Dual Resistance to Zidovudine and Lamivudine in Patients Treated with Zidovudine-Lamivudine Combination Therapy: Association with Therapy Failure

Veronica Miller, Andrew Phillips, Carsten Rottmann, Schlomo Staszewski, Rudi Pauwels, Kurt Hertogs, Marie-Pierre de Béthune, Sharon D. Kemp,* Stuart Bloor,* P. Richard Harrigan,* and Brendan A. Larder*

Human immunodeficiency virus type 1 (HIV-1) strains dually resistant to zidovudine and lamivudine (3TC) may arise during zidovudine-3TC combination therapy. The objective of this cross-sectional study (n = 43 patients) was to test the association between therapy response (clinical and immunologic) to zidovudine-3TC and the level of phenotypic zidovudine resistance and zidovudine resistance-associated genotype of 3TC-resistant isolates. Other variables included were baseline CD4+ cell count, baseline Centers for Disease Control and Prevention (CDC) classification, virus load, and time receiving zidovudine. Phenotypic resistance was assessed using a recombinant virus assay. Genotypic analysis was based on population sequencing of plasma HIV-1. In a univariate analysis using a logistic regression model, it was found that therapy response was significantly associated with phenotypic and genotypic zidovudine resistance, baseline CD4+ cell count, and virus load. After adjustment for all variables, phenotypic resistance to zidovudine remained the only significantly associated factor, independent of baseline CD4+ cell count, baseline CDC classification, and virus load.

The effectiveness of zidovudine-lamivudine (zidovudine-3TC) combination therapy was first demonstrated in four independent surrogate marker trials in antiretroviral-untreated [1, 2] and in zidovudine-pretreated patients [3, 4]. A significant clinical benefit in favor of 3TC-containing treatment arms was shown by a metaanalysis of these four trials [5], and more recently, in a clinical end-point trial comparing zidovudine-containing standard-of-care nucleoside analogue treatment combinations plus or minus 3TC in a patient population with more advanced disease [6]. A benefit for 3TC has also been demonstrated in triple combinations with human immunodeficiency virus type 1 (HIV-1) protease inhibitors [7] or nonnucleoside reverse transcriptase inhibitors [8].

Under conditions of continuing viral replication, a highly 3TC-resistant virus population containing a methionine-to-valine substitution at amino acid residue 184 in the catalytic site of reverse transcriptase (M184V) is selected [9–11] and has been detected 4–12 weeks after initiation of 3TC monotherapy or zidovudine-3TC combination treatment in virtually all HIV-1-infected patients tested [12, 13]. M184V is accompanied by increases of 100- to 500-fold in the IC50 of 3TC for patient-derived HIV-1 isolates or recombinant viruses [13, 14].

Resistance to zidovudine, on the other hand, develops gradually by the stepwise accrual of mutations, including substitutions at positions 41, 67, 70, 210, 215, and 219 [15–20]. The quantity and the pattern of mutations influence the level of phenotypic resistance [18–21].

The mechanisms proposed to explain the observed continued benefit of zidovudine-3TC combination therapy in spite of the high level of 3TC resistance include: a mutational interaction between M184V and zidovudine resistance-associated mutations, which results in a reversal of zidovudine resistance in virus strains carrying zidovudine resistance-associated mutations [11, 14]; delay of zidovudine resistance development in the presence of 3TC (and M184V) [14]; reduction of HIV-1 reverse transcriptase activity by M184V, resulting in a less replication-competent virus [22, 23]; and improved transcriptional fidelity of HIV-1 reverse transcriptase [24]. Evidence from clinical trials supporting the first two mechanisms has been published [14, 25, 26]. However, it has become evident that resistance to zidovudine may be expressed phenotypically in the presence of the M184V substitution in vitro [14] and in vivo [27–29].

