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Review

HIV-1 reverse transcriptase connection subdomain mutations involved in resistance to approved non-nucleoside inhibitors

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ABSTRACT

The human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) is a major target of antiretroviral intervention. Non-nucleoside RT inhibitors (NNRTIs) bind to a hydrophobic pocket located away from the DNA polymerase catalytic site of the RT. Approved NNRTIs are nevirapine, delavirdine, efavirenz, etravirine and rilpivirine. This review describes how these inhibitors affect RT function, the structural basis of NNRTI binding, and the role of specific amino acid substitutions at the NNRTI binding pocket in the acquisition of high-level drug resistance. However, two or more amino acid substitutions are required to achieve >20-fold decreased susceptibility to recently developed NNRTIs such as etravirine or rilpivirine, in phenotypic assays. While genotypic analysis of HIV-1 isolates in infected patients is usually restricted to residues 1–250 of the RT, recent reports indicate that several residues in the connection subdomain of the RT (comprising residues 319–426) could also modulate NNRTI resistance. Examples are Y318F or W, N348I, A376S and T369I or V. Tyr-318 participates in NNRTI binding, but other amino acid substitutions in the connection subdomain may affect resistance through an indirect mechanism. Studies on the effects of N348I and A376S on NNRTI resistance indicate that these changes could affect inhibitor binding by altering the interaction between RT subunits or between the RT and the template-primer. Moreover, those mutations could also modulate RNase H activity not only during DNA strand elongation, but also at the initiation of plus strand DNA synthesis as demonstrated for the N348I mutation.

Contents

1. Introduction

The reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) is a DNA polymerase responsible for the conversion of the viral genomic RNA into double-stranded proviral DNA. Nucleoside and non-nucleoside HIV-1 RT inhibitors constitute the backbone of highly active antiretroviral therapy (HAART) (Menéndez-Arias, 2010). Currently prescribed HAART regimens can be diverse, but usually include two nucleoside RT inhibitors combined with one non-nucleoside RT inhibitor (NNRTI) or alternatively, one ritonavir-boosted protease inhibitor or an integrase

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inhibitor. NNRTIs are a group of small (<600 Da) hydrophobic compounds that specifically bind HIV-1 RT, acting as non-competitive inhibitors with respect to either dNTP or nucleic acid substrates (Sluis-Cremer and Tachedjian, 2008). Unlike nucleoside RT inhibitors, NNRTIs do not require intracellular metabolism for activity.

There are five NNRTIs approved for clinical use: nevirapine, delavirdine, efavirenz, etravirine and rilpivirine (http://www.fda.gov/ForConsumers/byAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm118915.htm). First generation non-nucleoside inhibitors such as nevirapine and delavirdine were licensed by the US Food and Drug Administration in June 1996 and April 1997, respectively. Efavirenz, a more potent and selective NNRTI was approved in September 1998, and has been used in combination with nucleotide and nucleoside analogues such as tenofovir and emtricitabine, respectively, as part of a very potent and successful HAART therapy. However, the clinical use of nevirapine, delavirdine and efavirenz has been limited by their relatively low genetic barrier to resistance, their cross-resistance and tolerability issues. Some of these limitations have been overcome by diarylpyrimidine (DAPY) analogues such as etravirine and rilpivirine. These next-generation NNRTIs have been approved by the FDA in January 2008 (etravirine) and in May 2011 (rilpivirine).

2. HIV-1 RT structure and NNRTI inhibition of DNA polymerization

The HIV-1 RT is a heterodimeric enzyme composed of subunits of 66 kDa and 51 kDa, designated as p66 and p51, respectively. Both subunits share the same amino acid sequence, but p51 lacks residues 441–560 of p66 that form the RNase H domain. Crystal structures have shown that both subunits contain four common subdomains designated as 'fingers' (residues 1–85 and 118–155), 'palm' (residues 86–117 and 156–236), 'thumb' (237–318) and 'connection' (319–426) (Kohlstaedt et al., 1992). The folding of these subdomains relative to each other is different in each subunit. The DNA polymerase active site resides within p66 where the catalytic residues Asp-110, Asp-185 and Asp-186 are located. Two divalent cations (Mg^{2+}) are required for catalysis. The nucleic acid binding cleft is formed by the fingers, palm and thumb subdomains of p66 and the thumb subdomain of p51, that together with the connection subdomains of both subunits contribute to the "floor" of the cleft.

The NNRTI binding site is a hydrophobic pocket located in the palm subdomain of the 66-kDa subunit, at a distance of around 10 Å from the RT DNA polymerase catalytic site and 60 Å from the RT RNase H active site. Crystal structures of nevirapine bound to HIV-1 RT revealed that the binding pocket is formed by the movement of the side-chains of Tyr-181 and Tyr-188 that stabilize inhibitor binding. These interactions were found to be important for binding of other first-generation NNRTIs, such as MKC-442 or TNK-651 (El-Brollosy et al., 2002; Ren et al., 2001). Structural analysis of nevirapine-bound RT led authors to propose that NNRTI binding would restrict the relative subdomain movements required to complete the catalytic cycle of the enzyme (Kohlstaedt et al., 1992). Upon NNRTI binding, there is a change in the thumb subdomain that adopts a more open conformation in comparison with the closed conformation of the unliganded RT (Kohlstaedt et al., 1992; Rodgers et al., 1995). This rearrangement could affect the correct interaction between RT and template/primer. This suggested mechanism of nevirapine inhibition has been imaginatively called as 'molecular arthritis'.

Pre-steady-state kinetic studies have shown that NNRTIs act by slowing the rate of the chemical reaction of DNA polymerization catalyzed by the RT (Spence et al., 1995, 1996). These findings were consistent with structural data showing the differences between NNRTI bound and free forms of HIV-1 RT that affect the location of strands β4, β7 and β8 (Esnouf et al., 1995). These β-strands contain part of the polymerase active site including catalytic aspartic acid residues. It has been suggested that this conformational change affecting the active site of the RT could alter binding of divalent cations Mg^{2+}/Mn^{2+}, therefore weakening dNTP interactions. However, kinetic studies demonstrated that NNRTIs strengthen dNTP binding, while RT-template/primer-NNRTI complexes display a cation-dependent increase in dNTP binding affinity (Xia et al., 2007). These data argue against the proposal of NNRTI-shifted aspartates as being unable to bind divalent cations.

An alternative mechanism to explain RT inhibition by NNRTIs involves a conformational change affecting residues of the primer grip (residues 227–235), as a result of the contribution of the side-chains of the highly-conserved Trp-229 and Pro-236 in NNRTI binding (Hsiou et al., 1996). Since the primer grip is involved in the precise positioning of the DNA primer relative to the polymerase active site, its conformational change resulting from NNRTI binding would prevent the establishment of a catalytically competent ternary complex.

3. Structural basis of NNRTI binding and resistance

Crystal structures of HIV-1 RT bound to nevirapine, delavirdine, efavirenz, etravirine and rilpivirine have been determined (Table 1). The NNRTI binding site is located in the 66-kDa subunit of the RT. In general, NNRTI binding involves stacking interactions between aromatic rings of the inhibitors and the side-chains of Tyr-181, Tyr-188, Trp-229 and Tyr-318, electrostatic interactions involving the side-chains of Lys-101, Lys-103 and the p51 residue Glu-138, van der Waals interactions with Leu-100, Val-106, Val-179, Tyr-181, Glu-190, Trp-229, Leu-234, Pro-236 and Tyr-318, and hydrogen bonds between the NNRTI and the main chain of the RT. The formation of the NNRTI binding pocket is characterized by the movement of the side-chains of Tyr-181 and Tyr-188 from a 'down' to an 'up' position (Rodgers et al., 1995; Esnouf et al., 1997). Tyrosine residues 181 and 188 are important for nevirapine binding, and single amino-acid substitutions at those positions (e.g. Y181C, Y181I, Y188C and Y188I) are known to confer high-level resistance to the inhibitor (Fig. 1).

Larger first-generation inhibitors such as delavirdine, extend towards the flexible loop containing Pro-236, while maintaining stacking interactions with the tyrosine residues 181 and 188 and stabilizing hydrogen bonds with Lys-103 (Esnouf et al., 1997). On the other hand, stacking interactions are less important in the case of efavirenz binding. Hydrogen bonds between the protein backbone of Lys-101 and Lys-103 and the inhibitor are critical for efavirenz binding (Ren et al., 2000; reviewed in Ren and Stammers, 2008). K103N confers high-level resistance to efavirenz (Fig. 1). The crystal structure of the unliganded mutant RT revealed the formation of a hydrogen bond between the hydroxyl group of Tyr-188 and the Asn-103 amide that could stabilize the unliganded RT with the Tyr-188 side-chain in a 'down' position (Hsiou et al., 1996).

Efavirenz, as well as nevirapine and delavirdine, show a low genetic barrier for resistance. Single-nucleotide changes in the viral genome can confer high-level resistance to those inhibitors. NNRTI resistance mutations can be classified into three major groups based on their mechanisms of resistance: (i) loss of or change of key hydrophobic interactions of the inhibitor at the NNRTI binding site (e.g. Y181C and Y188L), (ii) steric hindrance affecting the central region of the NNRTI (e.g. L100I, V106A and G190A), or (iii) amino acid substitutions that occur at the rim of the NNRTI binding pocket and interfere with inhibitor entry (e.g. K101E and K103N) (Hsiou et al., 1996; Ren et al., 2007). Efavirenz binding occurs in a deeper position in the mutant K103N RT as compared with the

Table 1
Crystal structures of HIV-1 RT bound to approved NNRTIs.[a]

Inhibitor	RT	PDB code	Resolution (Å)	References
Nevirapine	Wild-type	3HVT	2.90	Smerdon et al. (1994)
		1VRT	2.20	Ren et al. (1995)
	Wild-type (in complex with P4Y)[b]	3QIP	2.09	Lansdon et al. (2011)
	M41L/D67N/K70R/M184V/T215Y[c]	1LWF	2.80	Chamberlain et al. (2002)
	M41L/T215Y	1LWE	2.81	Chamberlain et al. (2002)
	L100I	1S1U	3.00	Ren et al. (2004)
	K101E	2HND	2.50	Ren et al. (2006)
	K103N	1FKP	2.90	Ren et al. (2000)
	K103N (in complex with LP7)[d]	3LP0	2.79	Su et al. (2010)
	K103N (in complex with LP8) [e]	3LP1	2.23	Su et al. (2010)
	V108I	1S1X	2.80	Ren et al. (2004)
	E138K	2HNY	2.50	Ren et al. (2006)
	Y181C	1JLB	3.00	Ren et al. (2001)
	M184V	1LWC	2.62	Chamberlain et al. (2002)
	Y188C	1JLF	2.60	Ren et al. (2001)
	T215Y	1LW0	2.80	Chamberlain et al. (2002)
Delavirdine	Wild-type	1KLM	2.65	Esnouf et al. (1997)
Efavirenz	Wild-type	1FK9	2.50	Ren et al. (2000)
	K103N	1FKO	2.90	Ren et al. (2000)
	K103N/E478Q[f]	1IKV	3.00	Lindberg et al. (2002)
	Y181C	1JKH	2.50	Ren et al. (2001)
	E478Q	1IKW	3.00	Lindberg et al. (2002)
Etravirine	Wild-type	3MEC	2.30	Lansdon et al. (2010)
		3M8P	2.67	Kertesz et al. (2010)
	K103N	3MED	2.50	Lansdon et al. (2010)
	K103N/C280S	1SV5	2.90	Das et al. (2004)
Rilpivirine	Wild-type	3MEE	2.40	Lansdon et al. (2010)
	L100I/K103N/K172A/K173A/C280S	2ZE2	2.90	Das et al. (2008)
	K103N	3MEG	2.80	Lansdon et al. (2010)
	K103N/K172A/K173A/Y181C/C280S	3BGR	2.10	Das et al. (2008)
	K172A/K173A/C280S	2ZD1	1.80	Das et al. (2008)

