Multiple Drug Resistance to Nucleoside Analogues and Nonnucleoside Reverse Transcriptase Inhibitors in an Efficiently Replicating Human Immunodeficiency Virus Type 1 Patient Strain

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A human immunodeficiency virus type 1 (HIV-1)-seropositive patient was treated sequentially with the dideoxynucleoside (ddN) analogues zidovudine, didanosine, zalcitabine, stavudine, and lamivudine and the nonnucleoside HIV-1-specific reverse transcriptase inhibitor (NNRTI) loviride (α-APA). Accumulation of drug resistance mutations (mainly V75I, F77L, K103N, F116Y, Q151M, and M184V) eventually resulted in a strain that was genotypically and phenotypically resistant to all tested ddNs and the majority of NNRTIs. However, the multidrug-resistant virus retained wild type sensitivities to drugs such as foscarnet, phosphonomethoxethyl adenine, dextran sulfate, JM3100, saquinavir, and NNRTI TSAO-m'T. Drug-resistant isolates showed replication kinetics and infectivity in an in vitro peripheral blood mononuclear cell system similar to those of the wild type isolate from the same patient. The multi-ddN-resistant isolate was not eliminated in a competition culture with the wild type isolate. Sequential therapy did not prevent the appearance of multidrug-resistant virus with a conserved replication rate.

Drug resistance due to specific point mutations in the reverse transcriptase (RT) gene of human immunodeficiency virus type 1 (HIV-1), has been described for most antiviral compounds shortly after their introduction into the clinic [1–3]. Sequential or combination therapy with multiple drugs was expected to overcome the emergence of resistance; however, cross-resistance between different dideoxynucleoside (ddN) analogues (e.g., didanosine, zalcitabine, lamivudine) or nonnucleoside HIV-1-specific RT inhibitors (NNRTI) soon appeared as a major limitation for drug combination strategies. It was then speculated that these cross-resistant strains with multiple amino acid substitutions in the RT protein would display reduced or even a lack of polymerase activity. This generated the idea of so-called convergent combination therapy, associating multiple drugs acting at the same viral target (reviewed in [4]). Recently, several investigators found a set of five new drug resistance mutations (A62V, V75I, F77L, F116Y, Q151M) emerging under combination therapy with either zidovudine plus didanosine or zidovudine plus zalcitabine and conferring a much broader resistance pattern, which has been referred to as multiple nucleoside analogue resistance [5–9].

Materials and Methods

Case report. In 1989, a 52-year-old HIV-1-seropositive patient with cutaneous Kaposi's sarcoma (KS) was treated with zidovudine (Retrovir; Glaxo-Wellcome, Dartford, UK), 200 mg every 8 h, and interferon (IFN-α) (Intron A; Schering-Plough, Innishannon, Ireland), 20 × 10⁶ U every 24 h. After 2 months, zidovudine had to be withdrawn because of severe anemia, and IFN-α was maintained alone at 20 × 10⁶ U twice a week. In January 1992 (blood sample L1), zalcitabine (Hivid; Roche, Brussels) was added to the IFN-α treatment but was stopped 4 months later because of neurologic toxicity. Didanosine (Videx; Bristol-Myers Squibb, Evansville, IN) was then initiated (sample L2).

In March 1993, IFN-α was stopped and the patient was switched to zidovudine-didanosine combination therapy. Again, because of hematologic toxicity after <2 months, anti-HIV treatment was interrupted. After a new short course of zalcitabine, the patient developed fever and was hospitalized with a CD4 cell count of 10/μL. Stavudine (Zerit; Bristol-Myers Squibb) was given together with liposomal daunorubicin for relapsing KS (sample L3, 2 weeks after the start of stavudine therapy). Six months later, stavudine was stopped because of neurologic toxicity, and loviride (α-anilinophenylacetamide, α-APA) was given in monotherapy for 2 months (sample L4) and then in combination with lamivudine (Epivir; Glaxo-Wellcome) (sample L5, 1 month after the start of lamivudine therapy). Samples L6 and L7 were obtained during follow-up in February and April 1995, respectively, while the pa-
Figure 1. Evolution of CD4 cell count and antiretroviral treatment (INF, interferon; AZT, zidovudine; ddC, zalcitabine; ddl, didanosine; d4T, stavudine; α-APA, loviride; 3TC, lamivudine) over time. L1–L7 indicate time points when blood samples were collected.
check for the presence of a sufficient quantity of DNA (810-bp band) and the absence of nonspecific bands.