An association between resistance to zidovudine and therapy failure based on surrogate marker analysis [30–33] as well as between zidovudine resistance and clinical progression [34–40] has been established for HIV-1–infected patients being treated with zidovudine monotherapy or being switched to deoxyinosine (ddI). Resistance was measured in phenotypic
The association of zidovudine resistance development associated with zidovudine-3TC treatment with clinical progression has not been examined to date. In zidovudine-3TC-treated patients, a dissociation between the prognostic value for therapy failure of the presence of zidovudine resistance—associated mutations versus phenotypic resistance may be expected due to the M184V resistance effect. We therefore sought to analyze the genotypic and phenotypic patterns of resistance to zidovudine in a cross-sectional study of patients treated long-term with zidovudine-3TC who appeared to be having therapy failure on the basis of declining CD4 cell counts. To further define clinical failure, all HIV-related clinical events occurring while the patient was being treated with zidovudine-3TC and plasma load at the time of resistance analysis were used. Patients still benefitting from zidovudine-3TC therapy on the basis of CD4 cell count and clinical status were included for comparison. Our objectives were to establish whether and to what level phenotypic zidovudine resistance did occur in patients whose zidovudine-3TC combination therapy failed compared with patients still experiencing treatment benefit; to determine whether the quantity or specific patterns of zidovudine resistance—associated mutations correlated with phenotypic resistance; to determine whether the level of zidovudine resistance correlated with declining therapy benefit of zidovudine-3TC in this patient population; and to compare the association of genotypic and phenotypic resistance measurements with response to therapy.

Materials and Methods

Patient selection and follow-up. Patients selected for this study had been treated with zidovudine-3TC in the HIV Outpatient Center of the Frankfurt University Hospital (Frankfurt, Germany) during 1993–1996 as participants in controlled trials [2, 3] or as part of the 3TC compassionate use program. We included all participants of the NUCB3001 and 3002 trials for whom plasma samples were available at the time that resistance testing became available. Thus, we had 16 patients from the NUCB3002 trial (for zidovudine-pretreated patients) and 3 patients from the NUCB3001 trial (for therapy-inexperienced patients). At the time of sampling, the randomized and open-label follow-up trial periods were finished, and these patients were being treated with zidovudine-3TC within the compassionate use program as part of an ongoing therapy regimen. In addition, we included all patients who had begun 3TC treatment through the NUCB3004 protocol (compassionate use), for whom resistance testing had been performed as part of therapy monitoring because therapy changes were being considered (24 patients). Patients were seen every 4–8 weeks. Plasma samples were obtained and stored at −70°C for plasma HIV-1 RNA measurements and resistance analysis. Information regarding immunologic status and clinical progression was collected retrospectively from the case report forms and/or patients’ medical files. For the purposes of this analysis, the follow-up period was defined as starting at baseline (the date 3TC therapy was started (either added to a preexisting zidovudine therapy or as part of zidovudine-3TC combination therapy in previously naïve patients) and continuing to 4 weeks after the sample date for resistance analysis.

Resistance analysis on consecutive samples was done for 12 patients. Only the first sample from each patient was included in the analyses.

Preparation of recombinant HIV-1 from patient plasma. Recombinant HIV-1 for the HeLa CD4+ plaque-reduction assay was constructed as previously described [14, 19, 41]. The primers used were: OUT3 (5’-CATTTGCTTCCATTACTGTTATTTCTCATG-3’) for reverse transcription, OUT5 (5’-GTACAGTTTAGTAGGACCTACCTGTCAAACAT-3’) and OUT3 for the first-round polymerase chain reaction (PCR), and IN5 (5’-CATTTCCCATAGTCTATTGAAACTGTACCAG-3’) and IN3 (5’-TCTATTCATCTAAATATTATGACTTCTTCTGATTCC-3’) for the second-round PCR. This yields a fragment encompassing the complete coding region of reverse transcriptase.

These primer combinations were also used for the MITT-MT4 assay (Antivirogram; VIRCO Laboratories) [42]. RNA was extracted from 200 μL of plasma (QIAamp Viral RNA Extraction Kit; Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Reverse transcriptase mixtures (final reaction volume, 20 μL) contained 5 mM MgCl2, 1 mM dNTPs (Pharmacia, Uppsala, Sweden), 0.75 μM primer OUT3, 20 U of RNase inhibitor (Perkin-Elmer, Foster City, CA), 6.5 U of reverse transcriptase (Expand; Boehringer Mannheim, Mannheim, Germany), 4 μL of RNA solution, and 2 μL of reverse transcriptase reaction buffer (10×; Boehringer Mannheim). Reaction mixtures were incubated at 42°C, 30 min for cDNA synthesis; the reverse transcriptase was then inactivated at 99°C for 5 min. All reactions were carried out in a thermocycler (GenAmp 9600; Perkin-Elmer). The first round of nested PCR reaction mixture (final volume, 100 μL) contained 2.5 mM MgCl2, 200 mM dNTPs, 0.15 μM each primer, 5 U of Expand High-Fidelity polymerase mixture (Boehringer Mannheim), 10 μL of Expand reaction buffer (10×), and 20 μL of cDNA mixture. For the second-round PCR, 10 μL of the first-round reaction was mixed with 0.15 μM primers and other components as for the first-round reaction. All reactions were carried out in a Biometra Uno Thermocycler (Biometra, Göttingen, Germany). Reaction conditions for both PCR rounds were as follows: 95°C for 3 min followed by 30 cycles of 90°C for 1 min, 55°C for 30 s, and 72°C for 2 min. PCR products were purified (QIAquick PCR Purification Kit; Qiagen) and analyzed by 1% agarose gel electrophoresis. The amplification product (5 μg) and linearized reverse transcriptase–deleted proviral clone pHIV/ΔRT (10 μg) were transfected into MT4 cells using the Gene Pulser (Bio-Rad, Hercules, CA) in 0.4-cm electrode cuvettes at 250 μF and 300 V. After a 30-min incubation at room temperature, 10 μL of fresh culture medium was added, and the cells were incubated at 37°C in a humidified atmosphere. Stocks of chimeric viruses were harvested from the culture supernatant.

