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NNRTI-selected mutations at codon 190 of human immunodeficiency virus type 1 reverse transcriptase decrease susceptibility to stavudine and zidovudine

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Abstract

The non-nucleoside reverse transcriptase (RT) inhibitor (NNRTI)-binding pocket of HIV-1 RT spans codons 100–110, 180–190 and 220–240 and mutations in these domains are responsible for HIV-1 NNRTI resistance. Recombinant HIV-1 strains carrying G190S/A/E, G190S + T215Y, T215Y and K103N mutations were constructed to evaluate susceptibility to both NNRTIs and nucleoside RT inhibitors (NRTIs). In addition, purified recombinant RT enzymes were obtained to determine the degree of *in vitro* inhibition by drugs of both classes. High-level resistance to nevirapine and moderate level resistance to both stavudine and zidovudine were associated with G190S/A/E substitutions. The simultaneous presence of G190S and T215Y decreased stavudine and zidovudine susceptibility more than T215Y alone. On the other hand, G190S was associated with a marked decrease in RT catalytic efficiency, while T215Y showed a more limited effect. Interestingly, the simultaneous presence of G190S and T215Y was associated with a reduction in the impairment of the G190S-mutated enzyme. Mutations in the HIV-1 RT NNRTI binding pocket may be associated with cross-resistance to NRTI. Selection of double mutants, with further decrease in NRTI susceptibility, might be favoured by the compensatory effect of T215Y on the reduction of RT catalytic efficiency associated with G190S.

Keywords: HIV-1; RT mutations; NNRTI; NRTI; Drug resistance

1. Introduction

Highly active antiretroviral therapy (HAART) is the standard of care for treatment of HIV-1 infection in developed countries, and consists of the combined use of nucleoside (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), as well as protease and fusion inhibitors. NRTI are compounds structurally related to nucleosides or nucleotides, which act as alternative substrates, being incorporated by HIV-1 RT and leading to the termination of viral DNA synthesis. NNRTIs are potent inhibitors of HIV-1 replication, structurally unrelated to natural substrates of HIV-1 RT, binding to an allosteric site of the enzyme. Sub-optimal treatment with both NRTIs and NNRTIs results in rapid emergence of drug-resistant HIV-1 mutants.

NNRTIs inhibit HIV-1 RT by binding to a hydrophobic pocket adjacent to the active site of the enzyme (Smerdon et al., 1994). The NNRTI-binding pocket of HIV-1 RT includes amino acids (aa) 100–110, 180–190 and 220–240, and mutations in these RT regions can substantially decrease susceptibility to all drugs belonging to the NNRTI class (Huang et al., 2003). On the other hand, mutations in RT domains involved in nucleotide binding and processing are associated with resistance to NRTIs. Since the binding and processing domains for NRTIs and NNRTIs are well differentiated, interclass resistance was originally thought to be unlikely. In contrast with this assumption, it has been shown that Y181I/C substitutions selected by NNRTI treatment may be associated with NRTI stavudine cross-resistance (Baldanti et al., 2003). In addition, NNRTI may sequentially select mutations

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at position 190 of the RT gene (affecting NNRTI susceptibility) and then mutations at positions 74 and 75 (affecting NRTI susceptibility) (Kleim et al., 1996).

However, the amplitude and mechanisms of cross-resistance between NRTIs and NNRTIs are still unclear. Amino acid substitutions at codon 190 of HIV-1 RT are frequently selected during HAART regimens including NNRTI, accounting for 14.1% and 23.8% of mutants emerging during efavirenz and nevirapine monotherapy, respectively (Stanford HIV Drug Resistance Database).

The present study was aimed at investigating the impact of different aa changes at position 190 of HIV-1 RT on NNRTI and NRTI drug susceptibility in HAART-treated patients. In addition, the effects of different mutations and antiviral drugs on RT enzymatic activity *in vitro* were studied.