[a] Atomic coordinates available from the Protein Data Bank (PDB) at http://www.pdb.org.
[b] P4Y is 5,6-dihydroxy-2-[(2-phenyl-1*H*-indol-3-yl)methyl]pyrimidine-4-carboxylic acid, an RNase H inhibitor.
[c] M41L, D67N, K70R and T215Y are thymidine analogue resistance-associated mutations and M184V is a lamivudine resistance mutation.
[d] LP7 is ethyl 1,4-dihydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate, an RNase H inhibitor.
[e] LP8 is 3-cyclopentyl-1,4-dihydroxy-1,8-naphthyridin-2(1*H*)-one, an RNase H inhibitor.
[f] E478Q is an amino acid substitution that inactivates the RNase H activity of the RT.

wild-type enzyme (Ren et al., 2000), and is thus less effective in inhibiting the mutant enzyme. Kinetic studies carried out with nevirapine showed that K103N impacts the rate of inhibitor binding (k_{on}), while L100I and V106A influenced inhibitor dissociation (k_{off}). Major resistance mutations such as Y181I or Y188L have an impact on both parameters k_{on} and k_{off} (Maga et al., 1997).

Recently developed DAPY compounds, such as etravirine and rilpivirine, adopt a horseshoe shape in the NNRTI binding pocket (Das et al., 2004, 2008; Lansdon et al., 2010) (Fig. 2). Etravirine is bound to the HIV-1 RT with the central pyrimidine ring located between Leu-100 and Val-179, and establishing a key hydrogen bond with Lys-101. The benzonitrile moiety of etravirine locates in the pocket defined by Val-106, Pro-225, Phe-227, Leu-234, Pro-236 and Tyr-318, with its dimethylcyanophenyl group oriented towards Tyr-188, Phe-227 and Trp-229 (Das et al., 2004; Lansdon et al., 2010).

Rilpivirine shows a similar conformation in the NNRTI binding pocket, but the inhibitor slides closer to Glu-138 (p51) and protrudes further in the pocket defined by Phe-227 and Trp-229 (Lansdon et al., 2010). As shown for other NNRTIs, upon etravirine and rilpivirine binding, there is a shift in the location of the conserved YMDD motif (residues 183–186). Unlike nevirapine, delavirdine or efavirenz, DAPY derivatives can adopt different conformational modes while binding the RT enzyme. These compounds show remarkable torsional flexibility (“wiggling”) and ability to reposition (“jiggling”) within the NNRTI binding pocket, thereby optimizing their interactions with the enzyme (Das et al., 2004, 2008). In vitro studies have shown that high-level resistance to etravirine or rilpivirine is usually achieved by the accumulation of two or more amino acid substitutions as compared with the wild-type strain. Examples are K101E/K103N and K103N/Y181C in the case of etravirine (Andries et al., 2004). Single mutations do not prevent their binding to the enzyme but reduce the number of alternative binding configurations available for the inhibitor. However, recently published results of phase III clinical trials of rilpivirine in combination with tenofovir/emtricitabine showed a high prevalence of E138K (usually in combination with the emtricitabine resistance-associated mutation M184I) in individuals experiencing virological failure (Cohen et al., 2011; Molina et al., 2011), suggesting that the rilpivirine genetic barrier to resistance could be lower than previously thought.

4. Other effects of NNRTI binding on RT function

As described above, NNRTI binding impairs DNA polymerization. However, the kinetic constants for inhibition can vary more than 100-fold depending on the template/primer used in the assays. In addition, reverse transcription requires the coordinate action of the DNA polymerase and RNase H activities of the RT, particularly at strand transfer reactions required to complete minus strand DNA synthesis and to synthesize the full proviral DNA.

It has been shown that some NNRTIs (e.g. nevirapine, efavirenz) are able to inhibit 5′-RNA directed HIV-1 RNase H activity (or polymerase-independent RNase H activity), while stimulating 3′-DNA directed (i.e. polymerase-dependent) RNase H activity (Hang et al., 2007; Radzio and Sluis-Cremer, 2008). In agreement with these observations, it has been shown that efavirenz and other NNRTIs show a more potent effect in strand transfer assays than in DNA polymerization assays. In addition, NNRTI resistance mutations such as K103N, V106A, Y181C or P236L modulate RNase H activity by diminishing 5′-RNA and/or 3′-DNA directed cleavage

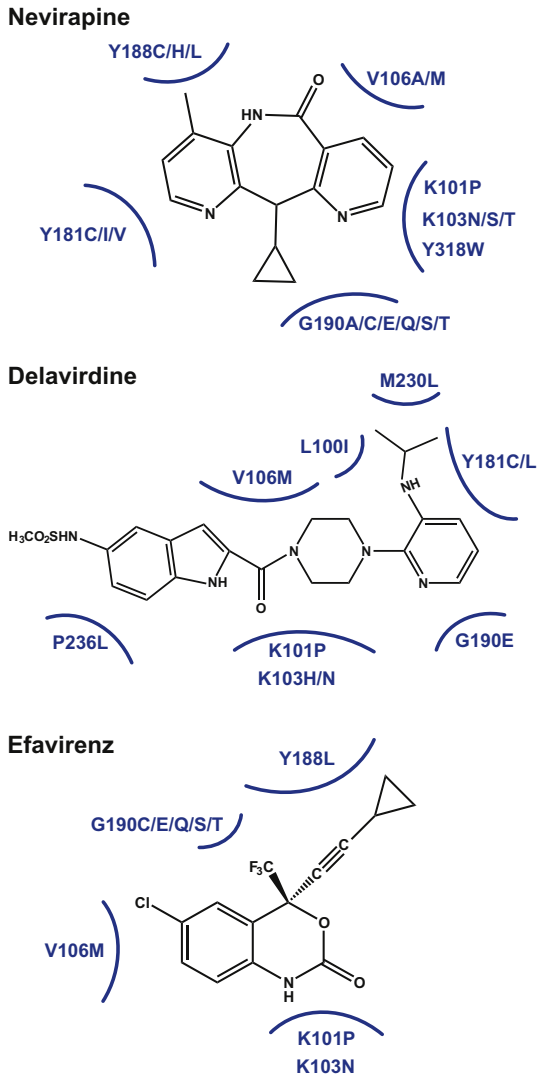

Fig. 1. Chemical structures of nevirapine, delavirdine and efavirenz and amino acid substitutions that by themselves confer a >40-fold increase in the inhibitory concentration (IC_{50}) relative to the wild-type virus in phenotypic assays using recombinant HIV-1 strains. Locations of amino acid residues relative to the NNRTI structures were obtained from the corresponding crystal structures of RT/NNRTI complexes, using Protein Data Bank (PDB) coordinates given in Table 1. NNRTI susceptibility data on the effects of single amino acid substitutions were taken from the following references: (i) nevirapine (Richman et al., 1991; Balzarini et al., 1993, 1994a,b; Richman et al., 1994; Ahgren et al., 1995; Buckheit et al., 1995a,b; Fujihashi et al., 1995; Kleim et al., 1997; Pelemans et al., 1997; Fujiwara et al., 1998; Balzarini et al., 2000; Petropoulos et al., 2000; Bacheler et al., 2001; Chan et al., 2001; Isaka et al., 2001; Brenner et al., 2003; Huang et al., 2003; Rao et al., 2004; Amiel et al., 2005; Harrigan et al., 2005; Parkin et al., 2006; Barreca et al., 2007; Kagan et al., 2009; Tambuyzer et al., 2009; Azijn et al., 2010), (ii) delavirdine (Dueweke et al., 1993; Balzarini et al., 1994a, 1996a,b; Olmsted et al., 1996; Demeter et al., 1997; Kleim et al., 1997; Fujiwara et al., 1998; Balzarini et al., 2000; Isaka et al., 2000; Petropoulos et al., 2000; Bacheler et al., 2001; Brenner et al., 2003; Harrigan et al., 2005; Parkin et al., 2006; Sato et al., 2006), and (iii) efavirenz (Young et al., 1995; Balzarini et al., 2000; Petropoulos et al., 2000; Bacheler et al., 2001; Brenner et al., 2003; Huang et al., 2003; Das et al., 2004; Amiel et al., 2005; Sato et al., 2006; Kagan et al., 2009; Tambuyzer et al., 2009; Azijn et al., 2010). For a comprehensive review on the effects of one or more amino acid substitutions on phenotypic resistance to NNRTIs, see Menéndez-Arias (2011).

(e.g. K103N, V106A, P236L), or increasing the rate and frequency of secondary cleavages in the viral RNA (e.g. Y181C) (Gerondelis et al., 1999; Archer et al., 2000).

In vitro studies have shown that NNRTIs are potent inhibitors of the initiation of plus-strand DNA synthesis under conditions in which there was little inhibition of minus strand DNA synthesis (Grobler et al., 2007). Plus-strand DNA synthesis involves binding of the RT to a template/primer containing a DNA template and an RNA polypurine tract (PPT) that is used as a primer. Biochemical studies showed that RT preferentially binds to RNA PPT hybrid duplexes in a polymerase-dependent mode (i.e. with the 3′ end of the primer located at the polymerase active site) (Palaniappan et al., 1998; Götte et al., 2000).

Fluorescent resonance energy transfer (FRET) experiments using fluorophores attached to one of the single strand overhangs of the DNA template and to the polymerase (either at the RT fingers subdomain or at the RNase H domain) have confirmed that the RNA PPT could adopt different orientations, with its 3′ end located either at the DNA polymerase catalytic site or at the RNase H active site (Abbondanzieri et al., 2008; Liu et al., 2008). In the absence of dNTPs, the RT has a stronger tendency to interact with the PPT primer in a polymerase-independent mode (Abbondanzieri et al., 2008) (Fig. 3). However, nucleotide addition occurs in the polymerase-dependent orientation. Extension of the PPT primer facilitates its removal because the RNA becomes more accessible to the RNase H activity of the RT. FRET-based single-molecule studies showed that nevirapine binding increases the flipping rate from the polymerase-competent to the RNase H-competent (or polymerase-independent) orientation (Abbondanzieri et al., 2008) (Fig. 3). Therefore, NNRTIs appear to facilitate primer removal while inhibiting DNA synthesis.

