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Resilience to Resistance of HIV-1 Protease Inhibitors: Profile of Darunavir

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Abstract

The current effectiveness of HAART in the management of HIV infection is compromised by the emergence of extensively cross-resistant strains of HIV-1, requiring a significant need for new therapeutic agents. Due to its crucial role in viral maturation and therefore HIV-1 replication and infectivity, the HIV-1 protease continues to be a major development target for antiretroviral therapy. However, new protease inhibitors must have higher thresholds to the development of resistance and cross-resistance. Research has demonstrated that the binding characteristics between a protease inhibitor and the active site of the HIV-1 protease are key factors in the development of resistance. More specifically, the way in which a protease inhibitor fits within the substrate consensus volume, or "substrate envelope", appears to be critical. The currently available inhibitors are not only smaller than the native substrates, but also have a different shape. This difference in shape underlies observed patterns of resistance because primary drug-resistant mutations often arise at positions in the protease where the inhibitors protrude beyond the substrate envelope but are still in contact with the enzyme. Since all currently available protease inhibitors occupy a similar space (in spite of their structural differences) in the active site of the enzyme, the specific positions where the inhibitors protrude and contact the enzyme correspond to the locations where most mutations occur that give rise to multidrug-resistant HIV-1 strains. Detailed investigation of the structure, thermodynamics, and dynamics of the active site of the protease enzyme is enabling the identification of new protease inhibitors that more closely fit within the substrate envelope and therefore decrease the risk of drug resistance developing. The features of darunavir, the latest FDA-approved protease inhibitor, include its high binding affinity ($K_d = 4.5 \times 10^{-12}$ M) for the protease active site, the presence of hydrogen bonds with the backbone, and its ability to fit closely within the substrate envelope (or consensus volume). Darunavir is potent against both wild-type and protease inhibitor-resistant viruses in vitro, including a broad range of over 4,000 clinical isolates. Additionally, in vitro selection studies with wild-type HIV-1 strains have shown that resistance to darunavir develops much more slowly and is more difficult to generate than for existing protease inhibitors. Clinical studies have shown that darunavir administered with low-dose ritonavir (darunavir/ritonavir) provides highly potent viral suppression (including significant decreases in HIV viral load in patients with documented protease inhibitor resistance) together with favorable tolerability. In conclusion, as a result of its high binding affinity for and overall fit within the active site of HIV-1 protease, darunavir has a higher genetic barrier to the development of resistance and better clinical efficacy against multidrug-resistant HIV relative to current protease inhibitors. The observed efficacy, safety and tolerability of darunavir in highly treatment-experienced patients makes darunavir an important new therapeutic option for HIV-infected patients. (AIDS Rev. 2008;10:131-42)

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Key words**HIV. Drug resistance. Darunavir. TMC114. Substrate recognition. HIV-1 protease.****Introduction**

Although HAART has successfully improved the management of HIV infection by reducing mortality and slowing the progression to AIDS, its widespread effectiveness is compromised with the emergence of extensively cross-resistant strains of HIV-1¹⁻⁴. The degree of cross-resistance to different drugs within a drug class has been shown to vary according to the number and type of mutations⁵. The transmission of drug-resistant HIV is also a growing problem, with 5-25% of newly infected individuals being infected with resistant virus⁶⁻⁹. Despite the use of HAART, incomplete viral suppression and persistent low-level viremia can be associated with the development of clinically significant resistance and poor long-term clinical outcome, even when overt virologic failure is not apparent¹⁰⁻¹². For protease inhibitors (PIs), Richman, et al. estimated that 41% of patients with HIV viral load > 500 copies/ml were resistant to one or more of the drugs in the class³.

Nevertheless, despite the challenges of potential drug resistance, HIV protease continues to be a prime target for antiretroviral therapy because of its essential role in viral replication^{13,14}. Nine HIV-1 PIs have been approved by the U.S. Food and Drug Association (FDA): amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir, and recently darunavir (previously TMC114). To be effective, a PI should combine the following characteristics: improved binding to the protease, prevention of the development of resistance, high potency against both wild-type and resistant HIV strains, effective and durable virologic suppression, favorable tolerability, and convenience. This article profiles the balance of substrate recognition and reviews the existing HIV-1 PIs, including tipranavir, in comparison with the unique *in vitro* and clinical properties of the latest FDA-approved PI, darunavir¹⁵⁻¹⁷.

The structural and dynamic determinants of substrate recognition in HIV-1 protease

During virion maturation, HIV-1 protease specifically cleaves the posttranslational precursor polyproteins

Gag and Gag-Pro-Pol in at least nine nonhomologous sites, releasing six structural proteins (matrix, capsid, p2, nucleocapsid, p1, and p6) and four enzymes (protease, reverse transcriptase, RNaseH, and integrase)¹⁸. This processing is essential for viral maturation and therefore HIV-1 replication and infectivity. Abolition of protease activity by either deletions or point mutations results in the production of noninfectious viral particles^{13,19}. The HIV-1 protease's extreme specificity and critical role in viral maturation makes it an attractive target for antiretroviral drugs^{13,14}.

The HIV-1 protease is a small aspartyl protease. Like other retroviral aspartyl proteases^{20,21} it is active as a symmetric homodimer, but each monomer is only 99 amino acids long. The structure is primarily β -sheet with the active site at the dimer interface. Each monomer contributes one critical aspartic acid²² to form the active enzyme. The dynamics of HIV-1 protease are also critical to function, as the substrate sites within the Gag and Gag-Pro-Pol polyproteins access the active site when two large flexible flaps open²³⁻²⁵ then close over the active site allowing proteolysis of the substrate to occur. The flaps then reopen and allow the cleaved products to be released.

The substrate sequences that HIV-1 protease cleaves are diverse in sequence and asymmetric; however, it is not a promiscuous enzyme, but rather is highly specific. The rate and order in which the sequences are cleaved is site-specific. Crystal structures of peptide complexes have been determined that correspond to the various cleavage sites in Gag and Gag-Pro-Pol²⁶⁻³³. Analysis of the structures of the substrate complexes revealed that although the sequences are diverse, the structures that they form pack into a conserved asymmetric shape³¹. This asymmetric shape appears to determine the enzyme's specificity rather than any particular amino acid sequence or hydrogen bonding pattern^{30,31}. The conservation of this shape was determined by the intersection of the van der Waals surfaces of the substrates, and this intersecting volume or consensus volume has been defined as the protease "substrate envelope", or the region within the active site that is most critical for substrate recognition (Fig. 1)^{15,34}.

