The effects of RNase H inhibitors and nevirapine on the susceptibility of HIV-1 to AZT and 3TC

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ABSTRACT

It was recently proposed that HIV RT mutations that decrease RNase H activity increase zidovudine (AZT) resistance by delaying the degradation of the RNA template, allowing more time for AZTMP excision from the 3′ end of the viral DNA. This predicts that suboptimal concentrations of an RNase H Inhibitor (RNHI), which would decrease RNase H activity, would decrease AZT susceptibility. Conversely, a suboptimal concentration of a nonnucleoside RT inhibitor (NNRTI) would decrease polymerase activity and increase AZT susceptibility. We determined the effect of several RHNIs and an NNRTI (nevirapine) on AZT and lamivudine (3TC) susceptibility with vectors that replicate using WT or AZT resistant RTS. Susceptibility to 3TC, which is not readily excised, did not change significantly. Nevirapine, and most RHNIs tested, had only small effects on the susceptibility of either HIV vector to AZT and 3TC. One RNHI, F0444-0019, increased the IC50 for AZT for either vector by ~5-fold, which may be a concern.

Introduction

The reverse transcriptase (RT) of HIV is the enzyme that converts the single stranded viral RNA genome into the double stranded linear DNA that is subsequently integrated into the host genome. RT has two enzymatic activities that are essential for viral replication: a polymerase activity that can copy either an RNA or a DNA template and an RNase H activity that degrades RNA when it is part of an RNA/DNA duplex. RNase H degrades the RNA template during the synthesis of the first DNA strand, setting the stage for the synthesis of the second DNA strand. RNase H is also required for the two strand transfer events that are needed for the synthesis of the viral DNA and for the removal of the two RNA primers that are used to initiate minus and plus strand DNA synthesis. Due to its critical role in the HIV life cycle, RT is an important target for anti-HIV drugs. However, despite the fact that there are two essential enzymatic activities, all of the currently approved anti-RT drugs inhibit the polymerase activity. Although the RNase H activity of RT is an attractive target for the development of new anti-HIV drugs, no RNase H inhibitors (RNHI) have made it into clinical trials.

There are two classes of approved drugs that inhibit the polymerase activity of RT: nucleoside reverse transcriptase inhibitors (NRTI) and nonnucleoside reverse transcriptase inhibitors (NNRTI). NRTIs are dNTPs analogs that inhibit polymerization by being incorporated into the growing DNA strand. Unlike the natural dNTP substrates, all of the currently approved NRTIs lack a 3′ hydroxyl group; thus the incorporation of an NRTI blocks the extension of the viral DNA strand, a process called chain termination. Mutations in RT that confer resistance to NRTIs either increase the discrimination between the triphosphate form of the NRTI and the dNTPs during DNA synthesis, of which the best studied example is 3TC/FTC resistance caused by the M184V/I mutations (Gao et al., 2000; Sarafianos et al., 1999), or, as with most common AZT resistance mutations, increase the ATP-dependent excision of AZTMP from the end of the viral DNA (Arion et al., 1998; Boyer et al., 2001; Meyer et al., 1998, 1999). In contrast to NRTIs, NNRTIs bind to a site near to the polymerase active site, distorting the protein, and inhibiting the chemical step of polymerization (Andries et al., 2004; Ding et al., 1995; Kohlstaedt et al., 1992; Ren et al., 1995; Rittinger et al., 1995; Spence et al., 1995; Tantillo et al., 1994).

Resistance to NNRTIs usually involves mutations in or near the NNRTI-binding site, which interferes with the binding of the drugs in the NNRTI-binding pocket (see (Sarafianos et al., 2004) or (Sarafianos et al., 2009) for a review). Although most of the primary mutations that give rise to NNRTI and NNRTI resistance are relatively near the sites where the drugs bind, there are reports that mutations in the connection (CN) subdomain, which is not close to the binding sites for either NRTIs or NNRTIs, can enhance resistance to both NRTIs and NNRTIs (Brehm et al., 2007; Ehteshami et al., 2008; Gupta et al., 2010; Hachiya et al., 2008; Nikolenko et al., 2007, 2010; Yap et al., 2007; Zelina et al., 2008).
Modern highly active anti-HIV therapy (HAART) usually involves two different NRTIs and either an NNRTI or a protease inhibitor. While this combination therapy greatly decreases morbidity, long-term treatment often has undesirable side effects, and HIV can develop resistance to all of the available drugs. Given the emergence of drug resistant variants of HIV, it is important to continue to develop new inhibitors that would be effective against the existing resistant variants. Because drugs that inhibit the polymerase activity of RT are the backbone of most HAART therapies, it is important to consider whether a new class of inhibitor, such as the RNHIs, would be expected to enhance or diminish the effectiveness of any of the commonly used polymerase inhibitors, and whether a new inhibitor would be expected to have a positive or negative impact on resistance to the existing drugs.