Drug susceptibility assays. Phenotypic susceptibilities to zidovudine and 3TC were assessed using the HeLa plaque-reduction plasma–derived recombinant virus assay (RVA) and the MTT-MT4 assay (Antivirogram) as previously described [41–43]. Results are expressed as the fold-resistance (fold-increase in IC50 relative to wild-type) or the log10 of the fold-resistance value (log10(R)). Resistance analyses were carried out in a blinded fashion with respect to therapy response.

Sequence analysis. The sequences of the reverse transcriptase–coding regions from recombinant viruses were deter-
Results

124 cells/mm³. At baseline, 7 patients were classified as CDC stage A, 22 as CDC stage B, and 14 as CDC stage C.

Zidovudine and 3TC resistance for the total population. Zidovudine and 3TC drug susceptibility assays were performed on all plasma-derived recombinant virus samples using the Antivirogram (MTT read-out) method. Samples from 30 patients were also analyzed using the RVA-HeLa plaque-reduction assay. There was excellent agreement between the zidovudine and 3TC susceptibility levels obtained with either method ($R = 0.8, P < 0.001$) [42].

Resistance to 3TC. Recombinant viruses obtained from all samples except 2 were fully resistant to 3TC ($IC_{50} > 100 \mu M$, in both assays when available). Genotypic analysis, available for samples from 40 of the 43 patients, confirmed that these recombinant viruses carried the M184V mutation in the reverse transcriptase coding region. Recombinant virus originating from 2 patients displayed a low level of 3TC resistance (13-fold and 5-fold in the MTT and HeLa plaque cell assays, respectively) and were genotypically wild-type. One of these 2 patients had interrupted 3TC therapy for 6 months but reported having received 3TC for the previous 5 months at the time the sample was taken. The second patient had discontinued 3TC treatment 4 months prior to the sample date.

Resistance to zidovudine. The zidovudine $IC_{50}$ values ranged from 0.2-fold to $>1062$-fold that of the wild-type $IC_{50}$ in the total sample population using the MTT read-out method (0.5-fold to $>250$-fold using HeLa plaque cell assay, data not shown). In 21 samples, a high level of resistance to zidovudine was detected ($IC_{50} > 100$-fold that of wild-type); 13 samples had an intermediate level of resistance ($IC_{50}, 10$- to 100-fold that of wild-type), and recombinant viruses from 9 samples were zidovudine-sensitive or had low-level resistance ($IC_{50} < 10$-fold higher than wild-type). Table 2 lists the baseline characteristics for the population, divided into the 3 groups on the basis of the level of zidovudine resistance. The median baseline CD4 cell count was 63 cells/mm³ for patients with high-resistance samples, 164 cells/mm³ for patients with samples showing intermediate resistance, and 233 cells/mm³ for patients with sensitive or low-resistance isolates. The patients with high-resistance isolates were also more advanced in terms of clinical status (CDC classification). The total time receiving zidovudine did not correlate with the level of phenotypic resistance.

In 12 patients, 2 consecutive samples were tested, with a time interval of 1–8 months. The second sample showed either an increased level or level of zidovudine resistance similar to the first (data not shown).

Under zidovudine monotherapy, the level of zidovudine resistance has been reported to vary according to the number and pattern of zidovudine-associated mutations [15, 16, 18, 19, 21]. We therefore examined the relation between the total number of zidovudine resistance–associated mutations (at positions 41, 67, 70, 210, 215, and 219) and phenotypic zidovudine resistance in this zidovudine-3TC–treated population. Genotypic data were available for 40 of the 43 patients.