2. Materials and methods

2.1. Generation of HIV-1 recombinant strains and evaluation of HIV-1 drug susceptibility

Recombinant viruses were constructed by introducing the wild-type RT gene from NL4-3 reference strain and the genes containing G190S/A/E, G190S + T215Y, T215Y and K103N mutations from HAART-treated patients into the pHXB2Δ2-261RT vector (kindly provided by C. Boucher, Utrecht, The Netherlands). RT genes from field HIV strains were selected among a collection of more than 4000 strains on the basis of the following criteria: (i) presence of G190A/S/E, T215Y and K103N as single drug-resistance associated mutation or G190S + T215Y as single combination of drug resistance mutations, (ii) the lowest number of aa polymorphisms with respect to XB2 HIV reference strain; (iii) the most similar pattern of aa polymorphisms among selected sequences (Fig. 1). Upon transfection of CD4+ HeLa cells with each of the pHXB2Δ2-261RT constructs (G190S/A/E, G190S + T215Y, T215Y, K103N and wild-type RT), infectious recombinant viruses were obtained (Paolucci et al., 2004).

Susceptibility of recombinant HIV-1 strains to two representative NRTIs (zidovudine, range 0.007–8.0 μM, and stavudine, range 0.008–132.0 μM) and one NNRTI (nevirapine, range 0.005–120.0 μM) was tested as previously reported (Paolucci et al., 2004). Recombinant HIV-1 strains carrying wild-type RT and resistance-associated mutations were assayed in parallel. The degree of viral replication inhibition was measured by determining the HIV-1 p24 antigen level (NEN Research Product, Boston, MA) in the supernatant of CD4+ HeLa cell cultures, and was expressed as the fold increase in the 50% inhibitory concentration (IC_{50}) for recombinant HIV-1 variants compared with IC_{50} for the wild-type recombinant variant (Paolucci et al., 2004). Each test was performed in triplicate.

2.2. Cloning and expression of recombinant HIV-1 RT, evaluation of RT activity and RT drug inhibition assays

The HIV-1 RT gene fragment spanning codons 2–261 from pHXB2Δ2-261RT constructs carrying G190S, T215Y and G190S + T215Y mutations was amplified by PCR, digested with Acc1 and Pvu2 and cloned into the expression plasmid pUC12/Hisp66(ΔAcc1/Pvu2) containing the wild-type RT gene (Boyer et al., 1998). The resulting pUC12/Hisp66 (G190S, T215Y and G190S + T215Y) expression vectors were used for the production in *E. coli* (BL21) and purification of recombinant his-tagged RT enzymes, as previously described (Maga et al., 1997). As judged from the Coomassie staining, the proteins were >90% pure. Identity of the polypeptides present in the final preparation was confirmed by Western blotting with anti-RT monoclonal antibodies.

Steady-state kinetic measurements of RT activity were performed under the conditions described for the HIV-1 RT RNA-dependent DNA polymerase activity assay (Maga et al., 1997). The time-dependent incorporation of radioactive deoxythymidine triphosphate (dTTP) into poly(rA)/oligo(dT) at different substrate concentrations was monitored by removing 25 μl-aliquots at different time points (0, 2, 4, 6, 10 and 20 min). Initial reaction velocities, determined by linear regression analysis of the data, were then plotted against the corresponding substrate concentrations. To determine the K_m and V_{max} values, an interval of substrate concentrations from $0.2K_m$ to $10K_m$ was used.

Incorporation of radioactive dTTP into poly(rA)/oligo(dT) was monitored in the presence of increasing amounts of AZT or d4T triphosphates (AZTTP or d4TTP, 0.004, 0.01, 0.1, 0.2, 1 and 5 μM) or NNRTIs (nevirapine, 0.001, 0.01, 0.025, 0.1,

Recombinant HIV strains	Amino acid RT mutations																					
	83	103	121	122	123	132	135	139	142	166	172	177	178	185	190	200	207	211	215	248
Gly190Ala					Glu				Val	Arg	Lys	Glu			**Ala**	Ala		Lys		
Gly190Glu					Glu	Val			Val	Arg	Lys	Glu			**Glu**	Ala		Lys		
Gly190Ser	Lys				Glu			Glu		Arg					**Ser**					Asn
Thr215Tyr	Lys						Leu							Asn				Lys	**Tyr**	
Gly190S+T215Tyr	Lys														**Ser**	Ala		Lys	**Tyr**	
Lys103Asn	Lys	**Asn**	Lys	Glu									Leu				Glu	Lys		

Fig. 1. Drug resistance-associated aa mutations (boldface character) and polymorphisms (plain character) in HIV-1 RT genes from HAART-treated patients utilized for (i) generation of recombinant HIV-1 variants for drug susceptibility testing and (ii) expression of purified RT enzymes for evaluation of RT activity.

