NOTES

The Nucleoside Analog-Resistant E89G Mutant of Human Immunodeficiency Virus Type 1 Reverse Transcriptase Displays a Broader Cross-Resistance That Extends to Nonnucleoside Inhibitors

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The alteration of a glutamic acid (E) to a glycine (G) amino acid residue at position 89 (E89G alteration) in the human immunodeficiency virus type 1 reverse transcriptase confers decreased susceptibility to several nucleoside analog inhibitors. Because the nonnucleoside inhibitor-binding pocket is adjacent to the deoxynucleoside triphosphate substrate-binding site, the impact of the E89G alteration on susceptibility to nonnucleoside inhibitors was studied. Our results indicate that E89G reverse transcriptase has decreased susceptibility to TIBO R82150, nevirapine, and to a lesser extent, delavirdine. Human immunodeficiency viruses bearing the same mutation displayed decreased susceptibility to inhibition by these compounds in a cell culture virus replication assay.

A majority of the approved drugs currently used in anti-human immunodeficiency virus (anti-HIV) therapy are structural analogs of deoxynucleosides directed against the viral reverse transcriptase (RT) (21, 22). These include 3'-azido, 2',3'-dideoxythymidine (AZT; zidovudine), ddI, ddC, 2',3'-dideoxy, 2',3'-didehydrothymidine, and the (–)-enantiomer of 2',3'-dideoxy,3'-thiacytidine (3TC). These inhibitors exert their action by competing with the deoxynucleoside triphosphate (dNTP) substrate as well as by chain termination (6). A second class of anti-RT inhibitors, collectively termed non-nucleoside inhibitors (NNIs), has also been identified. These compounds are structurally dissimilar, noncompetitive, and known to interfere with polymerization via binding to a hydrophobic pocket adjacent to the active site (15, 26, 31).

Monotherapy with both types of inhibitors leads to the appearance of resistant variants of HIV containing single or multiple mutations in RT, limiting the clinical effectiveness of these drugs. For example, treatment with AZT leads to the appearance of mutations consisting of a threonine to a tyrosine amino acid residue change at position 215 (T215Y), M41L, D67N, K70R, and K219Q which confer decreased susceptibility to AZT (16, 17); treatment with 3TC results in the accumulation of a variant with an M184V substitution which confers resistance to 3TC (4, 8, 34); M184V and L74V alterations can each confer decreased susceptibility to ddI (9, 32); mutations T69D and K65R result in decreased susceptibility to ddC (7, 10). Similarly, mutants with decreased susceptibilities to various NNIs have been isolated, including Y181C, K103N, E138K, and L236P (1–3, 24, 25).

Recently, there has been an emphasis on multidrug combination therapies. Among the clinical trials in progress, several involve combinations of nucleoside analogs and NNIs. Although their modes of inhibition are different, the physical proximities of their binding sites raise a question as to whether mutations that cause resistance to both types of inhibitors can arise. Since no such mutations have been reported, we wished to determine if an existing RT mutant known to possess decreased susceptibility to one class of compounds might also display decreased susceptibility to the other class. The RT of the E89G variant that we isolated previously (23) displays cross-resistance to all four standard dNTPs, AZT triphosphate (AZTTP), and phosphonoformic acid (foscarnet). Furthermore, in viruses selected for growth under 3TC selection in vitro (8) as well as those from patients treated with 3TC, the E89G mutation was found to coexist with the M184V mutation (35). Here we report for the first time that the RT of the E89G variant, a nucleoside analog-resistant RT, displays decreased susceptibility to NNIs.

RT assays were performed with purified preparations of recombinant HIV type 1 (HIV-1) RT to quantitate polymerase activity in the presence or absence of each of three NNIs, TIBO R82150, nevirapine, and delavirdine. The bacterial expression and purification of both wild-type and E89G RTs have been described previously (14). Increasing concentrations of NNIs were added to standard HIV-1 RT reaction mixtures (volume, 25 μ l) containing 50 mM Tris $\cdot$ Cl (pH 8.0), 10 mM dithiothreitol, 80 mM KCl, 6 mM MgCl₂, 1 μ M poly(rC) $\cdot$ oligo(dG) 3' ends (Pharmacia Biochemicals), and 12 μ M [α -³²P] dGTP (2.7 Ci/mmol). The reactions were initiated by the addition of 25 ng of wild-type or E89G RT heterodimers and were allowed to proceed for 15 min. The incorporation of dGMP into the DNA product was measured by spotting 10 μ l of the reaction mixture in duplicate onto a DEAE cellulose

