Selection of Zidovudine Resistance Mutations and Escape of Human Immunodeficiency Virus Type 1 from Antiretroviral Pressure in Stavudine-Treated Pediatric Patients

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The relationship between clinical changes in stavudine activity and stavudine resistance was investigated in 16 human immunodeficiency virus (HIV)–infected children who received stavudine monotherapy for 18 months. Seven patients responded well to stavudine therapy, 3 experienced transient reductions in virus load, and all others had no detectable virologic response. In both the responders and nonresponders, no changes in stavudine susceptibility or specific baseline/emergent mutations in reverse transcriptase were observed. Only posttherapy HIV isolates from transient responders had elevated IC50 values for stavudine. In 2 of the 3 transient responders, substitutions at codons 41, 210, and 215 were selected. The significance of these mutations was confirmed in viral competition experiments, site-directed mutagenesis, and in vitro selection. Selection of mutations previously associated with zidovudine resistance can be an important mechanism through which HIV may escape stavudine. The effect of these mutations on phenotypic stavudine susceptibility is relatively small but apparently large enough to be clinically significant.

Stavudine (d4T, 2',3'-didehydro-3'-deoxythymidine) is a nucleoside-analogue reverse-transcriptase inhibitor (NRTI) indicated for the treatment of human immunodeficiency virus (HIV) infection. Viral replication is inhibited by stavudine via direct competition between the phosphorylated drug (d4T-triphosphate) and endogenous deoxythymidine triphosphate and subsequent DNA chain termination [1–3].

Stavudine monotherapy improves various clinical, immunologic, and virologic parameters in antiretroviral-naive and zidovudine-experienced adults and children [4–10]. It is currently recommended for use in highly active antiretroviral regimens that combine 2 NRTIs with either a protease inhibitor or a non-NRTI, and studies have found stavudine to be effective in this capacity in both adult and pediatric patients [11–14].

A principal obstacle to the sustained efficacy of antiretroviral therapy with currently available drugs is the development of HIV drug resistance. Phenotypic changes in viral susceptibility to most NRTIs have been associated with genotypic changes in the HIV reverse transcriptase (RT) gene. Mutations conferring resistance to zidovudine, for example, have been mapped to codons 41, 67, 70, 210, 215, and 219 of the RT gene, and the accumulation of these mutations corresponds to a stepwise incremental reduction in zidovudine susceptibility [15–17].

The resistance profile of stavudine, however, differs from that of most other NRTIs in that genotypic mutations associated with stavudine resistance in vitro, specifically the I50T or V75T mutation [18, 19], are virtually never detected in patients treated with stavudine, even in cases of long-term therapy and stavudine failure [20–24]. Moreover, clinical HIV-1 isolates displaying stavudine-specific resistance have yet to be identified [22]. Nonetheless, a gradual loss of clinical response to stavudine has been observed following prolonged stavudine therapy, and possible genetic pathways to stavudine resistance or “multinucleoside resistance” have been described [4, 25–30]. These resistant variants arise through a specific pathway involving the selection of the Q151M mutation or double serine residue insertions between codons 67 and 70. However, only a fraction of isolates fit these descriptions, and additional mechanisms of stavudine resistance must exist [22, 27, 28]. In this study, we investigated the genotypic and phenotypic correlates of changes in
stavudine activity in antiretroviral therapy naive children receiving long-term stavudine monotherapy.

Patients, Materials, and Methods

Study population. The children in this study were part of a randomized, double-blind, multicenter study conducted by Pediatric AIDS Clinical Trials Group (PACTG) 240 to compare the safety and tolerability of zidovudine and stavudine in 212 HIV-1–infected children aged 3 months to 6 years [10]. Patients in our study were selected on the basis of randomization to the stavudine arm of PACT 240 (1 mg/kg every 12 h; maximum, 40 mg every 12 h), pharmacokinetic data suggesting compliance with therapy, and the availability of plasma samples collected prior to the initiation of therapy and after 18 months. Blood samples were evaluated for serum drug concentrations, plasma HIV-1 RNA levels, and the presence of phenotypic and genotypic stavudine resistance.

Viral RNA quantification. Plasma HIV-1 RNA was quantified by use of the Roche Diagnostics Amplicor HIV-1 Monitor assay (version 1.5) [31].