Several studies have shown that NNRTIs act as chemical enhancers of HIV-1 RT dimerization (Tachedjian et al., 2001; Venezia et al., 2006). Their binding pocket is located at the interface between the HIV-1 RT subunits p66 and p51, and includes residues such as Tyr-181 in p66 or Glu-138 in p51 (Menéndez-Arias et al., 2001). Efavirenz was found to be the most potent inducer of RT dimerization, whereas nevirapine has a weak effect and delavirdine has no effect at all (Tachedjian et al., 2001). Efavirenz is a tight-binding inhibitor capable of binding RT monomers (i.e. p51 or p66) or dimers (p51/p51, p66/p51 and p66/p66) (Figueiredo et al., 2006; Braz et al., 2010). Based on these observations, it has been suggested that efavirenz and probably other NNRTIs could promote Gag-Pol dimerization and enhance polyprotein processing due to a premature activation of the viral protease (Figueiredo et al., 2006). The compensatory effect of amino acid substitutions in the viral protease that restore viability of HIV clones containing defective RTs (Olivares et al., 2007) also argues in favour of tight regulation between RT dimerization and viral protease activation. In addition, other studies have shown that efavirenz impairs virus particle production by affecting RT-Gag interactions. This effect is suppressed by mutant RTs bearing the amino acid substitutions W401A/W402A that occur at the RT dimerization interface (Chiang et al., 2009).

5. Connection subdomain mutations related to NNRTI resistance

Current genotypic analysis of HIV-1 isolates generally focus on residues 1–250 of the RT, and therefore do not provide information on potential antiretroviral therapy-related mutations occurring in the thumb-connection and RNase H domains of the viral polymerase. Recent findings have revealed that mutations in the connection subdomain and in the RNase H domain of the RT can significantly amplify resistance to the nucleoside analogue zidovudine (AZT), by altering the balance between excision and RNA template degradation (Nikolenko et al., 2005; Delviks-Frankenberry et al., 2008; for recent reviews, see Menéndez-Arias, 2008; Delviks-Frankenberry et al., 2010). Suppression of AZT resistance by NNRTIs also suggests that there is an interaction between the

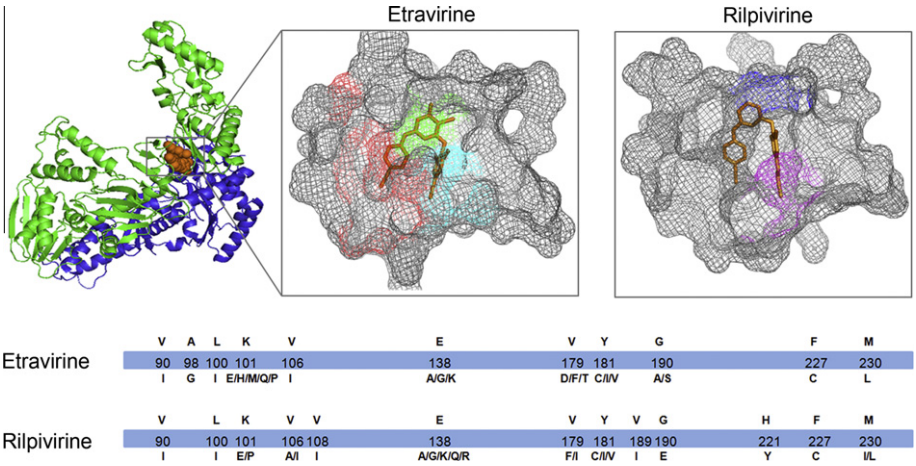

Fig. 2. Structures of etravirine and rilpivirine binding pockets and resistance-associated mutations. Top panel shows the HIV-1 RT bound to etravirine with green and blue ribbon diagrams showing the location of p66 and p51, respectively. The NNRTI binding pockets of etravirine and rilpivirine are shown using a mesh representation. NNRTIs are represented with orange sticks. In the etravirine complex, Leu-100 and Val-179 are shown in green, Val-106, Pro-225, Phe-227, Leu-234, Pro-236 and Tyr-318 in red, and Tyr-188 and Trp-229 in cyan. In the rilpivirine complex, highlighted residues are Glu-138 (blue) and Phe-227 and Trp-229 (purple). Coordinates were taken from PDB files 3MEC and 3MEE. The lower panel shows amino acid substitutions associated with phenotypic resistance to etravirine (Andries et al., 2004; Das et al., 2004; Tambuyzer et al., 2009) and rilpivirine (Azijn et al., 2010). *In vitro*, high-level resistance to both inhibitors requires the combination of two or more amino acid substitutions (for a comprehensive list of mutant HIV-1 strains with etravirine or rilpivirine susceptibility data, see Menéndez-Arias, 2011).

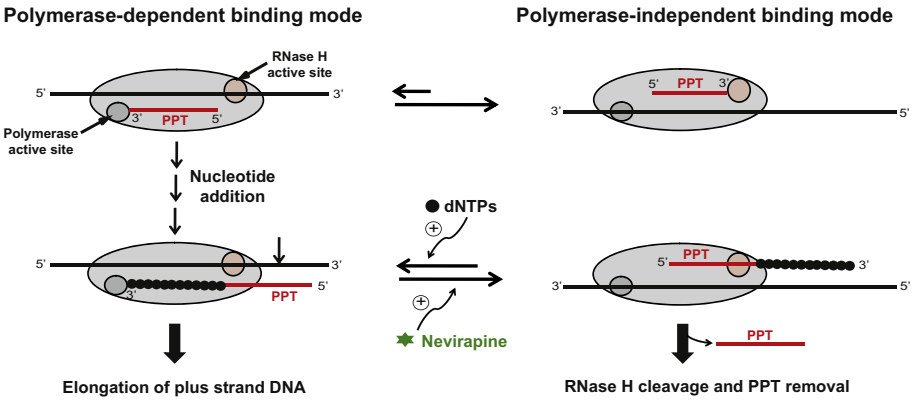

Fig. 3. Equilibrium between polymerase-competent and polymerase-incompetent RT orientations during initiation of plus strand DNA synthesis. Nevirapine facilitates the interaction of the DNA/PPT-containing complex in the polymerase-independent binding mode that facilitates PPT removal, through cleavage by the RNase H activity of the RT. The DNA is represented in black and the PPT RNA in red.

binding sites of AZT and NNRTIs. Furthermore, the NNRTI resistance mutation Y181C can suppress AZT resistance mediated by the combinations D67N/K70R/T215F/K219Q or D67N/K70R/T215Y/K219Q (reviewed in Menéndez-Arias, 2008).

Several mutations in the thumb and connection subdomains of the RT have been associated with resistance to NNRTIs. Among those occurring in the thumb, the amino acid substitution L283I produces an approximately 2-fold increase in the 50% inhibitory concentration (IC_{50}) of nevirapine and delavirdine, particularly when combined with I135L, I135M or I135T (Leigh Brown et al., 2000). Both amino acids locate at the p51 heterodimer interface of the RT, but do not participate in the NNRTI binding pocket. The N265D polymorphism has been identified in subtype A HIV-1 clones with NNRTI resistance mutations, but unexpectedly, in one clone it showed an antagonistic effect on K103N over nevirapine, delavirdine and efavirenz resistance, rendering HIV-1 susceptible to the drugs (Eshleman et al., 2006). Other HIV-1 subtype A variants selected in the presence of efavirenz contained mutations A288T or D312E/Q452L, which appeared associated with the NNRTI resistance mutation Y188C or with L234F, respectively (Lai et al., 2010). Another thumb subdomain mutation (V314I) has been selected in combination with E138K in HIV-1 subtype C strains grown in the presence of etravirine (Lai et al., 2010).

A number of amino acid substitutions in the RT connection subdomain have been associated with low-level but significant resistance to NNRTIs (Table 2). In some cases, the residues involved occur at the NNRTI binding pocket and participate in interactions with the inhibitor (e.g. Y318F and Y318W). However, in most cases, drug resistance mutations occur away from the NNRTI binding pocket. The molecular mechanisms involved in resistance mediated by these mutations are less clear and still being investigated.

6. NNRTI binding pocket mutations: effect of Y318F and Y318W on resistance

The prevalence of mutations at position 318 in large panels of HIV-infected antiretroviral-drug treated patients has been estimated at less than 2%, and about 5 to 11 times less frequent than major NNRTI resistance mutations such as K103N or Y181C (Harrigan et al., 2002). Tyr-318 is highly conserved among all HIV-1 and HIV-2 strains and is part of the binding pocket of all

Table 2
Effect of RT connection subdomain mutations on NNRTI susceptibility as determined in phenotypic assays.

Amino acid substitution	IC_{50} (fold-change)[a]				References
	Nevirapine	Delavirdine	Efavirenz	Etravirine	
E312Q	2.4	nr	0.8	nr	Hachiya et al. (2009)
Y318F	**1.5–7.8**	**17.3–42**	0.5–1.7	1.4	Pelemans et al. (1998), Harrigan et al. (2002), Vingerhoets et al. (2005), Sato et al. (2006) and Tambuyzer et al. (2009)
Y318W	**53.7**	0.3	nr	nr	Pelemans et al. (1998)
G333D	2.4	nr	1.4	nr	Hachiya et al. (2009)
G333E	nr	nr	nr	0.9	Gupta et al. (2011)
G335C	2.8	nr	0.6	nr	Hachiya et al. (2009)
N348I	**3.3–27**	**3.4–5.5**	1.7–2.7	1.4–1.6	Yap et al. (2007), Hachiya et al. (2008, 2009), Sluis-Cremer et al. (2010), Gupta et al. (2010, 2011) and Lengruber et al. (2011)
K358R	0.5	nr	nr	nr	Lengruber et al. (2011)
G359S	0.7	nr	nr	nr	Lengruber et al. (2011)
A360I	1.7	nr	0.8	nr	Hachiya et al. (2009)
A360V	0.8–2.2	nr	1.3	nr	Hachiya et al. (2009) and Lengruber et al. (2011)
V365I	2.9	nr	1.1	nr	Hachiya et al. (2009)
T369I	**8.4**	**5.2**	2.1–2.4	1.5	Zhang et al. (2007), and Gupta et al. (2010, 2011)
T369V	**8.6–9.2**	**5.5**	**3.1**	nr	Gupta et al. (2010) and Lengruber et al. (2011)
A371V	1.2	nr	nr	nr	Lengruber et al. (2011)
I375V	0.9	nr	nr	nr	Lengruber et al. (2011)
A376S	**3.7–4.3**	1.5	1.8–1.9	1.1	Hachiya et al. (2009) and Paredes et al. (2011)
T377M	1.2	nr	nr	nr	Lengruber et al. (2011)
T386A	nr	nr	1.6	0.5	Vingerhoets et al. (2005)
I393L	1.3	1.0	1.0	nr	Hachiya et al. (2008)
E399D	nr	nr	nr	1.2	Gupta et al. (2011)
Q509L[b]	**9.1**	nr	2.7	nr	Hachiya et al. (2009)
N348I/T369I[c]	**58.6**	**29.8**	**8.1**	1.7	Gupta et al. (2010, 2011)
N348I/I393L[c]	**26**	**7**	1.7	nr	Hachiya et al. (2008)

nr, not reported.
[a] Indicated values correspond to the ratio between the 50% inhibitory concentrations (IC_{50}) obtained with recombinant virus carrying the indicated mutation and a reference wild-type strain. Significant levels of resistance (>2.5-fold) are indicated in bold.
[b] Q509L is an RT RNase H domain mutation.
[c] Combination of RT connection subdomain mutations that confer significant resistance to one or more approved NNRTIs.

approved NNRTIs. Specifically, Tyr-318 makes extensive contacts with delavirdine through its piperazine moiety and the adjacent CO group. Non conservative substitutions of Tyr-318 in the RT lead to the loss of its DNA polymerase activity to levels below 5% of the wild-type enzyme (Pelemans et al., 1998). However Y318F and Y318W retain more than 60% of the wild-type RT activity.

