Genotypic Testing for HIV-1 Drug Resistance (11-30-03)

by

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I. Rationale for HIV-1 drug resistance testing

Nineteen antiretroviral drugs have been approved for the treatment of HIV-1 infection: seven nucleoside and one nucleotide reverse transcriptase inhibitors (NRTIs), seven protease inhibitors (PIs), three nonnucleoside RT inhibitors (NNRTIs), and one fusion inhibitor. In previously untreated individuals with drug-susceptible HIV-1 strains, combinations of three or more drugs from two drug classes (highly active antiretroviral therapy, HAART) can lead to prolonged virus suppression and immunologic reconstitution.

However, drug resistance remains as relevant in the post-HAART era as it was in the pre-HAART era. First, tens of thousands of individuals who began therapy in the early and mid-1990s already harbor multidrug-resistant viruses. Second, a significant proportion of new HIV infections result from the transmission of strains that are already resistant to one or more antiretroviral drugs. Third, as the epidemic continues to grow worldwide, increasing numbers of individuals are being treated. The margin of success for achieving and maintaining virus suppression is narrow. Extraordinary patient effort is required to adhere to drug regimens that are expensive, inconvenient, and often associated with dose-limiting side effects. Incomplete virus suppression due to these factors predisposes to the development of drug resistance, which threatens the success of future treatment regimens.

Drug resistance before starting a new drug regimen is an independent predictor of virologic response to that regimen (reviewed in (DeGruttola et al., 2000; Demeter & Haubrich, 2001; Hanna & D'Aquila, 2001; Haubrich & Demeter, 2001)). Several prospective controlled studies have also shown that patients whose physicians have access to drug resistance data, particularly genotypic resistance data, respond better to therapy than control patients whose physicians do not have access to these assays (Durant et al., 1999; Baxter et al., 2000; Cingolani et al., 2002; Cohen et al., 2002; Tural et al., 2002; Wegner et al., 2002; Vray et al., 2003). The accumulation of such retrospective and prospective data has led several expert panels to recommend the use of resistance testing in the treatment of HIV-1-infected patients (Hirsch et al., 2000; British HIV Association, 2001; EuroGuidelines Group for HIV Resistance, 2001; US Department of Health and Human Services Panel on Clinical Practices for Treatment of HIV Infection, 2003) (Table 1). The purpose of this review is to summarize the genetic basis of HIV-1 drug resistance and the implications of genotypic resistance data for choosing antiretroviral therapies.

II. Evolution of HIV-1 drug resistance

The evolution of HIV-1 drug resistance within an individual depends on the generation of genetic variation in the virus and on the selection of drug-resistant variants during therapy. HIV-1 genetic variability is a result of the inability of HIV-1 reverse transcriptase (RT) to proofread nucleotide sequences during replication (Mansky, 1998). This variability is compounded by the high rate of HIV-1 replication, the accumulation of proviral variants during the course of HIV-1 infection, and genetic recombination when viruses of different sequence infect the same cell. As a result, innumerable genetically distinct variants (quasispecies) evolve within an individual in the months following primary infection (Coffin, 1995).

Development of drug resistance depends on the extent to which virus replication continues during drug therapy, the ease of acquisition of a particular mutation (or set of mutations), and the effect of drug-resistance mutations on drug susceptibility and virus fitness. Some mutations selected during drug therapy confer measurable phenotypic resistance by themselves, whereas other mutations compensate for the diminished replicative activity that can be associated with drug resistance or cause resistance only when present in combination with other mutations.

It is estimated that every possible single point mutation occurs between $10^4$ and $10^5$ times per day in an untreated HIV-1-infected individual and that double mutants also occur commonly (Coffin, 1995). Therefore, most drug-resistance mutations are probably present prior to the start of therapy. However, viruses containing sufficient drug-resistance mutations to replicate in the presence of multiple drugs do not appear to exist in previously untreated persons infected with wildtype viruses. Indeed, once potent HIV-1 suppression is achieved in previously untreated persons, it usually persists indefinitely if therapy is not interrupted (Phillips et al., 2001).

Resistant virus strains can also be transmitted between individuals. In the United States and Europe about 10% to 20% of new infections are with HIV-1 strains harboring resistance to at least one of three classes of antiretroviral drugs (Boden et al., 1999; Yerly et al., 1999; Balotta et al., 2000; Salomon et al., 2000; Tamalet et al., 2000a; Briones et al., 2001; Duwe et al., 2001; UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance, 2001; Grant et al., 2002; Harzie et al., 2002; Little et
al., 2002; Simon et al., 2002; Bennett et al., 2003; Chaix et al., 2003; Grant et al., 2003; Mendoza et al., 2003; Wensing et al., 2003). These studies show a gradual increase in resistance to PIs and NNRTIs and a gradual increase in isolates with resistance to drugs in more than one drug class. In persons found to be newly diagnosed with HIV-1 in whom the year of infection is not known, rates of resistance are lower but still clinically significant ranging between 5% to 10% (Bennett et al., 2003; Chaix et al., 2003; Jayaraman et al., 2003; Pillay & Green, 2003; Wensing et al., 2003).

III. Identifying and characterizing drug resistance mutations

Definition of antiviral drug resistance

Antiviral drug resistance is defined by the presence of virus mutations that reduce drug susceptibility compared with the susceptibility of wild type viruses. Antiviral resistance can be mediated either by changes in the molecular target of therapy or in other virus proteins that indirectly interfere with a drug's activity. However, HIV-1 drug resistance is caused primarily by changes in the molecular targets of therapy. HIV-1 drug resistance should be distinguished from other causes of drug failure such as nonadherence, insufficient drug levels, and drug regimens with intrinsically weak antiviral activity.

The terms "drug resistance" and "reduced drug susceptibility" have similar meaning provided that each term is viewed as representing a continuum between susceptible and highly resistant. Because antiretroviral drugs differ in their potencies, reductions in susceptibility must be related to the activity of the drug against wild type viruses. Pre-existing resistant variants are often present in a small subset of wild type virus populations. Although many group O isolates are intrinsically resistant to NNRTIs, naturally occurring resistance against group M HIV-1 is uncommon for currently approved antiretroviral drugs.

Drug susceptibility testing

Drug susceptibility testing involves culturing a fixed inoculum of HIV-1 in the presence of serial dilutions of an inhibitory drug. The concentrations of drug required to inhibit virus replication by 50% (IC$_{50}$) or 90% (IC$_{90}$) are the most commonly used measures of drug susceptibility. Drug susceptibility results depend on the inoculum size of virus tested, the cells used for virus replication, and the means of assessing virus replication. Drug susceptibility assays are not designed to determine the exact amount of drug required to inhibit virus replication in vivo but rather to identify differences in the drug concentration required to inhibit a fixed inoculum of a virus relative to the concentrations required to inhibit wild type viruses. Virus susceptibility to a drug can be characterized by the range in susceptibility obtained testing wild type virus isolates (wild type susceptibility range) and the range in susceptibility obtained testing resistant virus isolates (dynamic susceptibility range).

Characterizing drug-resistance mutations

Drug resistant viruses are often first identified by in vitro passage experiments in which HIV-1 isolates are cultured in the presence of increasing concentrations of an antiviral compound. Isolates identified in this manner are sequenced to identify genetic changes arising during selective drug pressure and tested for drug susceptibility to confirm the development of resistance. In some cases, the specific mutations observed during in vitro passage experiments are placed in a wild type HIV-1 construct to confirm their role in causing drug resistance and to quantify their effect. However, the spectrum of mutations developing during in vitro passage experiments is narrower than the spectrum of mutations developing in virus isolates from treated patients, especially those receiving drugs in combination or in sequence. Therefore, mutations should also be linked to drug resistance by showing that they are selected in persons receiving an antiretroviral drug, that they reduce drug susceptibility in clinical isolates, or that they interfere with the virologic response to a new drug treatment (Table 2).

HIV-1 isolates from persons experiencing virologic failure provide insight into which mutations the virus uses to escape from drug suppression in vivo and are particularly important for elucidating the genetic mechanisms of resistance to drugs that are difficult to test in vitro. Drug susceptibility results quantify the impact of a mutation or combination of mutations in vitro. Finally, correlations between genotype and virologic response to a new regimen are essential for demonstrating the clinical significance of drug-resistance mutations. Many drug-resistance mutations compromise enzymatic function. Although the fitness of these variants can be tested in vitro, such tests cannot distinguish defects for which other genetic changes in the virus may readily compensate from defects that may be more crippling. How a
mutant virus responds to a new drug regimen in vivo therefore provides the most meaningful test of virus fitness.

**Conventions for describing drug-resistance mutations**

There is a standard numbering system for HIV-1 protease and RT based on their amino acid sequences. The most commonly used wild type reference sequence is the subtype B consensus sequence. This sequence was originally derived from alignments in the HIV Sequence Database at Los Alamos National Laboratory (Kuiken et al., 1999) and can also be found on the HIV RT and Protease Sequence Database (HIVRT&PrDB) (Rhee et al., 2003). Mutations are typically described using a shorthand notation in which a letter indicating the consensus B wild type amino acid is followed by the amino acid residue number followed a letter indicating the mutation (e.g. T215Y).

Because so many mutations in both the protease and RT have been associated with drug resistance, it has become customary to label some drug-resistance mutations as either "primary" or "major" and other mutations as "secondary" or "minor". Primary mutations are those that reduce drug susceptibility by themselves whereas secondary mutations reduce drug susceptibility in combination with primary mutations or improve the replicative fitness of virus isolates with a primary mutation. However, which mutations are considered primary and which are considered secondary are not strictly defined and some mutations might be considered to be primary for one drug but secondary for another drug.

**Reproducibility of genotypic testing by dideoxynucleoside sequencing**

The results of genotypic sequencing have become highly reproducible. A study in which two laboratories compared the reproducibility of RT and protease sequencing using cryopreserved plasma aliquots from 46 heavily treated HIV-1-infected persons, the rates of complete sequence concordance between the two laboratories was 99.1% (Shafer et al., 2001). Approximately 90% of the discordances were partial, defined as one laboratory detecting a mixture and the second laboratory detecting only one of the mixture's components. Therefore, only 0.1% of the nucleotides were discordant and these were significantly more likely to occur in plasma samples with lower plasma HIV-1 RNA levels. In every case in which one laboratory detected a mixture, the second laboratory detected the same mixture or detected one of the mixture's components. The high rate of concordance in detecting mixtures and the fact that most discordances were partial suggests that most discordances were caused by variation in sampling of the HIV-1 quasispecies, rather than sequencing errors.

**IV. Protease inhibitor (PI) resistance**

**HIV-1 protease**

The HIV-1 protease enzyme is responsible for the post-translational processing of the viral gag and gag-pol polyproteins to yield the structural proteins and enzymes of the virus. The enzyme is an aspartic protease composed of two non-covalently associated, structurally identical monomers 99 amino acids in length (Figure 1). Its active site resembles that of other aspartic proteases and contains the conserved triad, Asp-Thr-Gly, at positions 25-27. The hydrophobic substrate cleft recognizes and cleaves 9 different peptide sequences to produce the matrix, capsid, nucleocapsid, and p6 proteins from the gag polyprotein and the protease, RT, and integrase proteins from the gag-pol polyprotein (Figure 2). The enzyme contains a flexible flap region that closes down on the active site upon substrate binding.

The three-dimensional structures of wild type HIV-1 protease and several drug-resistant mutant forms bound to various inhibitors (Baldwin et al., 1995; Chen et al., 1995; Ala et al., 1997; Ala et al., 1998; Mahalingam et al., 1999; Mahalingam et al., 2001) and to the enzymes natural polypeptide substrates (Prabu-Jeyabalan et al., 2002) have been determined by crystallography. Mutations in the substrate cleft cause resistance by reducing the binding affinity between the inhibitor and the mutant protease enzyme. Mutations elsewhere in the enzyme either compensate for the decreased kinetics of enzymes with active site mutations or also cause resistance by altering enzyme catalysis, dimer stability, inhibitor binding kinetics, or by re-shaping the active site through long-range structural perturbations (Erickson et al., 1999; Barbour et al., 2002; Muzammil et al., 2003). Most substrate cleft mutations cause a 2-5-fold reduction in susceptibility in vitro to one or more PIs. However, additional mutations in the enzyme flap and in other parts of the molecule are usually required for resistance to emerge in vivo. This requirement for multiple mutations to overcome the activity of PI has been referred to as a "genetic barrier" to drug resistance (Condra et al., 1996; Molla et al., 1996; Kempf et al., 2001b).
Mutations at several of the protease cleavage sites are also selected during treatment with protease inhibitors (Doyon et al., 1996; Zhang et al., 1997; Mammano et al., 1998; Cote et al., 2001; Kaufmann et al., 2001; Kempf et al., 2001a; Maguire et al., 2002b; Prado et al., 2002). Protease cleavage site mutations improve the kinetics of protease enzymes containing PI-resistance mutations. Cleavage site mutations are compensatory rather than primary and there have been no reports that changes at cleavage sites alone can cause PI resistance. Most of the reported cleavage site mutations occur at the cleavage sites in the 3' part of the gag gene, the p7/p1 and p1/p6 cleavage sites. It is not known whether these are the most commonly mutated cleavage sites or whether mutations at these sites are just detected most commonly because they are convenient to sequence, being just 5' to the protease gene.

Protease Inhibitors (PIs)

There are seven FDA-approved PIs: amprenavir, indinavir, lopinavir (manufactured in combination with ritonavir), nelfinavir, ritonavir, saquinavir, and the recently approved compound atazanavir. The dynamic susceptibility range for each of the PIs is about 100-fold (Hertogs et al., 1998; Hertogs et al., 2000b; Petropoulos et al., 2000; Kempf et al., 2001b; Parkin et al., 2003b). The spectrum of mutations developing during therapy with indinavir, nelfinavir, saquinavir, ritonavir, and amprenavir have been well characterized (Condra et al., 1996; Molla et al., 1996; Schapiro et al., 1996; Boden & Markowitz, 1998; Craig et al., 1998; Patrick et al., 1998; Shafer et al., 1999b; Atkinson et al., 2000; Maguire et al., 2002a) but fewer data are available for lopinavir (Masquelier et al., 2002; Romano et al., 2002a) and atazanavir (Colombo et al., 2002). Fosamprenavir (GW433908) is a prodrug of amprenavir with improved bioavailability that was approved by the FDA in October 2003. Preliminary data suggest that the spectrum of mutations developing during therapy with fosamprenavir is similar to that developing with amprenavir (Macmanus et al., 2003).

Pharmacologic factors influence the clinical efficacy of PIs more than that of the other classes of antiretroviral drugs (Schapiro et al., 1996; Stein et al., 1996; Burger et al., 1998; Hoetelmans et al., 1998; Acosta et al., 1999; Murphy et al., 1999b; Durant et al., 2000; Hoetelmans, 2001; van Heeswijk et al., 2001). Virologic response is highly correlated with the inhibitory quotient (IQ) defined as the trough concentration divided by the inhibitory concentration of the drug (e.g. the IC50 in a standardized assay) (Hoetelmans, 2001; Shulman et al., 2002a; Marcelin et al., 2003b). Drug levels achieved during PI monotherapy can vary greatly among individuals, often resulting in low IQs (van Heeswijk et al., 2001). This has led to the practice of administering sub-therapeutic doses of ritonavir (a cytochrome P450 enzyme inhibitor) in combination with other PIs to increase, or “boost” their drug levels (van Heeswijk et al., 2001). Lopinavir is formulated in a fixed combination with ritonavir (Hurst & Faulds, 2000); and saquinavir, indinavir, and amprenavir are now usually administered with low-dose ritonavir (van Heeswijk et al., 2001). Boosted PIs require higher levels of resistance than PIs given as monotherapy before significant loss of antiviral activity and virologic rebound occur (Kempf et al., 2002; Shulman et al., 2002a; Marcelin et al., 2003b).

Protease substrate cleft mutations (Figures 1, 3)

V82A/T/F/S occur predominantly in HIV-1 isolates from patients receiving treatment with indinavir or ritonavir (Condra et al., 1996; Molla et al., 1996). V82A also occurs in isolates from patients receiving prolonged therapy with saquinavir following the development of the mutation G48V (Winters et al., 1998b; Sevin et al., 2000). By themselves, mutations at codon 82 confer reduced susceptibility in vitro to indinavir, ritonavir, and lopinavir (Condra et al., 1996; Molla et al., 1996; Sham et al., 1998; Kempf et al., 2001b) but not to nelfinavir, saquinavir, or amprenavir. However, when present with other PI mutations, V82A/T/F/S contribute phenotypic and clinical resistance to each of the PIs (Shafer et al., 1998; Sham et al., 1998; Winters et al., 1998b; Kempf et al., 2001b; Colombo et al., 2003; Marcelin et al., 2003b) (Table 3). V82A is the most common mutation at this position; V82S, the least common. The phenotypic and clinical significance of these differences and the differences between each of these mutations has not been studied. V82I occurs in about 1% of untreated individuals with subtype B HIV-1 and in 5-10% of untreated individuals with non-B isolates (Gonzales et al., 2001). Although V82I occasionally emerges during PI therapy (Maguire et al., 2002a), preliminary data suggest that V82I confers minimal or no resistance to the available PIs (King et al., 1995; Descamps et al., 1998; Brown et al., 2001; Rhee et al., 2003).

I84V has been reported in patients receiving indinavir, ritonavir, saquinavir, and amprenavir as their sole PI (Condra et al., 1996; Molla et al., 1996; Craig et al., 1998; Hertogs et al., 2000b; Sevin et al., 2000; Maguire et al., 2002a) and causes phenotypic (Partaledis et al., 1995; Tisdale et al., 1995; Condra et
al., 1996; Patick et al., 1996; Carrillo et al., 1998; Palmer et al., 1999; Kempf et al., 2001b; Prado et al., 2002; Colonno et al., 2003) and/or clinical (Zolopa et al., 1999b; Para et al., 2000; Kempf et al., 2002; Marcelin et al., 2003b) resistance to each of the PIs. I84V is rarely the first major PI-resistance mutation to develop, usually developing in isolates that already have the mutation L90M (Kantor et al., 2002; Wu et al., 2003). I84A and I84C are extremely rare mutations that are also associated with resistance to multiple PIs when present in combination with other PI-resistance mutations (Mo et al., 2003b).

G48V occurs primarily in patients receiving saquinavir and rarely in patients receiving indinavir. This mutation causes 10-fold resistance to saquinavir and about 3-fold resistance to indinavir, ritonavir, and nelfinavir (Jacobsen et al., 1995; Patick et al., 1996; Hertogs et al., 1998; Winters et al., 1998b). G48V has been reported to cause low-level biochemical resistance to amprenavir when present in site-directed mutants, but to interfere with amprenavir resistance when present together with more typical amprenavir-resistance mutations such as M46I, I47V, and I50V (Markland et al., 2000). Its affect on lopinavir and atazanavir is not known. G48V usually occurs with mutations at positions 54 and 82 (Shafer et al., 1998; Palmer et al., 1999; Schiffer et al., 2001; Wu et al., 2003).