Viral RNA isolation and amplification. Amplification of viral RT was done as follows: HIV-1 RNA was extracted from 100 μL of plasma by the method described by Boom et al. [32]. After viral RNA isolation, an equivalent of 10 μL of plasma was used to reverse transcribe and amplify the complete RT gene. For 5 patients with a low HIV-1 RNA concentration, we used an equivalent of 50–100 μL.

Reverse transcription and amplification were done by use of the GeneAmp XL RNA polymerase chain reaction (PCR) kit (Applied Biosystems International [ABI]) essentially as described by the manufacturer, with 14 pmol of the oligonucleotide 3′ RTout (5′-TCT ACT TGT CCA TGC ATG GCT TC-3′; Pharmacia) for reverse transcription. We added 10 pmol of oligonucleotide RT18 (5′-GGA AAC CAA AAA TGA TAG GGA AGA TTG GAG G-3′; nt 2376–2406; Pharmacia) for subsequent amplification. The amount of amplified product was further increased in a second (nested) amplification reaction containing 14 pmol of the oligonucleotide RT19 (5′-GGA CAT AAA GCT ATA GGT ACA GAG G-3′; Pharmacia) for correct recombination and the occurrence of selection. The amplified cell-free virus was harvested. The virus stocks were sequenced to determine the infectious virus titer (TCID50) by use of endpoint dilutions in MT2 cells [34]. Stavudine susceptibility of the recombinant RT viruses was determined in duplicate using an MTT assay [35]. The stavudine susceptibility of site-directed mutant viruses was also evaluated by using the PhenoSense HIV assay (ViroLogic) [36].

Viral competition assay. To determine the replication efficiencies of recombinant viruses containing patient mutant RT relative to patient wild-type RT, 50% viral mixtures were prepared and used to infect 1.10⁶ SupT1 cells at an MOI of 0.01 in the absence and presence of 5 μM stavudine. After 2 h of infection, the cells were washed with RPMI 1640 (Gibco BRL Life Technologies) and resuspended in fresh RPMI 1640 (supplemented with 10% fetal calf serum [Gibco] and antibiotics) in the absence and presence of 5 μM stavudine. During the experiment, the culture was monitored daily for the appearance of syncytia. When full-blown syncytia were observed, cell-free virus was harvested and was used for subsequent infection and the analysis of the relative amount of mutant virus as compared with wild-type virus. To do so, viral RNA was isolated, viral RT amplified, and population sequencing was done with the Taq Dye Deoxy Terminator cycle sequencing kit.

Stavudine in vitro selection analysis. Stavudine in vitro selection experiments were performed by using HIV-1 HXB2. The selection was initiated by infection of SupT1 cells (MOI, 0.01) in the absence of the drug. After 1 h of incubation at 37°C, cells were spread over 6 wells of a 24-well plate and supplemented with culture medium containing 2.5 μM stavudine. During the experiment, the culture was monitored daily for the appearance of syncytia. When full-blown syncytia were observed, cells and free virus were used to initiate the next cell culture passage. The drug concentration was raised during the subsequent passages: 5 μM in passage
2, 10 μM in passage 3, 20 μM in passages 4–6, 40 μM in passage 7, and 80 μM in subsequent passages. We used HIV-1 RNA from the virus supernatant of passages 9 and 75 for genotypic analysis of the complete RT gene.

Results

Sixteen HIV-1 infected children who had received stavudine monotherapy for >18 months (PACTG 240) were included in the present study. Pre- and posttherapy samples were analyzed for changes in virus load, the development of drug resistance–associated mutations in RT, and changes in phenotypic drug susceptibility. Twelve children were antiretroviral naive prior to the initiation of stavudine therapy, but 1 child’s mother had received zidovudine during pregnancy; 4 children had been treated with zidovudine monotherapy for 6 weeks. Pharmacokinetic data were available for all but 1 child during therapy and showed acceptable serum drug concentrations at all time points, suggesting compliance at these visits (data not shown).