Phenotypic assays using recombinant virus have demonstrated that Y318F produces a 17.3- to 42-fold increase in the IC_{50} for delavirdine, while conferring low-level resistance (1.5- to 3-fold increase) to nevirapine (Pelemans et al., 1998; Harrigan et al., 2002; Sato et al., 2006; Tambuyzer et al., 2009). In contrast, the available data indicate that Y318F has a minor impact on efavirenz and etravirine resistance (Vingerhoets et al., 2005; Tambuyzer et al., 2009), although selection experiments carried out in the presence of etravirine allowed for the identification of viruses having mutations V179I, Y181C, L234F and Y318F and showing high-level resistance to etravirine (Vingerhoets et al., 2005). In site-directed mutagenesis studies carried out with HIV-1 clones, Y318F was shown to increase by 2-fold efavirenz and etravirine resistance mediated by the combination of K103N and Y181C (Vingerhoets et al., 2005).

The Y318W substitution confers HIV-1 high-level resistance to nevirapine (i.e. >50-fold increase in the IC_{50}), while rendering the virus susceptible to delavirdine (Pelemans et al., 1998). However, the presence of this mutation in vivo has not been reported, since it requires two simultaneous nucleotide changes (TAT to TGG) to emerge and any intermediate single-nucleotide change would render a defective virus.

7. Mutations away from the NNRTI binding pocket: resistance mechanisms

As shown on Table 2, amino acid substitutions in the RT connection subdomain at residues located away from the NNRTI binding pocket confer low to moderate levels of resistance to nevirapine and delavirdine, and in a lesser extent to efavirenz. The most relevant are N348I, T369I or T369V and A376S. High-level resistance to nevirapine and delavirdine, and moderate levels of resistance to efavirenz have been observed in the case of the double-mutant N348I/T369I, in the absence of mutations affecting the NNRTI binding site (Gupta et al., 2010, 2011). Interestingly, these two amino acid substitutions can produce a significant increase of the resistance mediated by classical NNRTI resistance mutations such as L100I, K103N, Y181C or G190S, as demonstrated in drug susceptibility assays carried out with nevirapine, delavirdine, efavirenz and etravirine (Gupta et al., 2010, 2011). For etravirine, the introduction of the connection subdomain mutations N348I/T369I produces a 10-fold increase of IC_{50} obtained with viruses having Y181C, and renders a highly-resistant HIV-1 strain (Gupta et al., 2011).

7.1. N348I

The most extensively studied mutation of the connection subdomain is N348I. This amino acid substitution is selected early in response to antiretroviral therapy (Yap et al., 2007), but disappears soon after interrupting therapy due to its negative effects on the HIV-1 replicative capacity (Hachiya et al., 2008; Gupta et al., 2010; McCormick et al., 2011). N348I is usually associated with thymidine analogue resistance mutations (e.g. M41L and T215Y), and by itself confers dual resistance to AZT and nevirapine (Yap et al., 2007; Hachiya et al., 2008). An association between N348I and the accumulation of NNRTI resistance mutations has also been reported (Cane et al., 2007; Yap et al., 2007) and, specifically, the combination of K103N and N348I shows increased efavirenz and nevirapine resistance in comparison with mutants having K103N alone (Yap et al., 2007).

Yap et al. showed that N348I reduces the rate of RNA template degradation by RT in either a wild-type background or in the

presence of thymidine analogue resistance mutations (Yap et al., 2007). This property would facilitate the excision of AZT by giving RT more time to excise the blocking nucleoside analogue from the terminated primer (Nikolenko et al., 2005). A similar effect has been reported for Q509L, an RNase H domain mutation that decreases RNase H activity while increasing AZT resistance mediated by D67N/K70R (Brehm et al., 2008). Q509L confers 9.1- and 2.7-fold increased resistance to nevirapine and efavirenz in phenotypic assays (Hachiya et al., 2009).

However, it has been shown that N348I could also modulate the excision activity of the RT by an RNase H independent mechanism, since this mutation could increase the processivity of HIV-1 RT in the absence (Schuckmann et al., 2010) or in the presence of AZT resistance-associated mutations (Ehteshami et al., 2008). As for nevirapine, four major mechanisms have been proposed to explain the resistant phenotype conferred by N348I. Thus, this amino acid substitution could act (i) by decreasing inhibitor binding to the nevirapine binding pocket, (ii) by reducing the affinity of the RT for the template/primer in the presence of the NNRTI, (iii) by decreasing the RT RNase H activity, and (iv) by affecting the orientation of the RT relative to the nucleic acid substrate, a property that appears to be critical for PPT tract removal during plus strand DNA synthesis (Biondi et al., 2010; Nikolenko et al., 2010; Schuckmann et al., 2010) (Fig. 4). Asn-348 has no direct interaction with the NNRTI binding pocket and does not participate in p66/p51 subunit interactions.

Enzymatic studies have shown that N348I decreases catalytic efficiency (Schuckmann et al., 2010), thereby providing an explanation for the reduced fitness of viruses carrying the mutation. In addition, N348I improved affinity for nucleic acid and enhanced processivity of DNA synthesis, and these effects were also observed when the mutation was selectively introduced either in p66 or in p51. These properties had a long-range effect on inhibitor binding (K_d) by primarily decreasing the association rate (k_{on}) of the inhibitor (Schuckmann et al., 2010). Interestingly, the decreased RNase H activity conferred by N348I (Yap et al., 2007) was mapped to the 51-kDa subunit (Schuckmann et al., 2010).

A general model to explain dual resistance to nucleoside and nonnucleoside RT inhibitors that incorporates RNase H activity has been proposed (Nikolenko et al., 2010). In this model, reduced RNase H cleavages allow more time for dissociation of the NNRTI from the RT-template/primer-NNRTI complex leading to more efficient reinitiation of DNA synthesis. The experiments carried out with N348I mutants show that the dissociation rate for nevirapine is similar to that obtained with the wild-type RT (k_{off} 0.005 s^{-1}) (Schuckmann et al., 2010). In addition, increasing concentrations of NNRTIs can reverse N348I-mediated deficits in RNase H cleavage on regular RNA/DNA template/primers (Biondi et al., 2010). Nonetheless, N348I seems to reduce polymerase-independent RNase H cleavage during minus strand DNA synthesis, probably as a result of a decrease in the affinity for shorter double-stranded fragments (Ehteshami et al., 2008).

In agreement with the results of phenotypic assays (Table 2), Biondi et al. have shown that N348I has a more significant impact on susceptibility to nevirapine compared to efavirenz (Biondi et al., 2010). This differential behaviour has been related to the specific influence of the mutation on initiation of plus strand DNA synthesis. As discussed above, nevirapine (and efavirenz) facilitate PPT removal during initiation of plus strand DNA synthesis (Götte et al., 2000; Abbondanzieri et al., 2008). Premature removal of the PPT tract impairs reverse transcription. N348I counteracts the effects of nevirapine on this process by diminishing the primer removal reaction, without affecting RNase H activity in the presence of efavirenz (Biondi et al., 2010). Overall, these data suggest that long-range interactions involving RT subdomains can affect the orientation of the template/primer relative to the RT in the presence of NNRTIs, and this could have different effects depending on the reverse transcription step under consideration.

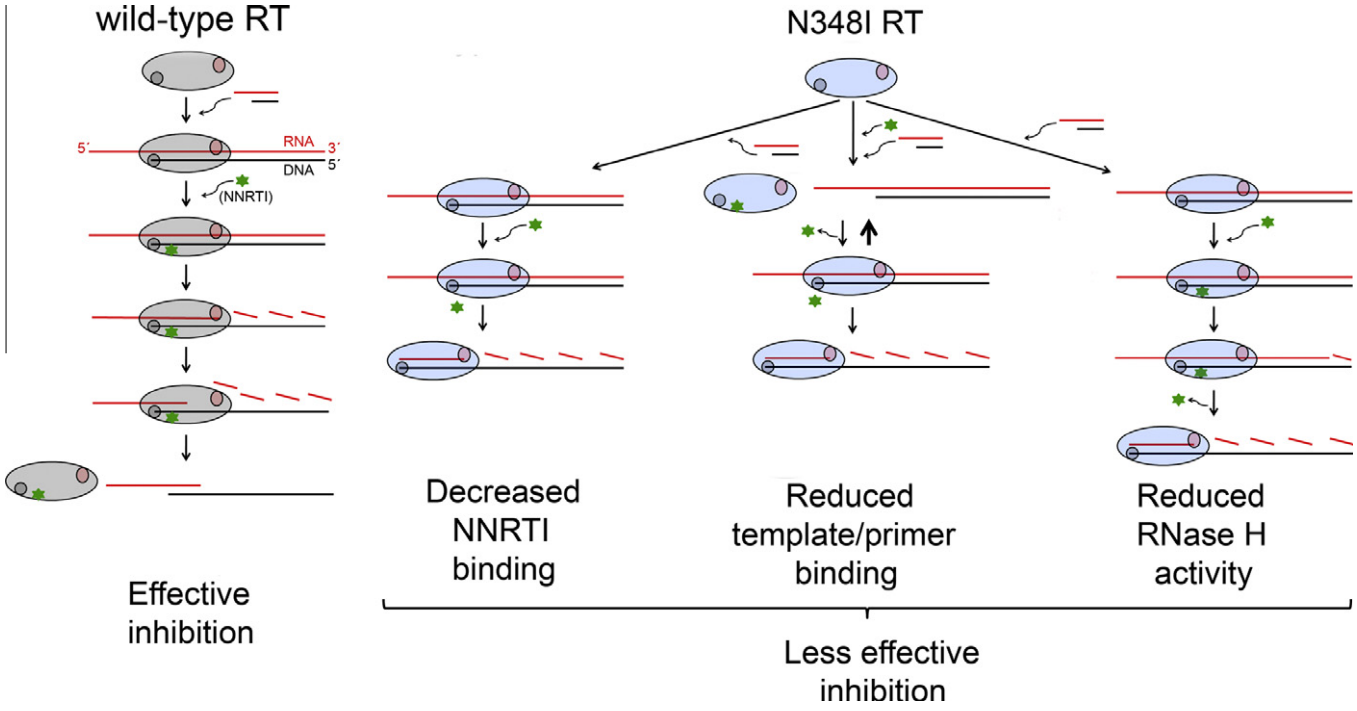

Fig. 4. Potential mechanisms to explain nevirapine resistance mediated by N348I. The amino acid substitution in the RT can affect interactions with nevirapine and the RNA/DNA template-primer and therefore, the coordination between the RNA-dependent DNA polymerase and RNase H activities of the enzyme during DNA elongation. The fourth mechanism acts on the initiation of plus strand DNA synthesis and is based on the reduced tendency of N348I RT to adopt a polymerase-independent orientation in the presence of nevirapine (see Fig. 3 for details).