D30N occurs solely in patients receiving nelfinavir and confers no \textit{in vitro} or clinical cross-resistance to the other PIs (Patick et al., 1996; Markowitz et al., 1998; Winters et al., 1998b; Zolopa et al., 1999a). D30N confers reduces nelfinavir susceptibility by 5-20 fold. D30N is often followed by the development of N88D and the combination reduces nelfinavir susceptibility by about 50-fold (Rhee et al., 2003). D30N usually does not develop in isolates containing other primary PI-resistance mutations (Kantor et al., 2002; Sugiuara et al., 2002; Wu et al., 2003).

I50V has been reported only in patients receiving amprenavir as their first PI (Maguire et al., 2002a). In addition to causing reduced amprenavir susceptibility, it causes reduced susceptibility to ritonavir and lopinavir (Partaleidis et al., 1995; Tisdale et al., 1995; Molla et al., 2001; Prado et al., 2002; Mo et al., 2003a; Parkin et al., 2003b). The development of I50V usually requires a specific compensatory cleavage site mutation (Maguire et al., 2002a; Prado et al., 2002). I50L occurs in patients receiving atazanavir as their first PI (Colonno et al., 2002). It reduces atazanavir susceptibility by 5-10 fold and causes hypersusceptibility to each of the remaining PIs (Colonno et al., 2002).

V32I occurs in patients receiving indinavir, ritonavir, or amprenavir. It usually occurs in association with other PI resistance mutations in the substrate cleft or flap and by itself appears to cause minimal resistance to any one drug. However, in combination with other mutations such as M46I/L, I47V, V82A, and I84V, high levels of resistance to multiple PIs, including lopinavir, have been reported (Parkin et al., 2003b).

R8K and R8Q are substrate cleft mutations that cause high-level resistance to one of the precursors of ritonavir (A-77003) (Ho et al., 1994; Gulnik et al., 1995) but they have not been reported with the current PIs.

\underline{Protease flap mutations (Figures 1, 3)}

The protease flaps (residues 33-62) extends over the substrate-binding cleft and must be flexible to allow entry and exit of the polypeptide substrates and products (Shao et al., 1997; Scott & Schiffer, 2000). The flap tips (residues 46-54) are particularly mobile and are the site of many drug-resistance mutations. In addition to mutations at positions 48 and 50, which extend into the substrate cleft, mutations at positions 46, 47, 53, and 54 make important contributions to drug resistance.

Mutations at position 54 (generally I54V, less commonly I54T/L/M/S) contribute resistance to each of the approved PIs (Condra et al., 1996; Molla et al., 1996; Kempf et al., 2001b; Maguire et al., 2002a; Colonno et al., 2003) and have been frequently reported during primary therapy with indinavir, ritonavir, amprenavir, and saquinavir, (Condra et al., 1996; Molla et al., 1996; Schapiro et al., 1996; Patick et al., 1998; Maguire et al., 2002a) and salvage therapy with lopinavir (Masquelier et al., 2002; Romano et al., 2002a; King et al., 2003a). I54L and I54M are particularly common in persons receiving amprenavir and have a greater effect on amprenavir than does I54V (Maguire et al., 2002a).

Mutations at position 46 (usually M46I/L, rarely M46V) contribute to resistance to each of the PIs except possibly saquinavir (Condra et al., 1996; Molla et al., 1996; Kempf et al., 2001b; Maguire et al., 2002a; Colonno et al., 2003) and have been frequently reported during primary therapy with indinavir, ritonavir, amprenavir, and nelfinavir (Molla et al., 1996; Schapiro et al., 1996; Patick et al., 1998; Condra et al., 2000; Maguire et al., 2002a) and during salvage therapy with lopinavir (Masquelier et al., 2002; Romano et al., 2002a).
I47V has been reported in patients receiving amprenavir, indinavir, and ritonavir, and often occurs in conjunction with the nearby substrate cleft mutation, V32I (Parkin et al., 2003b). I47A is an uncommon mutation that is associated with high-level resistance to lopinavir and intermediate resistance to amprenavir (Kagan et al., 2003).

F53L has been reported rarely in patients receiving PI monotherapy, but it occurs in more than 10% of patients treated with multiple PIs (Wu et al., 2003). In a multivariate analysis it has been associated with phenotypic resistance to lopinavir (Kempf et al., 2001b). F53Y is a less commonly occurring substitution at this position which occurs only in treated persons and probably has a similar role as F53L (Wu et al., 2003).

Protease mutations at other conserved residues (Figures 1, 3)

L90M has been reported from isolates from patients treated with saquinavir, nelfinavir, indinavir, and ritonavir. L90M either contributes to or directly confers in vitro and in vivo resistance to each of the seven approved PIs (Condra et al., 1996; Patrick et al., 1998; Lawrence et al., 1999; Hertogs et al., 2000b; Para et al., 2000; Dronda et al., 2001; Kempf et al., 2001b; Kempf et al., 2002; Colomno et al., 2003; Marcelin et al., 2003a) (Table 3). Crystal structures with and without the mutant have shown that the Leu90 side chain lies next to Leu24 and Thr26 on either side of the catalytic Asp25 (Mahalingam et al., 1999; Olsen et al., 1999; Mahalingam et al., 2001) but the mechanism by which L90M causes PI resistance is not known.

Mutations at codon 73, including G73C/S/T, have been reported in 10% of patients receiving indinavir and saquinavir as their only PI and less commonly in patients receiving nelfinavir as their only PI (Shafer et al., 1999b; Wu et al., 2003). However, this mutation occurs most commonly in patients failing multiple PIs, usually in conjunction with L90M (Kantor et al., 2002; Wu et al., 2003).

Mutations at position 88 (N88D and N88S) commonly occur in patients receiving nelfinavir and occasionally in patients receiving indinavir. By itself, a mutation at this position causes low-level resistance to nelfinavir and indinavir. However, mutations at this position cause high-level nelfinavir resistance in the presence of D30N or M46I (Colonno et al., 2000; Petropoulos et al., 2000; Ziermann et al., 2000). N88S (but not N88D) has been shown to hypersensitize isolates to amprenavir (Ziermann et al., 2000).

L24I has been reported primarily in HIV-1 isolates from patients receiving indinavir (Condra et al., 2000) and has not been shown to confer cross-resistance to other PIs, except possibly lopinavir (Kempf et al., 2001b).

L33F has been reported primarily in persons treated with ritonavir or amprenavir (Molla et al., 1996; Maguire et al., 2002a). Its effect on PI susceptibility levels has not been studied. However, it has gained attention recently because of its association with lack of response to the experimental PI, tipranavir (McCallister et al., 2003). In contrast, L33I/V are polymorphisms in untreated persons and their effect, if any, on drug resistance is not known.

Polymorphic sites contributing to resistance (Figures 1, 3)

Amino acid variants at several polymorphic positions also make frequent contributions to drug resistance but only in combination with drug-resistance mutations at non-polymorphic positions. Mutations at positions 10, 20, 36, and 71 each occur in up to 5 to 10% of untreated persons infected with subtype B viruses. However, in heavily treated patients harboring isolates with multiple other PI-resistance mutations, the prevalence of mutations at these positions increases dramatically. Mutations at positions 10 and 71 increase to 60 to 80%, whereas mutations at positions 20 and 36 increase to 30 to 40% (Hertogs et al., 2000b; Wu et al., 2003). Position 63 is the most polymorphic protease position. In untreated persons about 45% of isolates have 63L (considered the subtype B consensus), about 45% have 63P, and about 10% have other residues at this position. However, the prevalence of amino acids other than L increases to 90% in heavily treated patients (Yahi et al., 1999; Wu et al., 2003). Mutations at positions 77 and 93 increase in prevalence from about 25% in untreated persons to about 40% in heavily treated persons (Wu et al., 2003). I93L is statistically associated with multiple PIs; whereas V77I is statistically associated only with nelfinavir.

In some HIV-1 subtypes, mutations at codons 20, 36, and 93 occur at higher rates than they do in subtype B isolates (Cornelissen et al., 1997; Pieniazek et al., 2000; Gonzales et al., 2001). In contrast, mutations at positions 63 and 77 usually occur more commonly in subtype B than in non-B isolates. It has been hypothesized that individuals harboring isolates containing multiple accessory mutations may be at a greater risk of virologic failure during PI therapy (Perez et al., 2001; Perno et al., 2001b). However, most studies have not supported this hypothesis (Bossi et al., 1999; Harrigan et al., 1999a; Kuritzkes et al.,...
Additional treatment-associated mutations

In a recent analysis of 2,244 protease isolates from 1,919 persons, 45 protease positions were more likely to be mutant in isolates from treated compared with untreated persons, 17 positions exhibited polymorphisms that were unrelated to treatment, and 37 positions rarely, if ever varied (Wu et al., 2003). The 45 treatment-associated positions included 23 positions previously associated with drug resistance that are described above and 22 positions that had not previously been associated with drug resistance. Thirteen of the 22 newly described treatment-associated positions (positions 11, 22, 23, 45, 58, 66, 74, 75, 76, 79, 83, 85, 85) were highly conserved in untreated persons. Several of these mutations have also been described in other recent publications containing analyses of large databases (Parkin et al., 2003b; Wang et al., 2003). The phenotypic and clinical impact of these mutations is not yet known because they rarely occur in the absence of other known drug-resistance mutations and have not been studied in vitro (Wu et al., 2003).

PI cross-resistance patterns and salvage therapy

In a study of over 6000 HIV-1 isolates tested for susceptibility to indinavir, nelfinavir, ritonavir, and saquinavir, 59% to 80% of isolates with a 10-fold decrease in susceptibility to one PI also had a 10-fold decrease in susceptibility to at least one other PI (Hertogs et al., 2000b). In a study of 3000 HIV-1 isolates, resistance to indinavir, ritonavir, and lopinavir were highly correlated (Parkin et al., 2001). Isolates that were resistant to these drugs were generally also resistant to nelfinavir; however, isolates resistant to nelfinavir due to D30N were not resistant to other drugs.

Susceptibilities to saquinavir and amprenavir are somewhat less well correlated with one another and with susceptibilities to the other PIs (Race et al., 1999; Schmidt et al., 2000a; Kemper et al., 2001; Parkin et al., 2001), although isolates that are highly resistant to amprenavir are often cross-resistant to lopinavir (Parkin et al., 2003b). Atazanavir selects for a unique protease mutation in previously untreated persons, I50I, but most of the mutations that confer resistance to other PIs, appear to also confer atazanavir resistance (Colonna et al., 2003).

Patients in whom nelfinavir-resistant isolates arise after nelfinavir treatment often respond to a regimen containing a different PI because D30N confers little cross-resistance to other PIs (Zolopa et al., 1999b; Kemper et al., 2001) (Table 3). But because more than 20% of nelfinavir failures may be associated with mutations at positions 46 and/or 90, virologic failure during nelfinavir does not guarantee susceptibility to other PIs (Patick et al., 1998; Atkinson et al., 2000; Saah et al., 2003). Nelfinavir is usually unsuccessful as salvage therapy because most of the mutations that confer resistance to other PIs confer cross-resistance to nelfinavir (Lawrence et al., 1999; Hertogs et al., 2000b; Schmidt et al., 2000b; Walmisley et al., 2001a).

In a study of ritonavir/saquinavir salvage therapy using the hard gel capsule formulation of saquinavir (400-600 mg twice daily), the number of mutations at positions 46, 48, 54, 82, 84, and 90 predicted the virologic response at 4, 12, and 24 weeks (Table 3). Patients with three or more of these mutations had no virologic response to salvage therapy (Zolopa et al., 1999b). Decreased phenotypic susceptibility also predicted a reduced virologic response in this cohort (Zolopa et al., 1999b). However, nine patients with isolates having mutations at positions 82 and 90 and at either or both positions 46 and 54 had no virologic response to ritonavir/saquinavir salvage despite the fact that their isolates were found to be phenotypically susceptible to saquinavir or to have only low-level reductions of saquinavir susceptibility (Zolopa et al., 1999b; Zolopa et al., 2001).

There are few data on the genotypic predictors of response to indinavir/ritonavir salvage therapy. In two small published studies, adherence, indinavir levels, and the number of PI-resistance mutations at positions 46, 48, 54, 82, 84, 90 were predictive of virologic response (Shulman et al., 2002a; Campo et al., 2003).

In vitro susceptibility studies suggest that patients failing other PIs often have isolates that retain susceptibility to amprenavir (Race et al., 1999; Schmidt et al., 2000a). Data on the utility of amprenavir for salvage therapy, are shown in Table 3 (Falloon et al., 2000; Klein et al., 2000; Descamps et al., 2001; Duval et al., 2002). In the NARVAL ANRS 088 trial, the presence of fewer than four of the following mutations – L10I, V32I, M46IL, I47V, I54V, G73S, V82A/T/F/S, I84V, L90M – was associated with a 1.6 log_{10} RNA reduction 12 weeks after the administration of an amprenavir-containing regimen (Descamps et
The presence of exactly four mutations was associated with a 0.6 log_{10} RNA reduction. In another study, suppression of plasma HIV-1 RNA levels to <400 copies/ml during treatment with amprenavir/ritonavir was associated with having fewer than 6 of the following mutations (L10F/I/V, K20M/R, E35D, R41K, I54V, L63P, V82A/F/T/S, I84V) (Marcelin et al., 2003b). Of note, the mutations at positions 35 and 41 are common polymorphisms and have not been associated with PI resistance in any previous analyses.

In a study of salvage therapy with a regimen containing lopinavir and efavirenz, the number of mutations at positions 10, 20, 24, 46, 53, 54, 63, 71, 82, 84, and 90 predicted the level of phenotypic resistance and the virologic response after 24 weeks of therapy (Kempf et al., 2001b; Kempf et al., 2002) (Table 3). A decreased response to therapy was observed only in those patients that had six or more of the listed mutations. Subsequent analyses have suggested that mutations at positions 10, 20, 46, and 82 may be more predictive than the other mutations listed (Calvez et al., 2001; Molla et al., 2001) and that other mutations, including V32I, I47V/A, I50V, and G73S may contribute to resistance in patient cohorts with different antiretroviral treatment experience (Harrigan et al., 2001; Prado et al., 2002; Bongiovanni et al., 2003; Parkin et al., 2003b). Lopinavir has also proven highly effective as salvage therapy when combined with nevirapine in NNRTI-naive patients failing their first PI regimen (Benson et al., 2002).

During \textit{in vitro} passage experiments atazanavir-resistant isolates develop mutations at positions 32, 50, 84, and/or 88, a pattern of mutations that differs from but overlaps with the mutations developing in patients treated with other PIs (Gong et al., 2000). In patients receiving atazanavir as their first PI, the most common drug resistance mutation to develop, I50L, causes resistance to atazanavir alone, while hypersensitizing to other PIs. However, two of eight atazanavir failures had mutations at positions 46 and/or 82 in addition to I50L (Colonna et al., 2002) suggesting that susceptibility to other PIs may not be guaranteed. The usefulness of atazanavir for salvage therapy is currently being studied in phase III clinical trials (Badaro et al., 2003).

These studies suggest that because of the high cross-resistance between the approved PIs, the choice of a PI for salvage therapy depends primarily on the drug levels that are likely to be achieved. The presence of mutations known to preferentially affect one drug (e.g. G48V and saquinavir, I50V and amprenavir), will occasionally also influence the choice of salvage therapy. However, many combinations of mutations produce only subtle differences in susceptibility between available drugs. Clinical studies are needed to determine the usefulness of the protease genotype or phenotype at pointing to a preferred boosted PI for salvage.

**Investigational PIs**

Tipranavir and TMC114 are the investigational PIs at the most advanced stage of clinical development. The relative potency of tipranavir compared to other PIs either \textit{in vitro} or \textit{in vivo} has not been well-described because this drug has been studied entirely in salvage therapy settings (Schwartz et al., 2002; Cooper et al., 2003; Plsoker & Figgitt, 2003). However, tipranavir has a remarkably high genetic barrier to resistance. After prolonged in vitro passage, mutations at positions 32, 33, 45, 82, and 84 have been selected leading to a virus with 14-fold reduced susceptibility (Doyon et al., 2002). Most PI-resistant clinical isolates, even those with >10-fold resistance to the original four PIs (saquinavir, indinavir, ritonavir, and nelfinavir) rarely have more than 2-fold resistance to tipranavir (Larder et al., 2000).

Reduced susceptibility of clinical isolates obtained from persons with other PIs appears to require three of the following four mutations: L33I/V/F, V82A/F/L/T, I84V, L90M (Cooper et al., 2003). Phase II salvage therapy studies have shown that the optimal response to tipranavir occurs when 500 mg of tipranavir is administered with 200 mg of ritonavir twice daily (Yeni et al., 2003). In heavily treated persons harboring viruses resistant to most other PIs, 14 days of boosted tipranavir reduced plasma HIV-1 RNA levels by 1.2 logs provided baseline tipranavir resistance was <2-fold (Yeni et al., 2003). No virologic suppression was observed with viruses having >2-fold reduction in susceptibility.

TMC114 and its precursor compound TMC126 are highly potent in vitro (Yoshimura et al., 2002; King et al., 2003b). Like tipranavir, it has also been shown to have a high genetic barrier to resistance (Koh et al., 2003; Meyer et al., 2003b) and to be active, at least in the short term (14 days), as part of a salvage therapy regimen when boosted with ritonavir (Meyer et al., 2003c). Published data on the mechanisms of resistance to TMC114 \textit{in vivo} are not yet available.
V Nucleoside/nucleotide RT inhibitor (NRTI) resistance

HIV-1 RT

The RT enzyme is responsible for RNA-dependent DNA polymerization and DNA-dependent DNA polymerization. RT is a heterodimer consisting of p66 and p51 subunits. The p51 subunit is composed of the first 440 amino acids of the pol gene. The p66 subunit is composed of all 560 amino acids of the pol gene. Although the p51 and p66 subunits share 440 amino acids, their relative arrangements are significantly different. The p66 subunit contains the DNA-binding groove and the active site; the p51 subunit displays no enzymatic activity and functions as a scaffold for the enzymatically active p66 subunit. The general shape of the polymerase domain of the p66 subunit can be likened to a human hand with subdomains referred to as fingers, palm, and thumb. The remainder of the p66 subunit contains an RNaseH subdomain and a connection subdomain (reviewed in (Larder & Stammers, 1999; Sarafianos et al., 1999b)).

Most RT inhibitor resistance mutations are in the 5' polymerase coding regions, particularly in the "fingers" and "palm" subdomains (Figure 4). Structural information for RT is available from X-ray crystallographic studies of unliganded RT (Rodgers et al., 1995), RT bound to an NNRTI (Kohlstaedt et al., 1992), RT bound to double-stranded DNA (Jacobo-Molina et al., 1993), RT bound to double-stranded DNA and the incoming dNTP (ternary complex) (Huang et al., 1998), and an RT bound to double-stranded DNA containing an AZT-terminated DNA primer pre- and post-translocation (Sarafianos et al., 2002). There have been fewer structural determinations of mutant RT enzymes than of mutant protease enzymes (Ren et al., 1998; Sarafianos et al., 1999a; Sarafianos et al., 2001; Chamberlain et al., 2002).