0.2, 0.5, 1, 4, 10 and 20 μM). Inhibition constants (K_i) were calculated by fitting the data to a fully competitive (NRTIs) or fully non-competitive (NNRTIs) equation.

3. Results

3.1. Drug susceptibility in HIV-1 recombinant strains

High-level resistance (≥10-fold increase in IC_{50} with respect to wild-type RT) to nevirapine and moderate resistance (3–10-fold increase in IC_{50}) to both stavudine and zidovudine were associated with G190S/A/E mutations (Table 1). On the other hand, the simultaneous presence of G190S and T215Y mutations in HIV-1 RT was associated with higher stavudine and zidovudine resistance levels than those associated with T215Y alone (36.3- and 9.1-fold *versus* 9.9- and 6.7-fold increase in stavudine and zidovudine IC_{50} values, respectively). As expected, full susceptibility to stavudine and zidovudine, and a high level of resistance to nevirapine were associated with the K103N mutation (Table 1).

3.2. Characterization of in vitro expressed RT enzymes

In Table 2, the absolute levels of resistance (calculated as K_{imut}/K_{iwt}) to stavudine, zidovudine and nevirapine of G190S, T215Y and G190S + T215Y mutated enzymes with respect to wild-type RT are listed. As expected, the G190S mutant showed high-level resistance to NNRTIs, whereas the T215Y mutant displayed high levels of resistance to NRTIs. However, the G190S mutation alone was able to confer a low to moderate level of resistance (four- to five-fold) also to zidovudine and stavudine. Notably, the double mutant G190S + T215Y showed a marked increase in resistance levels with respect to the single mutants. When the selectivity parameter (K_i/K_m) was calculated (Table 2), it was clear that the reduced affinity for zidovudine and stavudine by the G190S mutant was paralleled by a proportional decrease in the affinity for the nucleotide substrate, so that the selectivity was unchanged with respect to the wild-type enzyme. Combination of the G190S + T215Y mutations caused a further drop in affinity for the nucleotide substrate, resulting in a two-fold increase in selectivity of the double mutant with respect to the single T215Y mutant.

As shown in Table 2, G190S was associated with a marked decrease of RT catalytic efficiency for nucleotide incorporation (261.9-fold reduction of its k_{cat}/K_m value with respect to wild-type RT). The effect of the T215Y mutation was more limited, resulting in a 10-fold reduction in the k_{cat}/K_m value of the mutated enzyme with respect to wild-type. Interestingly, the simultaneous presence of G190S and T215Y was associated with a recovery of the RT catalytic efficiency with respect to G190S mutant (36.6-fold *versus* 261.9-fold reduction in the k_{cat}/K_m value with respect to wild-type RT).

4. Discussion

NNRTIs and NRTIs are structurally unrelated and inhibit HIV-1 RT by binding at different enzyme domains and through

Table 1
IC_{50} and fold-increase values to HIV-1 RT inhibitors of recombinant HIV mutant viruses with respect to the wild-type recombinant strain

Drugs	IC_{50} values (fold-increase) with respect to wild-type recombinant HIV RT						
	WT	Gly190Ala	Gly190Ser	Gly190Glu	Lys103Asn	Thr215Tyr	Gly190Ser + Thr215Tyr
d4T[a]	3.6 ± 4.0 (NA[b])	14.7 ± 12.8 (4.1 ± 1.9)[c]	32.4 ± 11.7 (9.0 ± 7.1)	14.4 ± 0.7 (4.0 ± 2.7)	3.2 ± 1.3 (0.9 ± 1.1)	35.6 ± 4.2 (9.9 ± 4.7)	130.6 ± 0.3 (36.3 ± 0.5)
AZT	0.5 ± 0.7 (NA)	2.4 ± 0.9 (4.9 ± 5.3)	3.4 ± 2.7 (6.8 ± 3.9)	1.8 ± 0.07 (3.7 ± 2.3)	0.4 ± 0.5 (0.8 ± 1.04)	3.3 ± 1.3 (6.7 ± 1.9)	4.5 ± 0.9 (9.1 ± 0.1)
NVP	0.6 ± 0.7 (NA)	52.8 ± 0.7 (88.0 ± 1.5)	79.8 ± 0.7 (133.0 ± 0.8)	79.8 ± 1.4 (133.0 ± 0.9)	111.6 ± 1.8 (186 ± 1.4)	0.4 ± 0.07 (0.8 ± 0.04)	79.0 ± 0.3 (131.7 ± 1.7)

[a] d4T: stavudine; AZT: zidovudine; NVP: nevirapine.
[b] NA: not applicable.
[c] Data are from triplicate experiments.