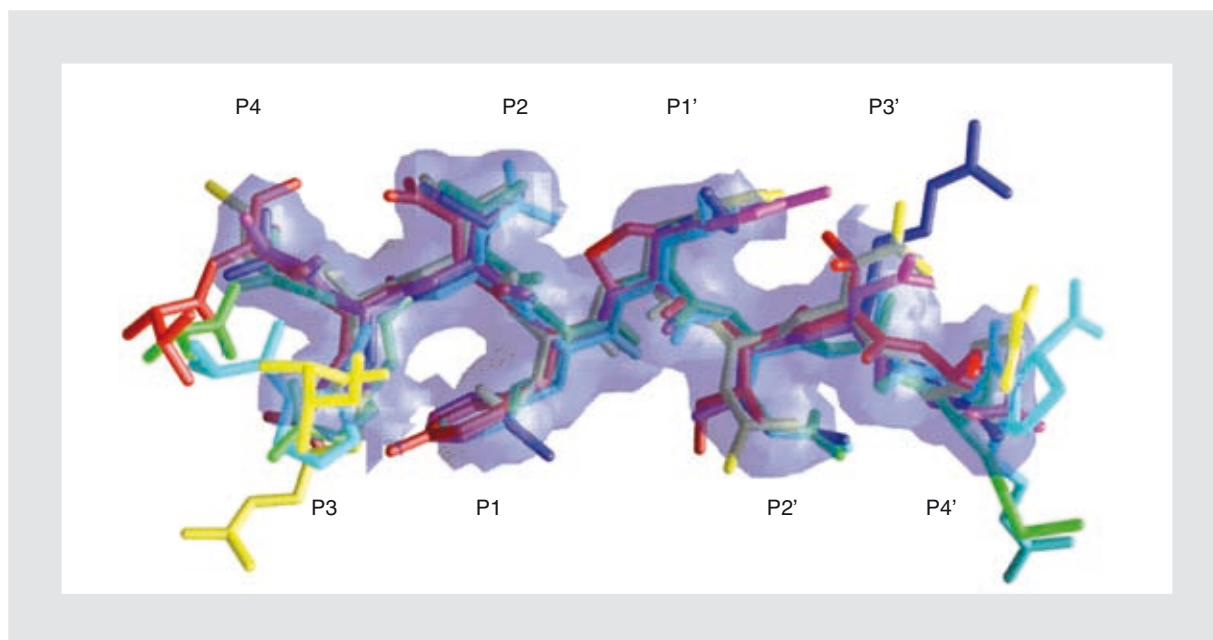


Figure 1. Substrate envelope of HIV protease. GRASP model generated from overlapping van der Waals volume of substrate peptides. Red: matrix capsid; green: capsid-p2; blue: p2-nucleocapsid; cyan: p1-p6; magenta: reverse transcriptase-ribonuclease H; yellow: ribonuclease H-integrase (reprinted from Chemistry & Biology, Vol. 11, Nancy M King, Moses Prabu-Jeyabalan, Ellen A Nalivaika, and Celia Schiffer, Combating susceptibility to drug resistance: lessons from HIV-1 protease, p1333-8, Copyright 2004, with permission from Elsevier³⁴).

Characterization of protease inhibitors – structures and thermodynamics

The HIV PIs were the first success of structure-based drug design²². All of the clinically successful ones are competitive active-site inhibitors. They interact with the active site in such a way as to keep the flaps of the enzyme tightly closed over the active site, thus mimicking the transition state and thereby effectively inactivating the enzyme. Most of the inhibitors, even those whose precursors were found through screening libraries, were optimized with successive co-crystal structures³⁵⁻⁴¹.

Chemically, these inhibitors have generally hydrophobic moieties that interact with the mainly hydrophobic S2-S2' pockets in the active site. Structurally, there are two types of HIV PI: peptidomimetic PI, which comprise the majority of approved PIs including darunavir, and non-peptidomimetic PI such as tipranavir⁴¹.

In addition to structure, isothermal titration calorimetry has been extensively utilized to characterize the thermodynamics of the binding of these inhibitors to HIV-1 protease^{34,42-47}. Under specified temperature and buffer conditions, thermodynamics allows for the enthalpy and entropy of a molecular interaction to be characterized. The inhibitors vary in binding affinity from nanomolar to picomolar. However, to achieve

these binding affinities there are a wide variety of different combinations of more favorable entropy or enthalpy. Many of the first-generation inhibitors, despite being designed on the basis of structural interactions, are actually enthalpically unfavorable and are driven into the active site by the entropically favorable action of burial of relatively rigid hydrophobic groups. Recent theories suggest that enthalpically driven inhibitors may be more resilient to resistant variants of HIV-1 protease^{34,46}; however, whether this is universal remains to be verified.

Recently approved protease inhibitors

The most recently approved PI, darunavir, is a peptidomimetic PI, containing a 3(R), 3a(S), 6a(R)-bis-tetrahydrofuranylethane (bis-THF) group. Darunavir is highly potent *in vitro* and *in vivo* against a broad range of HIV-1 strains, including wild-type virus and a variety of multidrug-resistant (MDR) clinical strains^{15,27,48-51}. Darunavir was identified in a unique research and development program, which assessed potential PI compounds by profiling their antiviral activity against a panel of recombinant HIV strains derived from highly PI-resistant and cross-resistant clinical isolates. Additionally, several thousand clinical isolates with varying degrees of PI resistance were also used for the evalu-

ation of the most interesting compounds^{16,51}. Two key evaluation criteria were high potency against both wild-type and MDR HIV-1 strains, and a favorable pharmacokinetic profile following oral administration. From this search, the prototype compound TMC126 was identified. To improve the pharmacokinetic profile of TMC126 while retaining its high potency, a series of closely related compounds, all of which had the bis-THF moiety for improved interaction in the P2-pocket, were synthesized and darunavir was identified as the lead compound for clinical development (Fig. 2).