7.2. A376S

Studies carried out with a large European cohort of HIV-infected patients showed that NNRTI-naïve individuals carrying the A376S substitution had a 10-fold increased risk of virological failure to nevirapine (Paredes et al., 2011). A376S confers low-level resistance to nevirapine in phenotypic assays, without affecting the RT susceptibility to other NNRTIs (Table 2). As in the case of N348I, the substitution A376S decreases nevirapine affinity (K_d) by two-fold despite the location of residue 376 more than 20 Å away from the NNRTI binding site (Paredes et al., 2011). These effects could result from the higher affinity for double-stranded DNA of the mutant A376S RT relative to the wild-type enzyme (Paredes et al., 2011), and could be a consequence of the contribution of Ala/Ser-376 in p66 to the dimerization interface in the RT heterodimer. Unlike in the case of N348I, the decreased nevirapine susceptibility does not seem to be related to effects on primer removal during initiation of plus strand DNA synthesis.

7.3. Other relevant substitutions

There are other amino acid substitutions in the RT connection subdomain whose involvement in NNRTI has been suggested. For example, T386A has been selected as a secondary mutation after passaging the virus in the presence of etravirine. This amino acid substitution increases etravirine resistance when classical NNRTI resistance mutations such as L100I and K103N are present in the virus (Vingerhoets et al., 2005). G359S has been also associated with the accumulation of classical NNRTI resistance mutations, although its real effect in resistance is not clear (Cane et al., 2007). Studies carried out with chimeric constructs containing the entire RT connection subdomains of HIV-1 subtype B isolates obtained from treatment-experienced patients showed the correlation between the presence of G335C and nevirapine resistance, or between the presence of A360I and resistance to both nevirapine and efavirenz. In these studies, isolates containing G335C and N348I were found to be susceptible to efavirenz (Nikolenko et al., 2010).

A correlation between etravirine susceptibility and the presence of mutations at codon 399 of the RT-coding region (e.g. E399G or E399D) has been observed in phenotypic assays carried out with RTs obtained from treated individuals (Poveda et al., 2008; Gupta et al., 2011). However, by themselves E399G and E399D have a minimal effect on etravirine susceptibility and their relevance in drug resistance is not clear. On the other hand, T369I and T369V are known to confer resistance to nevirapine and delavirdine in phenotypic assays and in the case of T369I, this substitution potentiates nevirapine, delavirdine and efavirenz resistance mediated by N348I (Table 2). T369I has also been selected in the presence of VRX-480773, an experimental NNRTI that inhibits efavirenz-resistant HIV-1 clones (Zhang et al., 2007). Thr-369 is close to the RT heterodimer interface but the molecular mechanisms involved in NNRTI resistance mediated by mutations at position 369 have not been investigated.

8. Conclusions

All described biochemical studies point towards nucleic acid binding affinity and RNase H activity as important modulators of NNRTI resistance. These studies also highlight the importance of interactions between distinct parts of the RT that play a significant role in drug resistance evolution. In addition, next-generation inhibitors (i.e. etravirine and rilpivirine) show more extensive interactions with the NNRTI binding pocket and require two or more mutations to achieve significant resistance. In this scenario, it is possible that RT connection subdomain and/or RNase H domain mutations could have a larger impact on resistance, and their relevance in clinical management of HIV drug resistance is likely to increase as the novel inhibitors integrate in current HAART therapies.

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References

Abbondanzieri, E.A., Bokinsky, G., Rausch, J.W., Zhang, J.X., Le Grice, S.F.J., Zhuang, X., 2008. Dynamic binding orientations direct activity of HIV reverse transcriptase. Nature 453, 184–189.

Ahgren, C., Backro, K., Bell, F.W., Cantrell, A.S., Clemens, M., Colacino, J.M., Deeter, J.B., Engelhardt, J.A., Hogberg, M., Jaskunas, S.R., Johansson, N.G., Jordan, C.L., Kasher, J.S., Kinnick, M.D., Lind, P., Lopez, C., Morin Jr., J.M., Muesing, M.A., Noreen, R., Oberg, B., Paget, C.J., Palkowitz, J.A., Parrish, C.A., Pranc, P., Rippy, M.K., Rydergard, C., Sahlberg, C., Swanson, S., Ternansky, R.J., Unge, T., Vasileff, R.T., Vrang, L., West, S.J., Zhang, H., Zhou, X.-X., 1995. The PETT series, a new class of potent nonnucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 39, 1329–1335.

Amiel, C., Desire, N., Schneider, V., Delphin, N., Race, E., Clavel, F., Piolot, T., Dam, E., Rozenbaum, W., Nicolas, J.C., 2005. A new insertion in the HIV-1 reverse transcriptase gene inducing major resistance to non-nucleoside reverse transcriptase inhibitors. AIDS 19, 1922–1924.

Andries, K., Azijn, H., Thielemans, T., Ludovici, D., Kukla, M., Heeres, J., Janssen, P., De Corte, B., Vingerhoets, J., Pauwels, R., de Béthune, M.-P., 2004. TMC125, a novel next-generation nonnucleoside reverse transcriptase inhibitor active against nonnucleoside reverse transcriptase inhibitor-resistant human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 48, 4680–4686.

Archer, R.H., Dykes, C., Gerondelis, P., Lloyd, A., Fay, P., Reichman, R.C., Bambara, R.A., Demeter, L.M., 2000. Mutants of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase resistant to nonnucleoside reverse transcriptase inhibitors demonstrate altered rates of RNase H cleavage that correlate with HIV-1 replication fitness in cell culture. J. Virol. 74, 8390–8401.

Azijn, H., Tirry, I., Vingerhoets, J., de Béthune, M.-P., Kraus, G., Boven, K., Jochmans, D., Van Craenenbroeck, E., Picchio, G., Rimsky, L.T., 2010. TMC278, a next generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. Antimicrob. Agents Chemother. 54, 718–727.

Bacheler, L., Jeffrey, S., Hanna, G., D'Aquila, R., Wallace, L., Logue, K., Cordova, B., Hertogs, K., Larder, B., Buckery, R., Baker, D., Gallagher, K., Scarnati, H., Tritch, R., Rizzo, C., 2001. Genotypic correlates of phenotypic resistance to efavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy. J. Virol. 75, 4999–5008.

Balzarini, J., Karlsson, A., Pérez-Pérez, M.J., Camarasa, M.J., Tarpley, W.G., De Clercq, E., 1993. Treatment of human immunodeficiency virus type 1 (HIV-1)-infected cells with combinations of HIV-1-specific inhibitors results in a different resistance pattern than does treatment with single-drug therapy. J. Virol. 67, 5353–5359.

Balzarini, J., Karlsson, A., Meichsner, C., Paessens, A., Riess, G., De Clercq, E., Kleim, J.P., 1994a. Resistance pattern of human immunodeficiency virus type 1 reverse transcriptase to quinoxaline S-2720. J. Virol. 68, 7986–7992.

Balzarini, J., Karlsson, A., Sardana, V.V., Emini, E.A., Camarasa, M.J., De Clercq, E., 1994b. Human immunodeficiency virus 1 (HIV-1)-specific reverse transcriptase (RT) inhibitors may suppress the replication of specific drug-resistant (E138K)RT HIV-1 mutants or select for highly resistant (Y181C→C181I)RT HIV-1 mutants. Proc. Natl Acad. Sci. USA 91, 6599–6603.

Balzarini, J., Pelemans, H., Aquaro, S., Perno, C.F., Witvrouw, M., Schols, D., De Clercq, E., Karlsson, A., 1996a. Highly favorable antiviral activity and resistance profile of the novel thiocarboxanilide pentenyloxy ether derivatives UC-781 and UC-82 as inhibitors of human immunodeficiency virus type 1 replication. Mol. Pharmacol. 50, 394–401.

Balzarini, J., Pelemans, H., Pérez-Pérez, M.J., San-Félix, A., Camarasa, M.J., De Clercq, E., Karlsson, A., 1996b. Marked inhibitory activity of non-nucleoside reverse transcriptase inhibitors against human immunodeficiency virus type 1 when combined with (−)2′,3′-dideoxy-3′-thiacytidine. Mol. Pharmacol. 49, 882–890.

Balzarini, J., De Clercq, E., Carbonez, A., Burt, V., Kleim, J.P., 2000. Long-term exposure of HIV type 1-infected cell cultures to combinations of the novel quinoxaline GW420867X with lamivudine, abacavir, and a variety of nonnucleoside reverse transcriptase inhibitors. AIDS Res. Hum. Retroviruses 16, 517–528.

Barreca, M.L., Rao, A., De Luca, L., Iraci, N., Monforte, A.M., Maga, G., De Clercq, E., Pannecouque, C., Balzarini, J., Chimirri, A., 2007. Discovery of novel benzimidazolones as potent non-nucleoside reverse transcriptase inhibitors active against wild-type and mutant HIV-1 strains. Bioorg. Med. Chem. Lett. 17, 1956–1960.

Biondi, M.J., Beilhartz, G.L., McCormick, S., Götte, M., 2010. N348I in HIV-1 reverse transcriptase can counteract the nevirapine-mediated bias toward RNase H cleavage during plus-strand initiation. J. Biol. Chem. 285, 26966–26975.

Braz, V.A., Holladay, L.A., Barkley, M.D., 2010. Efavirenz binding to HIV-1 reverse transcriptase monomers and dimers. Biochemistry 49, 601–610.

Brehm, J.H., Mellors, J.W., Sluis-Cremer, N., 2008. Mechanism by which a glutamine to leucine substitution at residue 509 in the ribonuclease H domain of HIV-1 reverse transcriptase confers zidovudine resistance. Biochemistry 47, 14020–14027.

Brenner, B., Turner, D., Oliveira, M., Moisi, D., Detorio, M., Carobene, M., Marlink, R.G., Schapiro, J., Roger, M., Wainberg, M.A., 2003. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to non-nucleoside reverse transcriptase inhibitors. AIDS 17, F1–F5.