Plasma HIV-1 RNA concentrations. Plasma HIV-1 RNA levels were determined at the start of stavudine therapy and at different time points during treatment (3, 6, 12, and 18 months). Patients were classified on the basis of their virologic responses to therapy at 2 time points (3 and 18 months). According to the literature, the cutoff value for both stavudine and zidovudine monotherapy is ~0.5 log [37, 38]. Responder patients are defined as having a response of >0.5 log at both time points, nonresponder patients as having a reduction of <0.5 log at both time points, and transient responders as having an initial response at 3 months of >0.5 log followed by a response of <0.5 log at 18 months. By this classification, 7 patients were classified as having a response of 0.5 log followed by a response of >0.5 log at 18 months. (P = 0.56, Spearman’s rank test).

Nonresponder patients were not associated with reduced stavudine susceptibility at baseline, as compared with the responders or transient responders (5.00 μM vs. 5.96 and 5.11 μM, respectively). In addition, no consistent RT mutations suggestive of resistance to stavudine or other NRTIs emerged during therapy in these patients, and there were no detectable changes in stavudine susceptibility over time (IC50 start, 5.00 μM; IC50 end, 4.49 μM).

Of interest, in the transient responder patients, increases in IC50 values for stavudine could be observed (IC50 start, 5.11 μM; 4.78 μM; IC50 end, 5.07 μM). In the 5 patients who did not demonstrate clear virus load reductions during stavudine therapy (nonresponders), lack of response was not associated with reduced stavudine susceptibility at baseline, as compared with the responders or transient responders (5.00 μM vs. 5.96 and 5.11 μM, respectively). In addition, no consistent RT mutations suggestive of resistance to stavudine or other NRTIs emerged during therapy in these patients, and there were no detectable changes in stavudine susceptibility over time (IC50 start, 5.00 μM; IC50 end, 4.49 μM).

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In the 5 children who received or were potentially exposed to zidovudine prior to stavudine therapy were in this patient group. Although 0 zidovudine resistance–associated mutations were detected at baseline in these patients, 2 developed the K70R substitution during stavudine therapy. Selection of this particular mutation, however, did not result in clinical stavudine failure or the development of detectable elevations in stavudine IC50 values.

Figure 1. Plasma human immunodeficiency virus (HIV) type 1 RNA load changes (log) during 18 months of stavudine monotherapy in children, by response group. Nonresponders, patients who have a reduction of <0.5 log at both time points; responders, patients who have a response of >0.5 log at both time points; and transient responders, patients who have an initial response at 3 months of >0.5 log followed by a response of <0.5 log at 18 months.
In addition, in 2 of the 3 patients who had an increase in IC\textsubscript{50}, multiple zidovudine resistance–associated changes (codons 41, 210, and 215) were selected. HIV-1 isolated from the third patient demonstrated a clear increase in stavudine resistance and harbored an I178L amino acid change in RT, as reported elsewhere, during stavudine monotherapy [21].

HIV-1 isolated from the patient excluded from classification also demonstrated a clear reduction in stavudine susceptibility (IC\textsubscript{50} start, 5.30 \textmu M; IC\textsubscript{50} end, 9.64 \textmu M) in the presence of a treatment-emergent mutation at codon 215. This patient had not been previously exposed to zidovudine.

Stavudine susceptibility of site-directed mutants. To examine the relationship between specific RT mutations and changes in stavudine susceptibility (IC\textsubscript{50} start, 5.30 \textmu M; IC\textsubscript{50} end, 9.64 \textmu M) in the presence of a treatment-emergent mutation at codon 215. This patient had not been previously exposed to zidovudine.

Stavudine susceptibility of site-directed mutants. To examine the relationship between specific RT mutations and changes in stavudine susceptibility, 5 different site-directed HIV-1 mutants were generated [39] (table 2). The K70R variant did not have reduced susceptibility to stavudine, which is in keeping with the observation of this mutation in 2 responders. Variants harboring other zidovudine resistance–associated mutations observed in our transient responder patients (T215Y alone or in combination with the M41L) demonstrated small decreases in stavudine susceptibility by both our in-house phenotypic assay and the commercial PhenoSense assay.

Viral competition experiments. To examine the replication capacity of the mutant virus population (M41L-R135T-L210W-T215Y) of patient 501907h relative to its wild-type virus population, we performed 50% mixture experiments between the wild-type and mutant recombinant virus in the absence and presence of 5 \textmu M stavudine. After 2 passages, no significant change in the virus population was observed in the absence of stavudine, whereas, in the presence of stavudine, the mutant population was dominantly present. This clearly demonstrates the selective advantage of the mutant virus population in the presence of stavudine and confirms the small decreases in stavudine susceptibility.