Table 2
Kinetic parameters and inhibition constants for *in vitro* expressed wild-type and mutated HIV-1 RT

Enzyme	TTP[a]			AZTTP[a]		d4TTP[a]		NVP[a]
	K_m[b]	k_{cat}[b]	k_{cat}/K_m[b]	K_i[b]	K_i/K_m	K_i[b]	K_i/K_m	K_i
Wild-type	2.0 ± 0.2 (NA)[c]	2.3 ± 0.2 (NA)	1.1 ± 0.1 (NA)	0.04 ± 1.0 (NA)	0.02 (NA)	0.025 ± 0.005 (NA)	0.012 (NA)	0.4 ± 0.01 (NA)
G190S	9.5 ± 0.5 (4.75)[d]	0.04 ± 0.001 (−57.5)	4.2 ± 0.3 × 10^{-3} (−261.9)	0.2 ± 0.2 (5.0)	0.02 (1)[e]	0.1 ± 0.02 (4.0)	0.01 (0.8)	6.0 ± 0.1 (15.0)[d]
T215Y	7.6 ± 0.6 (3.8)	0.9 ± 0.01 (−2.5)	0.11 ± 0.02 (−10.0)	4.0 ± 0.4 (100.0)	0.5 (25)	1.25 ± 0.02 (50.0)	0.16 (13.3)	0.9 ± 0.09 (2.2)[d]
G190S + T215Y	30.0 ± 2 (15.0)	0.95 ± 0.01 (−2.4)	0.03 ± 0.005 (−36.6)	6.4 ± 6.0 (160.0)	0.21 (10.5)	2.75 ± 0.05 (110.0)	0.09 (7.5)	10.0 ± 0.2 (25.0)[d]

[a] TTP: tymidine triphosphate; AZTTP: zidovudine triphosphate; d4TTP: stavudine triphosphate; NVP: nevirapine.
[b] K_m: Michelis constant for the nucleotide substrate (μM); k_{cat}: apparent rate of nucleotide incorporation (s^{-1}); k_{cat}/K_m: nucleotide substrate utilization efficiency value ($\mu M^{-1} s^{-1}$); K_i: inhibition constant (μM). Values are the mean of three independent replicates.
[c] NA: not applicable.
[d] Fold changes with respect to the wild-type enzyme defined as K_{mmut}/K_{mwt} or K_{imut}/K_{iwt}, for comparison with the substrates or inhibitors, respectively.
[e] Fold changes with respect to the wild-type enzyme $((K_i/K_m)_{mut}/(K_i/K_m)_{wt})$.

different mechanisms of action: interference with the catalytic step through displacement of critical residues for the NNRTIs *versus* competition with natural nucleotides for incorporation into nascent DNA strand and its termination for NRTIs. Thus, independent molecular bases for the selection and fixation in the viral population of NNRTI and NRTI resistance mutations were a long-standing dogma for both physicians and virologists. On the other hand, the cumulative impact of thymidine-analog associated mutations (TAMs) on NRTI resistance has been recently recognized. Indeed, the presence of five TAMs confers resistance to all NRTIs (analogs to either thymidine or other nucleotides) (Miller and Larder, 2001). Moreover, single aa changes in HIV-1 RT can confer resistance to multiple NRTIs (Loveday, 2001) or to both NRTIs and NNRTIs (Paolucci et al., 2004). Therefore, mutations in the RT coding sequence can modify the enzyme structure with unpredictable effects on its biological functions. In this respect, it is not surprising that NNRTI-selected mutations interfere with NRTI susceptibility. For example, several studies have shown the association of the NNRTI resistance mutation Y181C with the resensitization to zidovudine (Richman et al., 1994; Selmi et al., 2003; Van Laethem et al., 2001). On the other hand, we reported that Y181I/C substitutions could decrease stavudine susceptibility (Baldanti et al., 2003) by enhanced nucleotide substrate discrimination and phosphorolytic activity (Blanca et al., 2003). Thus, mutations in NNRTI binding pocket appear to influence RT activity in different ways.