Figure 1A shows that there was no significant correlation between the number of zidovudine mutations and the level of resistance to zidovudine in recombinant isolates with $>3$ mutations.
Table 1. Characteristics of HIV-1–infected patients treated with combined zidovudine and lamivudine (3TC).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Median No. of months of baseline</th>
<th>Range No. of months of pretreatment</th>
<th>Median No. of months of treatment</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>43</td>
<td>124–436</td>
<td>18</td>
<td>0–66</td>
</tr>
<tr>
<td>Zidovudine pretreated</td>
<td>42</td>
<td>19–66</td>
<td>17</td>
<td>6–30</td>
</tr>
<tr>
<td>Zidovudine naive</td>
<td>1</td>
<td></td>
<td>17</td>
<td>6–30</td>
</tr>
<tr>
<td>Zidovudine and 3TC only</td>
<td>32</td>
<td></td>
<td>17</td>
<td>6–30</td>
</tr>
<tr>
<td>Other nucleoside analogues</td>
<td>11</td>
<td></td>
<td>17</td>
<td>6–30</td>
</tr>
<tr>
<td>NNRTI</td>
<td>13</td>
<td></td>
<td>17</td>
<td>6–30</td>
</tr>
<tr>
<td>CDC clinical stage at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7</td>
<td>233–301</td>
<td>22</td>
<td>0–58</td>
</tr>
<tr>
<td>B</td>
<td>22</td>
<td>122–378</td>
<td>19</td>
<td>0–58</td>
</tr>
<tr>
<td>C</td>
<td>14</td>
<td>41–436</td>
<td>11</td>
<td>2–58</td>
</tr>
</tbody>
</table>

NOTE. NNRTI, nonnucleoside reverse transcriptase inhibitors.

\((P = .69)\). All samples with no or 1 mutation were phenotypically sensitive or hypersensitive to zidovudine. Phenotypic resistance in samples with two zidovudine resistance–associated mutations ranged between <10-fold and >100-fold. Figure 1B shows the level of phenotypic resistance in relation to the mutational pattern. Most isolates contained both the M41L and T215Y/F mutations; therefore, it was not possible to analyze the relationship between the presence of one or both of these mutations and phenotypic resistance. The level of zidovudine resistance in HIV-1 recombinants carrying both the 41 and 215 mutations ranged from 6-fold to 1062-fold. However, of 32 samples containing M41L + T215Y/F, 19 also carried the L210W mutation. The median level of zidovudine resistance was higher in the samples carrying all 3 mutations (median \log_{10} R = 2.16) compared with samples with mutations at position 41 and 215 but wild-type at position 210 (median \log_{10} R = 1.23). This difference was statistically significant \((P < .001)\).

No nonnucleoside reverse transcriptase inhibitor resistance-associated mutations known to influence phenotypic zidovudine resistance [45, 46] were detected.

Zidovudine resistance and response to zidovudine-3TC therapy. Of the 43 patients, 21 had clinical progression while receiving zidovudine-3TC, and 29 had therapy failure on the basis of CD4 cell count (for 2 patients, the baseline CD4 cell count was not available). Sixteen patients had therapy failure on the basis of both criteria.

We first examined the relationship between zidovudine phenotypic resistance and clinical progression (occurrence of new class B or C events or progression from class A to B and from class B to C), as shown in figure 2. Resistance to zidovudine in patients who were in category A at baseline (start of 3TC therapy) and remained in this category until the end of the follow-up period ranged from \(-0.4\) to \(0.95\) \log_{10} R, with a median \log_{10} R value of 0 (or 1-fold). Conversely, progression from CDC category A to B was associated with a median \log_{10} R of 1.89 \((P = .08)\). Similarly, resistance in patients who started in category B and remained there without experiencing new class B events was lower than in patients who progressed from category B to category C (medians, 1.15 and 2.08, respectively, \(P = .007)\). A difference was also seen in the median resistance in patients who were class B at baseline and who remained there but had new class B events compared with those without new class B events (median \log_{10} R 2.2 vs. 1.15, \(P = .02)\) and in patients who were class C at baseline who had new class C events compared with those without new class C events, although the latter difference was not statistically significant (median \log_{10} R 2.24 vs. 2.09, \(P = .30)\).

Table 2. Baseline characteristics of HIV-1–infected patients divided into 3 groups on the basis of the level of HIV-1 resistance to zidovudine.