Although darunavir has some chemical similarities to amprenavir, it binds approximately 100-times more tightly to wild-type protease ($K_d = 4.5 \times 10^{-12}$ M vs. $K_d = 3.9 \times 10^{-10}$ M) and 1,000-times more tightly than indinavir, nelfinavir, ritonavir, and saquinavir^{15,52}. The unique high binding affinity of darunavir may partly be due to the bis-THF moiety at P2¹⁷. This moiety forms strong hydrogen bonds with the backbone atoms of residues D29 and D30. These hydrogen bonds may be partially responsible for the very favorable binding enthalpy of darunavir to the wild-type protease (-12.1 kcal/mol)¹⁶ and very slow dissociation rate⁵². Together with further hydrogen bonds between darunavir and the backbone atoms at the base of the active site, the drug mimics the conserved hydrogen bonds of the natural substrates^{30,31}. In contrast, other PIs form more extensive interactions with the protease side-chains^{48,49}.

Tipranavir is another recently approved PI. Tipranavir is non-peptidomimetic (sulfonamide-containing 5,6-dihydro-4-hydroxy-2-pyrone) and as such is structurally unrelated to the other PIs⁵³. Identified through structure-based drug design, tipranavir is a potent inhibitor of the HIV-1 protease with a dissociation constant for inhibitor binding of $K_d = 1.9 \times 10^{-11}$ M⁵⁴. A study examining the thermodynamic basis of the potent antiviral activity of tipranavir against PI-resistant mutants found that in contrast to darunavir, the high potency of tipranavir results from a large entropy change (-14.6 kcal/mol) combined with a small enthalpy change (-0.7 kcal/mol)⁵⁴. The structure of tipranavir allows seven direct hydrogen bonds to be established with conserved residues of the protease enzyme, and makes fewer water-mediated hydrogen bonds compared with other PIs. Together with the retention of structural flexibility, these features allow enthalpy interactions to compensate for conformational entropy losses induced in the presence of resistance mutations. This is also thought to go some way towards explaining the activity of tipranavir against resistant viruses⁵⁴.

Protease inhibitor resistance and cross-resistance

In general, drug resistance occurs when mutations in the target protein enable it to retain function while no longer being effectively inhibited by the drug³⁴. The high viral replication rate combined with the highly error-prone reverse transcriptase and the selective pressure of therapy cause many drug-resistant mutants of HIV-1 to emerge^{43,55,56}. Since the introduction of PIs, drug-resistant mutations in the protease have become widespread. These mutations render the variant protease resistant to the inhibitor, while allowing it to maintain its function in cleaving its natural substrates^{5,34}. Mutations in at least 34 of the 99 residues of HIV-1 protease have been found to have clinical significance⁵⁶⁻⁵⁹. Although only a subset of these mutations (e.g. D30N, G48V, V82A, I84V, I50V, and I50L) affect inhibitor binding by an alteration of a direct point of contact within the active site, many others alter inhibitor binding by altering the balance between substrate recognition and inhibitor binding. The HIV-1 found in most highly PI-experienced patients has between 5-15 mutations in the protease gene^{56,57}. These are often in specific combinations of mutations both inside and outside the active site. Mutations outside the active site may not only impact inhibitor binding, but also compensate for the viability and fitness of the enzyme and thus increase the growth rate of the mutant virus^{60,61}.

Varying degrees of resistance to all of the PIs have been described and cross-resistance among the older PIs is common^{5,62,63}. In a phenotypic and genotypic analysis of antiretroviral drug resistance to indinavir, nelfinavir, ritonavir, and saquinavir in more than 6,000 patient isolates from Europe and the USA, phenotypic resistance to any single PI was found in 17-25% of isolates. Cross-resistance was found in 59-80% of those isolates that were resistant (greater than 10-fold increase in inhibitory concentration [EC_{50}]) to at least one PI⁶². Although certain predominantly primary active-site mutations are closely associated with a particular PI^{64,65}, such as D30N with nelfinavir, G48V with saquinavir, I50V with amprenavir, or I50L with atazanavir, others such as V82A and I84V impact almost all of the PIs. The active-site mutations are often combined with other common mutational patterns that contribute to PI cross-resistance, including combinations of mutations at L10I, I54V or T, A71V or T, V77I, and L90M^{56,57,62}. The structural similarities between darunavir and amprenavir may contribute to some shared determinants of resistance. Recent studies have indicated that