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TABLE 1. In vitro susceptibilities of wild-type and E89G mutant RTs to NNIs and AZTTP in biochemical assays

RT	IC ₅₀ (μM) ^a			
	Delavirdine	Nevirapine	TIBO R82150	AZTTP
Wild type	0.26 ± 0.005	0.1 ± 0.01	0.5 ± 0.02	0.045 ± 0.005
E89G	0.56 ± 0.05	0.9 ± 0.03	5.0 ± 0.1	0.315 ± 0.005

^a The values represent the means of three separate determinations of the IC₅₀s in all cases except for delavirdine (two times), and standard deviations are indicated. Biochemical assays were performed as described previously (14).

filter (DE-81; Whatman), washing three times with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and detecting the radioactivity incorporated by scintillation counting. The 50% inhibitory concentrations (IC₅₀s) were derived by plotting the numbers of picomoles of dGMP incorporated into the DNA product at increasing concentrations of the inhibitor. Table 1 provides the IC₅₀s obtained for each of the NNIs tested for wild-type and E89G RTs. Interestingly, the E89G RT showed a significant decrease (~10-fold) in susceptibility to both nevirapine and TIBO compared with that of the wild-type enzyme. In contrast, when tested for susceptibility to delavirdine, E89G displayed only a 2.2-fold decrease compared with that of the wild-type enzyme. To compare the susceptibility of the E89G RT to NNI and nucleoside analogs, we also measured the IC₅₀s of AZTTP using the same reaction conditions described above, except that the template primer was poly(rA)·oligo(dT) (Pharmacia Biochemicals). Our results showed that E89G RT was sevenfold less susceptible to AZTTP than the wild-type RT.

Next, we examined whether the E89G mutation, when introduced into a replicating HIV isolate, would also confer resistance to NNIs. High-titer HXB2 wild-type and HXB2 E89G viruses were generated by growth in MT4 cells. These virus supernatants were passaged twice in fresh peripheral blood mononuclear cells (PBMCs) prior to determinations of drug susceptibilities. The susceptibility of each of these viruses to TIBO, nevirapine, and delavirdine was measured by a PBMC-based assay as described previously (13). Our results indicated that HXB2 E89G virus displays decreased susceptibility to each of the NNIs. The degree of reduction in susceptibility was three- to fourfold (Table 2). These results were reproducible in multiple experiments and indicated that the E89G alteration in RT conferred decreased susceptibility to NNIs. In contrast, an HIV-1 isolate that contained the M184V alteration, which is known to confer decreased susceptibilities to ddI, ddC, and 3TC, displayed minimal changes in its susceptibility to delavirdine and nevirapine and was hypersusceptible to TIBO (Table 2). Furthermore, HXB2 E89G virus dis-

played a 10-fold decrease in susceptibility to AZT compared with that of the wild-type virus. Thus, the E89G alteration leads to a decreased susceptibility to both NNIs and a nucleoside analog not only in enzymatic assays but also in cell culture virus replication assays.

The drug susceptibility data presented here reveal the range of cross-resistance to drugs, including both nucleoside and nonnucleoside analogs, conferred by a single mutation. Insights into the mechanism of resistance of the E89G variant RT to nucleoside analogs have come from both biochemical work on the mutant enzyme and X-ray crystallography studies of wild-type HIV-1 RT complexed with DNA (12). Structural studies revealed that the E89 residue is proximal to the dNTP-binding pocket and is located in the β5a strand, which forms the "template grip." An electron density map of the E89 residue shows that the side chain of glutamic acid is in contact with the template at the penultimate base pair of the template-primer duplex (Fig. 1 of reference 12). Alteration of the 89th residue could affect the interaction between β5a and the sugar-phosphate backbone of the template, causing template repositioning, which in turn induces subtle changes in the geometry of the dNTP-binding pocket. This hypothesis has been proposed by another group of investigators (5) and is supported by previous studies. In a kinetic study, E89G RT demonstrated an increase in *K_i* for ddTTP and an increase in *K_m* for dTTP (14). Site-directed mutagenesis of codon 89 combined with biochemical studies of the resultant mutant RTs revealed that the presence of some neutral and hydrophobic residues at the 89th position led to the greatest decrease in susceptibility to ddGTP (30). Drug selection during in vitro passages led to the isolation of a foscarnet-resistant variant of HIV that contained an E89K substitution in RT (33). In addition to E89, the flanking residue mutations W88S and V90A have been reported to confer decreased susceptibility to foscarnet and dNTP analogs, respectively (11, 20). This may suggest that these changes on β5a, like E89G, lead to nucleotide triphosphate analog resistance via alterations in their interactions with the template.