NRTIs

The NRTIs are chain terminators that block further extension of the proviral DNA during reverse transcription. The FDA has approved seven nucleoside and one nucleotide analog. The nucleoside analogs include zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, and emtricitabine. Tenofovir disoproxil fumarate (DF) is the only approved nucleotide analog. It is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate, which requires initial diester hydrolysis to tenofovir. Both nucleoside and nucleotide analogs are prodrugs that must be phosphorylated by host cellular enzymes. Nucleosides must be tri-phosphorylated; nucleotides, because they already have one phosphate moiety, must be di-phosphorylated. Phosphorylated NRTIs compete with natural deoxynucleoside triphosphates (dNTPs) for incorporation into the newly synthesized DNA chains where they cause chain termination.

The requirement for triphosphorylation complicates the in vitro assessment of both NRTI activity and phenotypic resistance testing. Table 4 shows that there are significant differences between the relative in vitro and in vivo potency of the NRTIs. Zidovudine appears to be the most potent NRTI in vitro because the concentration of zidovudine that inhibits HIV-1 replication by 50% (IC50) is 10 to 100-fold lower than that of the other NRTIs. Yet, in patients, lamivudine, emtricitabine, abacavir, tenofovir, and didanosine are more potent than zidovudine at lowering plasma HIV-1 RNA levels. The basis for this discordance has been known since the early 1990's. In vitro susceptibility tests use activated lymphocytes because it is difficult to culture HIV-1 using resting lymphocytes. Activated lymphocytes triphosphorylate zidovudine at a higher rate than other NRTIs making zidovudine appear more active. In contrast, didanosine, for example, is converted to its active form, dDA-triphosphate at much lower rates in activated lymphocytes making it appear much weaker in vitro (Gao et al., 1993).

Differences in NRTI triphosphorylation rates between the cells used for susceptibility testing and the wider variety of cells infected by HIV-1 in vivo also appears to explain why resistance to some drugs is difficult to detect by in vitro susceptibility testing. Mutant isolates from patients failing therapy with zidovudine and lamivudine usually have high-level (often >100-fold) phenotypic drug resistance. In contrast, mutant isolates from patients failing therapy with each of the other NRTIs have much lower levels of phenotypic resistance. As explained in the next section, one of the two main mechanisms of NRTI resistance – primer unblocking - also depends on intracellular dNTP concentrations, which are highly dependent on the state of cell activation. This difficulty in detecting resistance to NRTIs such as didanosine, zalcitabine, stavudine, and tenofovir appears to be related to the high dNTP concentrations present in the activated cells used for in vitro susceptibility testing (Meyer et al., 2000; Lennerstrand et al., 2001).

Mechanisms of NRTI resistance
There are two biochemical mechanisms of NRTI drug resistance. The first mechanism is mediated by mutations that allow the RT enzyme to discriminate against NRTIs during polymerization, thereby preventing their addition to the growing DNA chain relative to the natural dNTP substrates (Huang et al., 1998; Larder & Stammers, 1999; Sarafianos et al., 1999b). The second mechanism is mediated by mutations that promote the hydrolytic removal of the chain-terminating NRTI and enable continued DNA synthesis (Arion et al., 1998; Meyer et al., 1998; Meyer et al., 1999; Arion et al., 2000) (Figure 4). This mechanism of resistance has also been referred to as pyrophosphorolysis, nucleotide excision, and primer unblocking. The hydrolytic removal requires a pyrophosphate donor, which in most cells is usually ATP (Meyer et al., 1998; Meyer et al., 1999; Boyer et al., 2001, 2002a, b). Mutations that discriminate against NRTIs are generally associated with decreased enzymatic replication in vitro. Primer unblocking mutations are associated with lesser enzymatic impairment.

Mutations at positions 41, 67, 70, 210, 215, and 219 (Figures 4, 5)
The most common mutations occurring in clinical HIV-1 samples obtained from patients receiving NRTIs were originally identified for their role in causing zidovudine resistance. During the past few years, many studies have shown that these mutations are associated with phenotypic (Table 5) (Whitcomb et al., 2003b) and clinical (Table 6) resistance to each of the other NRTIs. The six mutations reviewed in this section are also referred to as thymidine analog mutations (TAMs) because they are most often selected by zidovudine and stavudine-containing regimens.

Various combinations of these mutations at positions 41, 67, 70, 210, 215, and 219 (Larder & Kemp, 1989; Kellam et al., 1992; Harrigan et al., 1996; Hooker et al., 1996) have been shown to promote ATP-dependent hydrolytic removal of a dideoxynucleotide monophosphate (ddNMP) from a terminated cDNA chain (Arion et al., 1998; Meyer et al., 1998; Meyer et al., 1999; Meyer et al., 2000). Early biochemical studies suggested that D67N and K70R are the mutations most responsible for rescue of chain-terminated primers (Arion et al., 1998; Meyer et al., 1999) and that the main effect of T215Y/F might be to cause a compensatory increase in RT processivity (Caliendo et al., 1996; Arion et al., 1998; Arts et al., 1998). More recent structural and modeling studies have shown that codons 70 and 215 are close to the incoming dNTP (Huang et al., 1998; Chamberlain et al., 2002) and that T215Y/F are in a position that would increase the affinity of RT for ATP so that at physiologic ATP concentrations, excision is reasonably efficient (Boyer et al., 2001; Sarafianos et al., 2002; Sarafianos et al., 2003). Mutations at positions 41 and 210 appear to stabilize the interaction of 215Y/F with the dNTP binding pocket (Huang et al., 1998; Sarafianos et al., 2002).

In an NRTI-terminated primer, the presence of the dNTP that would have been incorporated next - had the primer been free for elongation - results in the formation of a stable “dead-end” catalytic complex between RT, primer, template, and dNTP (Tong et al., 1997; Boyer et al., 2001; Lennerstrand et al., 2001; Miller & Larder, 2001; Sarafianos et al., 2002; Sarafianos et al., 2003) (Figure 6). The formation of such a dead-end complex interferes with the ability of even a mutant RT to facilitate the resumption of virus DNA chain elongation. Several studies have suggested that the bulky azido group of zidovudine interferes with the formation of a dead-end catalytic complex by preventing translocation and the addition of the next dNTP (Boyer et al., 2001; Lennerstrand et al., 2001; Sarafianos et al., 2002). Therefore ATP-dependent rescue of zidovudine terminated primers is more likely to occur than rescue of other NRTI-terminated primers at the dNTP concentrations present in activated cells (Meyer et al., 2000). This observation helps explain why the primer unblocking mutations cause the highest levels of phenotypic resistance to zidovudine, but it also suggests that these mutations can cause cross-resistance to other NRTIs in cells where dNTP pools are low (Meyer et al., 2000; Lennerstrand et al., 2001).

The primer unblocking mutations are selected primarily in patients treated with zidovudine or stavudine either alone or in combination with other NRTIs (Richman et al., 1991; Boucher et al., 1992; Kozal et al., 1993; Lin et al., 1994; Shafer et al., 1995; Izopet et al., 1999; Pellegrin et al., 1999; Coakley et al., 2000; Montaner et al., 2000; Picard et al., 2001; Ross et al., 2001; Shulman et al., 2001c; Maxeiner et al., 2002). They also occur in about 10% of patients treated with didanosine monotherapy (Demeter et al., 1995; Winters et al., 1997; Winters et al., 2001) but do not appear to occur during abacavir monotherapy (Miller et al., 2000) or during combination regimens lacking zidovudine or stavudine.

T215Y/F results from a two base-pair mutation and causes intermediate (10 to 15-fold) zidovudine resistance. It arises in patients receiving dual NRTI therapy, as well as in those receiving zidovudine monotherapy (Shafer et al., 1995; Kuritzkes et al., 1996; Larder et al., 1996). T215S/C/D are transitional mutations between wild type and Y or F that do not cause reduced drug susceptibility but rather indicate the
presence of previous selective drug pressure (Larder et al., 1991; Yerly et al., 1998; de Ronde et al., 2001). They are also referred to as T215 revertants because they are commonly observed in persons who once had viruses containing T215Y/F but who discontinued therapy and in persons who have been infected with a drug-resistant virus. In a study of 603 recently infected untreated individuals, 2 had T215Y, 1 had T215F, and 20 (3.3% of total) had other mutations at this position including T215D (8), T215C (6), T215S (4), and T215E (1). (Garcia-Lerma et al., 2001). T215I/V are additional treatment-associated mutations at this position (Rhee et al., 2003).

K70R causes low-level (about 4-fold) zidovudine resistance and is usually the first drug-resistance mutation to develop in patients receiving zidovudine monotherapy (Boucher et al., 1992; de Jong et al., 1996). Mutations at positions 70 and 215 are antagonistic in their effect on zidovudine resistance and these two mutations rarely occur together unless additional TAMs are also present (Boucher et al., 1992; Gonzales et al., 2003). Mutations at positions 67 and 219 may occur with mutations at position 70 or with mutations at position 215. Mutations at positions 41 and 210 occur only with mutations at position 215 (Harrigan et al., 1996; Hooker et al., 1996; Yahi et al., 2000; Gonzales et al., 2003). In patients failing multiple dual-NRTI regimens it is not unusual for isolates to have four or five TAMs.

Clinical studies have shown that the primer unblocking mutations, particularly mutations at position 215, interfere with the clinical response to zidovudine (Kozal et al., 1993; Rey et al., 1998), stavudine (Shulman et al., 2001c; Calvez et al., 2002), abacavir (Lanier et al., 1999; Falloon et al., 2000; Khanna et al., 2000; Brun-Vezinet et al., 2003), didanosine (Japour et al., 1995; Holodniy et al., 1996; Molina et al., 2003), and most dual-NRTI regimens (Shafer et al., 1995; Holodniy et al., 1996; Havlir et al., 1998; Izopet et al., 1999; Montaner et al., 2000; Calvez et al., 2002). Complete loss of response to abacavir appears to require the combination of three or more TAMs together with the mutation M184V (Lanier et al., 1999; Katlama et al., 2000; Brun-Vezinet et al., 2003). The presence of 1-2 TAMs has little effect on the virologic response to the addition of didanosine to a stable regimen; 3 TAMs causes a reduction in response; complete loss of response appears to require four TAMs (Molina et al., 2003). In the presence of M41L, L210W, and T215Y, there is little virologic response to tenofovir (Barrios et al., 2003; McColl & Miller, 2003; Squires et al., 2003). In contrast, mutations at positions 67, 70, and 219, and the T215F substitution have less impact on tenofovir susceptibility and virologic response (Barrios et al., 2003; McColl & Miller, 2003; Squires et al., 2003).

Both K70R and T215Y cause reproducible reductions in zidovudine susceptibility regardless of the susceptibility assay used. Phenotypic resistance to other NRTIs generally requires multiple TAMs. The presence of four or more TAMs will typically cause >100-fold decreased susceptibility to zidovudine, 5 to 7-fold decreased susceptibility to stavudine, 5 to 7-fold decreased susceptibility to stavudine, didanosine, zalcitabine, and tenofovir (Mayers et al., 1994; Harrigan et al., 2000; Larder & Bloom, 2001; Lennert et al., 2001; Miller et al., 2001; Miller & Larder, 2001; Wainberg & White, 2001; Margot et al., 2002; Shulman et al., 2002b; Wolf et al., 2003). The TAMs cause low-level phenotypic lamivudine resistance but do not appear to compromise lamivudine activity. Regimens containing zidovudine, lamivudine, and a potent third drug are often highly effective even in the presence of multiple TAMs (Hirsch et al., 1999; Albrecht et al., 2001).

**M184V (Figures 4, 5)**

M184V emerges rapidly in patients receiving lamivudine monotherapy (Boucher et al., 1993; Tisdale et al., 1993; Kavlick et al., 1995; Schuurman et al., 1995; Wainberg et al., 1995). This mutation is also first to develop in isolates from patients receiving incompletely suppressive lamivudine-containing regimens (Holder et al., 1999; Descamps et al., 2000; Havlir et al., 2000; Maguire et al., 2000; Gallego et al., 2001; Mouroux et al., 2001; Staszewski et al., 2001). M184V is also selected during therapy with emtricitabine (Quinn et al., 2003), abacavir (Tisdale et al., 1997; Harrigan et al., 2000; Miller et al., 2000) and less commonly with didanosine (Gaunt et al., 1992; Shirasaka et al., 1993; Winters et al., 1997). M184I results from a G to A mutation (ATG [methionine] to ATA [isoleucine]) and usually develops before M184V in patients receiving lamivudine because HIV-1 RT is more prone to G to A substitutions than to A to G substitutions (ATG to GTG [valine]) (Ji & Loeb, 1994; Keulen et al., 1996; Keulen et al., 1997). Although M184I also causes high-level resistance to lamivudine, the enzymatic efficiency of M184I is less than that of M184V, and nearly all patients with mutations at this position eventually develop M184V (Frost et al., 2000). Steric conflict between the oxathiolone ring of lamivudine and the side chain of beta-branched amino acids such as valine and isoleucine at position 184 perturbs inhibitor binding, leading to a reduction in lamivudine incorporation (Sarafianos et al., 1999a).
M184V alone causes high-level (>100-fold) resistance to lamivudine and emtricitabine (Boucher et al., 1993; Tisdale et al., 1993; Larder et al., 1995; Whitcomb et al., 2003b). In the absence of other drug-resistance mutations, M184V causes a median 1.5-fold reduction in didanosine susceptibility and 3-fold reduction in abacavir susceptibility in the PhenoSense™ assay (ViroLogic, South San Francisco) (Rhee et al., 2003). In the presence of TAMs, M184V decreases susceptibility to didanosine, zalcitabine, and abacavir and increases susceptibility to zidovudine, stavudine, and tenofovir (Miller et al., 1999; Palmer et al., 1999; Naeger et al., 2001; Shulman et al., 2001a; Parkin et al., 2003a; Whitcomb et al., 2003b). Resensitization may be due to the ability of M184V to impair the rescue of chain-terminated DNA synthesis (Gotte et al., 2000; Boyer et al., 2002a) and probably explains the slow evolution of phenotypic zidovudine resistance in patients receiving the combination of zidovudine or stavudine and lamivudine (Larder et al., 1995; Masquelier et al., 1999; Kuritzkes et al., 2000b). Resensitization, however, can be overcome by the presence of four or more zidovudine resistance mutations (Tisdale et al., 1993; Whitcomb et al., 2003b).

Position 184 is in a conserved part of the RT close to the active site. The possibility that isolates containing M184V are compromised was suggested by the initial lamivudine monotherapy studies showing that plasma HIV-1 RNA levels remained about 0.5 log copies below their starting value in patients receiving lamivudine for 6 to 12 months despite the development of M184V and lamivudine resistance (Eron et al., 1995; Ingrand et al., 1995; Pluda et al., 1995). Data from multiple lamivudine-containing dual NRTI regimens also suggest that lamivudine continues to exert a beneficial even in patients whose virus isolates contain M184V (Miller et al., 2002b; Diallo et al., 2003a; Vray et al., 2003). The role of lamivudine in these situations may be to maintain selective pressure on the virus to retain M184V, which increases HIV-1 susceptibility to zidovudine, stavudine, and tenofovir.

M184V by itself does not significantly compromise virologic response to treatment with abacavir (Lanier et al., 1999; Katlama et al., 2000; Van Vaerenbergh et al., 2000b; Henry et al., 2001). However, M184V in combination with multiple zidovudine resistance mutations or in combination with mutations at positions 65, 74, or 115 leads to both in vitro and in vivo abacavir resistance (Lanier et al., 1999; Palmer et al., 1999; Harrigan et al., 2000; Katlama et al., 2000; Walter et al., 2002). Although M184V may also be selected by didanosine monotherapy (in viruses that also have L74V), M184V by itself has little, if any effect on the virologic response to didanosine. Two studies have shown that in heavily treated patients infected with isolates containing multiple TAMs and M184V, a change from lamivudine to didanosine was usually associated with an improved virologic response (Rusconi et al., 2001; Winters et al., 2003). Didanosine intensification in treating isolates containing M184V (and varying numbers of TAMs) led to a median plasma HIV-1 RNA reduction of 0.6 log copies/ml (Molina et al., 2003). Moreover, M184V is frequently observed to revert to wildtype in persons changing therapy from lamivudine to didanosine (Winters et al., 2003).

Several studies have shown that in vitro RT enzymes with M184V have increased fidelity (Wainberg et al., 1996; Oude Essink et al., 1997; Drosopoulos & Prasad, 1998) and others decreased processivity (Boyer & Hughes, 1995; Back et al., 1996; Back & Berkhout, 1997; Sharma & Crumpacker, 1999). The clinical significance of these biochemical studies is not known, and the increased fidelity does not appear to limit the ability of HIV to develop new mutations under continued selective drug pressure (Jonckheere et al., 1998; Keulen et al., 1999).

Mutations at positions 65, 69, 74, and 75

Positions 64-72 form a loop between the β2 and β3 strands in the “fingers” region of the RT, which makes important contacts with the incoming dNTP during polymerization (Huang et al., 1998; Sarafianos et al., 1999b). In addition to the TAMs at positions 67 and 70, this region contains several important NRTI-resistance mutations.

Substitutions at position 69 are the most commonly occurring NRTI resistance mutations other than the TAMs and M184V. T69D was initially identified as causing resistance to zalcitabine (Fitzgibbon et al., 1992) but substitutions at this position have since been reported after treatment with each of the available NRTIs. In site-directed mutagenesis studies, other mutations at this position including T69N/S/A have been shown to confer resistance to zidovudine, didanosine, zalcitabine, and stavudine (Winters & Merigan, 2001). Mutations at position 69 may contribute to resistance to each of the NRTIs when they occur together with TAMs (Bloor et al., 1998; Hertogs et al., 1998; Miller et al., 1999; Wainberg et al., 1999; Winters & Merigan, 2001). In a group of 23 zidovudine-treated children each with multiple TAMs (including T215Y/F), the development of T69D/N was associated with a poor response to subsequent
didanosine monotherapy (Naugler et al., 2002). In this study, mutations at position 69 were more likely to develop than mutations at position 74, the mutation that usually develops in isolates without TAMs during didanosine treatment.