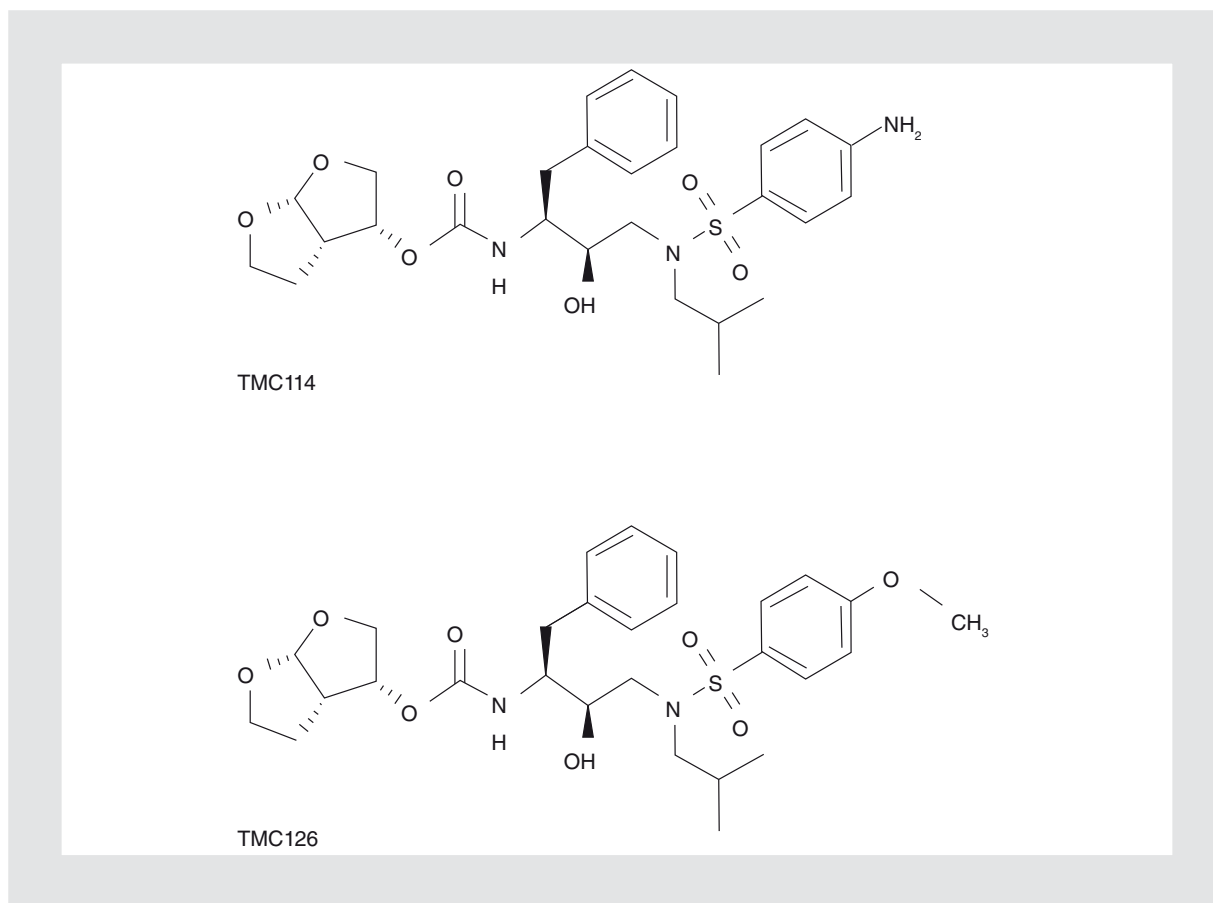


Figure 2. Chemical structure of darunavir (TMC114) and TMC126 (reproduced with permission¹⁵).

previous use of or resistance to (fos)amprenavir may be associated with a higher level of resistance to darunavir^{66,67}, although in phase IIb trials the impact on virologic response to darunavir was minimal⁶⁸. This commonality potentially limits the success of subsequent therapy following virologic failure of PI-containing regimens, since fewer new mutations may be required to produce viruses that are clinically resistant to the PI(s) in the salvage regimen⁶⁹.

Why is there so much cross-resistance between the PIs? Although there are clear chemical differences between all the currently available PI, when bound in the active site of HIV-1 protease they occupy a similar space and even have similar functional groups at similar positions (Fig. 3). As a result, atoms of different PIs frequently come into contact with the protease at the same residues³⁴. Thus, mutations at these residues in HIV-1 protease produce MDR viruses^{15,34,70}. The key molecular recognition question is how do the protease variants, with resistance mutations in their active sites that confer resistance to competitive inhibitors, retain the ability to recognize and cleave substrates and thus

maintain viable virus? This retention of activity often occurs without co-evolution of the substrate sequences. A partial answer to this puzzle was found when the inhibitor complexes were superimposed on the substrate complexes^{32,34} (Fig. 4). Not only are the inhibitors smaller than the substrates, but they also have a different shape than the substrates. The superimposition studies found that drug resistance mutations occur at positions where the inhibitors protrude beyond the substrate consensus volume (or substrate envelope) but are still in contact with the enzyme. This explains how resistance can occur without having a significant impact on function, as those residues are more important to inhibitor binding than they are for substrate recognition and thus are prime locations for resistant mutations.

Avoiding cross-resistance among protease inhibitors

For a PI to maintain effectiveness against resistance mutations, two characteristics are required: a high binding affinity for the wild-type protease, and resilience to the



Figure 3. The inhibitor envelope in red, as it fits within the active site of HIV-1 protease, calculated from overlapping van der Waals volume of five or more of eight inhibitor complexes. The colors of the inhibitors are yellow: nelfinavir; gray: saquinavir; cyan: indinavir; light blue: ritonavir; green: amprenavir; magenta: lopinavir; blue: atazanavir; red: darunavir (reprinted from Chemistry & Biology, Vol. 11, Nancy M King, Moses Prabu-Jeyabalan, Ellen A Nalivaika, and Celia Schiffer, Combating susceptibility to drug resistance: lessons from HIV-1 protease, p1333-8, Copyright 2004, with permission from Elsevier³⁴).

effect of mutations; i.e. the PI is able to maintain viable binding affinity and effective inhibition in the presence of those mutations^{32,34,71}. The traditional approach to combating drug resistance has included the characterization and targeting of each drug-resistant variant protein to enable the identification of new active inhibitors^{72,73}. However, this is not practical for HIV-1 protease as more than one-third of the 99 residues are known to mutate and contribute in various combinations to PI resistance^{56,57}. We have proposed a general strategy of structure-based design to decrease the probability of drug resistance developing, by designing inhibitors that interact only with those residues that are essential for function³⁴. For HIV-1 protease, this would mean developing a PI that is contained within the substrate envelope and thus interacts only with the same residues that are required for substrate recognition. Such an inhibitor would be less susceptible

to drug resistance, as any mutation that affects the inhibitor would simultaneously affect the recognition of a substantial proportion of the substrates.

In fact, darunavir and amprenavir more closely mimic substrate interactions because they fit within the substrate consensus volume or substrate envelope better than any of the other PIs (Fig. 5)^{15,34}. The combination of tight binding, mimicking substrate hydrogen bonds, and the overall fit within the substrate envelope should provide darunavir with a higher “genetic barrier” to the development of resistance, as the backbone interactions are not changed by mutation. Furthermore, the mutations that perturb darunavir binding will have a negative impact on substrate recognition and cleavage, and are either less likely to occur or will result in reduced viral replication. Thus, based on simple molecular interaction principles, this suggests that darunavir should have greater robustness in treating HIV-1 infection.