We observed that the fold increases in the IC₅₀s of NNIs observed for the E89G variant in the two assays were not identical. However, both assays revealed increases in IC₅₀s for the E89G variant. A dichotomy between the results of biochemical and virus replication assays has been observed for several other nucleoside analog resistance mutations. For example, the M184V substitution confers a 1,000-fold decrease in susceptibility to 3TC in cell culture virus replication assays (8), whereas in biochemical assays with the purified mutant RT, only a 40- to 50-fold decrease was observed (36). A second example can be found in studies of the influence of the L74V substitution on susceptibility to AZTTP. In in vitro studies of AZTTP inhibition of purified L74V mutant RT, Martin et al. (19) reported a fivefold decrease in susceptibility (an increase in the *K_i* value). However, St. Clair et al. (32) reported no change in susceptibility to AZT for HIV-1 variants containing the L74V substitution compared with that for wild-type HIV-1 isolates. Such a lack of correlation has not been observed for mutations that have so far been reported to confer decreased susceptibility to NNIs. All of those mutations, however, are located directly in the NNI-binding pocket, while E89G appears to affect susceptibility to NNIs in an indirect manner.

The results showing that the E89G mutation in HIV-1 RT confers resistance to AZT at the level of virus replication are in contrast to previous observations showing a lack of concordance between biochemical and cell culture viral replication assays for this mutant. In the original description of the E89G mutation, although decreased susceptibility to ddGTP was observed in biochemical reactions with the recombinant RT mu-

TABLE 2. Susceptibilities of wild-type and E89G mutant HIV strains to NNIs and AZT in a PBMC assay

Virus	IC ₅₀ (μg/ml) ^a			
	Delavirdine	Nevirapine	TIBO R82150	AZT
Wild type (HXB2)	0.01 ± 0.003	0.15 ± 0.03	0.05 ± 0.009	0.002 ± 0.0005
E89G	0.04 ± 0.01	0.44 ± 0.09	0.18 ± 0.02	0.02 ± 0.005
M184V	0.008 ± 0.002	0.2 ± 0.03	0.02 ± 0.0005	0.001 ± 0.0002

^a The values represent the means of three separate determinations of the IC₅₀s in all cases, and the standard deviations are indicated. PBMC assays were performed as described previously (13).

FIG. 1. The ribbon drawing of the catalytic site of HIV-1 RT p66 includes a portion of the template-primer (ribbons connected by vertical thin lines) that is near the dNTP-binding site and the NNI-binding pocket. The β 9- β 10, β 12- β 13, and β 6 strands form the hydrophobic, NNI-binding pocket. Of these, the β 9- β 10 hairpin also forms the primer grip. The template grip, which includes β 5a, contains a glutamic acid residue at position 89 (E89). Other residues involved in nucleoside analog resistance, M184, L74, and Q151, are also designated. Ribbons β 5a, β 6, β 9, β 10, β 12, and β 13 make up portions of the palm subdomain, and ribbons containing Q151 and L74 represent portions of the fingers subdomain. The figure was generated with the Quanta package (Molecular Simulations, Cambridge, Mass.). The coordinates were retrieved from the Brookhaven protein data bank, entry PDB1HMI (12). The 5'-terminal phosphate position was modeled on the basis of coordinates for type A DNA generated within the Quanta package.

tant, the virus derived from the HXB2 strain containing the E89G substitution displayed wild-type-like susceptibility to ddG (23). Similarly, mutations at the conserved residues of HIV-1 RT that lead to the loss of susceptibility to AZTTP in vitro did not confer a decrease in susceptibility to AZT when viruses bearing the same mutations were tested for resistance to AZT in cell culture (18). On the other hand, with respect to susceptibility to foscarnet, a concordance between the biochemical assays and cell culture virus replication assays was observed for E89G and the mutants described by Larder et al. (18) and Prasad et al. (23). We do not understand why susceptibility to AZT or AZTTP or to foscarnet is reproducible between the two assays, while susceptibility to ddG and ddGTP is not. Perhaps this is due to the different efficiencies at which ddG is converted to the active, triphosphorylated form in the target cells used. Alternately, this discrepancy may reflect differences in the sensitivities of the assays used.