Buckheit Jr., R.W., Fliakas-Boltz, V., Yeagy-Bargo, S., Weislow, O., Mayers, D.L., Boyer, P.L., Hughes, S.H., Pan, B.C., Chu, S.H., Bader, J.P., 1995a. Resistance to 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine derivatives is generated by mutations at multiple sites in the HIV-1 reverse transcriptase. Virology 210, 186–193.

Buckheit Jr., R.W., Kinjerski, T.L., Fliakas-Boltz, V., Russell, J.D., Stup, T.L., Pallansch, L.A., Brouwer, W.G., Dao, D.C., Harrison, W.A., Schultz, R.J., Bader, J.P., Yang, S.S., 1995b. Structure-activity and cross-resistance evaluations of a series of human immunodeficiency virus type-1-specific compounds related to oxathiin carboxanilide. Antimicrob. Agents Chemother. 39, 2718–2727.

Cane, P.A., Green, H., Fearnhill, E., Dunn, D., 2007. Identification of accessory mutations associated with high-level resistance in HIV-1 reverse transcriptase. AIDS 21, 447–455.

Chamberlain, P.P., Ren, J., Nichols, C.E., Douglas, L., Lennerstrand, J., Larder, B.A., Stuart, D.I., Stammers, D.K., 2002. Crystal structures of zidovudine- or lamivudine-resistant human immunodeficiency virus type 1 reverse transcriptases containing mutations at codons 41, 184, and 215. J. Virol. 76, 10015–10019.

Chan, J.H., Hong, J.S., Hunter 3rd, R.N., Orr, G.F., Cowan, J.R., Sherman, D.B., Sparks, S.M., Reitter, B.E., Andrews 3rd, C.W., Hazen, R.J., St Clair, M., Boone, L.R., Ferris, R.G., Creech, K.L., Roberts, G.B., Short, S.A., Weaver, K., Ott, R.J., Ren, J., Hopkins, A., Stuart, D.I., Stammers, D.K., 2001. 2-Amino-6-arylsulfonylbenzonitriles as non-nucleoside reverse transcriptase inhibitors of HIV-1. J. Med. Chem. 44, 1866–1882.

Chiang, C.C., Wang, S.M., Tseng, Y.T., Huang, K.J., Wang, C.T., 2009. Mutations at human immunodeficiency virus type 1 reverse transcriptase tryptophan repeat motif attenuate the inhibitory effect of efavirenz on virus production. Virology 383, 261–270.

Cohen, C.J., Andrade-Villanueva, J., Clotet, B., Fourie, J., Johnson, M.A., Ruxrungtham, K., Wu, H., Zorrilla, C., Crauwels, H., Rimsky, L.T., Vanveggel, S., Boven, K., on behalf of the THRIVE study group, 2011. Rilpivirine versus efavirenz with two background nucleoside or nucleotide reverse transcriptase inhibitors in treatment-naive adults infected with HIV-1 (THRIVE): a phase 3, randomised, non-inferiority trial. Lancet 378, 229–237.

Das, K., Clark Jr., A.D., Lewi, P.J., Heeres, J., De Jonge, M.R., Koymans, L.M., Vinkers, H.M., Daeyaert, F., Ludovici, D.W., Kukla, M.J., De Corte, B., Kavash, R.W., Ho, C.Y., Ye, H., Lichtenstein, M.A., Andries, K., Pauwels, R., De Béthune, M.P., Boyer, P.L., Clark, P., Hughes, S.H., Janssen, P.A., Arnold, E., 2004. Roles of conformational and positional adaptability in structure-based design of TMC125–R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. J. Med. Chem. 47, 2550–2560.

Das, K., Bauman, J.D., Clark Jr., A.D., Frenkel, Y.V., Lewi, P.J., Shatkin, A.J., Hughes, S.H., Arnold, E., 2008. High-resolution structures of HIV-1 reverse transcriptase/TMC278 complexes: Strategic flexibility explains potency against resistant mutations. Proc. Natl. Acad. Sci. USA 105, 1466–1471.

Delviks-Frankenberry, K.A., Nikolenko, G.N., Boyer, P.L., Hughes, S.H., Coffin, J.M., Jere, A., Pathak, V.K., 2008. HIV-1 reverse transcriptase connection subdomain mutations reduce template RNA degradation and enhance AZT excision. Proc. Natl. Acad. Sci. USA 105, 10943–10948.

Delviks-Frankenberry, K.A., Nikolenko, G.N., Pathak, V.K., 2010. The "connection" between HIV drug resistance and RNase H. Viruses 2, 1476–1503.

Demeter, L.M., Meehan, P.M., Morse, G., Gerondelis, P., Dexter, A., Berrios, L., Cox, S., Freimuth, W., Reichman, R.C., 1997. HIV-1 drug susceptibilities and reverse transcriptase mutations in patients receiving combination therapy with didanosine and delavirdine. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 14, 136–144.

Dueweke, T.J., Pushkarskaya, T., Poppe, S.M., Swaney, S.M., Zhao, J.Q., Chen, I.S., Stevenson, M., Tarpley, W.G., 1993. A mutation in reverse transcriptase of bis(heteroaryl)piperazine-resistant human immunodeficiency virus type 1 that confers increased sensitivity to other nonnucleoside inhibitors. Proc. Natl. Acad. Sci. USA 90, 4713–4717.

Ehteshami, M., Beilhartz, G.L., Scarth, B.J., Tchesnokov, E.P., McCormick, S., Wynhoven, B., Harrigan, P.R., Götte, M., 2008. Connection domain mutations N348I and A360V in HIV-1 reverse transcriptase enhance resistance to 3′-azido-3′-deoxythymidine through both RNase H-dependent and -independent mechanisms. J. Biol. Chem. 283, 22222–22232.

El-Brollosy, N.R., Jørgensen, P.T., Dahan, B., Boel, A.M., Pedersen, E.B., Nielsen, C., 2002. Synthesis of novel N-1 (allyloxymethyl) analogues of 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442, emivirine) with improved activity against HIV-1 and its mutants. J. Med. Chem. 45, 5721–5726.

Eshleman, S.H., Jones, D., Galovich, J., Paxinos, E.E., Petropoulos, C.J., Jackson, J.B., Parkin, N., 2006. Phenotypic drug resistance patterns in subtype A HIV-1 clones with nonnucleoside reverse transcriptase resistance mutations. AIDS Res. Hum. Retroviruses 22, 289–293.

Esnouf, R.M., Ren, J., Ross, C., Jones, Y., Stammers, D., Stuart, D., 1995. Mechanism of inhibition of HIV-1 reverse transcriptase by non-nucleoside inhibitors. Nat. Struct. Biol. 2, 303–308.

Esnouf, R.M., Ren, J., Hopkins, A.L., Ross, C.K., Jones, E.Y., Stammers, D.K., Stuart, D.I., 1997. Unique features in the structure of the complex between HIV-1 reverse transcriptase and the bis(heteroaryl)piperazine (BHAP) U-90152 explain resistance mutations for this non-nucleoside inhibitor. Proc. Natl. Acad. Sci. USA 94, 3984–3989.

Figueiredo, A., Moore, K.L., Mak, J., Sluis-Cremer, N., de Béthune, M.P., Tachedjian, G., 2006. Potent non-nucleoside reverse transcriptase inhibitors target HIV-1 Gag-Pol. PLoS Pathog. 2, e119.

Fujihashi, T., Hara, H., Sakata, T., Mori, K., Higuchi, H., Tanaka, A., Kaji, H., Kaji, A., 1995. Anti-human immunodeficiency virus (HIV) activities of halogenated gomisin J derivatives, new nonnucleoside inhibitors of HIV type 1 reverse transcriptase. Antimicrob. Agents Chemother. 39, 2000–2007.

Fujiwara, T., Sato, A., el-Farrash, M., Miki, S., Abe, K., Isaka, Y., Kodama, M., Wu, Y., Chen, L.B., Harada, H., Sugimoto, H., Hatanaka, M., Hinuma, Y., 1998. S-1153 inhibits replication of known drug-resistant strains of human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 42, 1340–1345.

Gerondelis, P., Archer, R.H., Palaniappan, C., Reichman, R.C., Fay, P.J., Bambara, R.A., Demeter, L.M., 1999. The P236L delavirdine-resistant human immunodeficiency virus type 1 mutant is replication defective and demonstrates alterations in both RNA 5′-end- and DNA 3′-end-directed RNase H activities. J. Virol. 73, 5803–5813.

Götte, M., Kameoka, M., McLellan, N., Cellai, L., Wainberg, M.A., 2000. Analysis of efficiency and fidelity of HIV-1 (+)-strand DNA synthesis reveals a novel rate-limiting step during retroviral reverse transcription. J. Biol. Chem. 276, 6711–6719.

Grobler, J.A., Dornadula, G., Rice, M.R., Simcoe, A.L., Hazuda, D.J., Miller, M.D., 2007. HIV-1 reverse transcriptase plus-strand initiation exhibits preferential sensitivity to non-nucleoside reverse transcriptase inhibitors in vitro. J. Biol. Chem. 282, 8005–8010.

Gupta, S., Fransen, S., Paxinos, E.E., Stawiski, E., Huang, W., Petropoulos, C.J., 2010. Combinations of mutations in the connection domain of human immunodeficiency virus type 1 reverse transcriptase: Assessing the impact on nucleoside and nonnucleoside reverse transcriptase inhibitor resistance. Antimicrob. Agents Chemother. 54, 1973–1980.

Gupta, S., Vingerhoets, J., Fransen, S., Tambuyzer, L., Azijn, H., Frantzell, A., Paredes, R., Coakley, E., Nijs, S., Clotet, B., Petropoulos, C.J., Schapiro, J., Huang, W., Picchio, G., 2011. Connection subdomain mutations in HIV-1 reverse transcriptase do not impact etravirine susceptibility and virologic responses to etravirine-containing regimens. Antimicrob. Agents Chemother. 55, 2872–2879.

Hachiya, A., Kodama, E.N., Sarafianos, S.G., Schuckmann, M.M., Sakagami, Y., Matsuoka, M., Takiguchi, M., Gatanaga, H., Oka, S., 2008. Amino acid mutation N348I in the connection subdomain of human immunodeficiency virus type 1 reverse transcriptase confers multiclass resistance to nucleoside and nonnucleoside reverse transcriptase inhibitors. J. Virol. 82, 3261–3270.

Hachiya, A., Shimane, K., Sarafianos, S.G., Kodama, E.N., Sakagami, Y., Negishi, F., Koizumi, H., Gatanaga, H., Matsuoka, M., Takiguchi, M., Oka, S., 2009. Clinical relevance of substitutions in the connection subdomain and RNase H domain of HIV-1 reverse transcriptase from a cohort of antiretroviral treatment-naïve patients. Antiviral Res. 82, 115–121.

Hang, J.Q., Li, Y., Yang, Y., Cammack, N., Mirzadegan, T., Klumpp, K., 2007. Substrate-dependent inhibition or stimulation of HIV RNase H activity by non-nucleoside reverse transcriptase inhibitors. Biochem. Biophys. Res. Commun. 12, 341–350.