This strength was demonstrated when comparing the binding of darunavir to wild-type protease with binding to a prototype MDR HIV-1 protease variant (I84V, V82T). Variant I84V is the most common resistance mutation that affects the binding of every currently used PI. The flexible *N*-isobutyl group of darunavir interacts with residue 84 (Fig. 5) in the protease active site and allows for conformational adjustments in the ligand and possible retention of binding affinity. However, the binding affinity of darunavir (measured by isothermal titration calorimetry) was reduced by a factor of 13.3. Nevertheless, despite the reduction in binding affinity to this variant HIV protease, darunavir still binds with a 60 pM binding affinity which is more than 33-fold higher than amprenavir and more than 1.5 orders of magnitude higher than older PIs¹⁵. Other recent studies have examined the structure and binding affinity with other resistant variants^{27,50} and found a maximum of 30-fold loss in binding affinity to the I50V variant, a known amprenavir-resistant mutation and a point of contact outside of the substrate envelope (Fig. 5). Thus, the high binding affinity of darunavir appears to provide resilience against the effect of resistance mutations in the HIV-1 protease at the molecular level.

Activity of darunavir against wild-type and resistant strains of HIV, and development of resistance in vitro

Darunavir has demonstrated strong potency against both wild-type and PI-resistant viruses *in vitro*, including a broad range of over 4,000 clinical isolates^{48,51}. *In vitro*, darunavir showed potent activity against wild-type HIV-1

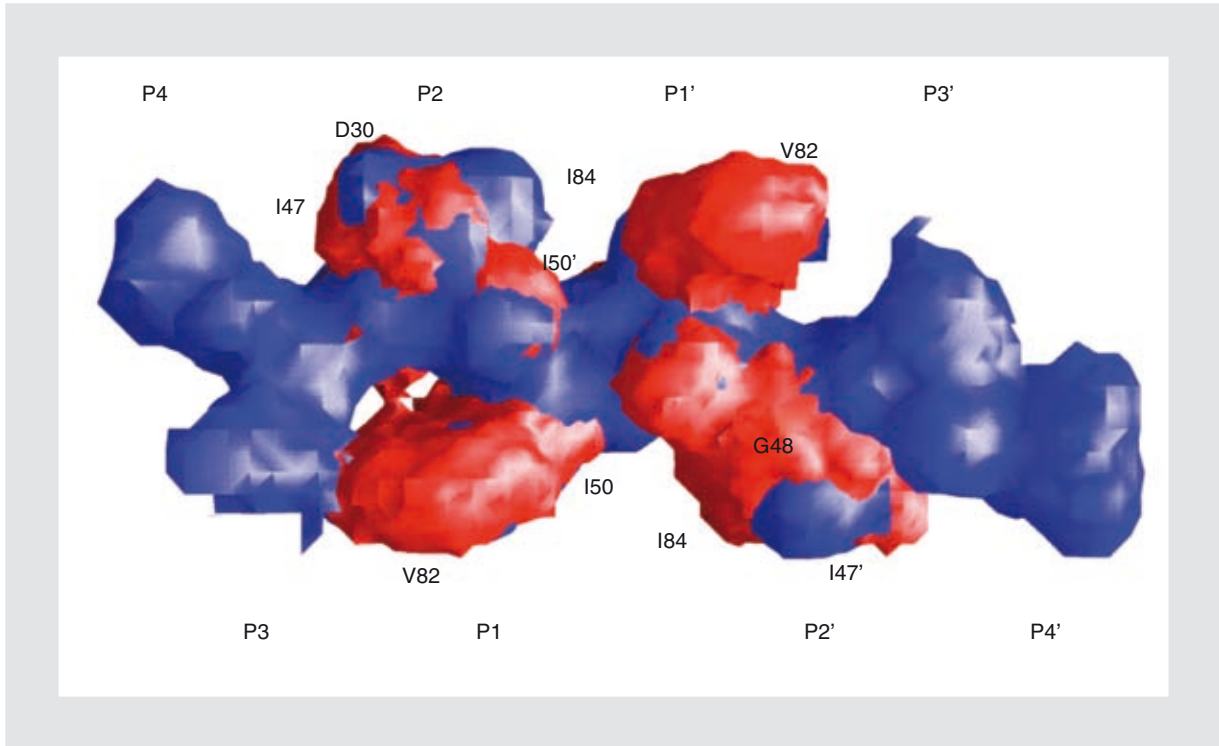


Figure 4. Superimposition of the substrate consensus volume (blue) with the inhibitor consensus volume (red). Residues that contact with the inhibitors where the inhibitors extend beyond the substrate volume and confer drug resistance when they mutate are labeled (reprinted from Chemistry & Biology, Vol. 11, Nancy M King, Moses Prabu-Jeyabalan, Ellen A Nalivaika, and Celia Schiffer, Combating susceptibility to drug resistance: lessons from HIV-1 protease, p1333-8, Copyright 2004, with permission from Elsevier³⁴).