<table>
<thead>
<tr>
<th>Level of zidovudine resistance</th>
<th>n</th>
<th>Baseline CD4 cell count (median)</th>
<th>Baseline CDC classification</th>
<th>Total time receiving zidovudine (median, months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (&gt;100-fold)</td>
<td>21</td>
<td>63/mm³</td>
<td>1 A; 9 B; 11 C</td>
<td>29</td>
</tr>
<tr>
<td>Intermediate (10- to 100-fold)</td>
<td>13</td>
<td>164/mm³</td>
<td>1 A; 10 B; 2 C</td>
<td>40</td>
</tr>
<tr>
<td>Sensitive-low (&lt;10-fold)</td>
<td>9</td>
<td>233/mm³</td>
<td>5 A; 3 B; 1 C</td>
<td>53</td>
</tr>
</tbody>
</table>
Figure 1. A. Phenotypic zidovudine (ZDV) resistance and no. of mutations. Mutations include zidovudine resistance–associated changes at positions 41, 67, 70, 210, 215, and 219 of HIV-1 reverse transcriptase. Lines indicate median log_{10}-R values for each group. B. Phenotypic zidovudine resistance and mutational pattern. For positions 41 or 215: zidovudine resistance–associated amino acid substitutions at one of these positions of HIV-1 RT only; 41 + 215: zidovudine resistance–associated amino acid substitutions at both of these positions but not at position 210; 41 + 210 + 215: zidovudine resistance–associated amino acid substitutions at all 3 positions. In all cases, changes at other positions not included in legend may have occurred. Lines indicate median log_{10}-R values for each group. (P < .001, Wilcoxon 2-sample test for 41 + 215 vs. 41 + 210 + 215).
We next analyzed the number of clinical events during follow-up as a function of zidovudine resistance (figure 3). The analysis shows that no events occurred in patients with a zidovudine resistance value <10-fold. In contrast, a total of 66 events per 21 patients (39 CDC class B and 27 CDC class C events) had occurred in the 21 patients with high-level zidovudine resistance. The intermediate-level group (13 patients) had a total of 10 clinical events (5 CDC class B and 5 CDC class C). The difference in the number of clinical events was statistically significant ($P < .001$, Kruskal-Wallis test).

The actual levels of zidovudine resistance per sample and the patient’s response to zidovudine-3TC treatment, defined as a composite measure of clinical and CD4 cell count responses, are illustrated in figure 4. The median log10-R for patients experiencing clinical progression and a negative CD4 curve (therapy response category FF) was 2.12 (range, 1.15–3.03), whereas the median log10-R for patients remaining clinically stable with a CD4 cell count still above baseline (therapy response category RR) was 0 (range, −0.7 to 1.67). Patients who had one of the two failure-defining criteria showed a median resistance value of 2.34 (therapy response category FR) and 1.23 (therapy response category RF). In the FF group, 4 patients had samples with intermediate-level zidovudine resistance, and 13 had samples with high-level zidovudine resistance. In contrast, samples from patients in the RR group did not yield any recombinant viruses with high-level zidovudine resistance. In this group, the majority (6/9) had samples with sensitive or low-level zidovudine resistance samples. The association of phenotypic resistance with therapy response was highly statistically significant ($P < .001$, Kruskal-Wallis nonparametric analysis).

Baseline plasma virus load data were not available for this patient population. However, we were able to determine the virus load for the time point at which the sample for resistance testing was taken for 41 of the 43 patients. Figure 5 shows that a statistically significant correlation exists between virus load at time of sampling and zidovudine resistance ($R = .59$, $P < .001$, Spearman’s rank correlation coefficient).

The association of resistance, baseline CD4 cell count, baseline CDC category, virus load, and total time receiving zidovudine treatment with therapy response (clinical plus CD4 cell count) was analyzed statistically using a logistic regression model with a three-level response variable (based on response categories FF, FR+RF, and RR, as defined for figure 4). The results of a univariate analysis are listed in table 3 (column 1,
unadjusted). The level of phenotypic resistance and the extent of genotypic changes associated with zidovudine resistance were both highly significantly associated with therapy response, with relative odds of a better response of 6.59 (confidence interval [CI], 2.63–16.6, \( P < .001 \)) and 2.03 (CI, 1.37–3.01, \( P < .001 \)), respectively. Virus load at sample time and the CD4 cell count at the start of zidovudine-3TC combination treatment were also statistically associated, with relative odds of 3.67 (CI, 1.65–8.20, \( P = .002 \)) and 2.23 (CI, 1.24–4.03, \( P = .008 \)), respectively. The association of baseline CDC classification with therapy response was not statistically significant (relative odds of 2.17; CI, 0.92–5.12, \( P = .08 \)).