The present data further confirm and extend previously observed interactions by showing that NNRTI-induced mutations at codon 190 of RT can reduce susceptibility to zidovudine and stavudine. Interestingly, the simultaneous presence of G190S and T215Y showed a stronger effect on stavudine and zidovudine resistance than T215Y alone. The analysis of *in vitro* expressed recombinant enzyme activity confirmed the drug susceptibility data obtained from recombinant viruses. The mutations did not impact the selectivity (i.e. the ability to discriminate between different substrates) of the RT but increased their absolute resistance to inhibition. Since the intracellular levels of nucleotides are finely regulated and usually in saturating amounts, whereas the intracellular concentration of triphosphorylated drugs is subjected to high variations, a drop in the absolute affinity of the enzyme for a given drug is expected to have a major impact, as shown here by the phenotypic tests.

Single mutations at positions 190 and 215 of RT were associated with reduction in RT catalytic efficiency. Mutations G190A/E/S are known to impair the positioning of the primer template and consequently the RT catalytic activity, and this deleterious effect may be mitigated also by the acquisition of the NRTI resistance mutation L74V and V75I (Fan et al., 1996; Boyer et al., 1998; Wang et al., 2006). Similarly, we observed that the marked impairment of RT activity associated with G190S appeared to be partially removed by the simultaneous presence of G190S and T215Y. Thus, acquisition of NRTI resistance mutations (T215Y, L74V and V75I) in HIV-1 strains carrying mutations at position 190 might have a compensatory effect, so that the emergence of double mutants in the viral population under drug pressure (NNRTI, NRTI or both) could be favoured both by an increase in drug resistance and recov-

ery in the catalytic efficiency of RT. Similarly, the greater viral replicative capacity of HIV-1 strains with zidovudine resistance mutations T215Y *versus* T215F has been proposed to explain the preferential T215Y selection *in vivo* (Hu et al., 2006). A role of T215Y in altering the geometry of the dNTP binding site has also been proposed. Based on crystal structures of binary and ternary complexes of HIV-1 RT, it has been proposed that a tyrosine residue at position 215 facilitates a proper orientation of the pyrophosphate molecule, and affects dNTP binding through significant conformational changes occurring during polymerization (Matamoros et al., 2004). Thus, it is conceivable that the rearrangements imposed by the T215Y mutation around the dNTP binding site might help to compensate for the defect of the G190S. However, a more precise definition of the effects of HIV-1 RT 190 mutations would require additional studies based on both suppression of such mutations in the context of enzymes carrying other aa substitutions (as it is usually observed in the clinics) and insertion of different aa substitutions at position 190 of RT of the same HIV-1 reference wild-type strain, in order to better dissect the contribution of individual 190 aa substitutions to drug resistance and RT activity.

The finding of a role for aa changes at domain 180–190 of HIV-1 RT in NNRTI-NRTI interclass resistance raises immediate diagnostic and clinical concerns: (i) genotypic interpretation algorithms should be revised on the basis of the new data; (ii) HAART regimens (namely rescue therapies) should take into account the risk of reduced NRTI efficacy when in the presence of NNRTI-selected mutations in this RT region.

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References

Baldanti, F., Paolucci, S., Maga, G., Labò, N., Hubscher, U., Skoblov, A.Y., Victorova, L., Spadari, S., Minoli, L., Gerna, G., 2003. Nevirapine-selected mutations Y181I/C of HIV-1 reverse transcriptase confer cross-resistance to stavudine. AIDS 17, 1568–1570.

Blanca, G., Baldanti, F., Paolucci, S., Yu Skoblov, A., Victorova, L., Hubscher, U., Gerna, G., Spadari, S., Maga, G., 2003. Nevirapine resistance mutation at codon 181 of the HIV-1 reverse transcriptase confers stavudine resistance by increasing nucleotide substrate discrimination and phosphorolytic activity. J. Biol. Chem. 278, 15469–15472.