### Table 1. Analysis of genotypic and phenotypic resistance after prolonged stavudine monotherapy (18 months).

<table>
<thead>
<tr>
<th>Patients</th>
<th>AZT pretreatment</th>
<th>Changes in complete HIV-1 reverse-transcriptase gene</th>
<th>Stavudine IC\textsubscript{50}, \textmu M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td></td>
<td></td>
<td>Start</td>
</tr>
<tr>
<td>380639e</td>
<td>—</td>
<td>I142V, K211V/Q, M245V/T, I293I/V</td>
<td>2.74</td>
</tr>
<tr>
<td>380686f</td>
<td>Mother during pregnancy</td>
<td>—</td>
<td>V245V/A, Y405Y/H</td>
</tr>
<tr>
<td>410206a</td>
<td>5 weeks</td>
<td>T69N, <strong>K70R</strong></td>
<td>S452S/L</td>
</tr>
<tr>
<td>420074d</td>
<td>4 weeks</td>
<td>—</td>
<td>No stock</td>
</tr>
<tr>
<td>430132</td>
<td>5 weeks</td>
<td>T69N, <strong>K70R</strong></td>
<td>R356R/K</td>
</tr>
<tr>
<td>690168j</td>
<td>—</td>
<td>—</td>
<td>K550K/R</td>
</tr>
<tr>
<td>700053f</td>
<td>—</td>
<td>K20K/R, I195L/L, K366K/R, R395R/K, T554T/A</td>
<td>6.29</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>5.96</td>
</tr>
<tr>
<td>Nonresponders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>380587g</td>
<td>—</td>
<td>D204E</td>
<td>Q174Q/K, L334L/Q, I435I/M, V466V/A</td>
</tr>
<tr>
<td>410243d</td>
<td>5 weeks</td>
<td>T376A</td>
<td>—</td>
</tr>
<tr>
<td>410252e</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>380626L</td>
<td>—</td>
<td>I108V</td>
<td>—</td>
</tr>
<tr>
<td>650137L</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>5.00</td>
</tr>
<tr>
<td>Transient responders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>501229e</td>
<td>I178L</td>
<td>T277S, R277R/K, L469L/I, N554N/S</td>
<td>3.24</td>
</tr>
<tr>
<td>440113</td>
<td>M142I, L210W, T/A215Y</td>
<td>R166K/R, Q524E</td>
<td>2.25</td>
</tr>
<tr>
<td>Mean</td>
<td>I371A</td>
<td>L214F/L, S215F/S, S359S/G, E404E/D</td>
<td>5.11</td>
</tr>
<tr>
<td>380526c</td>
<td>—</td>
<td>L214F/L, S215F/S, S359S/G, E404E/D</td>
<td>5.30</td>
</tr>
</tbody>
</table>

NOTE. Patients were participants in the Pediatric AIDS Clinical Trial Group 240. Amino acids that are known to be associated with zidovudine resistance are in boldface. A dash (—) indicates no amino acid substitutions. AZT, azidothymidine; HIV, human immunodeficiency virus.

### Table 2. Analysis of phenotypic stavudine susceptibility of human immunodeficiency virus (HIV) type 1 reverse-transcriptase (RT) mutants.

<table>
<thead>
<tr>
<th>HIV-1 RT genotype</th>
<th>Fold change in IC\textsubscript{50} vs. wild type (HXB2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K70R</td>
<td>1.1</td>
</tr>
<tr>
<td>T215Y</td>
<td>1.5</td>
</tr>
<tr>
<td>M41L</td>
<td>1.3</td>
</tr>
<tr>
<td>T215F</td>
<td>1.3</td>
</tr>
<tr>
<td>M41L-T215Y</td>
<td>1.3</td>
</tr>
<tr>
<td>M41L-T215F</td>
<td>1.3</td>
</tr>
</tbody>
</table>
In 4 other experiments, the V75I mutation was selected either alone or in combination with the K65K/R or the E122E/K. In the remaining experiment, an N147D amino acid change was observed. Genotypic analysis after 75 passages in the presence of 80 μM stavudine revealed a change in the codon 75 substitution from isoleucine to threonine, a finding reported elsewhere in in vitro selection experiments [19].