Our results indicate that the E89G mutation, which is known to confer decreased susceptibility to nucleoside analogs, can also result in decreased susceptibility to NNIs. The mechanism by which the E89G alteration leads to decreased susceptibility to NNI analogs is not well understood. NNIs do not appear to affect initial template-primer binding but interfere with the catalytic event involving dNTP incorporation. The NNIs are thought to bind to a hydrophobic pocket formed by the beta strands β 9- β 10, β 12- β 13, and β 6, of which β 9 and β 10 form a part of the primer grip (Fig. 1). Since the β 5a (template grip) and β 9- β 10 (primer grip) make contact on either side of the template-primer duplex, it is possible that mutations in β 5a, through their ability to cause template repositioning, induce conformational changes in the NNI-binding pocket. The repositioning of the template-primer resulting from the E89G mu-

tation not only brings about changes in ddNTP binding but may also play an important role in NNI binding.

The E89G mutation belongs to a class of nucleoside analog resistance mutations that indirectly influence the dNTP-binding pocket via template-primer interactions. Other mutations of this type include L74V (32), Q151M (27-29), and possibly, M184V. In light of our results, it is possible that some of these mutations may also alter the susceptibility of HIV-1 RT to NNI compounds. Thus, it will be important to document the cross-resistance of mutants in each class to both types of inhibitors in view of the impact that such cross-resistance could have on the outcome of combination therapies.

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REFERENCES

1. Balzarini, J., A. Karlsson, M.-J. Perez-Perez, L. Vrang, J. Walbers, H. Zhang, B. Oberg, A.-M. Vandamme, M.-J. Camarasa, and E. De Clercq. 1993. HIV-1 specific reverse transcriptase inhibitors show differential activity against HIV-1 mutant strains containing different amino acid substitutions in the reverse transcriptase. *Virology* 192:246-253.
2. Balzarini, J., A. Karlsson, V. Sardana, E. Emini, M. J. Camarasa, and E. De Clercq. 1994. Human immunodeficiency virus 1 (HIV-1)-specific reverse transcriptase (RT) inhibitors may suppress the replication of specific drug-resistant (E138K) RT HIV-1 mutants or select for highly resistant (Y181C→C181I) RT HIV-1 mutants. *Proc. Natl. Acad. Sci. USA* 91:6599-6603.
3. Balzarini, J., A. Karlsson, A.-M. Vandamme, M.-J. Perez-Perez, H.

