**Nonnucleoside RT inhibitor (NNRTI) resistance mutations**

The NNRTIs bind to a hydrophobic pocket in the RT located between the β6-β10-β9 and β2-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., 2003). 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 (Figure 1)**

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., 2001; Torti et al., 2001) 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., 2000a; 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., 2003).

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 al., 2001). 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 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; Bacheler et al., 2001). A98S is a common polymorphism that does not cause NNRTI resistance (Rhee et al., 2003).

NNRTI mutations between codons 179-190 (Figure 1)

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 (Calvez et al., 2000; Shulman et al., 2000; Walmsley et al., 2001). 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 (Fujiwara 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 (Bacheler 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; Fujiwara et al., 1998; Petropoulos et al., 2000; Bacheler et al., 2001). Y188C and Y188H are uncommon mutations at this position that cause intermediate-to-high levels of nevirapine resistance and low-leves 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 (Figure 1)
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; Bacheler 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., 2000b). 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 RNase H 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., 1998; Rhee et al., 2003). L234I cause resistance to the experimental NNRTI, capravirine but its effect on other NNRTIs is not known (Fujiwara et al., 1998). K238N/T occur in 1%-2% of NNRTI treated isolates. K238N causes intermediate resistance to nevirapine and delavirdine and low-level resistance to efavirenz. K238R is a polymorphism that is the consensus amino acid at this position in subtype E (CRF01_AE) viruses.

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 (Harrigan et al., 2002). 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 (Delaugerre et al., 2001; 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**

Some combinations of NRTI-resistance mutations hypersensitize HIV-1 to the currently approved NNRTIs (Shulman et al., 2001; Haubrich et al., 2002; Whitcomb et al., 2002). Although multidrug-resistance to both NRTIs and NNRTIs occur commonly (Emini et al., 1993; Larder et al., 1993; Shulman et al., 2001), antagonistic mutational interactions between the two drug classes suggest that the number of ways in which HIV-1 can develop simultaneous high-level resistance to both NRTIs and NNRTIs may be restricted. Interactions between NRTI and NNRTI mutations may also help explain the success of regimens containing two NRTIs plus an NNRTI not only as part of initial therapy but also in certain salvage therapy situations (Haubrich et al., 2000; Kuritzkes et al., 2000; Albrecht et al., 2001).

Y181C and L100I hypersensitize HIV-1 to zidovudine (Larder, 1992) and tenofovir (Parkin et al., 2003) presumably by interfering with primer unblocking (Selmi et al., 2003). Conversely, Y181C may lead to subtle reductions in stavudine susceptibility to stavudine (Baldanti et al., 2003; Blanca et al., 2003; Parkin et al., 2003).

**Figure 1. 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 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.

Figure 1 References


References


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