**Discussion**

The mechanisms that enable HIV-1 to escape the antiviral pressures of stavudine in vivo have yet to be thoroughly elucidated [20, 22–24]. For most NRTIs, clinical and phenotypic changes in viral susceptibility are clearly associated with genotypic changes in HIV RT. Mutations associated specifically with HIV-1 resistance to stavudine in vitro (V75T and I50T) are rarely detected in patients who receive prolonged stavudine therapy, even when stavudine therapy fails [22].

Clinical HIV-1 isolates that express intermediate- or high-level resistance to stavudine display 1 of 2 multinucleoside resistance phenotypes associated with the selection of the Q151M mutation (and associated secondary mutations) and the insertion of 2 serine residues between positions 67 and 70, respectively, usually in the presence of other NRTI resistance–associated mutations [27–30]. However, many of the isolates expressing stavudine resistance display neither mutation pattern. Extensive investigation by several teams revealed a wide array of genotypic changes that emerge during stavudine therapy, but no consistent series or pattern has been identified other than the 2 multinucleoside-resistance pathways described above. The combined in vivo and in vitro portions of the present study demonstrate that selection of the mutations previously associated only with zidovudine resistance M41L and T215F/Y is an important mechanism in the development of stavudine resistance.

In the present study, 7 of 16 patients responded very well to stavudine monotherapy, with an average HIV-1 RNA reduction of 0.6 log_{10} copies/mL after 18 months of treatment. These data are in agreement with several other studies that demonstrated improvement of various clinical, immunologic, and virologic parameters in both adults and children who received long-term stavudine therapy [4–14].

Not surprisingly, the responder patients showed no change in stavudine susceptibility during treatment. Three of the 4 children who received zidovudine for < 6 weeks before stavudine therapy and the child whose mother received zidovudine while pregnant were in this group of responder patients. Although no mutations associated with zidovudine could be detected at baseline in these patients, HIV-1 isolates from 2 of them developed the K70R mutation, which is associated with low-level zidovudine resistance. Since this HIV-1 variant differs from the wild-type sequence by only 1 nt, it is likely that it preexisted as 1 of many viral quasi species, as demonstrated in studies of persons not previously exposed to antiretroviral drugs [40, 41]. Analysis of the site-directed mutant K70R showed no detectable decrease in stavudine susceptibility in vitro, in agreement with the observation that this particular mutation did not result in stavudine resistance even when the site-directed specific 2-fold reduction in stavudine susceptibility in vitro, in agreement with the observation that this particular mutation did not result in stavudine failure or in development of detectable phenotypic stavudine resistance in our patients. Others have also demonstrated that the K70R does not confer clinical cross-resistance to stavudine [42].

Five patients (nonresponders) did not demonstrate a clear virologic response during stavudine treatment even though serum drug concentrations could be measured at all time points. In the nonresponder patients, no stavudine-resistant phenotype at baseline and no particular RT amino acid changes or decrease in drug susceptibility were observed during therapy. There are at least two possible explanations for these observations.

First, stavudine resistance in these patients may be achieved through changes that map outside the RT gene. This was suggested by Lin et al. [21], who described an HIV-1 isolate from a stavudine-treated patient that had a 12-fold decrease in stavudine susceptibility despite an RT gene that, when inserted into a wild-type chimeric recombinant, contributed to only a modest reduction in stavudine susceptibility. The same group has reported numerous clinical isolates with as high as 10-fold reductions in stavudine susceptibility, despite the absence of a single RT amino acid substitution [22].

A second potential mechanism is cellular resistance, which affects the metabolic conversion of NRTI by cellular kinases to their corresponding activated 5’-triphosphate forms. Although no cellular resistance has been reported for stavudine, a cellular thymidine kinase is involved in the phosphorylation of stavudine to its active molecule, and in vitro incubation of cell lines with zidovudine can induce a reduction in its activity [43–45]. Reduced thymidine kinase activity in stimulated peripheral blood mononuclear cells derived from HIV-1–infected patients after long-term zidovudine monotherapy or combination therapy has also been reported [44]. These findings suggest that therapy with thymidine analogue drugs may result in altered drug metabolism, which may account for clinical resistance in the absence of conventional antiretroviral drug resistance.

