**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 RNase H 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 (Roders et al., 1995), RT bound to an NNRTI (Kohlstaedt & Steitz, 1992), RT bound to double-stranded DNA (Jacob-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; Stammers 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 6 shows that there are significant discordances between the *in vitro* and *in vivo* activities 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% (IC$_{50}$) 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**

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 7) (Whitcomb et al., 2003) and clinical (Table 8) resistance to each of the other NRTIs: zidovudine, stavudine > abacavir, tenofovir > didanosine, zalcitabine > lamivudine, emtricitabine. 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; Stammers et al., 2001) 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
corporated 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 4a-c). 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., 2001b; Maxeiner et al., 2002). They also occur in about 10% of patients
receiving 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). T215I/V are additional treatment-
associated mutations at this position (Rhee et al., 2003). 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), T215E (1), and T215I (1) (Garcia-
Lerma et al., 2001).

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-nucleoside therapy 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., 2001b; 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 combinations (Izopet et
al., 1999; Mayers et al., 1999; Montaner et al., 2000; Costagliola et al., 2001). 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; Costagliola et al., 2001; 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 abacavir, and 2 to 5-fold decreased susceptibility to stavudine, didanosine, zalcitabine, and tenofovir (Mayers et al., 1994; Harrigan et al., 2000; Larder & Bloor, 2001; Lennerstrand et al., 2001; Miller et al., 2001; Miller & Larder, 2001; Shulman et al., 2001c; Wainberg & White, 2001; Margot et al., 2002; 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
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 usually the 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., 2001b; 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 (Gu 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., 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 also develop M184V (Frost et al., 2000). Steric conflict between the oxathiolane 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., 2003). 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 (Rhee et al., 2003). In the presence 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., 2003; Whitcomb et al., 2003). 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., 2000). Resensitization, however, can be overcome by the presence of four or more zidovudine resistance mutations (Tisdale et al., 1993; Whitcomb et al., 2003).

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 (Reviewed in (Miller et al., 2002b; Diallo et al., 2003a)). 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, NRTIs often used in combination with lamivudine.

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 ddI 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). Moreover, 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).

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., 1998; de Jong et al., 1999; Larder et al., 1999; Tamalet et al., 2000; Masquelier et al., 2001). 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., 2003). 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., 2001). 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., 2003). 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-Lerma 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., 2003) 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 occurs 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 \textit{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., 1998; 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., 2001). 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., 2001; 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., 2000), 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., 2002; 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., 2001).

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.

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., 2001a). G333E has been reported to facilitate zidovudine resistance in isolates from patients receiving zidovudine and lamivudine who also 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.

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 occurred 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 published.

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., 2001; Meyer et al., 2003). However, these polymorphisms are so common in untreated persons that they should not be considered drug resistance mutations.

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 (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, it 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 5). 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.
Figure 4. 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, and are shown in "front" (a) and "back" (b) views.

Figure 4 References

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 5 References**


**References**


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