A series of multivariate analyses was performed (table 3, columns 2–5). Columns 2 and 3 show that after adjusting for baseline CD4 cell count, baseline CDC category, virus load, and time receiving zidovudine, phenotypic resistance (column 2) and genotypic resistance (column 3) remained associated with therapy response at a statistically significant level, with relative odds of 6.00 (CI, 1.88–19.0, \( P = .002 \)) and 2.02 (CI, 1.21–3.35, \( P = .006 \)), respectively. In contrast, when adjusted for the other variables, baseline CD4 cell count and virus load were not associated with therapy response. Columns 4 and 5 show results of multivariate analyses, taking into consideration both types of resistance measurements. The association between therapeutic response and phenotypic resistance measurement remained statistically significant after adjusting for the extent of genotypic change and all other variables, with relative odds of 4.67 (CI, 1.09–19.9, \( P = .04 \)), whereas the number of genotypic changes was not associated with therapeutic response. Finally, as shown in column 5, an analysis including only the two types of resistance measurements demonstrated that phenotypic zidovudine resistance retains a highly significant association with therapeutic response, with relative odds of 5.84 (CI, 1.87–18.1, \( P = .003 \)) after adjustment for the extent of genotypic resistance, whereas genotypic resistance was not associated with therapeutic response after adjustment for phenotype. Similar results were obtained when the genotypic pattern (mutations at position 41–215 vs. 41–210 + 215 vs. other combinations) was used instead of number of mutations. The association of therapeutic response with phenotypic zidovudine resistance remained statistically significant (relative odds, 4.93, \( P = .003 \)), whereas the association with genotypic resistance was not significant (data not shown).

**Discussion**

In this cross-sectional analysis of phenotypic resistance to zidovudine and 3TC in plasma-derived recombinant HIV-1 from zidovudine-3TC–treated patients, we have demonstrated that dual resistance to these drugs can develop in vivo in zidov-
Therapy response and zidovudine (ZDV) resistance. FF = patients with class B or C clinical events and CD4 cell failure (for definition, see Materials and Methods) during follow-up; FR = patients with clinical events but CD4 cell response during follow-up; RF = patients with no clinical events but with CD4 cell failure during follow-up; RR = patients with no clinical events and CD4 cell response during follow-up. Lines indicate median log_{10} R values for each group. ($P < .001$, Kruskal-Wallis nonparametric analysis).

vudine-3TC–treated patients with therapy failure. None of the patients still responding to zidovudine-3TC therapy had high-level zidovudine resistance; conversely, none of the patients with both clinical and immunologic failure had zidovudine-sensitive isolates, but rather, 76% had highly resistant isolates.

Phenotypic resistance to zidovudine was associated with the presence of zidovudine-specific mutations; however, the range of phenotypic resistance was wide, and in samples with more than two zidovudine mutations, more mutations did not translate into a higher level of resistance.

Phenotypic resistance to zidovudine in 3TC-resistant isolates was significantly associated with clinical progression, with the number of clinical events occurring during follow-up, and with virus load at the time of sampling. On the basis of a composite measure of therapy response involving HIV-1–associated clinical events and immunologic response, both phenotypic and genotypic resistance to zidovudine were significantly associated with therapy failure, along with virus load and baseline CD4 cell count. Multivariate analysis showed that zidovudine resistance was the only significant factor associated with therapeutic response after adjustment for baseline CD4 cell count, baseline CDC status, plasma virus load, and time receiving zidovudine therapy in this zidovudine-3TC–treated patient population. There was no correlation between zidovudine resistance and the total time receiving zidovudine treatment.

Although the zidovudine resistance–associated genotype (based on changes at positions 41, 67, 70, 210, 215, and 219) was associated at a significant level with therapeutic response in univariate and multivariate analyses including the above variables, detection of resistance at the genotypic level lost significance after adjusting for the level of phenotypic resistance. In this last analysis, phenotypic zidovudine resistance was the only significant factor associated with therapeutic response.