Recent evidence suggests that HIV vectors that carry mutations that reduce the level of RNase H activity have a reduced susceptibility to AZT (Nikolenko et al., 2007); this result suggests that suboptimal concentrations of RNHIs could increase AZT resistance. Conversely, mutations that reduce the levels of the polymerase activity of RT are expected to increase susceptibility to AZT, which would suggest that a suboptimal concentration of an NNRTI should increase AZT susceptibility. However, we tested the NNRTI nevirapine (NVP) and found that suboptimal concentrations of NVP did not have a significant impact on the concentration of AZT required to inhibit HIV-1 replication by 50% (IC\textsubscript{50}). Although a suboptimal concentration of most of the RNHIs we tested did not significantly affect the IC\textsubscript{50} for AZT, the RNHI F0444-0019 did cause a significant decrease in AZT susceptibility (~5-fold), which is similar to the decrease in susceptibility to AZT seen when CN subdomain mutations were to added to NNRTI-resistant clinical isolates (Gupta et al., 2010) of HIV-1 and to the contribution to resistance made by CN subdomain mutations identified in clinical isolates (Lengruber et al., 2011).

**Results**

The addition of NVP has little effect on the IC\textsubscript{50} for AZT for either WT or AZT-R vectors

We first added suboptimal amounts of the NNRTI NVP to cells that were subsequently infected with HIV to ask whether reducing the amount of polymerase activity would change the IC\textsubscript{50} for AZT. The vectors used in these experiments either replicated using WT RT, which has a modest ability to excise AZTMP, or an excision proficient AZT-R RT (see Methods) (Boyer et al., 2001). The HIV vectors lack a functional Env coding region and were complemented with VSV-G, which limits the infections to a single cycle. The HIV vectors express a luciferase gene from the Nef reading frame; luciferase activity was used to measure ability of the virus to infect the cells. With these vectors, high level expression of luciferase requires that viral DNA is reverse transcribed and integrated into the host genome. Before studying the effect of NVP on AZT resistance, NVP was tested for its ability to block the replication of the vectors. The WT or AZT-R HIV vector was used to infect HOS cells in the presence of increasing concentrations of NVP. Supplemental Fig. 1 shows that NVP blocks HIV replication and has an IC\textsubscript{50} of 36±17 nM for a one-round vector expressing WT RT. The IC\textsubscript{50} of NVP for blocking replication of the AZT-R HIV vector was 27±1 nM (Supplemental Fig. 1). In subsequent drug combination assays, 40 nM NVP was used to reduce the infectivity of the virus stock by ~60%.

Using the same luciferase-based infectivity assay, the IC\textsubscript{50} of AZT was measured in the presence and absence of 40 nM NVP to determine whether a decrease in polymerase activity would affect AZT resistance. The IC\textsubscript{50} of AZT for WT HIV was 4±3 nM in the absence of NVP and 3±1 nM in the presence of 40 nM NVP, a negligible difference (Fig. 1 and Table 1). As shown in Fig. 1 and Table 1, using AZT-R HIV, the IC\textsubscript{50} for AZT was 41±38 nM and the IC\textsubscript{50} was reduced to 21±11 nM in the presence of 40 nM NVP, a difference of only 2-fold. Although the absolute value of the IC\textsubscript{50} for AZT did show some drift over a period of several months, and this drift was seen in independent experiments performed by several different people in the laboratory (which accounts for the large standard deviations shown here), the fold change in AZT IC\textsubscript{50} caused by the addition of either NVP or an RNHI (see below) was relatively constant.

Lamivudine (2’, 3’-dideoxy-3’-thiacytidine, commonly called 3TC), was used as a control in these experiments because it is not readily excised by RT (Boyer et al., 2001; Naeger et al., 2002). Because 3TC is not efficiently excised by either WT or AZT-R HIV, the addition of NVP would be expected to have a more modest effect on the IC\textsubscript{50} for 3TC than the IC\textsubscript{50} for AZT. Data presented here shows that the presence of NVP slightly lowered the 3TC IC\textsubscript{50} for either WT or AZT-R HIV (~2-fold reduction for both, see Table 1 and Fig. 2), but the differences were not statistically significant. Thus, inhibiting the polymerase by the amount needed to reduce the infectivity by ~60% does not appear to have a large or specific effect on the susceptibility of the vectors that replicate using WT or the AZT-R RT to either 3TC or AZT.