HIV-1 isolates from 3 patients (transient responders) who experienced transient virus load responses during stavudine monotherapy expressed phenotypic stavudine resistance (range, 1.9–2.8-fold). A 1.8-fold reduction in stavudine susceptibility was also measured in 1 patient who could not be classified by virologic response due to the absence of a 3-month plasma sample. In 3 of the 4 patients with reductions in stavudine susceptibility, mutations previously only associated with zidovudine resistance were selected. Posttreatment HIV-1 isolated from the fourth patient harbored a I178L amino acid change as previously identified in stavudine-treated patients and associated with a 2-fold reduction in stavudine susceptibility in site-directed mutagenesis experiments [21]. Of interest, the RT activity of this I178L mutant was increased, as compared with that of wild-type HIV-1, which may be the mechanism through which I178L mutants overcome the antiretroviral effect of stavudine [46].
As mentioned above, in 3 of 4 patients with phenotypic stavudine resistance, a reduction in stavudine susceptibility was related to the selection of the T215F/Y mutation with or without the M41L mutation. None of these children was treated with zidovudine prior to study entry or exposed to zidovudine in utero. Therefore, the zidovudine–resistance–associated mutations observed in the present study were selected exclusively during stavudine therapy. Several other studies have demonstrated the selection of zidovudine resistance–associated mutations during stavudine monotherapy, but only 3 HIV-1 isolates bearing the M41L mutation with or without T215F/Y have been obtained from subjects not previously exposed to zidovudine or other NRTIs [22]. However, in those 3 isolates, no reduction in stavudine susceptibility was observed. Conversely, the selection of zidovudine resistance–associated mutations observed in patients in whom stavudine therapy failed resulted in small, but apparently significant, increases in stavudine IC_{50} values. We confirmed these data in viral competition experiments that showed a clear replicative advantage of the mutant virus population harboring multiple zidovudine resistance–associated mutations as compared with the wild-type virus in the presence of stavudine.

This association was mirrored in drug passage/resistance selection experiments in which the T215Y mutation was selected following exposure to increasing concentrations of stavudine in vitro. Besides the selection of the T215Y substitution in 1 of 6 experiments, the V75I was selected in 4 of 6 experiments. Two other studies describing stavudine mutation selection did not demonstrate the selection of mutations previously associated with zidovudine resistance but did show the selection of the V75T mutation or the I50T substitution [18, 19]. The V75I amino acid change in our in vitro selection experiments is an intermediate of the V75T substitution, and, after 75 passages in the presence of 80 mM stavudine, the isoleucine mutation was replaced by a threonine. An explanation for the observation that the T215Y is less often observed in vitro than the V75T mutation may be that T215Y is a more “difficult” substitution (requiring 2 transversions) than the V75T mutation (which requires 2 transitions) [47]. The preferred selection of mutations previously associated with zidovudine resistance in vivo, however, may be explained by the recent work of Trouplin et al. [48], who determined that the probability of the 41 and 215 mutations emerging in vitro is highest at physiologic concentrations of stavudine.

It remains unknown why a large group of patients had no response to stavudine therapy. However, from data in this study, we conclude that in transient responder patients, selection of mutations previously only associated with zidovudine resistance is an important mechanism by which HIV-1 overcomes the antiretroviral pressure of stavudine. These RT amino acid changes are apparently involved in escape from the antiviral pressure exerted by the deoxycytidine analogues zidovudine and stavudine. This observation has important clinical consequences since stavudine is often used in the second line of antiretroviral combination therapy in patients already treated with zidovudine. Perhaps of most significance is our finding that even low-level (e.g., 2.2-fold) stavudine resistance associated with amino acid substitutions at positions 41 and 215 may be clinically significant in patients with an initial response to stavudine therapy. On the basis of our findings here and clinical studies demonstrating the limited effectiveness of stavudine in patients pretreated with zidovudine, further investigation of the effects of low-level stavudine resistance on clinical outcome in patients receiving combination therapy appears warranted.

Acknowledgments

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References