Insertions at position 69 occur in about 2% of heavily treated HIV-1-infected patients (Van Vaerenbergh et al., 2000a). By themselves, these insertions cause low-level resistance to each of the NRTIs, but isolates containing insertions together with T215Y/F and other TAMs have high-level resistance to each of the NRTIs (Winters et al., 1998a; de Jong et al., 1999; Larder et al., 1999; Tamale et al., 2000b; Masquelier et al., 2001b). Insertions at this position are associated with up to 20-fold resistance to tenofovir, which is the highest reported level of resistance to this drug (Miller et al., 2001). Insertions at this position act in a manner similar to the TAMs by causing ATP-mediated primer unblocking but they also destabilize the dead-end complex described above and thus cause more phenotypic resistance to the whole NRTI class than observed with the TAMs alone (Lennerstrand et al., 2001; Boyer et al., 2002b; Mas et al., 2002; Meyer et al., 2003a). Single amino acid deletions between codons 67-70 occur in <1% of heavily treated patients (Imamichi et al., 2000a; Imamichi et al., 2000b; Winters et al., 2000; Imamichi et al., 2001b). These deletions also contribute to resistance to each of the NRTIs in patients with viruses containing multiple NRTI mutations.

L74V occurs commonly during didanosine and abacavir monotherapy (Kozal et al., 1994; Shafer et al., 1994; Winters et al., 1997; Harrigan et al., 2000; Miller et al., 2000) and confers 2 to 5-fold resistance to didanosine and zalcitabine (St. Clair et al., 1991; Winters et al., 1997) and 2 to 3-fold resistance to abacavir (Tisdale et al., 1997). L74V is sufficient to cause virologic failure in patients receiving didanosine monotherapy (Kozal et al., 1994) and appears to prevent antiviral activity when didanosine is used for intensification (Molina et al., 2003), but additional mutations may be required to cause virologic failure of abacavir monotherapy (Miller et al., 2000). L74V causes hypersensitivity to zidovudine and is consequently rarely observed in patients receiving dual nucleoside therapy with didanosine/zidovudine (St. Clair et al., 1991; Shafer et al., 1994; Kojima et al., 1995; Shafer et al., 1995; Miller & Larder, 2001). It is also rarely observed with didanosine/stavudine (Pellegrin et al., 1999; Coakley et al., 2000) but it is unclear if it also increases susceptibility to stavudine or tenofovir (Parkin et al., 2003a). L74V has also been shown to cause decreased RT processivity in enzymatic studies and decreased replication in cell culture (Sharma & Crumpacker, 1997, 1999; Diallo et al., 2003b). L74I is a less commonly occurring mutation at this position; it is also associated with a 2 to 5-fold reduction in didanosine susceptibility (Rhee et al., 2003).

Position 65 interacts with the γ-phosphate of the bound dNTP. K65R improves discrimination between dNTPs and most NRTIs leading to intermediate levels of resistance to didanosine, abacavir, zalcitabine, lamivudine, emtricitabine, and tenofovir, and low-level resistance to stavudine (Gu et al., 1994; Zhang et al., 1994; Gu et al., 1995; Tisdale et al., 1997; Srinivas & Fridland, 1998; Wainberg et al., 1999; Miller et al., 2000; Petropoulos et al., 2000; Sluis-Cremer et al., 2000; Miller et al., 2001; Selmi et al., 2001b; Lanier et al., 2003; Parikh et al., 2003). K65R is selected in vitro by abacavir (Tisdale et al., 1997), tenofovir (Wainberg et al., 1999) and stavudine (Garcia-Lerm & Wainberg et al., 2003). It has been reported during monotherapy with didanosine (Winters et al., 1997), zalcitabine (Zhang et al., 1994), and abacavir (Miller et al., 2000), and during tenofovir intensification (Miller et al., 2002a). K65R hypersensitizes HIV-1 to zidovudine (Larder & Stammers, 1999; Lanier et al., 2003; Whitcomb et al., 2003b) and does not develop in patients receiving zidovudine-containing regimens (Rhee et al., 2003).

Although once rare, its prevalence in clinical settings has been increasing from about 1% to 4% of treated persons (Miller et al., 2003b). In previously untreated persons, it occurred in 2.7% of persons receiving tenofovir, lamivudine, and efavirenz, and in 0.6% receiving stavudine, lamivudine, and efavirenz (Miller et al., 2003a). It occurs even more commonly and appears to be associated with a much larger proportion of virologic failures in persons receiving triple nucleoside regimens lacking zidovudine such as stavudine, didanosine, and abacavir (Roge et al., 2003), tenofovir, abacavir, and lamivudine (Farthing et al., 2003; Gallant et al., 2003), and tenofovir, didanosine, and lamivudine.

K65R generally occurs in association with other mutations such as M184V and Q151M that discriminate NRTIs from the natural dNTP substrates rather than causing primer unblocking (Gonzales et al., 2003). Like other discriminatory mutations, K65R is associated with a decrease in replication capacity (Miller et al., 2003a). It also appears to increase the replication fidelity of HIV-1 RT (Arion et al., 1996; Shah et al., 2000).

V75T develops in isolates cultured in the presence of increasing concentrations of stavudine and causes about 5-fold resistance to stavudine, didanosine, and zalcitabine (Lacey & Larder, 1994).
Biochemical and structural modeling data suggest that mutations at this position cause drug resistance through nucleotide discrimination and possibly also through a non-ATP-mediated mechanism of primer unblocking (Lennerstrand et al., 2001; Selmi et al., 2001a). V75T occurs rarely in vivo, even in patients receiving stavudine. V75I generally occurs in isolates that also have the multinucleoside resistance mutation, Q151M. V75M/A are other NRTI-selected mutations that occur in 2.1% (M) and 0.6% (A) of persons receiving NRTIs and also appear to contribute to stavudine resistance (Bloor et al., 1998; Rhee et al., 2003).

**Multinucleoside resistance due to Q151M**

Q151M is a two base-pair change in a conserved RT region that is close to the first nucleotide of the single-stranded nucleotide template (Sarafianos et al., 1995; Shirasaka et al., 1995; Huang et al., 1998). Q151M causes resistance by decreasing the rate of incorporation of NRTIs relative to the natural dNTP substrates (Deval et al., 2002). This mutation develops in up to 5% of patients who receive dual NRTI therapy with didanosine in combination with zidovudine or stavudine (Shafer et al., 1994; Shafer et al., 1995; Kavlick et al., 1998; Schmit et al., 1999; Pellegrin et al., 1999; Coakley et al., 2000; Van Vaerenbergh et al., 2000a) but rarely with lamivudine-containing NRTI regimens. Q151M alone causes intermediate levels of resistance to zidovudine, didanosine, zalcitabine, stavudine, and abacavir (Shirasaka et al., 1995; Iversen et al., 1996; Shafer et al., 1996; Van Laethem et al., 2000). It is nearly always followed by mutations at positions 62, 75, 77, and 116. Isolates with V75I, F77L, F116Y, and Q151M have high-level resistance to each of these NRTIs, and low-level resistance to lamivudine and tenofovir (Palmer et al., 1999; Miller et al., 2000). HIV-1 isolates with Q151M usually contain few, if any, primer unblocking mutations (Gonzales et al., 2003). Q151M is a common genetic mechanism of NRTI resistance in HIV-2-infected persons (Rodes et al., 2000; Brandin et al., 2003).

**Other NRTI resistance mutations**

E44D/A and V118I each occur in about 1% of untreated individuals and in 10%-15% receiving NRTIs (Rhee et al., 2003). The prevalence of these two mutations is much higher in isolates obtained from patients receiving dual NRTI combinations, particularly in isolates containing multiple TAMs (Delaugerre et al., 2001a; Montes & Segondy, 2002; Gonzales et al., 2003; Rhee et al., 2003). Although these mutations were first shown to contribute low-level resistance to lamivudine (Hertogs et al., 2000a), they have since been shown to be selected by and to contribute low-level resistance to most of the other NRTIs (Montes & Segondy, 2002; Romano et al., 2002b; Walter et al., 2002; Houtte et al., 2003). E44D and V118I cause NRTI resistance by different mechanisms. E44D increases primer unblocking; V118I interacts with the incoming nucleotide to decrease NRTI incorporation (Girouard et al., 2003). By themselves, these mutations do not appear to limit the virologic activity of lamivudine-containing HAART regimens (Perno et al., 2001a).

Y115F is an uncommon mutation that occurs predominantly in patients receiving abacavir (Miller et al., 2000). It has also been reported in combination with Q151M, in patients receiving other NRTI combinations (Rhee et al., 2003). Position 115 is in close proximity to F116 and V118, two other residues that interact with the incoming dNTP.

In a recent analysis of RT sequences from 267 untreated persons and 857 persons treated with NRTIs, mutations at nine additional positions were significantly associated with NRTI treatment: K20R, T39A, K43E/Q/N, E203D/K, H208Y, D218E, H221Y, D223E/Q, L228H/R (Gonzales et al., 2003). The first three mutations are polymorphic in untreated persons occurring in 4%, 4%, and 1% of untreated persons. The remaining six occur only in treated persons and are particularly common in persons receiving multiple courses of NRTI therapy, perhaps explaining their delayed recognition. These newly identified mutations nearly always occur in combination with other previously characterized NRTI-resistance mutations suggesting that they act primarily as accessories to increase NRTI resistance or to compensate for the decreased replication associated with other NRTI-resistance mutations. The precise phenotypic effect of these mutations alone and in combination with other mutations has not yet been studied.

G333E is a polymorphism that has been reported in 4/70 (6%) untreated persons and 26/212 (12%) of persons receiving NRTIs (Gallego et al., 2002). G333E has been reported to facilitate zidovudine resistance in isolates from patients receiving zidovudine and lamivudine who have multiple TAMs (Kemp et al., 1998). However, dual resistance to zidovudine and lamivudine usually emerges without this change (Shafer et al., 1998; Masquelier et al., 1999; Stoeckli et al., 2002). There are no data suggesting that this mutation by itself reduces susceptibility to zidovudine or any other NRTI. Two reports have suggested...
that, in some isolates, the common polymorphisms R211K and L214F may facilitate dual zidovudine and lamivudine resistance in the presence of mutations at positions 41, 215, or dipeptide insertions at position 69 (Miller & Larder, 2001; Torti et al., 2001a; Meyer et al., 2003a).

P157A/S is a rare mutation associated with lamivudine resistance. This mutation was first identified in a feline immunodeficiency virus isolate cultured in the presence of lamivudine and has subsequently been shown to be associated with high-level lamivudine resistance even in isolates lacking M184V (Smith et al., 1998; Smith et al., 1999; Picard et al., 2001). There is only one published clinical HIV-1 isolate with this mutation (Picard et al., 2001). Q145M is another rare mutation (also reported in a single isolate) that has been reported to cause resistance to each of the NRTIs (Paolucci et al., 2003).

### NRTI cross-resistance patterns

There are four patterns of mutations associated with resistance to either all or nearly all of the approved NRTIs: (i) The most common pattern includes multiple TAMs and M184V often in combination with substitutions at positions 44, 69, 75, and 118. (ii) Occasionally, there is a dipeptide insertion rather than a substitution at position 69. This is often described as a separate mechanism of resistance; however, the dipeptide insertion usually occurs in the same mutational context as the first mechanism. (iii) Q151M and its associated mutations V75I, F77L, and F116Y usually occurs in the absence of TAMs. M184V, which is required for high-level lamivudine resistance, may also be present; (iv) K65R by itself causes intermediate levels of resistance to each of the NRTIs except zidovudine. As noted above, K65R has been emerging with increased frequency as a cause of virologic failure in triple NRTI HAART regimens lacking zidovudine and occurs in association with M184V and occasionally Q151M but not TAMs.

The extent of clinical cross-resistance between different NRTIs has been determined largely from the retrospective analysis of studies in which a single NRTI was substituted for a second NRTI or added to a failing regimen (Table 6). The main conclusions of these studies have been that the TAMs compromise the activity of each of the NRTIs except lamivudine and that M184V interferes with the activity of lamivudine but has much less impact on abacavir and didanosine. Unless three or more TAMs are present, abacavir, tenofovir, and didanosine each lead to reductions in plasma HIV-1 RNA when they are added to a failing regimen. The extent to which each of these drugs is acting alone as opposed to producing synergistic effects with the two other NRTIs that were already being used is not known.

The mutational antagonism between the TAMs and discriminatory mutations such as M184V and L74V probably explains the clinical synergism observed with several of the older dual NRTI combinations such as zidovudine/lamivudine, stavudine/lamivudine, zidovudine/didanosine, and stavudine/didanosine. Patients switching from one dual-NRTI combination to a second dual-NRTI combination will generally have some response as long as high-level resistance to the first combination has not yet emerged. High-level resistance to both drugs in these dual-NRTI combinations usually requires multiple TAMs and M184V. In contrast, combinations of NRTIs such as tenofovir, lamivudine, and abacavir that select only for discriminatory mutations – although consisting of drugs that are individually highly potent (Saag et al., 1998; Louie et al., 2003) – have a lower genetic barrier to resistance because a single point mutation such as K65R can cause resistance to all three drugs.

The most recently approved NRTI, emtricitabine (FTC, Emtriva), is structurally highly similar to lamivudine, which is also an oxathialone-cytosine analog. Preliminary data suggest that it is more potent than lamivudine in vitro (Richman, 2001) but that the cross-resistance profiles between the two drugs are likely to be the same. M184V is the most common mutation selected in vitro (Schinazi et al., 1993; Tisdale et al., 1993) and in vivo (Borroto-Esoda et al., 2003) by emtricitabine. Biochemical data suggest K65R also modestly reduces susceptibility to both drugs (Mulamba et al., 2003).

### VI Nonnucleoside RT inhibitor (NNRTI) resistance mutations

The NNRTIs bind to a hydrophobic pocket in the RT located between the β6-β10-β9 and β 2-β13-β14 sheets of the p66 subunit (Hsiou et al., 2001) (Figure 7). A small portion of the pocket is also formed by residues from the p51 subunit. The NNRTI-binding pocket is close to but not contiguous with, the active site. The NNRTIs inhibit HIV-1 replication allosterically by displacing the catalytic aspartate residues relative to the polymerase-binding site (Kohlstaedt et al., 1992; Esnouf et al., 1995; Spence et al., 1995). The hydrophobic NNRTI-binding pocket is less well conserved than the dNTP-binding site. Indeed, HIV-1 Group O and HIV-2 (Shih et al., 1991; Hizi et al., 1993; Yang et al., 1996; Descamps et al., 1997) are intrinsically resistant to most NNRTIs.
A single mutation in the NNRTI-binding pocket may result in high-level resistance to one or more of the NNRTIs. Resistance usually emerges rapidly when NNRTIs are administered as monotherapy or in the presence of incomplete virus suppression, suggesting that resistance is caused by the selection of a pre-existing population of mutant viruses within an individual (Wei et al., 1995; Havlir et al., 1996; Jackson et al., 2000; Conway et al., 2001). Several studies have shown that a single dose of nevirapine used to prevent mother-to-child HIV transmission can select for NNRTI-resistant mutants that are detectable for at least two months (Jackson et al., 2000; Cunningham et al., 2002; Kantor et al., 2003b). Like many of the PI and NRTI-resistance mutations, some of the NNRTI resistance mutations may also compromise viral replication. Two mechanisms of impaired replication have been proposed: changes in the conformation of the dNTP binding pocket (Kleim et al., 1994; Van Laethem et al., 2000) and changes in RNaseH activity (Gerondelis et al., 1999; Archer et al., 2000).

**NNRTIs**

There are three FDA-approved NNRTIs: nevirapine, delavirdine, and efavirenz. The dynamic susceptibility range for each of the NNRTIs is greater than 100-fold. Wild type HIV-1 Group M isolates tend to have greater inter-isolate variability in their susceptibility to NNRTIs than to NRTIs and PIs (Brown et al., 2000). However, preliminary data suggest that the moderate (<5-fold) decreases in NNRTI susceptibility that have been reported in the absence of previous NNRTI therapy or known NNRTI-resistance mutations do not interfere with the virologic response to an NNRTI-containing HAART regimen (Bacheler et al., 2000a; Harrigan et al., 2003a).

**NNRTI mutations between codons 98-108 (Figures 7, 8)**

K103N occurs more commonly than any other mutation in patients receiving NNRTIs (Bacheler et al., 2000b; Demeter et al., 2000; Hanna et al., 2000; Conway et al., 2001; Deeks, 2001; Delaugerre et al., 2001b; Torti et al., 2001b) and causes 20- to 50-fold resistance to each of the available NNRTIs (Young et al., 1995; Huang et al., 1999; Petropoulos et al., 2000; Bacheler et al., 2001). Although this degree of resistance is less than the highest levels of resistance observed with these drugs, K103N by itself appears sufficient to cause virologic failure with each of the NNRTIs (Casado et al., 2000; Demeter et al., 2000; Joly et al., 2000; Shulman et al., 2000). K103S occurs in about 1% of NNRTI-treated persons and causes about 10-fold resistance to efavirenz and delavirdine, and 30-fold resistance to nevirapine (Harrigan et al., 2003b). K103R, occurs in about 1% of untreated persons (Rhee et al., 2003). By itself it does not cause NNRTI resistance, but in combination with V179D it is associated with about 10-fold resistance to each of the NNRTIs (Petropoulos et al., 2003).

Residue 103 is located on the outer rim of the NNRTI-binding pocket and in the vicinity of the entrance to the pocket. Structural studies of HIV-1 RT with K103N in both unliganded and NNRTI-bound conformations have shown that this mutation only minimally changes the enzyme structure but that unliganded it forms a network of hydrogen bonds that are not present in the wild type enzyme (Hsiou et al., 2001). These changes appear to stabilize the closed pocket form of the enzyme and interfere with the ability of inhibitors to bind to the enzyme (Hsiou et al., 2001).

V106A causes more than 30-fold resistance to nevirapine, and 2-5 fold resistance to delavirdine and efavirenz (Byrnes et al., 1993; Emini et al., 1993; Larder et al., 1993; Young et al., 1995; Balzarini et al., 1998; Fujiwara et al., 1998; Huang et al., 1999; Petropoulos et al., 2000; Bacheler et al., 2001). V106M, although rare in subtype B isolates, occurs commonly in subtype C isolates from persons failing NNRTIs (Brenner et al., 2003; Grossman et al., 2003). This mutation causes about 20-fold resistance to nevirapine and 10-fold resistance to efavirenz in subtype B isolates (Rhee et al., 2003), although higher levels of resistance have been reported in subtype C isolates (Brenner et al., 2003). V106I is a polymorphism that occurs in 1% of treated and untreated persons and is not associated with NNRTI resistance (Rhee et al., 2003).

L100I causes intermediate resistance to efavirenz and delavirdine and low-level resistance to nevirapine (Byrnes et al., 1993; Byrnes et al., 1994; Young et al., 1995; Winslow et al., 1996; Fujiwara et al., 1998; Petropoulos et al., 2000). L100I usually occurs with K103N in patients receiving efavirenz and significantly increases efavirenz resistance in these isolates (Bacheler et al., 2000b). L100I also partially reverses T215Y-mediated zidovudine and tenofovir resistance (Byrnes et al., 1994; Larder, 1994; Parkin et al., 2003a).