Sequencing and sequence alignments were done as described [12]. To completely sequence both DNA strands, two internal fluoresceni-labeled sequencing primers, FITC-AV36 (sense, 5’-CAGTACTGGATGGGTGATG, nt 2868-2888 of HXB2) and FITC-AV44 (antisense, 5’-TACTAGGTATGGTAAATGCAGT, nt 2930-2951), were used in addition to the M13 sequencing primers.

**Viral cultures and phenotypic drug resistance.** p24 antigen in viral culture supernatant was determined (HIV p24 Core Profile ELISA; DuPont de Nemours, Dreieich, Germany) following the assay manufacturer’s recommendations. For several experiments, culture supernatant was ultracentrifuged before the p24 antigen assay and the supernatant (not virion-associated p24 antigen) discarded. The virus pellet-associated p24 antigen was considered a measurement of the number of virus particles in the sample. If necessary, virus stocks were diluted in 10-fold steps (up to 1/10,000) to remain in the linear range of the assay.

PBMC, MT2, MT4, C8166 (Medical Research Council [MRC], UK), MOLT-4 (clone 8), HUT-78, and CEM cells were infected at a high MOI (>1) with 4 virus isolates (L1, L2, L4, and L6). The amounts of p24 antigen in the culture supernatant and the supernatant of cytopathogenic effect (CPE) and syncytia were determined after 7 and 14 days.

Virus stocks from strains L1, L2, L4, and L6 were grown on phytohemagglutinin (PHA)-stimulated seronegative donor PBMC in RPMI 1640 containing 10 U/mL recombinant interleukin-2 (Boehringer Mannheim, Brussels), 15% heat-inactivated fetal calf serum, 2 mM L-glutamine, 2 μg/mL polybrene, and 50 μg/mL gentamicin. Culture supernatants were harvested after 7 days and ultracentrifuged at 90,000 g for 2 h; the virus pellet was assayed for p24 antigen. p24 antigen—normalized amounts of the virus stocks were used to acutely infect 10^6 seronegative PBMC (all from the same donor). p24 antigen level was determined in culture supernatant at days 4, 7, and 10. All tests were done in triplicate and repeated three times in independent experiments. Virus stock titration for infectivity (50% cell culture infective dose, CCIDso) was done according to AIDS Clinical Trial Group (ACTG) protocol [13].

Equal p24 antigen—normalized amounts of virus were used to acutely infect simultaneously 10^6 seronegative PBMC from the same donor with the following mixtures of wild type L1 and mutant virus (L4 or L6): 25% wild type/75% mutant, 50% wild type/50% mutant, and 75% wild type/25% mutant (a total of six mixtures). The cultures were maintained for up to 10 weeks with a weekly refeeding of fresh PHA-stimulated PBMC from a same donor. Virus stocks of isolates L1, L2, L4, and L6 were grown on C8166 cells and titrated for infectivity. The inhibitory effect of compounds on viral replication was monitored by the inhibition of virus-induced CPE in C8166 cells. Briefly, C8166 cells were resuspended at 3 X 10^5 cells/mL and infected with 1 of the patient isolates at 100 CCIDso/mL. Immediately after infection, 100 μL of the cell suspension was brought into each well of a flat-bottomed microtiter tray containing decreasing concentrations of each drug (triplicates of 5-fold dilutions from 250 to 0.00012 μg/mL). After 5 days of incubation at 37°C, the inhibition of CPE as seen microscopically was scored. Sensitivities to the antiviral compounds were calculated by the median effect equation and expressed as micromolar ICso. All tests were repeated two to five times in independent experiments on different days.