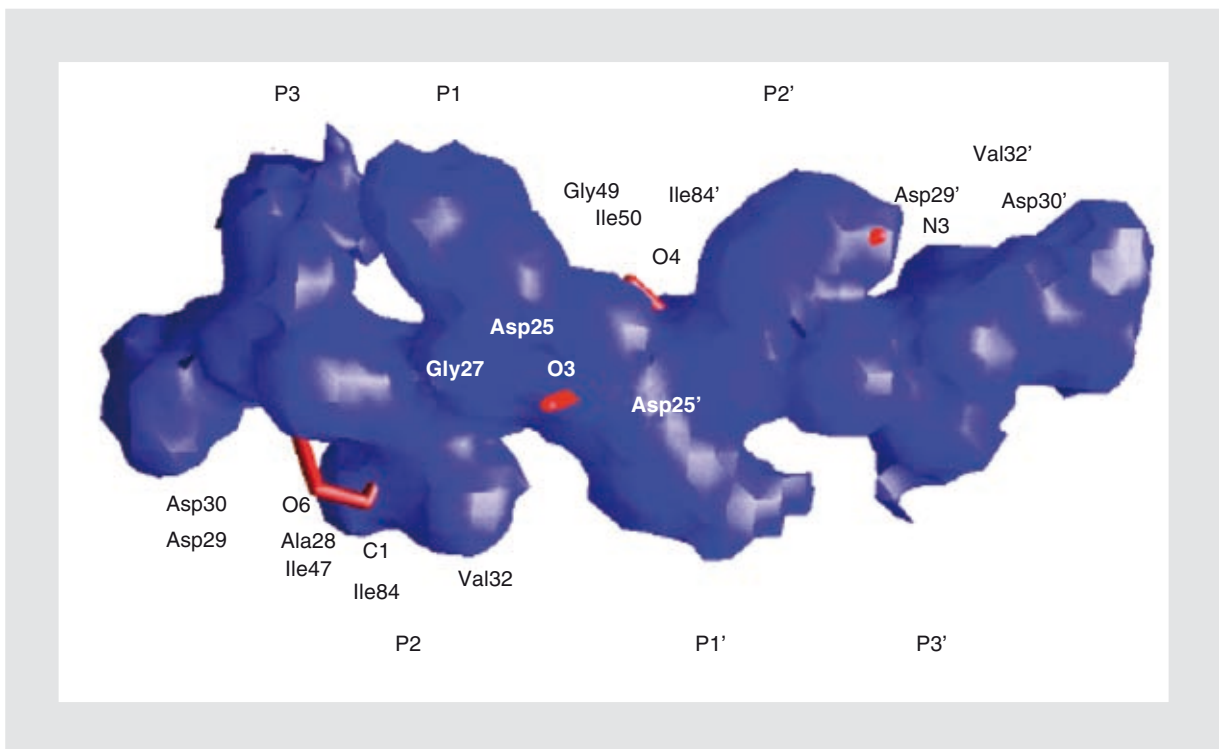


Figure 5. Crystal structure of darunavir superimposed on the structure of the substrate envelope derived from the substrate crystal structures. Atoms that protrude from the envelope are shown in red and labeled. Other labels highlight protease residues within van der Waals contact of the protruding atoms (reproduced with permission¹⁵).

and HIV-2, with an EC₅₀ of 1-5 nM and an EC₉₀ of 2.7-13 nM, with no observed cytotoxicity at concentrations up to 100 µM⁵¹. Darunavir also demonstrated good activity against 19 recombinant clinical isolates, with multiple protease mutations and phenotypic resistance to an average of five PIs. Except for one strain, darunavir showed EC₅₀ of < 10 nM for all isolates compared with EC₅₀ of > 100 nM for nelfinavir, indinavir, ritonavir, amprenavir, saquinavir, and lopinavir. Against 1,501 clinical isolates with a fourfold or greater change in EC₅₀ for one or more PIs, darunavir inhibited 75% of viruses with an EC₅₀ of < 10 nM and 21% of viruses with an EC₅₀ of 10-100 nM. Darunavir was more potent against these PI-resistant strains than any other PI tested (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir)⁴⁸.

Koh, et al. also demonstrated that darunavir has potent *in vitro* activity against wild-type HIV-1 and HIV-2 and MDR clinical HIV-1 strains⁴⁸. Compared with amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir, which suppressed the infectivity and replication of HIV-1 LAI with EC₅₀ of 17-47 nM, darunavir showed superior potency (EC₅₀ 3 nM). Darunavir also showed potent activity against HIV-1 Ba-L (EC₅₀ 3 nM) and two HIV-2 strains (EC₅₀ 3-6 nM), activity that was greater than that of the other PIs tested.

Preliminary data from *in vitro* selection studies with wild-type HIV-1 strains have shown that resistance to darunavir develops much slower and is more difficult to generate than for existing PIs^{16,51}. De Meyer, et al. showed that selection of resistant HIV with amprenavir, lopinavir, and nelfinavir was more rapid and easier than with darunavir, and resulted in the emergence of strains carrying clinically relevant PI resistance-associated mutations (Fig. 6)^{16,51}. The concentrations of these PI could readily be increased to > 1 µM and still allow virus replication. In contrast, the concentration of darunavir could not be increased rapidly and virus replication did not occur at concentrations > 200 nM (Fig. 6). Moreover, the mutations emerging in the selected strains (R41T and K70E) have never been associated with resistance to PI, and when introduced in a wild-type genetic background by site-directed mutagenesis, did not cause decreased susceptibility to darunavir⁷¹.

Clinical activity

In clinical studies, darunavir is administered together with low-dose ritonavir (darunavir/ritonavir). Published results from one phase IIa clinical study and two phase IIb studies indicate that darunavir/ritonavir has potent antiviral activity in PI-experienced patients⁷⁵⁻⁷⁷. In the

24-week, dose-finding portion of two large, randomized, controlled, 144-week phase IIb studies (POWER 1 [TMC114-C213] and POWER 2 [TMC114-C202]), patients were nucleoside reverse transcriptase inhibitor (NRTI)-experienced, nonnucleoside reverse transcriptase inhibitor (NNRTI)-experienced, and PI-experienced, had at least one primary PI mutation (according to the IAS-USA guidelines) and had viral load ≥ 1,000 copies/ml at screening^{76,77}. Both studies were of similar design. Patients were randomized to receive either investigator-selected PI(s) or one of four darunavir/ritonavir doses: 400/100 mg 1/d, 800/100 mg 1/d, 400/100 mg 2/d, or 600/100 mg 2/d; all regimens were given in combination with an optimized background regimen (OBR: at least two NRTIs with or without enfuvirtide). Patients were stratified by the number of primary PI mutations, by the use of enfuvirtide, and by screening viral load. The primary endpoint was the proportion of patients with a decrease in viral load of ≥ 1 log₁₀ HIV RNA copies/ml at week 24. Secondary endpoints included the decrease in viral load from baseline, the proportion of patients with HIV RNA < 50 copies/ml, and the mean change in CD4 cell count at week 24.

The baseline characteristics showed that patients in the POWER 2 study had somewhat more advanced disease (longer duration of infection, lower baseline CD4 and higher viral load), more patients had virus resistant to all commercially available PIs (71 vs. 63%), and a greater percentage of patients had three or more primary PI mutations (66 vs. 56%) than in the POWER 1 study⁷⁷. In both studies, darunavir/ritonavir plus OBR was shown to be significantly more effective than investigator-selected PI(s) plus OBR for both suppression of HIV-1 RNA and increase in CD4 cell count.