K101E causes about 10-fold resistance to nevirapine and 5-fold resistance to efavirenz and delavirdine but the clinical significance of this reduction is not known (Petropoulos et al., 2000; Bacheler et
K101Q is a common mutation at this position that causes 2-fold resistance to each of the NNRTIs (Rhee et al., 2003). K101P occurs in heavily treated persons failing NNRTIs. It is a 2-bp mutation that confers >20-fold resistance to each of the NNRTIs (Petropoulos et al., 2003).

A98G and V108I each cause about 2-fold resistance to each of the NNRTIs (Byrnes et al., 1994; Young et al., 1995; Huang et al., 1999; Petropoulos et al., 2000; Bacherel et al., 2001). A98S is a common polymorphism that does not cause NNRTI resistance (Rhee et al., 2003).

**NNRTI mutations between codons 179-190 (Figures 7, 8)**

Y181C/I causes more than 30-fold resistance to nevirapine and delavirdine and 2- to 3-fold resistance to efavirenz (Byrnes et al., 1993; Byrnes et al., 1994; Young et al., 1995; Petropoulos et al., 2000). Nonetheless, nevirapine-treated patients with isolates containing Y181C generally have only transient virologic responses to efavirenz-containing salvage regimens (Shulman et al., 2000; Delaugerre et al., 2001b). It is suspected that virologic failure in this setting is due not to low-level Y181C-mediated efavirenz resistance but rather to the more likely possibility that the virus population within patients developing isolates with Y181C is also enriched for other NNRTI-associated mutations, including K103N.

G190A causes high-level resistance to nevirapine and intermediate levels of resistance to efavirenz (Fujiiwara et al., 1998; Petropoulos et al., 2000; Huang et al., 2003). G190S causes high-level resistance to both nevirapine and efavirenz. Isolates containing G190A and G190S are hypersusceptible to delavirdine (Huang et al., 2003). Other mutations at position 190 such as G190E occur uncommonly (Bacherel et al., 2000b; Shulman et al., 2000). These mutations generally cause high-level resistance to efavirenz and nevirapine and low-level resistance to delavirdine and cause markedly reduced replication (Kleim et al., 1994; Huang et al., 2003).

Y188L causes high-level resistance to nevirapine and efavirenz and intermediate resistance to delavirdine (Byrnes et al., 1993; Young et al., 1995; Fujiiwara et al., 1998; Petropoulos et al., 2000; Bacherel et al., 2001). Y188C and Y188H are uncommon mutations at this position that cause intermediate-to-high levels of nevirapine resistance and low-levels of resistance to efavirenz and delavirdine.

V179D causes low-level (about 2-fold) resistance to each of the NNRTIs (Byrnes et al., 1993; Young et al., 1995; Kleim et al., 1996; Winslow et al., 1996). V179I is a common polymorphism that occurs in 2% of untreated persons and in 12% of persons receiving NNRTIs (Rhee et al., 2003). However it does not cause resistance to any of the approved NNRTIs (Rhee et al., 2003).

**NNRTI mutations between codons 225-238 (Figures 7, 8)**

Mutations in this region occur less commonly than those in the 98-108 and 179-190 regions. P225H occurs with K103N in patients receiving efavirenz (Pelemans et al., 1998; Huang et al., 1999; Bacherel et al., 2000b). K103N + P225H causes about 100-fold resistance to efavirenz and nevirapine and about 10-fold resistance to delavirdine because P225H hypersensitizes to delavirdine. M230L is an uncommon mutation that causes about 20-, 40-, and 60-fold decreased susceptibility to efavirenz, nevirapine, and delavirdine, respectively (Huang et al., 2000). P236L is an even rarer mutation that causes high-level resistance to delavirdine and hypersusceptibility to nevirapine (Dueweke et al., 1993; Huang et al., 1999; Demeter et al., 2000). P236L causes slowing of both DNA 3'-end- and RNA 5'-end-directed RNaseH cleavage possibly explaining the markedly decreased replication of isolates with this mutation (Gerondelis et al., 1999). F227L augments nevirapine resistance when present with V106A but does not cause resistance on its own or affect other NNRTIs (Balzarini et al., 1994) but does not cause resistance to the currently approved NNRTIs

**Other NNRTI resistance mutations**

Y318F is a mutation in the NNRTI-binding pocket which causes high-level resistance (about 40-fold) to delavirdine and low-level resistance (<3-fold) to nevirapine and efavirenz (Harrigan et al., 2002). This mutation rarely occurs in the absence of other NNRTI-resistance mutations. Mutations at codon 138 (e.g. E138K) that have been shown to confer resistance to an experimental group of NNRTIs called the TSAO inhibitors (Balzarini et al., 1994) but do not cause resistance to the currently approved NNRTIs
(Pelemans et al., 2001). This mutation exerts its effect via the part of the p51 subunit that contributes to the NNRTI binding pocket (Balzarini et al., 1994). Mutations at position 135 and 283 have been shown to cause low-level resistance to NNRTIs, particularly when present in combination but do not appear to influence the virologic response to NNRTI-containing regimens (Brown et al., 2000).

**NNRTI cross resistance**

High-levels of cross-resistance to the NNRTIs have been reported in clinical HIV-1 isolates from patients failing therapy with an NNRTI (Casado et al., 2000; Shulman et al., 2000; Delaugerre et al., 2001b; Walmsley et al., 2001b; Antinori et al., 2002; Falloon et al., 2002; Harrigan & Larder, 2002). Part of this cross-resistance results from the fact that most NNRTI-resistance mutations confer resistance to multiple drugs (Figure 8). Part of this cross-resistance may also result from the fact that a single drug may select for multiple different NNRTI-resistance mutations even if only one or two predominant mutations are detected during genotyping. Although some NNRTI mutations cause hypersusceptibility to at least one NNRTI (e.g. G190A/S and delavirdine), these uncommon cases of lack of cross-resistance and hypersusceptibility have not yet been shown to be clinically significant and no benefit of using NNRTIs either in combination or in sequence has been demonstrated (van Leth et al., 2003).

**NNRTI and NRTI mutation interactions**

There are two favorable interactions between NNRTI and NRTI resistance mutations that may explain the synergy observed when drugs from these classes are used in combination. In the early 1990s, it was shown that Y181C and L100I hypersensitize HIV-1 to zidovudine (Larder, 1992; Byrnes et al., 1993). This has been confirmed more recently and appears to also apply to tenofovir (Parkin et al., 2003a). This phenomenon has not been exhaustively studied and conceivably other NNRTI mutations may have a similar effect on NRTI susceptibility. The mechanism of action appears to be interference with primer unblocking (Selmi et al., 2003). The clinical significance of these interactions is not known.

The second favorable interaction was reported more recently. HIV-1 isolates containing multiple NRTI-resistance mutations are consistently more susceptible (hypersusceptible) to the currently approved NNRTIs than are isolates lacking NRTI-resistance mutations (Whitcomb et al., 2002). In the case of isolates without NNRTI-resistance mutations, this phenomenon results in an IC$_{50}$ that is about 3 to 5 times lower than a comparable isolate with the same NNRTI-resistance mutations but lacking NRTI-resistance mutations (resensitization) (van Leth et al., 2001b).

The biophysical basis for NNRTI hypersusceptibility is not known. However, two statistical analyses have provided some insight into the genetic basis for this phenomenon (Whitcomb et al., 2002; Shulman et al., 2003). Several primer unblocking mutations including M41L, D67N, L210W, and T215Y, and substitutions and insertions at position 69 have been associated with hypersusceptibility; whereas the role of other mutations, such as M184V has not yet been clarified (Whitcomb et al., 2002; Shulman et al., 2003).

In two studies, NNRTI hypersusceptibility was reported to be associated with improved clinical outcome when NNRTIs were used for salvage therapy (Hauri et al., 2002; Katzenstein et al., 2003). This is surprising considering that NNRTI hypersusceptibility results from NRTI resistance mutations and that conventional wisdom would suggest that patients whose virus isolates are susceptible both to NRTI and NNRTIs would have a better outcome than those whose viruses are susceptible (albeit hypersusceptible) to NNRTIs alone. Therefore, it is important that this finding be independently verified to exclude the possibility that the benefit of NNRTI hypersusceptibility in these studies reflects a covariate. For example, in patients failing an NRTI-containing regimen, the absence of any NRTI resistance (and associated lack of NNRTI hypersusceptibility) may be a marker for nonadherence to therapy. Moreover, the phenomenon of NNRTI resensitization (i.e. the presence of low-levels of phenotypic NNRTI resistance despite the presence of a major NNRTI resistance mutation such as K103N) has been shown not to be of clinical benefit (Shulman et al., 2001b).

In addition to these two common favorable interactions between NNRTI and NRTI resistance mutations, there are two rare unfavorable interactions. In one scenario, an NNRTI mutation – Y181C – leads to a subtle reduction in susceptibility to an NRTI (stavudine) (Baldanti et al., 2003; Blanca et al., 2003; Parkin et al., 2003a). In a different scenario, an NRTI-resistance mutation – L74V or V75I/L – facilitates the emergence of NNRTI-associated mutations at position 190.
Investigational NNRTIs

Capravirine and TMC125 are the investigational NNRTIs in the most advanced stages of clinical development. Capravirine (also known as AG1549 and formerly S-1153) appears to be about as potent as other NNRTIs, by itself reducing plasma HIV-1 RNA levels 1.7 logs over 10 days (Hernandez et al., 2000). It has been shown to retain nearly full in vitro susceptibility against HIV-1 isolates containing the RT mutation K103N (Fujiwara et al., 1998; Potts et al., 1999). However, other known NNRTI-resistance mutations such as Y181C and combinations of NNRTI-resistance mutations such as L100I + K103N or V106A + F227L have led to >10-fold reductions in capravirine susceptibility (Fujiwara et al., 1998; Potts et al., 1999). Moreover, a new NNRTI-resistance mutation, L234I has been observed during in vitro passage experiments (Fujiwara et al., 1998). Preliminary data suggest that capravirine may have residual activity in a subset of persons who have failed a previous NNRTI-containing regimen (Wolfe et al., 2001).

TMC125 is a highly potent compound that retains susceptibility against HIV-1 isolates containing all single NNRTI-resistance mutations possibly as a result of adopting more than one stable mode of binding to the NNRTI-binding pocket (Das et al., 2003; Vingerhoets et al., 2003). In a randomized, double-blind, placebo-controlled study, 12 previously untreated HIV-1-infected patients receiving TMC125 had a mean plasma HIV-1 RNA reduction of 2.0 logs within seven days (Gruzdev et al., 2003). This rate of plasma HIV-1 RNA reduction is similar to what had previously been observed with a seven-day course of therapy with five antiretroviral drugs belonging to three drug classes (Sankatsing et al., 2002). In patients who failed previous nevirapine or efavirenz-containing regimens and had documented NNRTI-resistance mutations, a seven-day course of TMC125 reduced plasma HIV-1 RNA levels >0.5 log in 12/16 patients and >1.0 log copies/ml in 7/16 patients. The genetic mechanisms of resistance to TMC125 either in vitro or in vivo and the long-term activity of this drug for treating patients failing other NNRTIs have not yet been described.

VII. HIV-1 fusion inhibitors

The HIV-1 envelope glycoprotein consists of two noncovalently associated subunits: a surface glycoprotein (gp120) and a transmembrane glycoprotein (gp41). Portions of gp120 bind to both the CD4 receptor and to one of the chemokine receptors on target cells. After gp120-CD4-coreceptor binding, the gp41 subunit undergoes a conformational change that promotes fusion of viral and cellular membranes, resulting in entry of the viral core into the cell. This conformational change results in a transient species, termed the pre-hairpin intermediate in which gp41 exists simultaneously as a membrane protein in both the viral and cellular membranes (Eckert et al., 1999).

Crystallographic studies of gp41 fragments show that in the process of fusion, two heptad repeat domains (HR1 and HR2) form a helical bundle containing trimers of each domain (Chan et al., 1998). The first successful inhibitors of viral entry were synthetic peptides designed to inhibit the interaction of HR1 and HR2 by mimicking either HR1 or HR2. One of these peptides, T20 (enfuvirtide; Trimeris, Durham, NC), corresponds to part of HR2 (residues 127 to 162 of gp41). When this peptide was administered intravenously over a two-week period the median plasma HIV-1 RNA levels of subjects receiving the higher dose levels (100 mg twice daily) declined by 2.0 logs (Kilby et al., 1998). In two phase 3 studies that led to its FDA approval, the addition of enfuvirtide to an optimized antiretroviral regimen reduced plasma HIV-1 RNA levels by about 1.5 logs in persons who had previously failed therapy with each of the three original drug classes (Lalezari et al., 2003; Lazzarin et al., 2003). In contrast, a reduction of only 0.7 logs occurred in the control patients receiving an optimized antiretroviral regimen in the absence of enfuvirtide.

The extracellular portion of gp41 is the most conserved region in the HIV-1 envelope glycoprotein (Hanna et al., 2002; Roman et al., 2002; Xu et al., 2002; Villahermosa et al., 2003). Nonetheless, there is about 10-fold variation in the enfuvirtide susceptibility among isolates from untreated persons, with an IC50 that is generally between 0.1 uM to 1.0 uM (Labrosse et al., 2003; Sista et al., 2003). This naturally occurring variation is due to gp41 polymorphisms (Stanfield-Oakley et al., 2003) and to other parts of the envelope or genome that influence cell fusion. Enfuvirtide can target gp41 during a kinetic window that appears to be opened by CD4 binding and closed by co-receptor engagement; factors that influence these two processes logically impact enfuvirtide sensitivity (Reeves et al., 2002). One group reported that the IC50 of HIV-1 isolates tropic for CCR5 are about 0.3 - 0.6 logs higher than those of CXCR4 tropic isolates (Derdeyn et al., 2000; Derdeyn et al., 2001), but this finding has not been replicated (Whitcomb et al., 2003a). Moreover, variation in baseline enfuvirtide susceptibility did not appear to affect virologic response in the TORO trials (Sista et al., 2003).
HIV-1 isolates resistant to enfuvirtide have been identified by culturing HIV-1 in the presence of increasing enfuvirtide concentrations and in persons receiving the inhibitor. Sequences of resistant isolates developing during in vitro passage experiments have demonstrated mutations between positions 36 to 38 in gp41 (Rimsky et al., 1998); whereas sequences from patients receiving enfuvirtide have demonstrated either single or double mutations between positions 36 to 45 (Mink et al., 2002; Sista et al., 2002; Wei et al., 2002). In site-directed mutagenesis experiments, isolates with a single mutation display 1 to 21-fold reductions in susceptibility; whereas isolates with two mutations display 15 to 500-fold reductions in susceptibility (Mink et al., 2002). In patients discontinuing enfuvirtide for four months, drug-resistant mutants in plasma were replaced by wildtype isolates suggesting that drug-resistant mutants compromise virus replication in the absence of therapy.

T-1249 is another fusion inhibitor developed by Trimeris that has a longer half-life and retains activity against enfuvirtide-resistant isolates. It is a 39 amino acid peptide that overlaps with enfuvirtide but includes a region of HR2 that binds into a deep pocket of HR1 (Kilby & Eron, 2003). HIV-1 isolates that are resistant to enfuvirtide are generally still susceptible to T-1249 in vitro (Greenberg et al., 2002). Virologic responses have been observed in patients harboring enfuvirtide-resistant viruses who have been treated with T-1249 for 10 days (Miralles et al., 2003).

**VIII. Intersubtype variability**

During its spread among humans, group M HIV-1 has evolved into multiple subtypes that differ from one another by 10% to 30% along their genomes (Korber et al., 2000; Robertson et al., 2000). The antiretroviral drugs used to treat HIV-1 were developed using biophysical and biochemical studies targeting subtype B isolates – the predominant subtype in North America and Western Europe – and the vast majority of data on the genetic mechanisms of HIV-1 drug resistance have been generated from observations on subtype B viruses. However, HIV-1 subtype B viruses account for only ~12% of the global HIV pandemic (Osmanov et al., 2002) and as therapy is introduced into developing countries the numbers of persons with non-B virus starting therapy will increase dramatically.

HIV-1 subtypes differ from one another by 10% to 12% of their nucleotides and 5% to 6% of their amino acids in the protease and RT (Gonzales et al., 2001). Although most inter-subtype variation is caused by synonymous nucleotide substitutions, there are subtype-specific amino acid patterns. Naturally occurring polymorphisms in the different protease subtypes often occur at sites that are accessory mutations in subtype B isolates. However, as noted above, these mutations at positions 20, 36, 93 – as well as V82I – do not appear to interfere with the response to protease inhibitor therapy. Naturally occurring polymorphisms in different RT subtypes generally do not occur at sites associated with drug resistance.

An increasing number of observational studies, both in vitro and in vivo, suggest that the currently available protease and RT inhibitors are probably as active against wildtype non-B viruses as they are against wildtype subtype B viruses (Shafer et al., 1997; Apetrei et al., 1998; Del Amo et al., 1998; Alaeus et al., 1999; Shafer et al., 1999a; Tanuri et al., 1999; Adje et al., 2001; Cane et al., 2001; Grossman et al., 2001; Weidle et al., 2001; Alexander et al., 2002; Frater et al., 2002; Kebba et al., 2002; Pillay et al., 2002; Toni et al., 2002; Vergne et al., 2002; Weidle et al., 2002; Landman et al., 2003). However, some in vitro and in vivo observations suggest that there may be differences in susceptibility among the different subtypes (Descamps et al., 1998; Palmer et al., 1998; Caride et al., 2000; Caride et al., 2001; Velazquez-Campoy et al., 2001; Loemba et al., 2002; Velazquez-Campoy et al., 2002).

Preliminary data have shown that all drug resistance mutations that have been described in subtype B have also been observed in at least one non-B subtype (Kantor et al., 2003a). However, it is not known whether drug resistance mutations may occur in other subtypes that have not been recognized in subtype B. Moreover, the patterns of mutations in non-B viruses from persons failing therapy occasionally differ from the pattern observed in subtype B viruses. For example, several groups have reported that, although both protease mutations D30N and L90M occur in non-B viruses during nelfinavir therapy, D30N occurs more commonly in subtype B viruses, whereas L90M occurs more commonly in subtypes C, G and CRF01_AE (Cane et al., 2001; Gomes et al., 2002; Grossman et al., 2002; Sugiura et al., 2002). Two groups also reported that the NNRTI-resistance mutation V106M occurs more commonly in subtype C than subtype B viruses of persons treated with NNRTIs (Brenner et al., 2003; Morris et al., 2003).
**IX. Interpretation of genotypic resistance tests**

The interpretation of HIV-1 genotypic resistance tests is complicated for the following reasons: First, there are many HIV-1 drug resistance mutations and they emerge in complex patterns. Second, HIV-1 drug resistance is rarely an all-or-none phenomenon. Clinicians treating infected patients usually need the answers to the following two questions: (i) Does the result suggest that the patient will respond to a drug in a manner comparable to a patient with a wild-type isolate? (ii) Does the result suggest that the patient will obtain any antiviral benefit from the drug? Third, the results of resistance testing must be integrated with a sound understanding of the principles of antiretroviral therapy as outlined in the frequently updated published treatment guidelines (US Department of Health and Human Services Panel on Clinical Practices for Treatment of HIV Infection, 2003).