Harrigan, P.R., Salim, M., Stammers, D.K., Wynhoven, B., Brumme, Z.L., McKenna, P., Larder, B., Kemp, S.D., 2002. A mutation in the 3′ region of the human immunodeficiency virus type 1 reverse transcriptase (Y318F) associated with nonnucleoside reverse transcriptase inhibitor resistance. J. Virol. 76, 6836–6840.

Harrigan, P.R., Mo, T., Wynhoven, B., Hirsch, J., Brumme, Z., McKenna, P., Pattery, T., Vingerhoets, J., Bacheler, L.T., 2005. Rare mutations at codon 103 of HIV-1 reverse transcriptase can confer resistance to non-nucleoside reverse transcriptase inhibitors. AIDS 19, 549–554.

Hsiou, Y., Ding, J., Das, K., Clark Jr., A.D., Hughes, S.H., Arnold, E., 1996. Structure of unliganded HIV-1 reverse transcriptase at 2.7 Å resolution: implications of conformational changes for polymerization and inhibition mechanisms. Structure 4, 853–860.

Huang, W., Gamarnik, A., Limoli, K., Petropoulos, C.J., Whitcomb, J.M., 2003. Amino acid substitutions at position 190 of human immunodeficiency virus type 1 reverse transcriptase increase susceptibility to delavirdine and impair virus replication. J. Virol. 77, 1512–1523.

Isaka, Y., Miki, S., Kawauchi, S., Suyama, A., Sugimoto, H., Adachi, A., Hayami, M., Yoshie, O., Fujiwara, T., Sato, A., 2000. Isolation and characterization of simian immunodeficiency virus variants that are resistant to nonnucleoside reverse transcriptase inhibitors. Arch. Virol. 145, 2481–2492.

Isaka, Y., Miki, S., Kawauchi, S., Suyama, A., Sugimoto, H., Adachi, A., Miura, T., Hayami, M., Yoshie, O., Fujiwara, T., Sato, A., 2001. A single amino acid change at Leu-188 in the reverse transcriptase of HIV-2 and SIV renders them sensitive to non-nucleoside reverse transcriptase inhibitors. Arch. Virol. 146, 743–755.

Kagan, R.M., Sista, P., Pattery, T., Bacheler, L., Schwab, D.A., 2009. Additional HIV-1 mutation patterns associated with reduced phenotypic susceptibility to etravirine in clinical samples. AIDS 23, 1602–1605.

Kertesz, D.J., Brotherton-Pleiss, C., Yang, M., Wang, Z., Lin, X., Qiu, Z., Hirschfeld, D.R., Gleason, S., Mirzadegan, T., Dunten, P.W., Harris, S.F., Villasenor, A.G., Hang, J.Q., Heilek, G.M., Klumpp, K., 2010. Discovery of piperidin-4-yl-aminopyrimidines as HIV-1 reverse transcriptase inhibitors. N-Benzyl derivatives with broad potency against resistant mutant viruses. Bioorg. Med. Chem. Lett. 20, 4215–4218.

Kleim, J.P., Winkler, I., Rösner, M., Kirsch, R., Rübsamen-Waigmann, H., Paessens, A., Riess, G., 1997. In vitro selection for different mutational patterns in the HIV-1 reverse transcriptase using high and low selective pressure of the nonnucleoside reverse transcriptase inhibitor HBY 097. Virology 231, 112–118.

Kohlstaedt, L.A., Wang, J., Friedman, J.M., Rice, P.A., Steitz, T.A., 1992. Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. Science 256, 1783–1790.

Lai, M.T., Lu, M., Felock, P.J., Hrin, R.C., Wang, Y.J., Yan, Y., Munshi, S., McGaughey, G.B., Tynebor, R.M., Tucker, T.J., Williams, T.M., Grobler, J.A., Hazuda, D.J., McKenna, P.M., Miller, M.D., 2010. Distinct mutation pathways of non-subtype B HIV-1 during in vitro resistance selection with nonnucleoside reverse transcriptase inhibitors. Antimicrob. Agents Chemother. 54, 4812–4824.

Lansdon, E.B., Brendza, K.M., Hung, M., Wang, R., Mukund, S., Jin, D., Birkus, G., Kutty, N., Liu, X., 2010. Crystal structures of HIV-1 reverse transcriptase with etravirine (TMC125) and rilpivirine (TMC278): Implications for drug design. J. Med. Chem. 53, 4295–4299.

Lansdon, E.B., Liu, Q., Leavitt, S.A., Balakrishnan, M., Perry, J.K., Lancaster-Moyer, C., Kutty, N., Liu, X., Squires, N.H., Watkins, W.J., Kirschberg, T.A., 2011. Structural and binding analysis of pyrimidinol carboxylic acid and N-hydroxy quinazolinedione HIV-1 RNase H inhibitors. Antimicrob. Agents Chemother. 55, 2905–2915.

Leigh Brown, A.J., Precious, H.M., Whitcomb, J.M., Wong, J.K., Quigg, M., Huang, W., Daar, E.S., D'Aquila, R.T., Keiser, P.H., Connick, E., Hellmann, N.S., Petropoulos, C.J., Richman, D.D., Little, S.J., 2000. Reduced susceptibility of human immunodeficiency virus type 1 (HIV-1) from patients with primary HIV infection to nonnucleoside reverse transcriptase inhibitors is associated with variation at novel amino acid sites. J. Virol. 74, 10269–10273.

Lengruber, R.B., Delviks-Frankenberry, K.A., Nikolenko, G.N., Baumann, J., Santos, A.F., Pathak, V.K., Soares, M.A., 2011. Phenotypic characterization of drug resistance-associated mutations in HIV-1 RT connection and RNase H domains and their correlation with thymidine analogue mutations. J. Antimicrob. Chemother. 66, 702–708.

Lindberg, J., Sigurdsson, S., Lowgren, S., Andersson, H.O., Sahlberg, C., Noreen, R., Fridborg, K., Zhang, H., Unge, T., 2002. Structural basis for the inhibitory efficacy of efavirenz (DMP-266), MSC194 and PNU142721 towards the HIV-1 RT K103N mutant. Eur. J. Biochem. 269, 1670–1677.

Liu, S., Abbondanzieri, E.A., Rausch, J.W., Le Grice, S.F.J., Zhuang, X., 2008. Slide into action: dynamic shuttling of HIV reverse transcriptase on nucleic acid substrates. Science 322, 1092–1097.

Maga, G., Amacker, M., Ruel, N., Hübscher, U., Spadari, S., 1997. Resistance to nevirapine of HIV-1 reverse transcriptase mutants: loss of stabilizing interactions and thermodynamic or steric barriers are induced by different single amino acid substitutions. J. Mol. Biol. 274, 738–747.

McCormick, A.L., Parry, C.M., Crombe, A., Goodall, R.L., Gupta, R.K., Kaleebu, P., Kityo, C., Chirara, M., Towers, G.J., Pillay, D., 2011. Impact of the N348I mutation in HIV-1 reverse transcriptase on nonnucleoside reverse transcriptase inhibitor resistance in non-subtype B HIV-1. Antimicrob. Agents Chemother. 55, 1806–1809.

Menéndez-Arias, L., 2008. Mechanisms of resistance to nucleoside analogue inhibitors of HIV-1 reverse transcriptase. Virus Res. 134, 124–146.

Menéndez-Arias, L., 2010. Molecular basis of human immunodeficiency virus drug resistance: an update. Antiviral Res. 85, 210–231.

Menéndez-Arias, L., 2011. Chapter 4: sensitivity to reverse transcriptase and protease inhibitors of recombinant HIV clones harboring resistance mutations: *in vitro* studies. In: Clotet, B., Menéndez-Arias, L., Schapiro, J.M., Kuritzkes, D., Burger, D., Rockstroh, J., Soriano, V., Telenti, A., Brun-Vezinet, F., Geretti, A.M., Boucher, C.A., Richman, D.D. (Eds.), The HIV & Hepatitis Drug Resistance and PK Guide, 11th ed., Fundació de Lluita contra la SIDA, Barcelona, Spain, pp. 171–333. Available from: <http://www.flsida.org/theguide/>.

Menéndez-Arias, L., Abraha, A., Quiñones-Mateu, M.E., Mas, A., Camarasa, M.-J., Arts, E.J., 2001. Functional characterization of chimeric reverse transcriptases with polypeptide subunits of highly divergent HIV-1 group M and O strains. J. Biol. Chem. 276, 27470–27479.

Molina, J.-M., Cahn, P., Grinsztejn, B., Lazzarin, A., Mills, A., Saag, M., Supparatpinyo, K., Walmsley, S., Crauwels, H., Rimsky, L.T., Vanveggel, S., Boven, K., on behalf of the ECHO study group, 2011. Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naive adults infected with HIV-1 (ECHO): a phase 3 randomised double-blind active-controlled trial. Lancet 378, 238–246.

Nikolenko, G.N., Palmer, S., Maldarelli, F., Mellors, J.W., Coffin, J.M., Pathak, V.K., 2005. Mechanism for nucleoside analog-mediated abrogation of HIV-1 replication: balance between RNase H activity and nucleotide excision. Proc. Natl. Acad. Sci. USA 102, 2093–2098.

Nikolenko, G.N., Delviks-Frankenberry, K.A., Pathak, V.K., 2010. A novel molecular mechanism of dual resistance to nucleoside and nonnucleoside reverse transcriptase inhibitors. J. Virol. 84, 5238–5249.

Olivares, I., Mulky, A., Boross, P.I., Tözsér, J., Kappes, J.C., López-Galíndez, C., Menéndez-Arias, L., 2007. HIV-1 protease dimer interface mutations that compensate for viral reverse transcriptase instability in infectious virions. J. Mol. Biol. 372, 369–381.

Olmsted, R.A., Slade, D.E., Kopta, L.A., Poppe, S.M., Poel, T.J., Newport, S.W., Rank, K.B., Biles, C., Morge, R.A., Dueweke, T.J., Yagi, Y., Romero, D.L., Thomas, R.C., Sharma, S.K., Tarpley, W.G., 1996. (Alkylamino) piperidine bis(heteroaryl)piperizine analogs are potent, broad-spectrum nonnucleoside reverse transcriptase inhibitors of drug-resistant isolates of human immunodeficiency virus type 1 (HIV-1) and select for drug-resistant variants of HIV-1IIIB with reduced replication phenotypes. J. Virol. 70, 3698–3705.

Palaniappan, C., Kim, J.K., Wisniewski, M., Fay, P.J., Bambara, R.A., 1998. Control of initiation of viral plus strand DNA synthesis by HIV reverse transcriptase. J. Biol. Chem. 273, 3808–3816.