In the POWER 1 study, 77% of patients receiving darunavir/ritonavir 600/100 mg 2/d (the dose selected for further development in treatment-experienced patients) achieved at least a 1.0 log₁₀ reduction in viral load relative to baseline, compared with only 25% of control patients (p < 0.001)⁷⁶. Similarly, significantly more darunavir/ritonavir patients had viral load < 50 copies/ml at week 24 compared with control patients (53 vs. 18%; p < 0.001). Increases in mean CD4 cell count were substantially greater at week 24 versus baseline for darunavir/ritonavir compared with the control group (124 vs. 20 cells/mm³; p < 0.001).

Similar results were observed in the POWER 2 study, which recruited more advanced patients; significantly more patients on darunavir/ritonavir 600/100 mg 2/d achieved at least a 1.0 log₁₀ reduction in viral load relative to baseline compared with control patients (62 vs.

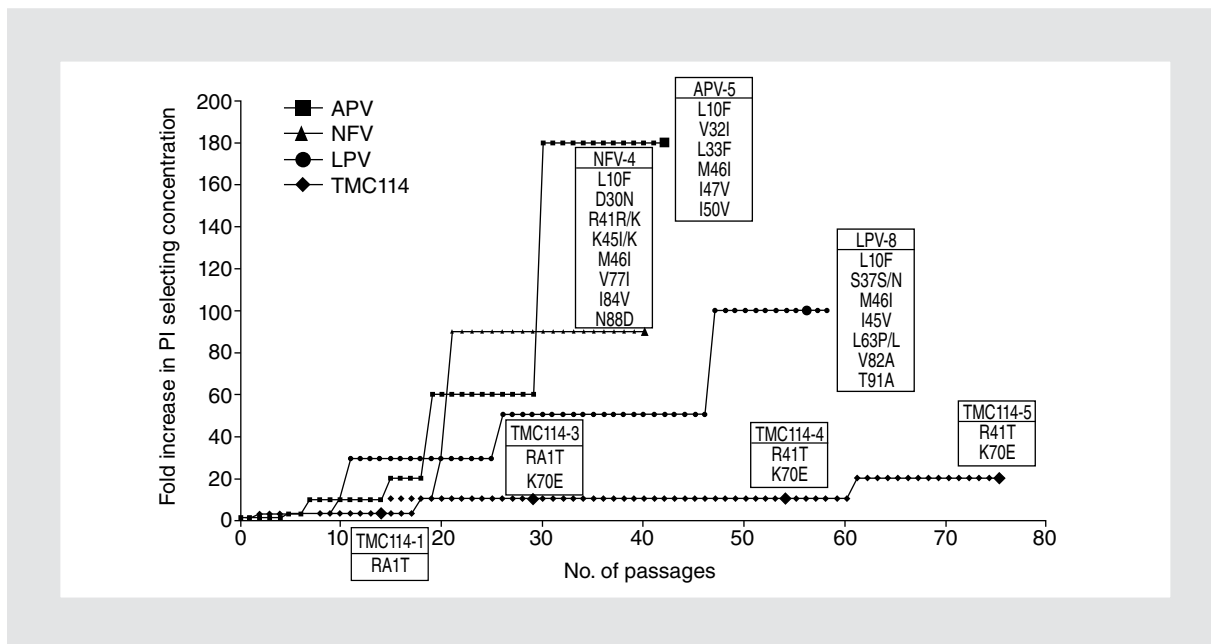


Figure 6. In vitro selection of resistant HIV from wild-type in the presence of amprenavir, lopinavir, nelfinavir, or darunavir (TMC114) in sequential passage experiments. Each passage represents 3-4 days, with passage 75 representing a total of 260 days. Enlarged symbols indicate genotypes of viruses selected at defined times and list all genotypic changes from the original HIV-1 LAI strain. Curves are normalized and starting concentrations were 10, 20, 100, and 100 nM for darunavir (TMC114), lopinavir, amprenavir, and nelfinavir, respectively (reproduced with permission⁵¹). APV: amprenavir; NFV: nelfinavir; LPV: lopinavir; TMC114: darunavir.

14%; $p < 0.001$)⁷⁷. Patients on the recommended dose of darunavir/ritonavir were also more likely to achieve a viral load < 50 copies/ml than control patients (39 vs. 7%; $p < 0.001$). The mean increase in CD4 cell count at week 24 versus baseline was significantly greater for patients on darunavir/ritonavir 600/100 mg compared with control patients (59 vs. 12 cells/mm³; $p < 0.01$).

At week 24, the overall mean changes versus baseline in log₁₀ viral load (NC = F) for the darunavir/ritonavir 600/100 mg 2/d treatment groups of the POWER 1 and 2 studies were -2.03 and -1.71 copies/ml, respectively^{76,77}. In the control groups, the mean changes versus baseline in log₁₀ viral load were -0.63 and -0.29 copies/ml, respectively^{76,77}. Importantly, the magnitude of the differences in change in viral load between the darunavir/ritonavir 600/100 mg 2/d groups and the control groups of the POWER 1 and 2 studies were comparable: a difference of 1.35 and 1.37 copies/ml, respectively^{76,77}.

Subgroup analyses demonstrated that the inclusion of a greater number of active antiretrovirals (including NRTI and enfuvirtide) in the OBR was associated with better virologic outcomes in all treatment groups. In the POWER 1 study, 63% of patients receiving darunavir/ritonavir 600/100 mg 2/d and using enfuvirtide for the first time achieved suppression < 50 copies/ml at week

24 compared to 56% of patients who did not receive enfuvirtide (for control: 22 and 19%, respectively)⁷⁶. In the POWER 2 study, this was 64 and 30% of patients, respectively (for control: 7 and 4%, respectively)⁷⁷. This apparent difference was likely related to the number of other viable agents in their OBR. On the recommended dose, the population evaluated in the POWER 1 study had a greater proportion of patients with one or more susceptible NRTIs than POWER 2 (77 vs. 62%)^{76,77}. Taken together, these findings underscore the value of using at least two potent antiretroviral agents in heavily treatment-experienced patients. Similar results were obtained with a comparable patient population receiving the darunavir/ritonavir 600/100 mg 2/d dose, in a non-randomized integrated analysis of two trials (POWER 3 [TMC114-C215 and C208])⁷⁸.