Because few clinicians can be expected to remember all that is known about the biological and clinical significance of these mutations, genotypic test results generally include not only a list of mutations but also an accompanying interpretation. The fact that genotypic interpretation is independent of the process of genotyping makes genotypic interpretation an ideal application for a computerized expert system that accepts either a string of nucleotides or a list of mutations and returns a predicted level of resistance to different antiretroviral drugs, a list of comments about the mutations in the sequence, or both. However, genotypic reports are not yet capable of recommending specific drugs because they do not take into account potential resistance to drugs that a person received in the past but to which drug resistance is not evident on the submitted sequence, they do not take into account a person’s risk of drug toxicity, and they do not incorporate the logic of which drugs should and should not be used in combination.

There are two basic approaches to genotypic interpretation: (i) rules-based algorithms, and (ii) machine-learning algorithms. Rules-based algorithms are developed by experts or expert committees based upon distillation of large amounts of published data on the phenotypic impact and clinical significance of drug-resistance mutations. Machine-learning algorithms contain rules that are discovered by a computer program by analyzing data linking genotype to phenotype or clinical outcome.

Most algorithms in current clinical use are based on rules. These algorithms have three advantages relative to machine learning algorithms. First, most machine-learning algorithms can operate only on a homogeneous data set such as correlations between genotype and phenotype but not one that considers each of the three types of data summarized in Table 2. Second, most data sets contain insufficient data linking genotype to other forms of data to fully exploit the learning ability of those algorithms with the most predictive power. In contrast, experts can extrapolate based upon a wide variety of knowledge to create rules for mutations that are represented at low frequency in a database. Finally, whereas the logic of rules-based algorithms is transparent and can be examined by the clinician, the logic of machine-learning algorithms is often not transparent.

**Rules-based algorithms**

Bayer Diagnostics (TRUGENE™) and Celera Diagnostics (ViroSeq™) have each developed FDA-approved kits for HIV-1 genotypic testing and interpretation. The Bayer Diagnostics algorithm is based on a set of more than 70 rules (Reid et al., 2002). The Celera Diagnostics is based on assigning scores to drugs based on the presence of specific drug-resistance mutations.

The online Stanford HIVdb algorithm accepts either a user-submitted sequence or list of mutations and returns inferred levels of resistance to 17 FDA-approved RT and protease inhibitors (Betts & Shafer, 2003; Ravela et al., 2003; Rhee et al., 2003). Each drug resistance mutation is assigned a drug penalty score and adding the scores associated with each mutation derives the total score for a drug. Using the total drug score, the program reports one of the following levels of inferred drug resistance: susceptible, potential low-level resistance, low-level resistance, intermediate resistance, and high-level resistance. The HIVRT&PrDB web site contains a detailed explanation of the rules as well as each of the drug penalty scores and comments used by the algorithm.

The Agence Nationale de Recherche sur le SIDA (ANRS) algorithm is updated on a regular basis by a panel of expert researchers on the basis of published studies, as well as ANRS clinical trials. It has been used in several clinical trials and retrospective studies (Rousseau et al., 2001; Meynard et al., 2002) and can be found at http://www.hivfenchresistance.org/tab2003.html. An algorithm created by researchers at the Rega Institute in Leuven, Belgium, has been evaluated using a data set of 240 HIV-1-infected persons and was found to be a significant independent predictor of virologic response to therapy at 3 months after starting a new regimen (Van Laethem et al., 2002). The Retrogram™ algorithm was shown to
be useful to clinicians in the HAVANA study (Tural et al., 2002). Finally, many large commercial laboratories in the United States typically develop their own algorithms for HIV-1 genotypic interpretation.

**Machine-learning algorithms**

Machine-learning algorithms learn from a training data set and then test their performance using a test data set. This process is necessary to prevent these algorithms from learning concepts that are too specific to the training set and thus not applicable to other sets of data. Of the well-known learning algorithms, decision trees are the fastest to construct. Decision trees are robust to missing data, outliers, and irrelevant data, and produce interpretable data. However, they rarely can provide predictive accuracy comparable to the best that can be achieved with the available data (Hastie et al., 2001; Beerenwinkel et al., 2002). Categorical analysis and regression trees (CART) is a statistical method that is somewhat similar to decision trees. CART has been applied to genotypic data to predict phenotype (Sevin et al., 2000) and virologic response to therapy (Quigg et al., 2002). Neural networks are especially effective in problems with a high signal-to-noise ratio and in settings in which prediction without interpretation is the goal. However, they are particularly vulnerable to missing values, outliers, and overfitting. There is one published report of using a neural network to predict lopinavir resistance (Wang & Larder, 2003).

The *Virtual Phenotype™* (Virco; Cambridge UK, and Mechelen, Belgium) is a proprietary algorithm that uses correlations between genotypes and phenotypes in the Virco database. The exact procedure has not been published but the basic concept is as follows. A list of mutations or differences from a consensus reference sequence is submitted to the program. The program then identifies sequences in the database that have a match to the submitted mutations. The phenotypes of the matching sequences are then analyzed to determine the median and range in the levels of fold-resistance. If the median is above the drug-resistance cut-off for a drug, the virus is considered to be resistant. In one prospective study, the *Virtual Phenotype™* was shown to be as useful as the Virco’s phenotypic assay, the Antivirogram™ for guiding salvage therapy (Mazzotta et al., 2003).

**Inter-algorithm comparisons and algorithm validation**

Two types of studies have been designed to examine the performance of algorithms for interpreting protease and RT genotypes: studies of inter-algorithm concordance and studies comparing the performance of one or more algorithms on a data set in which genotype has been linked to the virologic response to a new treatment regimen (Schmidt et al., 2002; De Luca & Perno, 2003). For these types of studies, it is has been necessary to normalize the results of algorithms that report more than three levels of susceptibility with those algorithms that report just three levels of susceptible (susceptible, intermediate, resistant).

A study of the first type applied four rules-based algorithms to sequences from 2045 individuals (Ravela et al., 2003). Drug resistance interpretations were classified as S for susceptible, I for intermediate, and R for resistant. The results of 30,675 interpretations (2045 sequences x 15 drugs) were as follows: 4.4% were completely discordant, with at least 1 algorithm assigning an S and another an R; 29.2% were partially discordant, with at least 1 algorithm assigning an S and another an I, or at least 1 algorithm assigning an I and another an R; and 66.4% displayed complete concordance, with all 4 algorithms assigning the same interpretation (Figure 9). Discordances between NRTI interpretations usually resulted from several simple, frequently occurring mutational patterns. Discordances between PI interpretations resulted from a larger number of more complex mutation patterns. Discordances between NNRTI interpretations were uncommon and resulted from a small number of individual drug-resistance mutations.

Three additional studies compared the *Virtual Phenotype™* to one or more rules-based algorithm and have generally reported high-levels of concordance except for the nucleoside RT inhibitors didanosine, stavudine, and zalcitabine which were more likely to be called susceptible by the *Virtual Phenotype™* (Puchhammer-Stockl et al., 2002; Kijak et al., 2003; Torti et al., 2003).

The validation of algorithms using a virologic outcome data set is not straightforward. First, because multiple drugs are typically used for salvage therapy, it is necessary to create a predicted level of activity for each drug that takes into account both the intrinsic activity of the drug as well as the predicted loss of activity due to drug resistance. Second, the predictive ability of different algorithms will depend heavily on the precise definition of success: whether success is treated as a binary or continuous variable, and whether success is defined as plasma HIV-1 RNA levels becoming undetectable or decreasing by at least a certain amount at a specific point in time after the initiation of salvage therapy.
There have been two published studies that retrospectively compared the performance of one or more algorithms on the ability to predict virologic response to a new treatment regimen (De Luca et al., 2003; Torti et al., 2003). In the study by De Luca and colleagues, the genotypes of 261 individuals were examined for their ability to predict plasma HIV-1 RNA levels at 12 and 24 weeks. All analyzed interpretation systems were significantly predictive of the virologic response, with odds ratios ranging from 1.35 to 2.04 at 3 months and 1.44 to 2.10 at 6 months. However, only 3 of 10 interpretation systems showed significant prediction of the 3- or 6-month response in a multivariable model that included characteristics of patient history, baseline features such as HIV-1 RNA levels and number of resistance mutations, and characteristics of the salvage regimen (number of new and total number of antiretroviral drugs and use of a new drug class). Not surprising, the algorithms were significantly more predictive in adherent than in nonadherent patients. In the study by Torti et al, the TRUGENE™ and Retrogram™ algorithms were more predictive than the VirtualPhenotype™.

X. Genotype-phenotype discordances

Physicians ordering simultaneous genotype and phenotype tests often get back interpretations that appear to be discordant. This section reviews six causes for these apparent discordances: (i) genotypic mixtures, (ii) transitional mutations, (iii) antagonistic mutations, (iv) the effect of TAMS on didanosine, stavudine, and tenofovir susceptibility, (v) atypical mutations, and (vi) complex patterns of mutations. The first three causes of discordance are intrinsic limitations of testing the susceptibility of a virus quasispecies and reflect the fact that phenotypic tests measure the average susceptibility of a population of viruses whereas genotypic tests provide a panoramic assessment of resistance within the virus population. The fourth reflects an artifact of NRTI susceptibility testing caused by the fact that the NRTIs are prodrugs that are triphosphorylated at different rates in vivo and in the cells used for susceptibility testing. The fifth and sixth reflect limitations of genotypic assays that can only be overcome by studies linking genotypic and phenotypic results.

HIV-1 mixtures

About 1% of all nucleotide positions in the RT and protease isolates from persons receiving antiretroviral therapy have detectable mixtures by population-based sequencing (Shafer et al., 2001). However, in persons receiving antiretroviral therapy, that proportion of mixtures at codons associated with drug resistance is considerably higher (>5%) because these positions are under selective drug pressure. If an isolate contains a mixture of a mutation and a wildtype residue at a position associated with drug resistance, genotypic algorithms will consider the mutation to be present and will infer the presence of resistance. Phenotypic assays, however, will not detect resistance if the mutation is present in a minority of the virus quasispecies (Parkin et al., 2002).

Transitional mutations

The canonical transitional mutations have been referred to as T215 revertants. These are mutations at RT position 215 such as T215S/C/E/D that are found as HIV-1 transitions from wildtype to mutant (Larder et al., 1991) or as HIV-1 reverts from mutant to wildtype in the absence of drug pressure (Goudsmit et al., 1996; Yerly et al., 1998; de Ronde et al., 2001). According to phenotypic assays, isolates containing T215 revertants will be fully drug susceptible. In contrast, most genotypic interpretations assign an intermediate level of resistance to isolates containing T215 revertants.

The concept of a transitional mutation can be generalized to mean any mutation that by itself does not cause resistance but that indicates evolving resistance. This is particularly common for protease drug-resistance mutations. For example, protease mutations at position 82 and 90 are generally considered by genotypic algorithms to reduce responsiveness to indinavir and saquinavir, respectively. However, these mutations alone often do not reduce susceptibility in phenotypic assays because accessory mutations (often at highly polymorphic sites) may be required (Parkin et al., 2002). However, the presence of these mutations indicates that the genetic barrier to resistance has been greatly lowered and that with the development of the accessory mutations phenotypic resistance will also be detectable.
**Antagonistic mutations**

As noted in the sections above on interactions between mutations, mutations causing resistance to one drug commonly hypersensitize HIV-1 to a second drug. The two most well studied examples are the antagonistic effect of the RT mutations M184V, L74V, L100I, and Y181C on zidovudine and tenofovir (and stavudine in the case of M184V) and of the NRTI-resistance mutations on the NNRTIs. When antagonistic mutations are present, phenotypic assays may not detect resistance to the drug being resensitized. Genotypic assays may reduce the extent of inferred resistance to the resensitized drug or may provide a comment about the potential for resensitization, but will usually provide an interpretation that indicates the presence of drug-resistance mutations indicative of either active or latent drug resistance.

**Thymidine analog mutations (TAMs)**

As noted in the NRTI section, differences in NRTI triphosphorylation rates between the cells used for susceptibility testing and the wider variety of cells infected by HIV-1 in vivo appears to explain why resistance to some drugs is difficult to detect by in vitro. The primer unblocking mutations, particularly the TAMs, are known to compromise the clinical response to all NRTIs but the phenotypic effect of these mutations on susceptibility to didanosine, stavudine, zalcitabine, and tenofovir is often minimal, and is below the level of technical reproducibility of probably all assays except for the highly reproducible PhenoSense™ assay.

**Atypical mutations**

Most genotypic resistance interpretation algorithms classify a mutation as a drug resistance mutation if the mutation has been shown to reduce susceptibility in site-directed mutants or in large numbers of clinical isolates. Mutations at about 55 positions in protease and RT are classified as drug-resistance mutations. However, many additional mutations at these 55 positions that occur commonly in persons receiving antiretroviral drugs have not been studied. The phenotypic effect of these mutations will be detected by phenotypic assays but some genotypic algorithms may not even report these mutations.

**New drugs and complex patterns of mutations**

The genetic mechanisms of resistance to a new antiretroviral drug are initially developed based on data from in vitro passage experiments and early clinical studies. For example, the initial genotypic predictors of lopinavir resistance were based on data from an early cohort of about 100 patients (Kempf et al., 2001b; Kempf et al., 2002). A subsequent study containing data on about 1,000 HIV-1 isolates, however, showed that the genotypic predictors of lopinavir resistance could be further improved by studying the correlation between genotype and phenotype on a larger number of isolates from additional patients with a wider range of prior treatments and protease mutation patterns (Parkin et al., 2003b).

**XI. Limitations of drug resistance testing**

The clinical usefulness of both genotypic and phenotypic drug resistance testing is limited by the following factors: (i) The relationship between drug resistance and clinical failure is complex. Drug resistance is not the only cause of treatment failure. Nonadherence, the use of insufficiently potent treatment regimens, pharmacokinetic factors that decrease the levels of one or more drugs in a treatment regimen, and possibly even host genomic factors also contribute to treatment failure. Conversely, a drug may have some benefit even in the setting of resistance, because many drug-resistant variants are less fit than drug-susceptible variants. In addition, all antiretroviral drugs are used in combinations, many of which are synergistic. Reduced susceptibility to a drug may not interfere with the drug's beneficial effect on the antiretroviral activity of another drug used in the same regimen. (ii) HIV-1 exists as a complex quasispecies in which many different subpopulations of drug-resistant variants may be archived in latently infected cells. The complexity of this quasispecies may influence the success of therapy in ways that cannot be predicted by any single drug resistance test. (iii) Cross-resistance within each of the three commonly used drug classes is common and often limits treatment options even if the information conveyed by a resistance test is accurate and predictive.

**Complex relationship between drug resistance and clinical failure**

The natural history of HIV-1 infection is highly variable and dependent on a complex set of host-virus interactions (O'Brien et al., 2000; Greene & Peterlin, 2002). In the absence of therapy some patients progress to advanced immunodeficiency within three years following infection, whereas other patients
remains healthy for more than 15 years. It is likely that the same host-virus interactions that so greatly influence disease progression in the absence of drug therapy also influence the risk of virologic failure in patients receiving antiretroviral therapy.

Two consistent observations underscore the complexity of the relationship between drug resistance and disease progression. Patients developing virologic failure on their first treatment regimen are often found to have HIV-1 isolates with resistance to only one of the drugs in the regimen (Murphy et al., 1999a; Descamps et al., 2000; Havlir et al., 2000; Maguire et al., 2000; Mouroux et al., 2000; Gallego et al., 2001; Miller et al., 2003a). The drugs to which resistance most commonly develops in this situation are lamivudine and the NNRTIs; resistance to PIs and NRTIs other than lamivudine is less common in patients with initial virologic failure. The observation that virus becomes detectable and replication ensues despite the fact that the replicating virus remains sensitive to at least two drugs in the treatment regimen suggests that virologic failure is multifactorial with factors in addition to drug resistance contributing to failure. Possibly the remaining drugs in the regimen are not potent enough to fully suppress virus even though they remain active. Alternatively, one of the presumably effective drugs in the regimen may have been present at insufficient levels because of nonadherence or pharmacokinetic factors.

In vitro experiments have consistently shown that isolates containing protease and/or RT drug-resistance mutations replicate less well in cell culture and that purified enzymes with these mutations usually have less activity than wild-type enzymes (reviewed in (Nijhuis et al., 2001; Quinones-Mateu & Arts, 2002)). Drug-resistance mutations are also often replaced in vivo by wild-type variants within weeks to months after removal of selective drug pressure (Goudsmit et al., 1996; Goudsmit et al., 1997; Devereux et al., 1999; Verhoefstede et al., 1999; Devereux et al., 2001). A potential clinical corollary is that virologic failure in patients receiving HAART is not always followed by immunologic and clinical deterioration (Belec et al., 2000; Deeks et al., 2001; Lecossier et al., 2001; Piketty et al., 2001). This may be because the immunologic benefits of virus suppression persist beyond the period of virus suppression or because multidrug-resistant viruses may be less virulent, particularly when they first emerge and are associated with fewer compensatory mutations (Picchio et al., 2000; Devereux et al., 2001). Indeed, two randomized controlled clinical trials have confirmed that there is often a benefit of continuing antiretroviral therapy even in the face of persistent viremia and drug resistance because discontinuation of therapy is often accompanied by higher levels of viremia and loss of CD4 cells (Deeks et al., 2001; Lawrence et al., 2003).

Quasispecies nature of HIV-1

A recognized limitation of HIV-1 drug susceptibility testing by either genotypic or phenotypic methods is the unreliability of these tests in detecting minority HIV-1 variants in the virus population of the patient tested. This limitation is particularly troublesome in patients with complicated treatment histories or those who have discontinued one or more antiretroviral drugs (Devereux et al., 1999; Verhoofstede et al., 1999; Hance et al., 2001). In some patients, the treatment history can be used to infer the presence of archived drug-resistance mutations. For example, if a patient previously received lamivudine as part of an incompletely suppressive treatment regimen, it is likely that M184V exists within the virus population of that patient even if it is not detected at the time of genotyping. The same principle would apply to patients who received NNRTIs as part of an incompletely suppressive treatment regimen. In contrast, patients receiving lamivudine and NNRTIs as part of completely suppressive treatment regimens are not expected to harbor variants resistant to these drugs.

If a patient once harbored drug-resistant variants, these variants may persist at low levels in latently infected cells even if a subsequent treatment regimen brings about complete virus suppression (Wong et al., 1997; Finzi et al., 1999; Martinez-Picado et al., 2000; Hance et al., 2001; Hermankova et al., 2001; Imamichi et al., 2001a; Masquelier et al., 2001a; Martinez et al., 2003). In patients in whom previous resistance tests have documented extensive drug resistance, the clinical usefulness of repeated resistance testing is likely to be minimal, because many resistant variants selected by previous treatment regimens will go undetected in future tests, yet are likely to emerge during attempts at salvage therapy.