**Test compounds.** 8-chloro-4,5,6,7-tetrahydroimidazo[4,5,1­jk][1,4]-benzodiazepin-2(1H)-one (TIBO) derivative R86183 [14] and α-APA compound R89439 ([(+)-(S)-4,5,6,7-tetrahydro-8-chloro-5-methyl-6-(3-methyl-2-butyl)imidazo[4,5,1-jk][1,4]-benzodiazepin-2(1H)-thione] [15] were provided by K. Andries (Janssen Research Foundation, Beerse, Belgium). Nevirapine (Bl-987, 11-cyclopropyl-5,11-dihydro-4-methyl-6-furfurydipyr0[3,2- b:2',3',e]diazepin-6-one) [16] was from Boehringer Ingelheim (Ridgefield, CT) and HEPT (1-(2-hydroxyethoxymethyl)-6-(phenylthio)thymine) derivative MKC-422, formerly I-EBU (1-(ethoxy­methyl)-6-benzyl-5-isopropyluracil) [17], was a gift of M. Baba (Kagoshima University, Kagoshima, Japan). The bis(heterocycle)­piperazine (BHAP) derivative U-90152 (delavirdine) [18] was synthesized by R. Kirsch (Hoechst, Frankfurt, Germany), and the thio­carbocyanilide derivative UC-82 by chemists at Uniroyal Chemical (Guelph, Canada). The 2',5'-bis-(ter-butyldimethylsilyl)­3'-spiro-5'-(4' -amino-1',2'-oxathiole-2',2'-dioxide) pyrimidine nucleoside analogue TSAO-m'T [19, 20] was provided by M.-J. Camarasa (Instituto de Quimica Medica, Madrid). Dextran sulfate (M, 5000) [21, 22], didanosine (ddl, 2',3',di- deoxyinosine), zalcitabine (ddc, 2',3'-dideoxyctydine), stavudine (d4T, 2',3'-didehydro-2',3'-dideoxythymidine), and phosphono­formic acid (PFA, foscarinet) were obtained from Sigma (St. Louis). The bicyclam derivative JMI3100 (1,1-[1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] octahydrochloride dihydrate) [23] was synthesized at Johnson Matthey (West Chester, PA). Zidovudine (AZT, 3'-azido-3'-deoxythymidine) and lamivudine (3TC, 3'-deoxy-3'-thiocytidine) were obtained from Glaxo-Wellcome. Saquinavir (Ro-31-8959) was provided by N. Roberts (Roche Products, Welwyn Garden City, UK). The acyclic nucleoside analogues PMEA (GS393, 9-[2-phosphonomethoxyethyl]adenine), PMEDAP (9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine), and (R)PMPDAP (R-(9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine) were synthesized by A. Holy (Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague) [24, 25].

**Results.**

**Sequencing of the RT gene.** Table 1 summarizes the amino acid substitutions in the RT protein (aa 1–259) as deduced from the nucleotide sequence of 7 successive patient isolates (L1–L7) compared with the sequence of reference strain HXB2. In addition, all strains (L1–L7) contained the following mutations: A98S, I135T, and G196E. Strain L1 also had a mutation (E174L) that disappeared in the follow-up samples.

**Phenotypic drug resistance.** Isolates L1, L2, L4, and L6 were tested for their sensitivity to the ddN analogues, NNRTI compounds, and several experimental drugs (table 2). Isolate L1 behaved as does the wild type in its drug sensitivities. Isolate L2 had mainly wild type susceptibility, except for a 5-fold reduced sensitivity to zalcitabine. Isolate L4 could be considered multi-ddN-resistant. Isolate L6 had additionally acquired resistance to NNRTIs except for TSAO. The rather low ICso value for lamivudine (only a 25-fold increase) in
Table 1. Amino acid substitutions in the RT protein of successive patient HIV-1 isolates compared with the wild type sequence HXB2.

<table>
<thead>
<tr>
<th>Codon</th>
<th>Strain 35</th>
<th>62</th>
<th>68</th>
<th>69</th>
<th>70</th>
<th>75</th>
<th>77</th>
<th>103</th>
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<th>151</th>
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<td>T</td>
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<td>G</td>
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<td>Y</td>
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NOTE: aa sequence has been deduced from nt sequence of RT coding region (codon 1-259). A, alanine; F, phenylalanine; G, glycine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; Q, glutamine; S, serine; T, threonine; V, valine; Y, tyrosine.

isolate L6 is explained by the partial reversal of V184 to wild type M184 during the growth of the virus stock, as confirmed by sequencing of viral RNA amplified from the inoculum. All strains remained additionally sensitive to foscamet (IC_{50}: 10.5-52 mM), acyclic nucleoside phosphonates (PMEA, PMEDAP, and (R)PMPDAP; IC_{50}: 2.2-11 nM), dextran sulfate (M_r: 5000; IC_{50}: 0.016-1 nM), bicyclam derivative JM3100 (nC: 3.6-130 nM), and protease inhibitor saquinavir (IC_{50}: 4.8-14.3 nM).