The pooled POWER 1 and 2 48-week analysis showed darunavir/ritonavir provided durable efficacy in this highly treatment-experienced population⁷⁹. More recently, treatment with darunavir/ritonavir 600/100 mg 2/d was shown to be statistically non-inferior and superior to lopinavir/ritonavir 400/100 mg 2/d for the endpoint of viral load < 400 copies/ml in early treatment-experienced patients at 48 weeks in the phase III TITAN trial⁸⁰. Treatment with darunavir/ritonavir 800/100 mg 1/d in treatment-naïve patients was also shown to be

non-inferior to lopinavir/ritonavir 800/200 mg (either 1/d or 400/100 mg 2/d) for the endpoint of viral load < 50 copies/ml in the phase III ARTEMIS trial at 48 weeks, and was more effective in patients with baseline HIV-1 RNA above 100,000 copies/ml⁸¹.

In terms of safety, darunavir showed no cytotoxicity at concentrations of up to 100 μ M in *in vitro* studies⁵¹. In patients, darunavir/ritonavir has been shown to be well tolerated in a phase IIa clinical study and the phase IIb POWER 1 and POWER 2 controlled studies⁷⁵⁻⁷⁷. In a combined analysis of all darunavir/ritonavir dose groups from POWER 1 and POWER 2, the most common adverse events (occurring in $\geq 10\%$ of patients, regardless of severity and causality) were similar in darunavir/ritonavir-treated patients and in the control arms; in decreasing order of incidence, these were: diarrhea, headache, nausea, fatigue, nasopharyngitis, upper respiratory tract infection, insomnia, cough, herpes simplex, and pyrexia⁸². The combined safety analysis revealed that there were more discontinuations in the control group (81% total: 67% for virologic failure, 5% for adverse events) than in the darunavir/ritonavir groups (25% total: 12% for virologic failure, 8% for adverse events) resulting in a shorter treatment duration for control patients. Finally, the safety results of the POWER 1 and POWER 2 studies showed no apparent relationship between darunavir dose and adverse event incidence, and no overall difference compared to PI used in the control groups. The safety results of POWER 3 were similar to those of POWER 1 and POWER 2⁷⁸. The pooled 48-week analysis of POWER 1 and 2 safety and tolerability results⁷⁹, along with the more recent 48-week data from the two phase III TITAN⁸⁰ and ARTEMIS⁸¹ trials, confirmed the favorable tolerability of darunavir/ritonavir treatment.

Darunavir/ritonavir can be used in patients with mild to moderate liver impairment. However, increased aspartate and alanine aminotransferase monitoring should be considered in patients with underlying chronic hepatitis, cirrhosis, or in patients who have pretreatment elevations of transaminases, especially during the first several months of treatment.

Darunavir resistance in the clinic

The most predictive factor for virologic outcome in the POWER 1, 2, and 3 studies was the fold change in EC₅₀ value for darunavir at baseline. The determinants of increased fold change for darunavir were researched by analyzing the influence of both the number and the type of protease mutations on the virologic response

at week 24, as well as the mutations that emerged upon virologic failure while on darunavir treatment.

Mutations present at study baseline that were associated with a diminished virologic response to darunavir/ritonavir were V111I, V32I, L33F, I47V, I50V, I54L/M, G73S, L76V, I84V, and L89V. Isolates from patients harboring these mutations tended to have a higher number of PI resistance-associated mutations, as compared with patients who did not harbor these mutations at baseline. Even in the presence of these mutations, however, virologic response in the darunavir/ritonavir arm was higher than for the control arm.

Mutations that developed on-study in $\geq 10\%$ of patients who failed virologically were V32I, L33F, I47V, I54L, and L89V. However, when introduced in a wild-type genetic background by site-directed mutagenesis, mutations V32I, L33F, I47V, or I54L (alone or in combination with one or two other PI resistance-associated mutations) did not cause decreased susceptibility to darunavir (mutation L89V is currently being studied). Interestingly, darunavir makes contacts outside of the substrate envelope at V32, I47, I50, and I84 (Fig. 5) making several of these sites of mutation consistent with the substrate envelope theory. Finally, isolates from patients experiencing virologic failure by rebound showed a median 8.14-fold change increase for darunavir at endpoint compared with baseline. In the same group of patients, however, no such fold change increase (median increase of 0.82) was found for tipranavir, suggesting limited cross-resistance between these two PI. However, this is based on a small number of observations. The fold change increase could not be studied for the other PIs, since the baseline isolates were already resistant to these PIs.

Thus, mutations developing upon virologic failure in this population of highly treatment-experienced patients with extensive PI resistance conferred resistance to darunavir only in the presence of a high number of PI resistance-associated mutations⁸³.

Conclusions

As resistance continues to be a problem with the currently available antiretrovirals, there is a significant need for new agents. In the case of PIs, these must have a higher threshold to the development of resistance. This should help limit the development of cross-resistance. The binding characteristics between a PI and the active site of the HIV-1 protease have been shown to be important factors in the development of resistance and cross-resistance. In particular, how PIs fit within

the substrate envelope (or substrate consensus volume) seems to be crucial, since primary drug-resistant mutations frequently occur at positions in the protease that are contacted by PI atoms that protrude beyond it. In addition, these specific residues correspond to those where most MDR mutations occur. Insight into the structure, thermodynamics, and dynamics of the active site should enable the design of new PIs that mostly fit within the substrate envelope and thus reduce the potential for the development of drug resistance.

Darunavir is a novel PI with improved potency and binding characteristics compared with most currently available PIs. Important distinguishing features include high binding affinity for the protease binding site, the presence of hydrogen bonds with the backbone at the active-site cleft, and fitting primarily within the substrate envelope or substrate consensus volume. Darunavir/ritonavir has demonstrated potent *in vitro* activity against wild-type and PI-resistant viruses and proves difficult to select for resistance *in vitro*. In clinical studies, darunavir/ritonavir has demonstrated highly potent viral suppression, including highly significant decreases in HIV viral load in both treatment-naïve and treatment-experienced patients with documented PI resistance, favorable tolerability, and the potential for both twice-daily and once-daily dosing. Due to its high binding affinity for and the closeness and flexibility of fit within the protease, darunavir has a higher genetic barrier to the development of resistance and improved clinical efficacy against MDR HIV compared with most current PIs, making it a valuable option for the treatment of HIV-infected patients.

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