**Decreased RNase H activity may decrease AZT susceptibility**

We next studied the effects of RNHIs on AZT susceptibility. To try to minimize the possibility that an effect on AZT susceptibility was due to an ancillary effect of the RNHI and not to the effect of the compound on RNase H activity, we tested several different RNHIs. The RNHI used in these experiments are shown in Fig. 3. All of these RNHIs have been tested with recombinant HIV-1 RT in vitro and have been shown to inhibit the RNase H activity, and are either less potent or inactive against the polymerase activity of RT (Table 2).

Because cytotoxicity can affect HIV replication and drug susceptibility, the RNHIs were tested for cytotoxicity in HOS cells using both XTT and ATP based assays (see Methods). XTT, a tetrazolium dye, is reduced by viable cells to a formazan dye that can be quantified. Similarly, ATP levels can be determined using a luciferase based assay. As shown in Fig. 1 and Table 1, assays were performed 7 times with WT HIV and 5 times with AZT-R HIV. The average IC\textsubscript{50} values ±SD can be found in Supplemental Fig. 1. Assays were performed 7 times with WT HIV and 5 times with AZT-R HIV.

![Fig. 1. Cell based luciferase assay measuring the effect of 40 nM NVP on the IC\textsubscript{50} of AZT in HOS cells infected with WT or AZT-R HIV. The average IC\textsubscript{50} values ±SD can be found in Table 1. Assays were performed 7 times with WT HIV and 5 times with AZT-R HIV.](image-url)

![Table 1. The Effect of NVP on the IC\textsubscript{50} of AZT and 3TC in HOS Cells infected with WT and AZT-R HIV-1.](table-url)

<table>
<thead>
<tr>
<th>Virus</th>
<th>AZT IC\textsubscript{50} (nM ± SD)</th>
<th>Fold decrease\textsuperscript{a}</th>
<th>3TC IC\textsubscript{50} (nM ± SD)</th>
<th>Fold decrease\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>4±3</td>
<td>3±1</td>
<td>8±3</td>
<td>4±1</td>
</tr>
<tr>
<td>AZT-R</td>
<td>41±38</td>
<td>21±11</td>
<td>24±14</td>
<td>15±3</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Not statistically significant (p>0.05).
derivative, is reduced to an orange colored product by mitochondrial enzymes; the amount of orange product, quantified using a standard microplate absorbance reader, is proportional to the number of metabolically active cells. The concentration of RNHIs that would kill half of the HOS cell population (CC50) was determined to be between 16 and 370 μM (see Table 3 and Supplemental Fig. 2). Because the ability of the cells to convert AZT to AZTTP depends on ATP, the effect of the RNHIs on the ATP levels was determined using a luciferase assay in which the amount of light produced is proportional to the cellular concentration of ATP. The ATP based luciferase assay indicated that the CC50 for these RNHI were between 14 and 160 μM, as shown in Table 3 and Supplemental Fig. 3.

Before studying the effect of the RNHI compounds on AZT resistance, the IC50 values of each RNHI was determined in HOS cells for the WT and the AZT-R vectors using the aforementioned luciferase assay. Not surprisingly, the IC50s were similar for the two types of vectors, as shown in Table 3 (see Supplemental Figs. 4 and 5). In most cases the IC50s for these compounds were similar to the CC50 values obtained in the cytotoxicity assays, and it is possible that some of the decrease in infectivity is an indirect effect of the compound on the cell rather than a direct effect on HIV replication. To help control for any indirect effects of the RNHIs on HIV-1 replication and/or NRTI susceptibility, we also tested their effects on 3TC susceptibility. Because we were concerned that the cytotoxicity of the compounds might contribute to the anti-viral effect, we analyzed the effects of five RNHIs. Because these are distinct compounds, we think it unlikely that they would all have similar indirect effects on viral replication.