Boyer, P.L., Gao, H.Q., Hughes, S.H., 1998. A mutation at position 190 of human immunodeficiency virus type 1 reverse transcriptase interacts with mutations at positions 74 and 75 via the template primer. Antimicrob. Agents Chemother. 42, 447–452.

Fan, N., Rank, K.B., Slade, D.E., Poppe, S.M., Evans, D.B., Kopta, L.A., Olmsted, R.A., Thomas, R.C., Tarpley, W.G., Sharma, S.K., 1996. A drug resistance mutation in the inhibitor binding pocket of human immunodeficiency virus type 1 reverse transcriptase impairs DNA synthesis and RNA degradation. Biochemistry 35, 9737–9745.

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.

Hu, Z., Giguel, F., Hatano, H., Reid, P., Lu, J., Kuritzkes, D.R., 2006. Fitness comparison of thymidine analog resistance pathways in human immunodeficiency virus type 1. J. Virol. 80, 7020–7027.

Kleim, J.P., Rosner, M., Winkler, I., Paessens, A., Kirsch, R., Hsiou, Y., Arnold, E., Riess, G., 1996. Selective pressure of a quinoxaline nonnucleoside inhibitor of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) on HIV-1 replication results in the emergence of nucleoside RT-inhibitor-specific (RT Leu-74->Val or Ile and Val-75->Leu or Ile) HIV-1 mutants. Proc. Natl. Acad. Sci. U.S.A. 93, 34–38.

Loveday, C., 2001. International perspectives on antiretroviral resistance. Nucleoside reverse transcriptase inhibitor resistance. J. Acquir. Immune Def. Syndr. 26 (Suppl. 1), S10–S24.

Maga, G., Amacker, M., Ruel, N., Hubscher, 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.

Matamoros, T., Franco, S., Vazquez-Alvarez, B.M., Mas, A., Martinez, M.A., Menendez-Arias, L., 2004. Molecular determinants of multi-nucleoside analogue resistance in HIV-1 reverse transcriptases containing a dipeptide insertion in the fingers subdomain: effect of mutations D67N and T215Y on removal of thymidine nucleotide analogues from blocked DNA primers. J. Biol. Chem. 279, 24569–24577.

Miller, V., Larder, B.A., 2001. Mutational patterns in the HIV genome and cross-resistance following nucleoside and nucleotide analogue drug exposure. Antivir. Ther. 6 (Suppl. 3), 25–44.

Paolucci, S., Baldanti, F., Maga, G., Cancio, R., Zazzi, M., Zavattoni, M., Chiesa, A., Spadari, S., Gerna, G., 2004. Gln145Met/Leu changes in human immunodeficiency virus type 1 reverse transcriptase confer resistance to nucleoside and nonnucleoside analogs and impair virus replication. Antimicrob. Agents Chemother. 48, 4611–4617.

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

Selmi, B., Deval, J., Alvarez, K., Boretto, J., Sarfati, S., Guerreiro, C., Canard, B., 2003. The Y181C substitution in 3′-azido-3′deoxythymidine-resistant human immunodeficiency virus, type 1, reverse transcriptase suppresses the ATP-mediated repair of the in 3′-azido-3′deoxythymidine 5′-monophosphate-terminated primer. J. Biol. Chem. 42, 40464–40472.

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. U.S.A. 91, 3911–3915.

Van Laethem, K., Witvrouw, M., Pannecouque, C., Van Remoortel, B., Schmit, J.C., Esnouf, R., Kleim, J.P., Balzarini, J., Desmyter, J., De Clercq, E., Vandamme, A.M., 2001. Mutations in the non-nucleoside binding-pocket interfere with the multinucleoside resistance phenotype. AIDS 15, 553–561.

Wang, J., Dykes, C., Domaoal, R.A., Koval, C.E., Bambara, R.A., Demeter, L.M., 2006. The HIV-1 reverse transcriptase mutants G190S and G190A, which confer resistance to non-nucleoside reverse transcriptase inhibitors, demonstrate reductions in RNase H activity and DNA synthesis from $tRNA^{Lys,3}$ that correlate with reductions in replication efficiency. Virology 348, 462–474.