These results derive from analysis of a small, primarily zidovudine-pretreated patient population. As this was neither a longitudinally followed nor a randomized patient population, no general conclusions as to the incidence of dual resistance during zidovudine-3TC therapy can be drawn. It will be of interest to examine the response to zidovudine-3TC–containing combination therapies in zidovudine-pretreated patients based on virus load and zidovudine resistance status at the start of 3TC therapy. For our patient population, however, baseline samples were not available for virologic investigations.
Figure 5. Zidovudine (ZVD) resistance and plasma HIV RNA. Virus load measurements were from plasma samples taken at end of follow-up period. ($R = .59$, $P < .001$, Spearman’s rank correlation coefficient).

Table 3. Association of therapy response with resistance, baseline CD4 cell counts, clinical stage, plasma HIV-RNA level, and time receiving zidovudine.

<table>
<thead>
<tr>
<th></th>
<th>Relative odds of better response (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted $n$ varies</td>
</tr>
<tr>
<td>Zidovudine resistance, phenotypic (per $1 \log_{10}$ lower)</td>
<td>6.59 (2.62–16.6)</td>
</tr>
<tr>
<td></td>
<td>$P &lt; .001$</td>
</tr>
<tr>
<td>Zidovudine resistance, genotypic (per 1 less mutation)</td>
<td>2.03 (1.37–3.01)</td>
</tr>
<tr>
<td></td>
<td>$P &lt; .001$</td>
</tr>
<tr>
<td>CD4 cell count at start of zidovudine/lamivudine therapy (per 100 cells/mm$^3$ higher count)</td>
<td>2.23 (1.24–4.03)</td>
</tr>
<tr>
<td></td>
<td>$P = .008$</td>
</tr>
<tr>
<td>CDC clinical stage at start of zidovudine/lamivudine therapy (per stage less advanced)</td>
<td>2.17 (0.92–5.17)</td>
</tr>
<tr>
<td></td>
<td>$P = .08$</td>
</tr>
<tr>
<td>Plasma HIV RNA at sampling time (per $1 \log_{10}$ lower)</td>
<td>3.67 (1.64–8.2)</td>
</tr>
<tr>
<td></td>
<td>$P = .002$</td>
</tr>
<tr>
<td>No. of months receiving zidovudine at sampling time</td>
<td>1.03 (0.99–1.06)</td>
</tr>
<tr>
<td></td>
<td>$P = .11$</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval.
In our study, an association of zidovudine resistance with the immediately preceding rather than subsequent therapeutic response was observed; therefore, the role of resistance in therapy failure cannot be defined. Nevertheless, we have shown that such an association is relevant for zidovudine-3TC therapy. Other studies have demonstrated an association of zidovudine resistance with subsequent clinical progression in patients treated with zidovudine monotherapy [34–40]. Although development of zidovudine resistance was correlated with baseline clinical status and CD4 cell count, the association of resistance and clinical development during zidovudine therapy was independent of these variables [35, 37, 38]. Zidovudine resistance in our population of patients with 3TC-resistant viruses was also associated with a lower clinical and immunologic status at baseline (in this case, start of 3TC therapy), while the response to therapy was associated with resistance independently of these baseline variables. In addition, phenotypic resistance to zidovudine as well as therapy response based on clinical and immunologic status were significantly associated with virus load at the end of the follow-up period, as would be expected [47–50].

On the basis of in vitro observations of mutational interactions, virus isolates containing zidovudine resistance–associated mutations at the six positions examined in this study would be expected to remain susceptible. This would imply that additional mutations must contribute to the development of dual resistance to zidovudine and 3TC. Preliminary data from sequence analysis of the complete reverse transcriptase coding region indicate that changes in other positions do occur with a higher frequency in dually resistant samples, and some of these have been shown to contribute to dual resistance by site-directed mutagenesis [51]. The significance and contribution of these changes to dual resistance and therapeutic response to zidovudine-3TC therapy are currently being investigated. A recent report, based on an analysis of 4 patients, found that a minimum of three zidovudine resistance–associated mutations were required for zidovudine resistance to develop in HIV-1 from 3TC-pretreated patients [52]. Our data indicate that in some cases, two of the well-characterized zidovudine mutations may be sufficient for high-level zidovudine resistance and point to the role of background genotype in determining the level of zidovudine resistance.

Using plasma-derived viral material, the incidence of M184V in HIV from 3TC-treated patients was 98% in the present study (95% in a previous study [14]). In only 1 of 54 samples from patients reportedly receiving 3TC therapy at the time of sampling did we detect 3TC susceptibility, albeit slightly decreased. Differences with other published incidences (e.g., 70% after 12 weeks of therapy [29]) may be due to methodologic differences and may reflect differences in kinetics of detectable resistance in proviral versus plasma RNA–derived material [13].