AZT susceptibility assays were performed in the presence the RNHIs at concentrations that were near the in vivo IC50 for each compound (20 μM for F0444-0019 and F0888-0058; 50 μM for F0915-1507; 70 μM for F3253-0041 and F3284-8495). F0444-0019 caused a moderate and significant increase on the AZT IC50 for WT and AZT-R HIV by ~5-fold; F0444-0019 also caused a small but significant increase in the IC50 for 3TC with WT HIV by ~3-fold (see Table 4; Figs. 4 and 5). The effect of F0444-0019 on the 3TC IC50 in cells infected with AZT-R HIV was insignificant. F0888-0058 had no significant effect on the IC50 of AZT (see Table 4) for WT HIV infected cells. There was a significant, but small (2-fold), effect of F0888-0058 on the AZT IC50 with AZT-R HIV infected cells; a very small, but statistically significant effect on 3TC susceptibility in cells infected with the WT HIV vector (~1.7-fold). We did not see a significant effect on the IC50 for 3TC when the experiments were repeated with the AZT-R HIV vector. F0915-1507 had no significant effect on the IC50 for AZT (see Table 4) for WT HIV infected cells; a similar lack of effect was seen with 3TC. F3253-0041 and F3284-8495 had little to no effect on the AZT IC50 with WT HIV and decreased the AZT IC50 in AZT-R infected cells; however these assays were performed only one or two times due to limited availability of the compounds and the small effects on AZT susceptibility (Table 4). Because the impact of F3253-
0041 and F3284-8495 on AZT resistance was small, we did not test its effects on 3TC susceptibility.

Because these RNHI were cytotoxic and treatment reduced the level of ATP in the cells, it is possible that some or all of the RNHIs reduced the level of ATP, which in turn led to a reduction in the amount of AZTTP in the cells. This could mean that part or all of the decrease in AZT susceptibility, seen with compound F0444-0019, could be due to an effect on the concentration of AZTTP rather than an effect on the RNase H activity of RT. To test this possibility, we used the mitochondrial toxin sodium azide (NaN₃) to reduce the level of ATP in the HOS cells. The cytotoxicity of NaN₃ in HOS cells was determined using both XTT and ATP based luciferase assays, as shown in Supplemental Fig. 6. The addition of 0.5 mM NaN₃ reduced the level of ATP in the HOS cells by ~70% (see Table 5). The concentration of most RNHI compounds used in the AZT (and 3TC) experiments reduced the levels of ATP by ~60% relative to untreated cells, as shown in Table 5, with two compounds reducing ATP levels by about 10%. The addition of 0.5 mM NaN₃ to HOS cells prior to HIV infection had no significant effect on the IC₅₀ for AZT with WT or AZT-R HIV (Fig. 6; Table 6). Thus, the data suggest that the effect of the one RNHI that had a significant effect on the IC₅₀ of AZT (F0444-0019) is not due to a

Table 4
The effect of RNHI on the efficacy of AZT and 3TC in HOS cells infected with an HIV vector that replicates using either WT or AZT-R RT.

<table>
<thead>
<tr>
<th>Drug</th>
<th>+F0444-0019</th>
<th>+F0888-0058</th>
<th>+F0915-1507</th>
<th>+F3253-0041</th>
<th>+F3284-8495</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZT IC₅₀</td>
<td>4±3</td>
<td>19±17</td>
<td>8±7</td>
<td>8±8</td>
<td>5³</td>
</tr>
<tr>
<td></td>
<td>WT</td>
<td>AZT-R</td>
<td>WT</td>
<td>AZT-R</td>
<td>WT</td>
</tr>
<tr>
<td>3TC IC₅₀</td>
<td>41±38</td>
<td>207±92</td>
<td>83±58</td>
<td>66±45</td>
<td>9.6²</td>
</tr>
<tr>
<td></td>
<td>WT</td>
<td>AZT-R</td>
<td>WT</td>
<td>AZT-R</td>
<td>WT</td>
</tr>
</tbody>
</table>

³ Indicates that the RNHI has a significant effect on the AZT or 3TC IC₅₀ (p<0.05) as determined by performing a Mann–Whitney Rank Sum Test.
² These experiments were performed only one or two times due to either a small effect or limited availability of the RNHI compounds.

Fig. 4. Cell based luciferase assay measuring the effect of 20 μM F0444-0019 on the IC₅₀ of AZT in HOS cells infected with WT (A) or AZT-R (B) HIV. The IC₅₀ values ± SD can be found in Table 4. Assays were performed 6 times with WT HIV and 4 times with AZT-R HIV.