- Zhang, L. Vrang, B. Oberg, K. Backbro, T. Unge, A. San-Felix, S. Velazquez, M.-J. Camarasa, and E. De Clercq. 1993. Human immunodeficiency virus type 1 (HIV-1) strains selected for resistance against the HIV-1-specific [2',5'-bis-*O*-(tert-butylidimethylsilyl)-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide)]- β -D-pentofuranosyl (TSAO) nucleoside analogues retain sensitivity to HIV-1 specific nonnucleoside inhibitors. *Proc. Natl. Acad. Sci. USA* **90**:6952-6956.
4. Boucher, C. A. B., N. Cammack, P. Schipper, R. Schuurman, P. Rouse, M. A. Wainberg, and J. M. Cameron. 1993. High-level resistance to (-) enantiomeric 2'-deoxy-3'-thiacytidine in vitro is due to one amino acid substitution in the catalytic site of human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **37**:2231-2234.
5. Boyer, P. L., C. Tantillo, A. Jacobo-Molina, R. G. Nanni, J. Ding, E. Arnold, and S. H. Hughes. 1994. Sensitivity of wild-type human immunodeficiency virus type 1 reverse transcriptase to dideoxynucleotides depends on template length; the sensitivity of drug-resistant mutants does not. *Proc. Natl. Acad. Sci. USA* **91**:4882-4886.
6. Cheng, Y.-C., G. E. Dutschman, K. F. Bastow, M. G. Sarngadharan, and R. Y. C. Ting. 1987. Human immunodeficiency virus reverse transcriptase. *J. Biol. Chem.* **262**:2187-2189.
7. Fitzgibbon, J. E., R. M. Howell, C. A. Haberzettl, S. J. Sperber, D. J. Gocke, and D. T. Dubin. 1992. Human immunodeficiency virus type 1 *pol* gene mutations which cause decreased susceptibility to 2',3'-dideoxycytidine. *Antimicrob. Agents Chemother.* **36**:153-157.
8. Gao, Q., Z. Gu, M. A. Parniak, J. Cameron, N. Cammack, C. Boucher, and M. A. Wainberg. 1993. The same mutation that encodes low-level human immunodeficiency virus type 1 resistance to 2',3'-dideoxyinosine and 2',3'-dideoxycytidine confers high-level resistance to (-) enantiomer of the 2',3'-dideoxy-3'-thiacytidine. *Antimicrob. Agents Chemother.* **37**:1390-1392.
9. Gao, Q., Z. Gu, M. A. Parniak, X. Li, and M. A. Wainberg. 1992. In vitro selection of variants of human immunodeficiency virus type 1 resistant to 3'-azido-3'-deoxythymidine and 2',3'-dideoxyinosine. *J. Virol.* **66**:12-19.
10. Gu, Z., Q. Gao, H. Fang, H. Salomon, M. A. Parniak, E. Goldberg, J. Cameron, and M. A. Wainberg. 1994. Identification of a mutation at codon 65 in the IKKK motif of reverse transcriptase of human immunodeficiency virus resistant to 2',3'-dideoxycytidine and 2',3'-dideoxy-3'-thiacytidine. *Antimicrob. Agents Chemother.* **38**:275-280.
11. Im, G. J., E. Tramantano, C. J. Gonzalez, and Y. C. Cheng. 1993. Identification of an amino acid in the human immunodeficiency virus type 1 reverse transcriptase involved in pyrophosphate binding of antiviral nucleoside triphosphate analogs and phosphonoformate. *Biochem. Pharmacol.* **46**:2307-2313.
12. Jacobo-Molina, A., J. Ding, R. G. Nanni, A. D. J. Clark, X. Lu, C. Tantillo, R. L. Williams, G. Kamer, A. L. Ferris, P. Clark, A. Hizi, S. Hughes, and E. Arnold. 1993. Crystal structure of human immunodeficiency virus reverse transcriptase complexed with double stranded DNA at 3.0 Å resolution shows bent DNA. *Proc. Natl. Acad. Sci. USA* **90**:6320-6324.
13. Japour, A. J., D. L. Mayers, V. A. Johnson, D. R. Kuritzkes, L. A. Beckett, J. M. Arduino, J. Lane, R. J. Black, P. S. Reichelderfer, R. T. D'Aquila, and C. S. Crumpacker. 1993. Standardized peripheral blood mononuclear cell culture assay for determination of drug susceptibilities of clinical human immunodeficiency virus type 1 isolates. *Antimicrob. Agents Chemother.* **37**:1095-1101.
14. Kew, Y., Q. Song, and V. Prasad. 1994. Subunit selective mutagenesis of Glu89 residue in human immunodeficiency virus reverse transcriptase. *J. Biol. Chem.* **269**:15331-15336.
15. Kohlstaedt, L. A., J. Wang, J. M. Friedman, P. A. Rice, and T. A. Steitz. 1992. Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* **256**:1783-1790.
16. Larder, B. A., G. Darby, and D. D. Richman. 1989. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* **243**:1731-1734.
17. Larder, B. A., and S. D. Kemp. 1989. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). *Science* **246**:1155-1158.