Paredes, R., Puertas, M.C., Bannister, W., Kisic, M., Cozzi-Lepri, A., Pou, C., Bellido, R., Betancor, G., Bogner, J., Gargalianos, P., Bánhegyi, D., Clotet, B., Lundgren, J., Menéndez-Arias, L., Martinez-Picado, J., and the EuroSIDA Study Group, 2011. A376S in the connection subdomain of HIV-1 reverse transcriptase confers increased risk of virological failure to nevirapine therapy. J. Infect. Dis. 204, 741–752.

Parkin, N.T., Gupta, S., Chappey, C., Petropoulos, C.J., 2006. The K101P and K103R/V179D mutations in human immunodeficiency virus type 1 reverse transcriptase confer resistance to nonnucleoside reverse transcriptase inhibitors. Antimicrob. Agents Chemother. 50, 351–354.

Pelemans, H., Esnouf, R., Dunkler, A., Parniak, M.A., Vandamme, A.M., Karlsson, A., De Clercq, E., Kleim, J.P., Balzarini, J., 1997. Characteristics of the Pro225His mutation in human immunodeficiency virus type 1 (HIV-1) reverse transcriptase that appears under selective pressure of dose-escalating quinoxaline treatment of HIV-1. J. Virol. 71, 8195–8203.

Pelemans, H., Esnouf, R.M., Jonckheere, H., De Clercq, E., Balzarini, J., 1998. Mutational analysis of Tyr-318 within the non-nucleoside reverse transcriptase inhibitor binding pocket of human immunodeficiency virus type 1 reverse transcriptase. J. Biol. Chem. 273, 34234–34239.

Petropoulos, C.J., Parkin, N.T., Limoli, K.L., Lie, Y.S., Wrin, T., Huang, W., Tian, H., Smith, D., Winslow, G.A., Capon, D.J., Whitcomb, J.M., 2000. A novel phenotypic drug susceptibility assay for human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 44, 920–928.

Poveda, E., de Mendoza, C., Pattery, T., González, M.M., Villacian, J., Soriano, V., 2008. Phenotypic impact of resistance mutations on etravirine susceptibility in HIV patients with prior failure to nonnucleoside analogues. AIDS 22, 2395–2398.

Radzio, J., Sluis-Cremer, N., 2008. Efavirenz accelerates HIV-1 reverse transcriptase ribonuclease H cleavage, leading to diminished zidovudine excision. Mol. Pharmacol. 73, 601–606.

Rao, A., Balzarini, J., Carbone, A., Chimirri, A., De Clercq, E., Monforte, A.M., Monforte, P., Pannecouque, C., Zappalà, M., 2004. 2-(2,6-Dihalophenyl)-3-(pyrimidin-2-yl)-1,3-thiazolidin-4-ones as non-nucleoside HIV-1 reverse transcriptase inhibitors. Antiviral Res. 63, 79–84.

Ren, J., Stammers, D.K., 2008. Structural basis for drug resistance mechanisms for non-nucleoside inhibitors of HIV reverse transcriptase. Virus Res. 134, 157–170.

Ren, J., Esnouf, R., Garman, E., Somers, D., Ross, C., Kirby, I., Keeling, J., Darby, G., Jones, Y., Stuart, D., Stammers, D., 1995. High resolution structures of HIV-1 RT from four RT-inhibitor complexes. Nat. Struct. Biol. 2, 293–302.

Ren, J., Milton, J., Weaver, K.L., Short, S.A., Stuart, D.I., Stammers, D.K., 2000. Structural basis for the resilience of efavirenz (DMP-266) to drug resistance mutations in HIV-1 reverse transcriptase. Structure 8, 1089–1094.

Ren, J., Nichols, C., Bird, L., Chamberlain, P., Weaver, K., Short, S., Stuart, D.I., Stammers, D.K., 2001. Structural mechanisms of drug resistance for mutations at codons 181 and 188 in HIV-1 reverse transcriptase and the improved resilience of second generation non-nucleoside inhibitors. J. Mol. Biol. 312, 795–805.

Ren, J., Nichols, C.E., Chamberlain, P.P., Weaver, K.L., Short, S.A., Stammers, D.K., 2004. Crystal structures of HIV-1 reverse transcriptases mutated at codons 100, 106 and 108 and mechanisms of resistance to non-nucleoside inhibitors. J. Mol. Biol. 336, 569–578.

Ren, J., Nichols, C.E., Stamp, A., Chamberlain, P.P., Ferris, R., Weaver, K.L., Short, S.A., Stammers, D.K., 2006. Structural insights into mechanisms of non-nucleoside drug resistance for HIV-1 reverse transcriptases mutated at codons 101 or 138. FEBS J. 273, 3850–3860.

Ren, J., Nichols, C.E., Chamberlain, P.P., Weaver, K.L., Short, S.A., Chan, J.H., Kleim, J.P., Stammers, D.K., 2007. Relationship of potency and resilience to drug resistance mutations for GW420867X revealed by crystal structures of inhibitor complexes for wild-type, Leu100Ile, Lys101Glu, and Tyr188Cys mutant HIV-1 reverse transcriptases. J. Med. Chem. 50, 2301–2309.

Richman, D., Shih, C.K., Lowy, I., Rose, J., Prodanovich, P., Goff, S., Griffin, J., 1991. Human immunodeficiency virus type 1 mutants resistant to nonnucleoside inhibitors of reverse transcriptase arise in tissue culture. Proc. Natl. Acad. Sci. USA 88, 11241–11245.

Richman, D.D., Havlir, D., Corbeil, J., Looney, D., Ignacio, C., Spector, S.A., Sullivan, J., Cheeseman, S., Barringer, K., Pauletti, D., Shi, C.-K., Myers, M., Griffin, J., 1994. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J. Virol. 68, 1660–1666.

Rodgers, D.W., Gamblin, S.J., Harris, B.A., Ray, S., Culp, J.S., Hellmig, B., Woolf, D.J., Debouck, C., Harrison, S.C., 1995. The structure of unliganded reverse

transcriptase from the human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA 92, 1222–1226.
Sato, A., Hammond, J., Alexander, T.N., Graham, J.P., Binford, S., Sugita, K., Sugimoto, H., Fujiwara, T., Patick, A.K., 2006. In vitro selection of mutations in human immunodeficiency virus type 1 reverse transcriptase that confer resistance to capravirine, a novel nonnucleoside reverse transcriptase inhibitor. Antiviral Res. 70, 66–74.
Schuckmann, M.M., Marchand, B., Hachiya, A., Kodama, E.N., Kirby, K.A., Singh, K., Sarafianos, S.G., 2010. The N348I mutation at the connection subdomain of HIV-1 reverse transcriptase decreases binding to nevirapine. J. Biol. Chem. 285, 38700–38709.
Sluis-Cremer, N., Tachedjian, G., 2008. Mechanisms of inhibition of HIV replication by non-nucleoside reverse transcriptase inhibitors. Virus Res. 134, 147–156.
Sluis-Cremer, N., Moore, K., Radzio, J., Sonza, S., Tachedjian, G., 2010. N348I in HIV-1 reverse transcriptase decreases susceptibility to tenofovir and etravirine in combinations with other resistance mutations. AIDS 24, 317–319.
Smerdon, S.J., Jager, J., Wang, J., Kohlstaedt, L.A., Chirino, A.J., Friedman, J.M., Rice, P.A., Steitz, T.A., 1994. Structure of the binding site for nonnucleoside inhibitors of the reverse transcriptase of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA 91, 3911–3915.
Spence, R.A., Kati, W.M., Anderson, K.S., Johnson, K.A., 1995. Mechanism of inhibition of HIV-1 reverse transcriptase by nonnucleoside inhibitors. Science 267, 988–993.
Spence, R.A., Anderson, K.S., Johnson, K.A., 1996. HIV-1 reverse transcriptase resistance to nonnucleoside inhibitors. Biochemistry 35, 1054–1063.
Su, H.P., Yan, Y., Prasad, G.S., Smith, R.F., Daniels, C.L., Abeywickrema, P.D., Reid, J.C., Loughran, H.M., Kornienko, M., Sharma, S., Grobler, J.A., Xu, B., Sardana, V., Allison, T.J., Williams, P.D., Darke, P.L., Hazuda, D.J., Munshi, S., 2010. Structural basis for the inhibition of RNase H activity of HIV-1 reverse transcriptase by RNase H active site-directed inhibitors. J. Virol. 84, 7625–7633.
Tachedjian, G., Orlova, M., Sarafianos, S.G., Arnold, E., Goff, S.P., 2001. Nonnucleoside reverse transcriptase inhibitors are chemical enhancers of dimerization of the HIV type 1 reverse transcriptase. Proc. Natl. Acad. Sci. USA 98, 7188–7193.
Tambuyzer, L., Azijn, H., Rimsky, L.T., Vingerhoets, J., Lecocq, P., Kraus, G., Picchio, G., de Béthune, M.-P., 2009. Compilation and prevalence of mutations associated with resistance to non-nucleoside reverse transcriptase inhibitors. Antivir. Ther. 14, 103–109.
Venezia, C.F., Howard, K.J., Ignatov, M.E., Holladay, L.A., Barkley, M.D., 2006. Effects of efavirenz binding on the subunit equilibria of HIV-1 reverse transcriptase. Biochemistry 45, 2779–2789.
Vingerhoets, J., Azijn, H., Fransen, E., De Baere, I., Smeulders, L., Jochmans, D., Andries, K., Pauwels, R., de Béthune, M.-P., 2005. TMC125 displays a high genetic barrier to the development of resistance: evidence from in vitro selection experiments. J. Virol. 79, 12773–12782.
Xia, Q., Radzio, J., Anderson, K.S., Sluis-Cremer, N., 2007. Probing nonnucleoside inhibitor-induced active-site distortion in HIV-1 reverse transcriptase by transient kinetic analyses. Protein Sci. 16, 1728–1737.
Yap, S.-H., Sheen, C.-W., Fahey, J., Zanin, M., Tyssen, D., Lima, V.D., Wynhoven, B., Kuiper, M., Sluis-Cremer, N., Harrigan, P.R., Tachedjian, G., 2007. N348I in the connection domain of HIV-1 reverse transcriptase confers zidovudine and nevirapine resistance. PLoS Med. 4, 1887–1900.
Young, S.D., Britcher, S.F., Tran, L.O., Payne, L.S., Lumma, W.C., Lyle, T.A., Huff, J.R., Anderson, P.S., Olsen, D.B., Carroll, S.S., Pettibone, D.J., O'Brien, J.A., Ball, R.G., Balani, S.K., Lin, J.H., Chen, I.-W., Schleif, W.A., Sardana, V.V., Long, W.J., Byrnes, V.W., Emini, E.A., 1995. L-743,726 (DMP-266): a novel, highly potent nonnucleoside inhibitor of the human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 39, 2602–2605.
Zhang, Z., Xu, W., Koh, Y.-H., Shim, J.H., Girardet, J.-L., Yeh, L.-T., Hamatake, R.K., Hong, Z., 2007. A novel nonnucleoside analogue that inhibits human immunodeficiency virus type 1 isolates resistant to current nonnucleoside reverse transcriptase inhibitors. Antimicrob. Agents Chemother. 51, 429–437.