,524, while different from that of a third protease inhibitor, Ro 31-8959. Vacca et al. have demonstrated that the  $K_i$  of the I84V mutant protease is 10-fold higher for L-735,524 than that of the wild-type protease (33).

*In vitro* selection experiments using Ro 31-8959 have identified G48V and L90M as changes that are important in con-

ferring increased resistance to this agent (15). We have constructed a G48V mutant virus and found it to be sensitive to ABT-538 (data not shown). These data support the premise that there are both overlapping and nonoverlapping patterns of resistance to the various protease inhibitors. A clear understanding of resistance patterns may well allow a more logical use of these agents in combination. Our current findings also serve to emphasize the importance of expanding these experiments to include other protease inhibitors in anticipation of designing cogent strategies to maximize the potential benefits of this new class of antiretroviral agents.

In summary, we have selected for HIV-1 variants with reduced sensitivity to ABT-538. The I84V mutation is the major determinant of resistance. Addition of V82F confers even greater resistance to ABT-538. These substitutions occur in close proximity to the active site of the enzyme and likely result in reduced inhibitor binding. The clinical significance of these findings should become clear shortly, as we are currently studying the antiviral effect of ABT-538 as a single agent in HIV-1-infected persons.

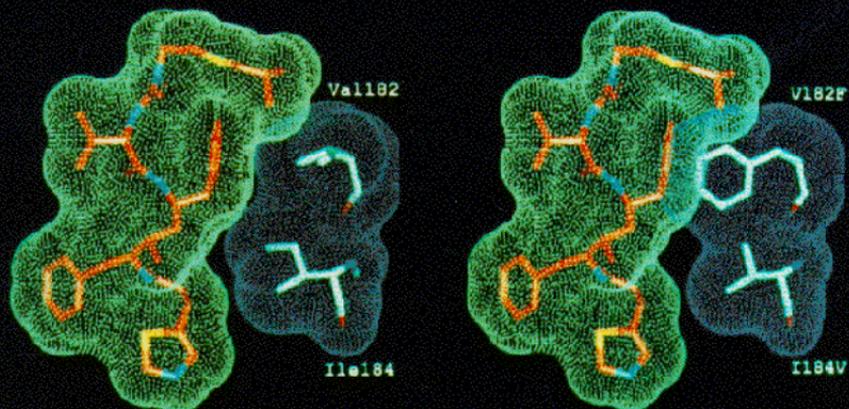
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