18. Larder, B. A., S. D. Kemp, and D. J. M. Purifoy. 1989. Infectious potential of human immunodeficiency virus type I reverse transcriptase mutants with altered inhibitor sensitivity. *Proc. Natl. Acad. Sci. USA* **86**:4803-4807.
19. Martin, J. L., J. E. Wilson, R. L. Haynes, and P. A. Furman. 1993. Mechanism of human immunodeficiency virus type 1 to 2',3'-dideoxyinosine. *Proc. Natl. Acad. Sci. USA* **90**:6135-6139.
20. Mellors, J., H. Bazmi, R. Schinazi, B. Roy, Y. Hsiou, E. Arnold, J. Weir, and D. Mayers. 1995. Novel mutations in reverse transcriptase of human immunodeficiency virus type 1 reduce susceptibility to foscarnet in laboratory and clinical isolates. *Antimicrob. Agents Chemother.* **39**:1087-1092.
21. Mitsuya, H., and S. Broder. 1987. Strategies for antiviral therapy in AIDS. *Nature (London)* **325**:773-778.
22. Mitsuya, H., R. Yarchoan, and S. Broder. 1990. Molecular targets for AIDS therapy. *Science* **249**:1533-1544.
23. Prasad, V. R., I. Lowy, T. De Los Santos, L. Chiang, and S. P. Goff. 1991. Isolation and characterization of a dideoxyguanosine triphosphate-resistant HIV-1 reverse transcriptase expressed in bacteria. *Proc. Natl. Acad. Sci. USA* **88**:11363-11367.
24. Richman, D., C.-K. Shih, I. Lowy, J. Rose, P. Prodanovich, S. Goff, and J. Griffin. 1991. Human immunodeficiency virus type 1 mutants resistant to nonnucleoside inhibitors of reverse transcriptase arise in tissue culture. *Proc. Natl. Acad. Sci. USA* **88**:11241-11245.
25. Richman, D. D., D. Havlir, J. Corbell, D. Looney, C. Ignacio, S. A. Spector, J. Sullivan, S. Cheeseman, K. Barringer, D. Pauletti, C.-K. Shih, M. Myers, and J. Griffin. 1994. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. *J. Virol.* **68**:1660-1666.
26. Rittinger, K., G. Divita, and R. S. Goody. 1995. Human immunodeficiency virus reverse transcriptase substrate-induced conformational changes and the mechanism of inhibition by nonnucleoside inhibitors. *Proc. Natl. Acad. Sci. USA* **92**:8046-8049.
27. Shafer, R. W., M. J. Kozal, M. A. Winters, A. K. N. Iversen, D. A. Katzenstein, M. V. Ragnei, W. A. Meyer III, P. Gupta, S. Rasheed, R. Coombs, M. Katzman, S. Fiscus, and T. C. Merrigan. 1994. Combination therapy with zidovudine and didanosine selects for drug-resistant human immunodeficiency virus type 1 strains with unique patterns of *pol* gene mutations. *J. Infect. Dis.* **169**:722-729.
28. Shirasaka, T., M. F. Kavlick, T. Ueno, W. Gao, E. Kojima, M. L. Alcaide, S. Chokekijchai, B. M. Roy, E. Arnold, R. Yarchoan, and H. Mitsuya. 1995. Emergence of human immunodeficiency virus type 1 variants with resistance to multiple dideoxynucleosides in patients receiving therapy with dideoxynucleosides. *Proc. Natl. Acad. Sci. USA* **92**:2398-2402.
29. Shirasaka, T., R. Yarchoan, M. C. O'Brien, R. N. Husson, B. D. Anderson, E. Kojima, T. Shimada, S. Broder, and H. Mitsuya. 1993. Changes in drug sensitivity of human immunodeficiency virus type 1 during therapy with azidothymidine, dideoxycytidine, and dideoxyinosine: an *in vitro* comparative study. *Proc. Natl. Acad. Sci. USA* **90**:562-566.
30. Song, Q., G. Yang, S. P. Goff, and V. R. Prasad. 1992. Mutagenesis of Glu⁸⁹ residue in human immunodeficiency virus type 1 (HIV-1) and HIV-2 reverse transcriptases: effects on nucleoside analog resistance. *J. Virol.* **66**:7568-7571.
31. Spence, R. A., W. M. Kati, K. S. Anderson, and K. A. Johnson. 1995. Mechanism of inhibition of HIV-1 reverse transcriptase by nonnucleoside inhibitors. *Science* **267**:988-993.
32. St. Clair, M. H., J. L. Martin, G. Tudor-Williams, M. C. Bach, C. L. Vavro, D. M. King, P. Kellam, S. D. Kemp, and B. A. Larder. 1991. Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase. *Science* **253**:1557-1559.
33. Tachedjian, G., D. J. Hooker, A. D. Gursinghe, H. Bazmi, N. J. Deacon, J. Mellors, C. Birch, and J. Mills. 1995. Characterization of foscarnet-resistant strains of human immunodeficiency virus type 1. *Virology* **212**:58-68.
34. Tisdale, M., S. D. Kemp, N. R. Parry, and B. A. Larder. 1993. Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in YMDD region of reverse transcriptase. *Proc. Natl. Acad. Sci. USA* **90**:5653-5666.
35. Wainberg, M. A. Unpublished data.
36. Wainberg, M. A., et al. Unpublished data.