Cross-resistance

Most mutations arising during drug therapy contribute resistance to multiple drugs within the same drug class. Because there are only four drug classes available, and because combinations of drugs from at least two classes are usually required to achieve durable HIV-1 suppression, cross-resistance significantly limits treatment options. Resistance assays frequently do not identify enough fully active non-cross-resistant drugs to completely block HIV-1 replication and many patients changing regimens because of
virologic failure will have to use a regimen containing drugs that are partially compromised at the start of therapy. Although cross-resistance is not a direct limitation of resistance testing, it limits its utility, particularly in heavily treated patients.

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Table 1. Expert Panel Recommendations on HIV Drug Resistance Testing

| Consensus opinions (DHHS (US Department of Health and Human Services Panel on Clinical Practices for Treatment of HIV Infection, 2003) and IAS-USA (Hirsch et al., 2003)) |
|---|---|
| Prior to starting therapy (acute and established infection) | - Genotypic testing was originally strongly recommended only for persons with acute HIV-1 infection because rates of resistance to one or more drug classes in this setting have been 10%-20% in the U.S. and Europe.  
- However, although pre-existing resistance is lower in chronically infected persons than in persons with acute HIV-1 infection, it is still significant, with most recent studies reporting resistance rates of 5%-10% in the U.S. and Europe.  
- Genotypic testing is particularly sensitive for detecting drug resistance mutations in this setting because transmitted drug resistance often consists of mutations (e.g. T215 revertants) that do not cause phenotypic resistance but that indicate the likely transmission of a resistant virus.  
- The detection of transmitted resistance can help in selecting therapy. For example, the presence of an NRTI or NNRTI-resistance mutation would make an otherwise excellent regimen such as two NRTIs and efavirenz inappropriate. In contrast, a lopinavir-containing regimen would still be expected to be highly effective because of the high genetic barrier to resistance with most ritonavir-boosted regimens. |
| First regimen failure | - The extent of drug-resistance is a clue to the cause of virologic failure. The presence of little or no resistance strongly suggests nonadherence or, less likely, a purely pharmacokinetic explanation for lack of drug exposure.  
- In patients with a first regimen failure, it should be possible to create a second regimen capable of achieving and maintaining long-term virologic suppression.  
- The specific pattern of mutations may also influence the choice of salvage treatment. |
| Multiple regimen failures | - In patient’s with multiple virologic failures, the past treatment history and any past genotype results should be considered together with a recent genotype, because drug-resistance mutations that were once present may no longer be detectable.  
- In patients with multiple regimen failures, the decision to change therapy is more complex. Changing therapy carries the potential benefit of an improved virologic outcome but the potential risk of rapid virologic failure with the development of additional drug resistance.  
- A phenotypic assay may provide useful supplementary data (e.g. selecting the most active boosted PI), if a major change in therapy such as adding enfuvirtide is considered. |
| Pregnancy | - Resistance testing should be done to select optimal treatment for suppressing maternal RNA levels, particularly near the time of delivery, and for choosing prophylactic neonatal treatment. |
| Post-exposure prophylaxis (PEP) | - Resistance testing should be done to optimize PEP in occupationally and non-occupationally exposed persons. However, PEP should not be delayed pending the results of testing. |

**Footnote:** IAS-USA: International AIDS Society - USA, DHHS - Department of Health and Human Services, BHIVA – British HIV Association
## Table 2. Sources of Knowledge on HIV Drug Resistance Mutations

1. **Which mutations are selected by drug therapy?**
   
   a. *In vitro.* The pre-clinical evaluation of a new drug often involves culturing a wild type laboratory HIV-1 isolate in the presence of increasing drug concentrations and identifying mutations that allow the virus to continue to replicate. Site-directed mutagenesis experiments are done to confirm that the mutations arising during virus passage confer drug resistance when introduced into a wild type virus. Drug resistance mutations identified by this process acquire widespread acceptance as the predominant mutations responsible for resistance to the drug under evaluation, and are often referred to as "canonical" resistance mutations.

   b. *In vivo.* Sequences of HIV-1 isolates from patients failing antiretroviral therapy are critical observations of HIV evolution that show which virus mutations are most significant *in vivo.* Such data are also essential for elucidating the genetic mechanisms of resistance to drugs that are difficult to test *in vitro.* The spectrum of mutations emerging *in vivo* is usually much greater than the spectrum emerging *in vitro.*

2. **Which mutations affect in vitro drug susceptibility?**
   
   a. Laboratory isolates. *In vitro* drug susceptibility data on laboratory isolates makes it possible to quantify the effect of a specific mutation or combination of mutations in an isogenic background. However, it is possible to study only a limited number of mutation patterns in this manner.

   b. Clinical isolates. Clinical isolates often contain more complicated patterns of mutations than laboratory isolates. The complexity of sequences obtained on clinical isolates often precludes site-directed mutagenesis. Instead statistical associations between drug resistance mutations and *in vitro* resistance are required to elucidate the role of specific mutations or mutation patterns in causing drug resistance.

3. **Which mutations interfere with the virologic response to a new treatment regimen?**
   
   Data correlating genotype and clinical response to subsequent antiretroviral therapy would ideally be the most clinically relevant type of data about a mutation. Unfortunately, such data are usually obtained from small underpowered studies and are rarely made publicly available. The correlations between a mutation and pattern of mutations is also dependent on multiple covariates including the starting virus load and CD4 count, the past treatment history, the choice of post-genotype treatment regimen, and patient adherence. Therefore, all three sources of data described in this table are essential to interpreting HIV-1 genotypic resistance tests.
Table 3. Correlations Between HIV-1 Protease Inhibitor (PI) Resistance Mutations and Response to a New PI-Containing Regimen

<table>
<thead>
<tr>
<th>Reference</th>
<th>Previous PI</th>
<th>Follow-up PI</th>
<th>No. Patients</th>
<th>Weeks</th>
<th>Effect of Baseline Mutations on Response to the Follow-up Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harrigan (Harrigan et al., 1999b)</td>
<td>≥1 PI</td>
<td>SQV/RTV</td>
<td>67</td>
<td>24</td>
<td>• In the papers by Harrigan and Zolopa, the number of mutations at codons 46, 48, 54, 82, 84, and 90 correlated with a lack of response to SQV/RTV.</td>
</tr>
<tr>
<td>Deeks (Deeks et al., 1998)</td>
<td>IDV or RTV</td>
<td>SQV/RTV</td>
<td>18</td>
<td>24</td>
<td>• In the paper by Zolopa, virologic response occurred in patients with ≤3 of these mutations. The presence of D30N did not affect response to SQV/RTV.</td>
</tr>
<tr>
<td>Tebas (Tebas et al., 1999)</td>
<td>NFV</td>
<td>SQV/RTV</td>
<td>24</td>
<td>24</td>
<td>• In the paper by Tebas, 17/24 patients who had been previously treated with NFV, had RNA levels &lt;500 copies/ml at week 24.</td>
</tr>
<tr>
<td>Zolopa (Zolopa et al., 1999b)</td>
<td>≥1 PI</td>
<td>SQV/RTV</td>
<td>54</td>
<td>26</td>
<td>• In the paper by Deeks, only 4/18 patients had a sustained decrease in HIV-1 RNA of 0.5 log_{10} at week 24.</td>
</tr>
<tr>
<td>Marcelin (Marcelin et al., 2003a)</td>
<td>≥1 PI</td>
<td>SQV/RTV</td>
<td>72</td>
<td>NA</td>
<td>• In the abstract by Marcelin, 0, 1, and ≥2 mutations at positions 24, 62, 82, 84, and 90 were associated with a median decrease in plasma HIV-1 RNA levels of -2.2, -1.2, and -0.3 log copies/ml.</td>
</tr>
<tr>
<td>Para (Para et al., 2000)</td>
<td>SQV</td>
<td>IDV</td>
<td>89</td>
<td>8</td>
<td>• In the paper by Para, mutations at codons 10, 20, 48, 82, 84, 90 predicted a poor response to IDV salvage therapy</td>
</tr>
<tr>
<td>Saah (Saah et al., 2003)</td>
<td>NFV</td>
<td>IDV</td>
<td>29</td>
<td>48</td>
<td>• In the paper by Saah, L90M predicted a higher risk of virologic failure than D30N</td>
</tr>
<tr>
<td>Shulman (Shulman et al., 2002a)</td>
<td>IDV ± PIs other than RTV</td>
<td>IDV/RTV</td>
<td>31</td>
<td>48</td>
<td>• In the paper by Shulman, 10/14 vs 3/14 subjects with ≥3 mutations at the following positions responded to RTV boosting: 10, 20, 30, 32, 33, 36, 46, 47, 48, 50, 54, 71, 73, 77, 82, 84, 88, 90.</td>
</tr>
<tr>
<td>Campo (Campo et al., 2003)</td>
<td>≥1 PI</td>
<td>IDV/RTV</td>
<td>28</td>
<td>24</td>
<td>• In the paper by Campo, adherence was more important that any particular pattern of mutations or phenotypic drug resistance in predicting virologic response.</td>
</tr>
<tr>
<td>Lawrence (Lawrence et al., 1999)</td>
<td>SQV</td>
<td>NFV</td>
<td>16</td>
<td>24</td>
<td>• In the paper by Lawrence, L90M predicted virologic failure</td>
</tr>
<tr>
<td>Walmsley (Walmsley et al., 2001a)</td>
<td>≥1 PI</td>
<td>NFV</td>
<td>63</td>
<td>24-48</td>
<td>• In the paper by Walmsley, 41% and 22% had HIV-1 RNA declines of ≥0.5 logs at 24 and 48 weeks respectively. The presence of mutations at codons 48, 82, 84, and 90 correlated with a poor virologic response</td>
</tr>
<tr>
<td>Cosado (Casado et al., 2001)</td>
<td>IDV ± RTV</td>
<td>NFV/SQV</td>
<td>31</td>
<td>26-52</td>
<td>• In the paper by Cosado, 35% and 56% of patients had HIV-1 RNA &lt; 50 copies/mL after 6 and 12 months. L90M decreased the rate of response (43% without L90M vs 0% with L90M). V82A did not affect rate of response (36% vs 38%).</td>
</tr>
<tr>
<td>Author</td>
<td>Mutations Required</td>
<td>Treatment</td>
<td>Week 12 Response</td>
<td>Week 24 Response</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------</td>
<td>-----------------</td>
<td>------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>Klein (Klein et al., 2000)</td>
<td>≥1 PI</td>
<td>APV</td>
<td>51</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Falloon (Falloon et al., 2000)</td>
<td>≥1 PI</td>
<td>APV</td>
<td>10</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Descamps (Descamps et al., 2001)</td>
<td>≥1 PI</td>
<td>APV</td>
<td>46</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Duval (Duval et al., 2002)</td>
<td>≥1 PI</td>
<td>APV vs APV/RTV</td>
<td>22</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Marcelin (Marcelin et al., 2003b)</td>
<td>≥1 PI</td>
<td>APV/RTV</td>
<td>49</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Kempf (Kempf et al., 2002)</td>
<td>≥1 PI</td>
<td>LPV/RTV</td>
<td>50</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Masquelier (Masquelier et al., 2002)</td>
<td>≥2 PIs</td>
<td>LPV/RTV</td>
<td>68</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Bongiovanni (Bongiovanni et al., 2003)</td>
<td>≥1 PI</td>
<td>LPV/RTV</td>
<td>134</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

- In the abstract by Klein, I84V and L90M predicted virologic failure; D30N did not.
- In the paper by Falloon, 9 heavily treated patients harboring PI mutations at codons 82 and 90 together with mutations at codons 46 and/or 54 had no virologic response to salvage therapy with an APV-containing regimen.
- In the abstract by Descamps, the presence of ≥ 4 of the following mutations differentiated those with an HIV-1 RNA decrease of ≥ 1.0 log at week 12: L10I, V32I, M46I/L, I47V, I54V, G73S, V82A/T/F/S, I84V, L90M.
- In the paper by Duval, 13/14 receiving APV/RTV vs 2/8 receiving APV had HIV-1 RNA <200 at week 24. Most patients had ≥ 1 mutation at position 46, 54, 82, or 90.
- In the paper by Marcelin, 70% of 49 patients had plasma HIV-1 RNA <400 copies/ml. Lack of response was associated with ≥6 of the following mutations (L10F/I/V, K20M/R, E35D, R41K, I54V, L63P, V82A/F/T/S, I84V).
- In the paper by Kempf, mutations at 11 positions were associated with drug resistance (10, 20, 24, 46, 53, 54, 63, 71, 82, 84, 90). Among 50 NNRTI-naive patients, 21/23 with 0-5 of the above mutations, 15/21 with 6-7 mutations, and 2/6 with 8-10 mutations had HIV-1 RNA <400 copies/mL at week 72.
- In the paper by Masquelier, 34% of persons had plasma HIV-1 RNA <400 copies/ml. Lack of response was associated with the baseline mutations M46I, I54V, and V82A and with ≥5 of the mutations described by Kempf et al.
- In the paper by Bongiovanni, 22/134 (16%) patients had ≥6 of the above mutations of whom 5 had undetectable virus at week 12. 71/112 with <5 mutations had undetectable virus at week 12.

**Abbreviations:** APV: amprenavir; EFV: efavirenz; IDV: indinavir; LPV: lopinavir; NFV: nelfinavir; NNRTI: nonnucleoside RT inhibitors; NRTI: nucleoside RT inhibitors; PI: protease inhibitor; RTV: ritonavir; SQV: saquinavir.
<table>
<thead>
<tr>
<th>Drug</th>
<th>ViroLogic (PhenoSense&lt;sup&gt;TM&lt;/sup&gt;)</th>
<th>Virco (Antivirogram&lt;sup&gt;TM&lt;/sup&gt;)</th>
<th>In vivo potency (approximate mean plasma HIV-1 RNA log&lt;sub&gt;10&lt;/sub&gt; reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (uM)</td>
<td>In vitro potency relative to AZT</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (uM)</td>
</tr>
<tr>
<td>AZT</td>
<td>0.03</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>ddC</td>
<td>0.7</td>
<td>0.04</td>
<td>0.5</td>
</tr>
<tr>
<td>d4T</td>
<td>0.7</td>
<td>0.04</td>
<td>1.0</td>
</tr>
<tr>
<td>ABC</td>
<td>1.7</td>
<td>0.02</td>
<td>1.8</td>
</tr>
<tr>
<td>3TC</td>
<td>1.8</td>
<td>0.02</td>
<td>0.7</td>
</tr>
<tr>
<td>TDF</td>
<td>1.7</td>
<td>0.02</td>
<td>1.8</td>
</tr>
<tr>
<td>ddI</td>
<td>4.6</td>
<td>0.007</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table 5. Drug Susceptibility Data on Isolates with Common Combinations of Thymidine Analog Mutations

<table>
<thead>
<tr>
<th>Mutational Pattern</th>
<th>AZT (fold-resistant)</th>
<th>ABC (fold-resistant)</th>
<th>d4T (fold-resistant)</th>
<th>ddI (fold-resistant)</th>
<th>ddC (fold-resistant)</th>
<th>TDF (fold-resistant)</th>
<th>3TC (fold-resistant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K70R</td>
<td>5.3</td>
<td>1.0</td>
<td>1.3</td>
<td>1.2</td>
<td>0.9</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>T215Y</td>
<td>10</td>
<td>1.2</td>
<td>1.5</td>
<td>1.4</td>
<td>1.0</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>D67N/K70R/K219Q</td>
<td>10</td>
<td>1.5</td>
<td>1.5</td>
<td>1.0</td>
<td>1.0</td>
<td>NA</td>
<td>1.2</td>
</tr>
<tr>
<td>M41L/T215Y</td>
<td>19</td>
<td>2.6</td>
<td>1.6</td>
<td>0.9</td>
<td>1.1</td>
<td>NA</td>
<td>2.0</td>
</tr>
<tr>
<td>M41L/210W/T215Y</td>
<td>64</td>
<td>2.6</td>
<td>2.6</td>
<td>1.4</td>
<td>1.3</td>
<td>3.6</td>
<td>3.2</td>
</tr>
<tr>
<td>D67N/K70R/T215F/K219Q</td>
<td>&gt;100</td>
<td>3.6</td>
<td>2.4</td>
<td>1.5</td>
<td>1.4</td>
<td>4.1</td>
<td>4.7</td>
</tr>
<tr>
<td>M41L/D67N/L210W/T215Y/K219Q</td>
<td>&gt;100</td>
<td>6.6</td>
<td>4.2</td>
<td>1.6</td>
<td>4.8</td>
<td>5.9</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Footnote: AZT: zidovudine, ABC: abacavir, d4T: stavudine, ddI: didanosine, ddC: zalcitabine, TDF: tenofovir, 3TC: lamivudine. Each entry consists of at least one or the median of more than one susceptibility assay performed on isolates containing the mutations shown in the first column and no other known RT inhibitor mutations. The data were obtained from the HIV RT and Protease Sequence Database (Rhee et al., 2003) and are based on data obtained using ViroLogic's Phenosense™ assay (Petropoulos et al., 2000). Similar but slightly higher levels of fold-resistance have been reported using either Virco’s Antivirogram™ (Hertogs et al., 1998) or the original plaque reduction assay (Larder et al., 1990). The addition of the mutation M184V to each of these patterns would lead to slightly lower levels of resistance to AZT, d4T, and TDF, slightly higher levels of resistance to ddI, ddC, and ABC, and very high levels (>200-fold resistance) to 3TC (Whitcomb et al., 2003b). Few published susceptibility data for emtriva (FTC) are available.
Table 6. Correlations Between HIV-1 NRTI Resistance Mutations and Response to a Treatment Regimen