Virus load. Plasma samples L5, L6, and L7 showed consistently high virus loads (6.18, 5.94, and 6.72 log RNA copies/mL, respectively).

Host range and syncytium-inducing capacity. All virus isolates showed a broad host cell range and could replicate in the following cells, as shown by p24 antigen production in the culture supernatant: PBMC, MT2, MT4, CEM, MOLT-4 (clone 8), HUT-78, and C8166. Virus-induced syncytia and CPE were detected in all cell lines except MT4, MOLT-4, and HUT-78.

Viral replication and infectivity. Figure 2 shows viral replication, as measured by p24 antigen in culture supernatant. No significant decrease in viral replication was noted for the mutant virus strains compared with the wild type strain. The infectivity of the resistant isolates (L4, L6) increased moderately compared with that of nonresistant preceding isolates L1 and L2 (table 3).

Viral competition cultures. Competition cultures between wild type L1 and multi-ddN-resistant L4 showed persistence of mutant virus and loss of wild type in the 3 cultures representing the three ratios of initial mixtures between L1 and L4 (25%, 50%, and 75% of L1) at 5 and 10 weeks of cultivation. On the other hand, for the competition between strains L1 and L6, we found only pure wild type virus after 5 weeks at all three ratios of the competition experiment. As a control, all virus isolates were also separately cultured for 4 weeks. Sequencing confirmed the persistence of all drug resistance mutations except for M184V in isolate L6.

Discussion

We report the progressive accumulation of multiple drug resistance mutations in the RT gene of successive HIV-I isolates from a patient under sequential anti-HIV drug therapy. This strain successively acquired not only mutations at codons 62, 75, 77, 116, and 151 but also the lamivudine resistance mutation, M184V, and the NNRTI resistance mutation, K103N. This resulted in severe phenotypic resistance to ddN analogues.

Table 2. Phenotypic sensitivity (IC_{50}) for different HIV-1 inhibitors (dideoxynucleoside analogues and nonnucleoside HIV-1-specific reverse transcriptase inhibitors).

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Zidovudine</th>
<th>Didanosine</th>
<th>Zalcitabine</th>
<th>Stavudine</th>
<th>Lamivudine</th>
<th>TIBO (R 86183)</th>
<th>Loviride (a-APA, R 89439)</th>
<th>Nevirapine (3R-RG-587)</th>
<th>TSAO-m^2T</th>
<th>Delavirdine (BHAP, L-90152)</th>
<th>MKC-422</th>
<th>UC-82</th>
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<tr>
<td>HIV-1_{100}</td>
<td>0.004 (0.003-0.005)</td>
<td>0.8 (0.5-0.8)</td>
<td>0.2 (0.2-0.2)</td>
<td>0.014 (0.014-0.043)</td>
<td>0.21 (0.21-0.28)</td>
<td>0.001 (0.001-0.003)</td>
<td>0.025 (0.013-0.023)</td>
<td>0.03 (0.03-0.038)</td>
<td>0.027 (0.027-0.027)</td>
<td>0.015 (0.009-0.022)</td>
<td>0.001 (0.001-0.001)</td>
<td>0.003 (0.001-0.004)</td>
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<td>L1</td>
<td>0.006 (0.004-0.012)</td>
<td>4.2 (2.2-4.2)</td>
<td>0.2 (0.1-0.2)</td>
<td>0.1 (0.1-0.14)</td>
<td>0.21 (0.14-0.28)</td>
<td>0.007 (0.005-0.026)</td>
<td>0.006 (0.007-0.016)</td>
<td>0.03 (0.03-0.038)</td>
<td>0.081 (0.047-0.081)</td>
<td>0.029 (0.022-0.058)</td>
<td>0.004 (0.004-0.005)</td>
<td>0.005 (0.004-0.007)</td>
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<tr>
<td>L2</td>
<td>0.009 (0.005-0.024)</td>
<td>4.2 (2.5-6.4)</td>
<td>0.9 (0.7-1.4)</td>
<td>0.2 (0.2-0.3)</td>
<td>0.07 (0.07-0.07)</td>
<td>0.020 (0.014-0.080)</td>
<td>0.091 (0.051-0.015)</td>
<td>0.15 (0.12-0.15)</td>
<td>0.135 (0.135-0.135)</td>
<td>0.145 (0.116-0.145)</td>
<td>0.011 (0.008-0.014)</td>
<td>0.027 (0.018-0.027)</td>
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<tr>
<td>L4</td>
<td>3.8 (2.2-18.7)</td>
<td>21.2 (2.1-42.3)</td>
<td>1.9 (1.4-2.4)</td>
<td>1.0 (1.0-1.0)</td>
<td>1.7 (1.7-1.7)</td>
<td>0.050 (0.037-0.087)</td>
<td>0.114 (0.080-0.342)</td>
<td>0.45 (0.30-0.60)</td>
<td>0.400 (0.400-0.400)</td>
<td>0.44 (0.44-0.64)</td>
<td>0.016 (0.016-0.016)</td>
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<td>L6</td>
<td>18.7 (0.9-28.0)</td>
<td>21.2 (12.7-42.3)</td>
<td>3.3 (2.1-4.0)</td>
<td>1.0 (0.7-1.4)</td>
<td>5.2 (2.8-5.2)</td>
<td>0.62 (0.62-1.86)</td>
<td>8.55 (5.70-8.55)</td>
<td>7.59 (7.59-13.16)</td>
<td>0.270 (0.200-0.340)</td>
<td>6.52 (4.35-8.69)</td>
<td>10.15 (10.15-10.15)</td>
<td>10.15 (10.15-10.15)</td>
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</table>