Reversal of zidovudine resistance by M184V and delay or inhibition of zidovudine resistance development have been documented in vitro and implicated in the 3TC-associated benefit of zidovudine-3TC combination therapy [14, 25, 26]. In the absence of baseline samples, we were not in a position to demonstrate M184V-mediated reversal of zidovudine resistance in this patient population. However, zidovudine susceptibility was observed in patients with zidovudine resistance–associated genomic changes. Although the ddI resistance–associated genotypic change at position 74 (Leu→Val) also has been shown to reverse zidovudine resistance in vitro [53], the incidence of this mutation under zidovudine-ddI therapy is not as frequent as the M184V under 3TC [54]. Furthermore, the ability of 74V to reverse zidovudine resistance is only minimal when mutations at both positions 41 and 215 are present [55].

Whether zidovudine resistance is associated with a poor prognosis when patients are switched to other combination therapies, such as triple combinations including protease inhibitors, remains to be determined. The ACTG 116B/117 trial demonstrated that high-level zidovudine resistance represented a relative risk of 1.74 (95% CI, 1.00–3.03) for clinical progression independent of a therapy switch to ddI monotherapy; on the other hand, a switch to ddI after a minimum of 16 weeks of zidovudine monotherapy was beneficial, independent of whether the isolates were highly resistant to zidovudine or not [37]. In that study, it may not have been possible to detect a relationship based on a more differentiated measurement of resistance. Similarly, in an accompanying mutational analysis [38], a significant association with clinical progression was found if the patients’ isolates contained mutations at positions 41 and 215 but not if a mutation was present at position 215 only, independent of whether patients continued to receive zidovudine monotherapy or were switched to ddI.

Other studies have shown an association between the presence of the T215F/Y mutation in isolates from zidovudine monotherapy–treated patients and a subsequent poor response to ddI [56]. An association between the level of phenotypic zidovudine resistance and cross-resistance to dideoxycytidine (ddC) and ddI has been reported [57]. In a study of M184V-mediated cross-resistance to ddC and ddI in zidovudine-3TC–treated patients, we have also observed an association of resistance to ddC and ddI with the level of phenotypic resistance to zidovudine [58]. These considerations may in part explain the “zidovudine resistance–independent” benefit of a switch to ddI in the ACTG 116B/117 trial as well as the treatment assignment–independent association of poor prognosis with zidovudine resistance. This would indicate that a certain level of zidovudine resistance would lead to poor therapeutic responses when patients are switched to combinations in which nucleoside analogues are the major component.

The methods for phenotypic resistance detection used in this study are based on direct amplification of circulating HIV-1 reverse transcriptase. The RVA allows a determination of resistance compared with wild-type on an individual sample level rather than population means or baseline samples. This methodology has several advantages over the classic peripheral blood mononuclear cell coculture-based assays: It measures the resistance associated with plasma-derived viral RNA rather than proviral RNA and therefore reflects the resistance kinetics.
of circulating HIV-1; the assay is not dependent on successful virus isolation by culture; selection due to culture is avoided; the use of a cell line affords greater standardization than the use of primary peripheral blood mononuclear cells; and the method is applicable for large-scale, high-throughput analysis. The RVA-HeLa CD4+ plaque-reduction assay has been compared directly with a standard peripheral blood mononuclear cell method, and it was found that the RVA was more sensitive, less variable, and better able to distinguish between fully resistant and partially resistant isolates [54].

Taken together with the data presented here, it would appear that phenotypic resistance measurements based on RVA systems are at present best suited to discern the clinical significance of zidovudine resistance. Whether this is true for resistance to other antiretroviral agents remains to be determined; however, in other situations in which mutational cross-talk may play a role, as in zidovudine-nonnucleoside reverse transcriptase inhibitor [45, 46] combinations, a phenotypic measurement may provide more relevant results than genotyping.

In summary, our results, based on a cross-sectional analysis with a relatively small sample size, indicate that a high level of phenotypic resistance to zidovudine is strongly correlated with zidovudine-3TC therapy failure. They also suggest that zidovudine resistance does not have to be high for an association with clinical progression to be evident. The prognostic resistance threshold under combination therapy remains to be determined. This analysis is exploratory, and the results need to be confirmed using a larger patient population from randomized clinical trials. It will be of interest to evaluate the impact of zidovudine resistance on the response to other combination therapies. Our results have implications for antiretroviral therapy strategies. Clearly, combinations that delay or inhibit the development of resistance should be strived for.

References