<table>
<thead>
<tr>
<th>Reference</th>
<th>Previous Rx</th>
<th>Follow-up Rx</th>
<th>No. Patients</th>
<th>Weeks</th>
<th>Effect of Baseline Mutations on Response to the Follow-up Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japour (Japour et al., 1995)</td>
<td>AZT</td>
<td>AZT or ddI</td>
<td>188</td>
<td>52</td>
<td>• 215Y/F was associated with increased risk of disease progression but this did not reach statistical significance in a model that also included CD4, syncytium inducing phenotype, AIDS diagnosis, and treatment assignment. 41L and 215Y/F were associated with an increased risk of disease progression regardless of treatment assignment.</td>
</tr>
<tr>
<td>Yerly (Yerly et al., 1996)</td>
<td>AZT</td>
<td>ddI</td>
<td>121</td>
<td>12</td>
<td>• T215Y/F was associated with a poorer CD4 count response to ddI monotherapy.</td>
</tr>
<tr>
<td>Shulman (Shulman et al., 2001c)</td>
<td>AZT</td>
<td>d4T</td>
<td>31</td>
<td>8</td>
<td>• K70R alone did not prevent a subsequent virologic response to d4T • All other TAMS did interfere with a subsequent response.</td>
</tr>
<tr>
<td>Holodniy (Holodniy et al., 1996)</td>
<td>AZT</td>
<td>AZT/ddI</td>
<td>4</td>
<td>30</td>
<td>• 215Y was associated with lack of virologic response.</td>
</tr>
<tr>
<td>Izopet (Izopet et al., 1999)</td>
<td>AZT + ddC</td>
<td>d4T/ddI</td>
<td>20</td>
<td>24</td>
<td>• The 13 patients containing TAMs (11/13 had 215Y ± 41L ± 210W) had significantly lower RNA suppression (-0.5 and –0.1 logs) at week 12 and 24 compared with the 7 lacking TAMs (-1.6 and –2.0 logs).</td>
</tr>
<tr>
<td>Montaner (Montaner et al., 2000)</td>
<td>AZT</td>
<td>d4T/3TC</td>
<td>48</td>
<td>4</td>
<td>• 215Y/F was associated with an odds ratio of failure to achieve a virologic response of 23 in a multivariate model.</td>
</tr>
<tr>
<td>Calvez (Calvez et al., 2002)</td>
<td>AZT ± ddI or ddC</td>
<td>d4T/3TC</td>
<td>26</td>
<td>24</td>
<td>• T215Y/F together with two additional TAMs was associated with a poor virologic response.</td>
</tr>
<tr>
<td>Kuritzkes (Kuritzkes et al., 2000a)</td>
<td>AZT</td>
<td>AZT/3TC/RTV</td>
<td>40</td>
<td>48</td>
<td>• In the studies by Kuritzkes and Gulick, the presence of TAMs did not appear to limit the effectiveness of AZT + 3TC + RTV and AZT + 3TC + IDV • In the study by Havlir, T215Y was strongly associated with virologic failure during the AZT/3TC maintenance phase. • In the study by Descamps and Joly, ≥ 2 TAMs were present in 123/155 patients. Virologic failure (RNA &gt; 5000) occurred in 7/24 classified as susceptible to AZT and in 26/131 classified as resistant by the ANRS algorithm.</td>
</tr>
<tr>
<td>Gulick (Gulick et al., 2000)</td>
<td>AZT</td>
<td>AZT/3TC/IDV</td>
<td>33</td>
<td>156</td>
<td>•</td>
</tr>
<tr>
<td>Havlir (Havlir et al., 1998)</td>
<td>AZT ± (ddI, ddC, d4T)</td>
<td>AZT/3TC/IDV followed by AZT/3TC</td>
<td>107</td>
<td>≥24</td>
<td>•</td>
</tr>
</tbody>
</table>

In the study by Descamps and Joly, ≥ 2 TAMs were present in 123/155 patients. Virologic failure (RNA > 5000) occurred in 7/24 classified as susceptible to AZT and in 26/131 classified as resistant by the ANRS algorithm. The
<table>
<thead>
<tr>
<th>Study</th>
<th>Combination</th>
<th>Description</th>
<th>N</th>
<th>Week</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Descamps, Joly (Descamps et al., 2002; Joly et al., 2002)</td>
<td>AZT±(ddI, ddC)</td>
<td>AZT/3TC/IDV or d4T/3TC/IDV</td>
<td>155</td>
<td>24</td>
<td>proportion of patients with HIV-1 RNA &gt;50 at week 24 did not differ between the AZT- and d4T-containing arms</td>
</tr>
<tr>
<td>Lanier (Lanier et al., 1999)</td>
<td>NRTI,NNRTI, PI</td>
<td>Addition of ABC</td>
<td>117</td>
<td>12-24</td>
<td>In the abstract by Lanier, the presence of ≥3 TAMs, particularly when present with M184V was associated with a poor virologic response</td>
</tr>
<tr>
<td>Katlama (Katlama et al., 2000)</td>
<td>NRTI, NNRTI, PI</td>
<td>Addition of ABC</td>
<td>185</td>
<td>16</td>
<td>In the paper by Katlama, M184V did not preclude an antiviral response; 73% of subjects with M184V had a ≥ 1.0 log₁₀ reduction in HIV-1 RNA</td>
</tr>
<tr>
<td>Brun-Vezinet (Brun-Vezinet et al., 2003)</td>
<td>NRTI, NNRTI, PI</td>
<td>ABC as part of a new HAART regimen</td>
<td>175</td>
<td>12</td>
<td>In the paper by Brun-Vezinet, of 175 patients treated with ABC, virologic response was −0.2 logs, -0.7 logs, and −1.6 logs in persons containing 5-6, 4, or &lt;4 mutations at the following positions: 41, 67, 210, 215, 74, and 184.</td>
</tr>
<tr>
<td>Miller (Miller et al., 2002a)</td>
<td>NRTI, NNRTI, PI</td>
<td>Addition of TDF</td>
<td>332</td>
<td>48</td>
<td>The presence of 41L, 210W, and 215Y, were inversely associated with virologic benefit. Mutations at positions 67, 70, and 219 did not affect response.</td>
</tr>
<tr>
<td>Barrios (Barrios et al., 2003)</td>
<td>NRTI, NNRTI, PI</td>
<td>TDF as part of a new HAART regimen</td>
<td>153</td>
<td>24</td>
<td>The presence of 41L, 210W, and 215Y were inversely associated with virologic benefit. M184V did not affect response.</td>
</tr>
<tr>
<td>Albrecht (Albrecht et al., 2000)</td>
<td>AZT±(ddI or ddC)</td>
<td>Addition or substitution of 3TC</td>
<td>195</td>
<td>8-48</td>
<td>Addition or substitution of 3TC was associated with a 0.5 log RNA reduction at week 8 but most of virologic benefit was lost by week 48 suggesting that 3TC has activity in the presence of TAMs but that using 3TC in maximally suppressive regimens is preferred</td>
</tr>
<tr>
<td>Perno (Perno et al., 2001a)</td>
<td>None</td>
<td>3TC-containing HAART regimen</td>
<td>9</td>
<td>≥24</td>
<td>In 9 untreated persons, 118I was not associated with an increased risk of virologic failure in persons receiving a 3TC-containing HAART regimen</td>
</tr>
<tr>
<td>Molina (Molina et al., 2003)</td>
<td>NRTI, NNRTI, PI</td>
<td>Addition of ddI</td>
<td>110</td>
<td>4</td>
<td>Patients with 0-1 TAMs had 0.8-1.0 log RNA decrease (n=40); those with 2 TAMs had 0.7 log RNA decrease (n=10); those with 3 TAMs had 0.5 log RNA decrease (n=25); those with 4 TAMs had 0.2 log RNA decrease (n=21).</td>
</tr>
<tr>
<td>Rusconi (Rusconi et al., 2001)</td>
<td>NRTI, PI±NNRTI</td>
<td>Change from 3TC to ddI</td>
<td>16</td>
<td>8</td>
<td>Median log RNA decrease in the presence of 184V (n=92) was 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Median log RNA decrease in the presence of L74V (n=9) was 0.1</td>
</tr>
</tbody>
</table>

In 6 of 8 patients with M184V + multiple TAMs, change from 3TC to ddI resulted in HIV-1 RNA decline lowered by ≥ 0.5 logs.
<table>
<thead>
<tr>
<th>Albrecht, Winters (Albrecht et al., 2001; Winters et al., 2003)</th>
<th>NRTI change and addition of NFV, EFV, or NFV/EFV</th>
<th>195</th>
<th>16-48</th>
</tr>
</thead>
</table>

- In patients without isolates containing M184V, addition of 3TC was associated with an improved virologic response
- In patients with isolates containing M184V, substitution of ddi for 3TC was associated with an improved virologic response

**Abbreviations:** ABC: abacavir; AZT: zidovudine; ddi: didanosine; d4T: stavudine; 3TC: lamivudine; TDF: tenofovir DF; IDV: indinavir; NFV: nelfinavir; NNRTI: nonnucleoside RT inhibitors; NRTI: nucleoside RT inhibitors; EFV: efavirenz, NVP: nevirapine; PI protease inhibitor; TAMS: thymidine analog mutations.
### Table 7. Common Causes of Discordance Between Genotypic and Phenotypic Test Results

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Phenotypic result (change in IC&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>Phenotypic interpretation</th>
<th>Genotypic interpretation</th>
<th>Expected virologic response to drug(s) in question</th>
<th>Explanation for differences between interpretations</th>
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<tr>
<td><strong>Genotypic mixture (e.g. NNRTI)</strong></td>
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<tr>
<td>K103N</td>
<td>25-fold</td>
<td>Resistance</td>
<td>Resistance</td>
<td>No response</td>
<td>The mutant form of the virus is rapidly selected once NNRTI treatment is started</td>
</tr>
<tr>
<td>K103K/N</td>
<td>1 to 25-fold</td>
<td>Possible resistance (depending on the phenotypic cut-off)</td>
<td>Resistance</td>
<td>No response</td>
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<tr>
<td><strong>Transitional mutations (e.g. zidovudine)</strong></td>
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<tr>
<td>T215S/C/D</td>
<td>None</td>
<td>Susceptible</td>
<td>Possibly resistant</td>
<td>Initial response</td>
<td>215S develops in isolates developing 125Y/F. 215C/D/E develop in isolates that once had 215Y/F. In both cases, it is likely that 215Y/F is present.</td>
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<td><strong>Transitional mutations (e.g. saquinavir)</strong></td>
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<tr>
<td>L90M</td>
<td>1 to 5-fold</td>
<td>Possible resistance (if &gt;2-3-fold)</td>
<td>Possibly resistant</td>
<td>Decreased response</td>
<td>L90M has been shown to compromise the response to saquinavir and ritonavir/saquinavir</td>
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<td><strong>Antagonistic mutation (e.g. efavirenz)</strong></td>
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<tr>
<td>Miscellaneous polymorphisms</td>
<td>3 to 5-fold</td>
<td>Susceptible</td>
<td>Susceptible</td>
<td>Response</td>
<td>K103N causes about 25-fold resistance by itself, but is associated with only 3-5-fold resistance when present with certain combinations of NRTI resistance mutations. Nonetheless, patients with such isolates have not responded virologically to NNRTI therapy (Shulman et al., 2001b).</td>
</tr>
<tr>
<td>M41L + L210W + T215Y + K103N</td>
<td>3 to 5-fold</td>
<td>Possible resistance (depending on the phenotypic cut-off)</td>
<td>Resistant</td>
<td>No response</td>
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<tr>
<td><strong>Antagonistic mutation (e.g. zidovudine)</strong></td>
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<tr>
<td>M41L + T215Y</td>
<td>10-fold</td>
<td>Resistant</td>
<td>Resistant</td>
<td>No Response</td>
<td>M184V partially reverses T215Y-mediated zidovudine resistance. However, high-level zidovudine resistance can emerge rapidly either by loss of M184V or the acquisition of two additional RT mutations (Larder et al., 1995).</td>
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<tr>
<td>M41L + T215Y + M184V</td>
<td>2-fold</td>
<td>Susceptible</td>
<td>Resistant</td>
<td>Partial response</td>
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<td><strong>TAMs (e.g. stavudine)</strong></td>
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<td>Mutation/Combination</td>
<td>Resistance Level</td>
<td>Interpretation</td>
<td>Response</td>
<td>Notes</td>
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<tr>
<td>M41L + T215Y</td>
<td>~1.5-fold</td>
<td>Low level resistance may be detected by the PhenoSense™ assay but not by the Antivirogram™</td>
<td>Probably resistant</td>
<td>Partial or no response</td>
<td>Stavudine, didanosine, and tenofovir susceptibilities are difficult to measure. The biological and clinical cut-offs overlap with the reproducibility cut-offs.</td>
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<td><strong>Atypical mutation (e.g. efavirenz)</strong></td>
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<td>K101P, K103S</td>
<td>&gt;15-fold for each mutation</td>
<td>Resistant</td>
<td>Possibly resistant</td>
<td>Presumably poor response</td>
<td>Most genotypic interpretation algorithms have rules triggered by specific mutations (e.g. K103N). Therefore, the presence of an atypical mutation may not trigger a report of resistance.</td>
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FIGURES

Figure 1

Structural model of HIV-1 protease homodimer labeled with protease inhibitor resistance mutations. The polypeptide backbone of both protease subunits (positions 1-99) is shown. The active site, made up of positions 25-27 from both subunits, is displayed in ball and stick mode. The protease inhibitor resistance mutations are shown for the subunit on the left but not for the mirror-image subunit on the right. The protease was co-crystallized with indinavir, which is displayed in space-fill mode. This drawing is based on a structure published by Chen et al (Chen et al., 1994).
Figure 2

Schematic representation of the how the protease recognizes nine cleavage sites to create the structural proteins from the \textit{gag} gene and enzymes from the \textit{pol} gene. The inset shows the peptides recognized by the HIV-1 protease. Compensatory changes at these cleavage sites occur commonly in viruses containing certain protease mutations.
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- **High-level resistance**
- **Intermediate resistance**
- **Low-level resistance**
- **Contributes to resistance**
- **No Resistance**
- **Hypersensitivity**
Figure 3. Protease inhibitors (PIs) and PI-resistance mutations. APV: amprenavir, ATV: atazanavir, IDV: indinavir, LPV: lopinavir (in combination with ritonavir), NFV: nelfinavir, RTV: ritonavir, SQV: saquinavir. Contribution to resistance is proportional to the darkness of the blue rectangles. Notes: (i) The first block of mutations consist of major PI-resistance mutations; the second block consists of the next most important set of mutations, those in the enzyme flap; the third block consists of mutations at nonpolymorphic residues which are probably somewhat less important than the flap mutations; the fourth block consists of polymorphic residues that contribute to resistance when present with other PI-resistance mutations. (ii) Multiple mutations are usually required to cause high-level resistance when PIs are used with RTV-boosting. (iii) Although specific mutations are not shown, nearly all differences from consensus B are associated with resistance and have similar consequences with a few notable exceptions: As indicated by the presence of two rows, I50V and I50L have very different effects on PI susceptibility. V82I occurs in untreated isolates, particularly in non-B isolates and causes little, if any, PI resistance; I54M/L cause a higher level of resistance to APV than other substitutions at this position; I47A has recently been reported to cause high-level LPCV resistance but its effect on other inhibitors is not known. N88S but not N88D has been shown to cause hypersusceptibility to APV. L33F is a nonpolymorphic substitution selected by several PIs. L33V/I are polymorphic substitutions that may have less impact on PI susceptibility.
Structural model of HIV-1 reverse transcriptase (RT) labeled with nucleoside RT inhibitor (NRTI) resistance mutations. The polypeptide backbone of the fingers and palm domain (positions 1-235), and DNA template strand and 3' primer terminus are shown in orange, in space fill mode. The active site positions (110, 185, 186) are displayed in purple, in ball and stick mode. The incoming nucleotide (dNTP) is displayed in yellow, in space-fill mode. These drawings are based on the structure published by Huang, et al. (Huang et al., 1998), and are shown in “front” (a) and “back” (b) views.
Figure 5

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- Purple: High Level Resistance
- Blue: Intermediate Resistance
- Light Blue: Low Level Resistance
- Light Gray: Contributes to Resistance
- White: No Resistance
- Yellow: Unknown
- Red: Hypersensitivity
Figure 5. Nucleoside and nucleotide RT inhibitors (NRTIs) and NRTI-resistance mutations. AZT: zidovudine, d4T: stavudine, TDF: tenofovir DF, ABC: abacavir, ddl: didanosine, 3TC: lamivudine. Zalcitibine is not shown because it not used commonly and has a resistance profile similar to ddl. The resistance profile of FTC appears so far to be identical to that of 3TC. Contribution to resistance is proportional to the darkness of the blue rectangles. "*" indicates that mutations at this position cause hypersusceptibility; "?" indicates insufficient data. Mutations are ordered according to their order in the text: TAMs; M184V; mutations in the β2-β3 loop region; Q151M-associated multinucleoside resistance mutations; insertions at position 69; accessory mutations. Although specific mutations are not shown, nearly all differences from consensus B are associated with resistance and have similar consequences with a few notable exceptions: T215S/C/D are transitional mutations which do not cause drug resistance. V75I does not cause NRTI resistance by itself; whereas other changes at this position, notably V75T, and probably V75M/A cause resistance to d4T and ddl.
A model for interactions between HIV-1 RT and the chain-terminated primer/template adopted from (Meyer et al., 1999). There are two alternate pathways following the addition of a chain-terminating NRTI: (i) Primer-rescue (nucleotide excision) leading to ongoing virus replication, and (ii) Dead-end complex (DEC) formation caused by the addition of the dNTP that is complementary to the next base. Open black boxes represent natural deoxynucleosides; closed boxes, chain-terminating nucleosides; open red boxes, ribonucleosides.
Figure 7

Structural model of HIV-1 reverse transcriptase (RT) labeled with non-nucleoside RT inhibitor (NNRTI) resistance mutations. The polypeptide backbone of the "fingers", "palm", and "thumb" subdomains of the p66 subunit (positions 1-300), and DNA primer and template strands are shown. This drawing is based on the structure provided by Kohlstaedt et al. (Kohlstaedt et al., 1992) in which the RT is co-crystallized with nevirapine, which is displayed in space-fill mode. The active site is shown in purple ball and stick mode. The positions associated with NNRTI resistance are shown surrounding the hydrophobic pocket to which nevirapine and other NNRTIs bind.
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- **High Level Resistance**
- **Intermediate Resistance**
- **Low-Level Resistance**
- **Contributes to Resistance**
- **No Resistance**
- **Hypersensitivity**
Figure 8. Non-nucleoside RT inhibitors (NNRTIs) and NNRTI-resistance mutations. DLV: delavirdine, EFV: efavirenz, NVP: nevirapine. Contribution to resistance is proportional to the darkness of the blue rectangles. "*" indicates that mutations at this position cause hypersusceptibility.
Nature of discordances between four HIV genotypic resistance interpretation algorithms according to drug (Ravela et al., 2003). The figure summarizes the results of 30,675 interpretations: 2,045 sequences x 15 drugs. Sequences for which all algorithms assigned a susceptible interpretation (S) are shown in green; those for which at least one algorithm assigned an S and another an intermediate interpretation (I) are shown in pale green, those for which at least one algorithm assigned an S and another resistance (R) are shown in red; those for which at least one algorithm assigned an I and another an R are shown in purple; those for which all algorithms assigned an R (or rarely an I) are shown in black.
References


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Sankatsing S, Weaverling GJ, van't Klooster G, Prins JM, Lange J. 2002. TMC125 monotherapy for 1 week results in a similar initial rate of decline of HIV-1 RNA as therapy with a 5-drug regimen [abstract 5]. 9th Conference on Retroviruses and Opportunistic Infections. Seattle, WA.


Winters MA, Schapiro JM, Lawrence J, Merigan TC. 1998b. Human immunodeficiency virus type 1 protease genotypes and in vitro protease inhibitor susceptibilities of isolates from individuals who were switched to other protease inhibitors after long-term saquinavir treatment. Journal of Virology 72:5303-5306.