Fig. 5. Cell based luciferase assay measuring the effect of 20 μM F0444-0019 on the IC₅₀ of 3TC in HOS cells infected with WT (A) or AZT-R (B) HIV. The IC₅₀ values ± SD can be found in Table 4. Assays were performed 3 times with WT HIV and 4 times with AZT-R HIV.
decrease in the level of ATP, which, in turn, led to decrease in the concentration of AZTTP. The interpretation that F0444-0019 has an effect on AZT susceptibility that is not simply due to an effect on the ATP concentration (and its impact on NRTI phosphorylation) is also supported by the fact that this RNHI has a greater effect on AZT susceptibility than it has on 3TC susceptibility; like AZT, 3TC must be converted to its triphosphate to be active.

Discussion

Combination drug therapy is the standard of care for HIV infections. Any new compounds that are developed will be used in combination with the existing first-line drugs. For this reason it is important to understand whether a new drug will interact with the existing drugs, either in a positive or a negative way, and to understand the mechanisms by which HIV can evade the drug. For instance, it has been reported that NNRTIs and NRTIs act in a synergistic manner to inhibit HIV replication (Basavapathruni et al., 2004; King et al., 2002), thus making AZT a more effective anti-HIV drug if it is given in combination with an NNRTI. The recent data showing that there is interplay between the levels of RNase H activity and polymerase activity can affect the efficiency of AZT excision and, by extension, AZT resistance could potentially offer an explanation for this reported synergy (Brehm et al., 2007; Ehteshami et al., 2008; Nikolenko et al., 2007, 2010).

The model proposed by Nikolenko et al. (Nikolenko et al., 2007) postulates that a decrease in the polymerase activity (including a decrease caused by an NNRTI) would allow the RNase H activity to degrade the template before the polymerase could excise the AZTMP from the end of the template. The model also suggests what affect RNase H inhibitors will have on AZT excision. Recent work showing that the mutations in the CN subdomain of RT can enhance AZT resistance (Brehm et al., 2007;
AZT. Although we found that one of the compounds we tested did cause CN subdomain mutations, and cause a reduction in the susceptibility to infections, be a good, not a bad, outcome. The more serious concern is virus to the drug; this would, from the point of view of treating HIV can be excised by mutant RTs, would increase the susceptibility of the however, is not that combining an NNRTI with AZT, or other NRTIs that activity as well as the polymerase activity could potentially affect the excision. Others have shown that, in addition to inhibiting the chemical and/or excision relative to RNA degradation. Even if the effects of NVP on we saw a similar small effect when the same experiments were performed with 3TC. Because, in contrast to AZT, 3TC is poorly excised by both WT RT and AZT-R RT, this effect cannot be easily explained as an effect on the rate of polymerization and/or excision relative to the degradation of the RNA template that would lead to a decrease in 3TC excision. The 3TC data raise the possibility that the same unknown mechanism could explain the small effect of NVP on the IC50 for AZT and 3TC, and by extension, the possibility that the small effect seen here with AZT does not involve the NVP altering the rates of polymerization and/or excision relative to RNA degradation. Even if the effects of NVP on AZT susceptibility are due to a differential effect on excision that is caused by a decrease in the polymerase activity relative to RNase H activity, the effects on AZT resistance are relatively small, suggesting that reducing polymerase activity has only a modest effect on AZT excision. Others have shown that, in addition to inhibiting the chemical step of polymerization, NNRTIs can also affect RNase H cleavage specificity (Palaniappan et al., 1995) and/or activity (Gopalakrishnan and Benkovic, 1994; Hang et al., 2007; Radzio and Sluis-Cremer, 2008; Shaw-Reid et al., 2005): thus, NNRTIs that have an effect on the RNase H activity as well as the polymerase activity could potentially affect the synergy between NNRTIs and NNRTIs in a complex way. The real concern, however, is not that combining an NNRTI with AZT, or other NNRTIs that can be excised by mutant RTs, would increase the susceptibility of the virus to the drug; this would, from the point of view of treating HIV infections, be a good, not a bad, outcome. The more serious concern is that a suboptimal dose of an NNHI might mimic the effects of some of the CN subdomain mutations, and cause a reduction in the susceptibility to AZT. Although we found that one of the compounds we tested did cause a significant decrease in susceptibility to AZT, the magnitude of the effect was modest (about 5-fold). However, even if an increase in the IC50 for AZT of 5-fold is less than what has been reported for some of the CN subdomain mutations (Nikolenko et al., 2007), it is still an undesirable outcome, particularly because a 5-fold loss in AZT susceptibility can cause clinical resistance. In terms of what is the expected amount of the reduction in AZT susceptibility from a CN subdomain mutation, when clinical isolates of drug-resistant RTs are modified to contain specific CN subdomain mutations the change in AZT susceptibility varies, and it would appear that the fold-change in susceptibility depends on the exact nature of the drug-resistance mutations that the clinical isolates carry (Gupta et al., 2010). The fold-increase in AZT resistance caused by the addition of either the T369I or the T369F mutation ranged from approximately 3.5-fold to more than 25-fold (Gupta et al., 2010). A similar observation was made when CN subdomain mutations that were associated with other AZT resistance mutations were placed in the context of WT or AZT-R HIV (Lengrubier et al., 2011). If these are representative numbers, the 5-fold decrease in AZT susceptibility we report for F0444-0019 could be considered to be a similar to the effect of adding one of the CN subdomain mutations to the clinical isolates. The relative modest effects of nevirapine on the IC50 for AZT also suggests that perturbing the ratio of RNase H and polymerase activity does not, by itself, necessarily cause a large change in the susceptibility of HIV to NNRTIs.