NOTE: Data (in µM) are median (25th-75th percentiles) of 2-5 independent tests done in triplicate.
Figure 2. Viral replication kinetics in peripheral blood mononuclear cells for 4 successive patient virus isolates as measured by p24 antigen production in culture supernatants. Data are representative of 3 independent experiments.

and most NNRTIs. Commonly detected mutations for resistance to zidovudine, didanosine, and zalcitabine at codons 41, 65, 67, 70, 74, 215, or 219 were not found in the isolates from our patient.

The Q151M substitution, which appeared first in this patient (L2), is thought to be crucial for the subsequent development of multiple ddN analogue resistance [7, 8]. Recent structural data on the HIV-1 RT protein locate the highly conserved Q151 residue at a region called the template grip, which is responsible for the correct template-primer orientation and the substrate dNTP binding during polymerization [26, 27]. In contrast to other cases of multi-ddN analogue resistance, our patient had not received combination therapy but only sequential drug treatment directed against the RT at the time Q151M or mutations V751, F77L, and F116Y emerged. Thus, multiple ddN analogue resistance is not restricted to patients receiving combination therapy but may also be found in patients getting sequential drug treatment.

Q151M alone did not cause significant phenotypic resistance to ddN analogues, except for zalcitabine (5-fold increase of IC\textsubscript{50}). Shirasaka et al. [5] recently showed by site-directed mutagenesis that Q151M alone produced a moderate 10-fold increase in IC\textsubscript{50} to zidovudine, 5-fold to didanosine, and 22-fold to zalcitabine when tested in a MOLT-4 cell system and that high-level resistance occurred only in the presence of mutations V75I, F77L, and F116Y. Discrepancies with our results may be due to the different cell system used for drug testing [28] or to a different genetic background in which the mutations appeared. Mutation A62V, often reported in association with this new set of multi-ddN resistance mutations, was detected in our patient only transiently as a mixture with wild type virus. The same mutation has been described in the presence of the five classical zidovudine resistance mutations [29].

In addition to the multi-ddN resistance mutations, the virus, with lamivudine treatment, acquired the resistance mutation M184V, first as a mixture, then as a pure virus population after only 1 month of \(\alpha\)-APA/lamivudine treatment. Under continuous lamivudine treatment, mutation M184V was confirmed in several follow-up samples (L5–L7); however, when grown in vitro in the absence of drugs, isolate L6 reverted to wild type at codon 184 after only a few days. In contrast to the antagonism found between M184V and T215Y/F, which results in phenotypically sensitive strains to zidovudine [30], no phenotypic reversal of zidovudine resistance was found in our patient’s isolates containing the M184V mutation.

Under in vivo treatment with \(\alpha\)-APA, the NNRTI resistance mutation, K103N, was additionally acquired after the M184V mutation was already present. In our case, the presence of Val at position 184 did not prevent the acquisition of the NNRTI resistance mutation, as might have been expected from a recent report [31]. In vivo and in vitro selection of K103N has been reported for several NNRTI compounds, and the relevance of this mutation for resistance has been confirmed by site-directed mutagenesis [32]. Together with K103N, the K70S mutation appeared. To our knowledge, the K70S mutation has not yet been described. It is different from mutation K70R, responsible for low-level zidovudine resistance [33], and its relevance for resistance has to be further investigated.

Several other mutations were found in the RT gene, most having been described sporadically in isolates from treated patients. V35I and I202V are commonly seen in zidovudine-treated patients. I135T, G196E, and R211G occur naturally in some subgroups of HIV-1. Mutation at codon 98 has been linked to NNRTI resistance, but the usual substitution is A98G and not A98S, as in our patient. S68G has been reported increasingly in patients treated with ddN analogues [29] (unpublished data). 1178M has been detected in 2 patients and linked

<table>
<thead>
<tr>
<th>Strain</th>
<th>Infectious titer (CCID\textsubscript{50} (\times 10^6)/mL)</th>
<th>p24 antigen (ng/mL)</th>
<th>RNA (log\textsubscript{10} copies/mL)</th>
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<tr>
<td>L1</td>
<td>213 ± 177</td>
<td>219 ± 195</td>
<td>9.73</td>
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<tr>
<td>L2</td>
<td>84 ± 27</td>
<td>296 ± 160</td>
<td>9.48</td>
</tr>
<tr>
<td>L4</td>
<td>570 ± 137</td>
<td>88 ± 58</td>
<td>7.88</td>
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<tr>
<td>L6</td>
<td>850 ± 282</td>
<td>133 ± 35</td>
<td>9.36</td>
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NOTE: Values for 50% cell culture infective dose (CCID\textsubscript{50}) and p24 antigen are averages for 3 independent tests ± SDs.
to a rapid decline in CD4 cell counts [34]. For all of these mutations, the relevance for drug resistance remains obscure.

Phenotypic drug resistance assays confirmed the genotypically predicted drug resistance patterns and allowed the study of experimental drugs. Treatment possibilities are limited in multidrug-resistant strains. All the Food and Drug Administration (FDA)–approved ddi analogues (i.e., zidovudine, didanosine, zalcitabine, stavudine, and lamivudine) were highly inefficient. The virus also showed high-level resistance to most NNRTIs, except for the TSAO-m3T compound (∼10-fold). Balzarini et al. [19] have shown that sensitivity to TSAO derivatives is not affected by the presence of K103N and that resistance to TSAO is related to a specific mutation (E138K). It should also be mentioned that the thiocarbamidoxime derivative UC-82 and TIBO kept antiviral activity in the nanomolar range. Of interest, the virus remained highly sensitive to the pyrophosphate analogue foscarnet and to the acyclic nucleoside phosphonates PMEA, PMEDAP, and (R)PMPDAP. These derivatives, as well as the bicyclic JM3100 or the protease inhibitors, may be considered prime candidate compounds to be included in a treatment schedule for multiply resistant virus strains.

Our patient had a very high virus load (≥6 log RNA copies/mL). Moreover, the successive isolates replicated efficiently in several T cell lines and had syncytium-inducing capacities. These circumstances might predispose to the accumulation of drug resistance mutations, as has been shown for zidovudine resistance [35]. Viral replication kinetics showed that our patient’s multidrug-resistant isolates retained a normal replication capacity despite the accumulation of multiple mutations in the RT enzyme. This is consistent with a recent report showing conserved replication in a recombinant virus with the same set of multi-ddN resistance mutations [8]. Moreover, our results suggest that mutant strains acquired during drug treatment can have a higher infectivity than the wild type strain. This may be due to mutations outside the RT gene. Finally, competition cultures between mutant strains and wild type strains, probably a more sensitive assay for replication abilities, showed fast elimination of the L6 isolates by the wild type in all cultures. In contrast, the multi-ddN–resistant isolate (L4) persisted in all cultures after 10 weeks. Globally, these results may be interpreted as an argument against the theory that multiply mutated virus will have serious selective disadvantages compared with wild type strains.

Our data, although limited to a single case, suggest that HIV-1 can circumvent drug treatment by acquiring concomitant drug resistance mutations resulting in phenotypic resistance to multiple ddi analogues and NRTI compounds without a reduction in virus infectivity or replication rate. The multidrug-resistant strains described here were acquired during sequential therapy, arguing against this strategy. Whether these mutations have a chance to arise simultaneously during combination therapies that start with concurrent treatment with multiple drugs remains to be investigated.

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References