We think it is likely that at least some of the CN mutations can have a direct effect on AZT resistance either in addition to, or instead of, their impact on RNase H activity because the recent publication from Delviks-Frankenberg et al. (Delviks-Frankenberg et al., 2008) reported that some of the effects of the CN domain mutations on AZT resistance could be attributed to an effect on the polymerase site (that is to a direct effect on AZT-MP excision) rather than to an effect that depended solely on a reduction in RNase H activity. That could help to explain what is otherwise a rather puzzling observation: the secondary mutations that have been observed in viruses isolated from patients that enhance AZT resistance are found in the CN subdomain, but not in the RNase H domain. We have no simple explanation for the smaller, but significant, impact of F0444-0019 on 3TC susceptibility. As has already been discussed, 3TC is poorly excised, and we do not believe that the change in 3TC susceptibility was caused by a change in the ATP levels. If F0444-0019 caused a 5-fold change in the AZT susceptibility of HIV, and if this is the kind of effect on AZT susceptibility an RNHI should cause, why then did we not see similar effects with all of the other NNHIs? It is important to note that, unlike most of the RNHIs tested in this study, F0444-0019 also has some inhibitory activity against DNA polymerase activity of RT, albeit much less than that against the RT-RNase H activity (Table 3). Although it is possible that the inhibitory effect of F0444-0019 on the polymerase activity of RT could have affected the results we obtained, as has already been discussed, a reduction in the polymerase activity of RT would be expected to enhance the susceptibility of the virus to AZT, not reduce it. We also suspect, but cannot prove, that the toxicity of some of the compounds may play a factor in the observed phenotype. Reducing the RNase H activity of RT has been shown to reduce the susceptibility of HIV to AZT (Nikolenko et al., 2007); however, as the NaN3 experiments showed, treating the cells with a toxic compound does not. The primary reason that we performed experiments with several NNHIs was because we knew the compounds were toxic; by testing several compounds we could ask if any of the compounds would significantly shift the IC50 for AZT.

From the point of view of drug development, the primary issue is the observation that one of the compounds tested caused a five-fold decrease in AZT susceptibility. Although this increase in AZT resistance is less than has been reported for some of the CN subdomain mutations, it is still sufficient to cause a clinically significant resistance to AZT, and is, for that reason, a potentially serious concern, particularly given the importance of combination therapy in the treatment of HIV infections.

**Methods**

**Reagents**

The human embryonic kidney cell line 293T was obtained from the GenHunter Corporation (Nashville, TN). The human osteosarcoma cell line (HOS) was obtained from Dr. Richard Schwartz (Michigan State University, Lansing MI). Cells were maintained in Dulbecco’s modified...
Eagle’s medium supplemented with 3.5% (vol/vol) fetal bovine serum, 3.5% (vol/vol) newborn calf serum, and penicillin (100 μg ml⁻¹) plus streptomycin (100 μg ml⁻¹) (Quality Biological, Gaithersburg, MD). AZT and 3TC were obtained from Moravek (Brea, CA). NVP was provided by Dwight Nissley (NCI-Frederick). The compounds that were identified as inhibiting RNase H activity were purchased from Life Chemicals (Burlington, ON, Canada).

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References


