

NOTES

Synergistic Inhibition of Replication of Human Immunodeficiency Virus Type 1, Including That of a Zidovudine-Resistant Isolate, by Zidovudine and 2',3'-Dideoxycytidine In Vitro

JOSEPH J. ERON, JR.,^{1†*} VICTORIA A. JOHNSON,^{1‡} DEBRA P. MERRILL,¹ TING-CHAO CHOU,²
AND MARTIN S. HIRSCH¹

Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114,¹ and Laboratory of Biochemical Pharmacology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received 27 January 1992/Accepted 24 April 1992

The combination of zidovudine (AZT) and 2',3'-dideoxycytidine synergistically inhibits human immunodeficiency virus type 1 (HIV-1) replication in vitro with AZT-sensitive and AZT-resistant clinical isolates and HIV-1_{IIIB}. Synergy was determined by the median-effect principle and isobologram techniques. Cytotoxicity of the agents was not observed. Clinical trials are ongoing to define the combination's role in HIV-1 therapy.

Several nucleoside reverse transcriptase inhibitors, including zidovudine (AZT), 2',3'-dideoxycytidine (ddC), and 2',3'-dideoxyinosine (ddI), have activity against human immunodeficiency virus type 1 (HIV-1) replication in vitro (25-28) and in vivo (6, 9-11, 20, 24, 32, 34, 36). Nevertheless, progression of disease continues to occur despite treatment, and HIV-1 replication is incompletely suppressed by AZT monotherapy (9, 15). HIV-1 isolates resistant to AZT in in vitro assays (21) can be obtained from most patients receiving prolonged AZT therapy (30). Viruses with decreased susceptibility to ddI or ddC have been noted (7, 13, 31). Cross resistance to ddC has not been reported in viruses with AZT resistance mutations, and molecularly cloned viruses with ddC resistance were not cross resistant to AZT (13, 21, 31).

Combination antiretroviral therapy may prolong drug efficacy against HIV-1 (18, 27, 35) and may reduce the emergence of resistant viruses. Combination therapy may also help decrease toxicity if lower doses of each agent can be used and if their toxicities are largely not overlapping, as is the case with AZT and ddC (8, 23, 24). A preliminary phase I clinical trial of this combination has shown promising results (23).

We performed a series of experiments to define interactions between AZT and ddC on the inhibition of HIV-1 replication by using HIV-1_{IIIB} and clinical isolates that had previously been shown to exhibit AZT-sensitive and -resistant phenotypes in vitro (19). The resistant isolate contains reverse transcriptase mutations as the dominant genotype (19) at AZT resistance-conferring codons (i.e., codons 67, 70, 215, and 219) previously described (2, 21, 22).

(This paper was presented in part at The Seventh International Conference on AIDS, Florence, Italy, June 1991.)

H9 cells provided by R. C. Gallo (National Cancer Institute, Bethesda, Md.) (29) were maintained as described

previously (14). Peripheral blood mononuclear cells (PBMC) from individual HIV-1-seronegative donors were obtained by Ficoll-Hypaque density gradient centrifugation of heparinized venous blood for each experiment in which PBMC were used. Cells were treated with 10 µg of phytohemagglutinin (Difco Laboratories, Detroit, Mich.) per ml and propagated in medium supplemented with 10% interleukin 2 (Pharmacia Diagnostics, Silver Springs, Md.) as previously described (19). HIV-1_{IIIB} was provided by R. C. Gallo (29) and propagated in H9 cells; the 50% tissue culture infectious dose per milliliter of cell-free supernatant was determined as described previously (14, 17). Paired HIV-1 clinical isolates were derived from an HIV-1-seropositive individual before (14a-4/87) and after (14a-6/89) 26 months of AZT monotherapy and were propagated and titrated as previously described (19).

AZT was obtained from P. A. Furman (Wellcome Research Laboratories, Research Triangle Park, N.C.), and ddC was obtained from I. Sim (Hoffmann-La Roche, Nutley, N.J.).

Three pairs of experiments were performed (Table 1). In experiments 1 and 2, 0.4×10^6 H9 cells per ml of medium were exposed to 500 50% tissue culture infective doses of HIV-1_{IIIB} as inoculum per 10^6 cells in flasks containing either single agents or the combination of AZT and ddC. In experiments 3 through 6, 10^6 uninfected 4-day phytohemagglutinin stimulated PBMC per ml of medium were exposed to 1,000 50% tissue culture infective doses of the HIV-1 isolate (either 14a-4/87 [experiments 3 and 4] or 14a-6/89 [experiments 5 and 6]) per 10^6 cells in flasks containing drug. Untreated infected controls were maintained in duplicate, and drug-containing flasks were maintained in singleton. Uninfected drug-treated toxicity controls were maintained. Culture medium was changed twice weekly, maintaining original drug concentration(s). At medium changes, cell-free culture supernatant fluids were harvested for HIV-1 p24 antigen production starting on day 7 in culture. Cell proliferation and viability were assessed by the trypan blue dye exclusion method. Cell-free culture supernatant fluids were assayed by an HIV-1 p24 antigen enzyme-linked immuno-

* Corresponding author.

† Present address: Division of Infectious Diseases, University of North Carolina, Chapel Hill, NC 27599.

‡ Present address: Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL 35294.

TABLE 1. CIs for two-drug combination regimens of AZT and ddC against HIV-1 replication in vitro^a

Expt	Concn (μM) of:		HIV-1 isolate	p24 antigen assay day	CI at HIV-1 inhibition of:			
	AZT	ddC			50%	75%	90%	95%
1	0.04, 0.16, 0.64, 2.56	0.00125, 0.005, 0.02, 0.08	HIV-1 _{IIIB}	11	0.83	0.64	0.50	0.43
2	0.04, 0.16, 0.64, 2.56	0.00125, 0.005, 0.02, 0.08	HIV-1 _{IIIB}	10	0.59	0.46	0.38	0.35
3	0.0025, 0.005, 0.01, 0.02	0.02, 0.04, 0.08, 0.16	14a-4/87	7	0.91	0.67	0.50	0.41
4	0.0025, 0.005, 0.01, 0.02	0.02, 0.04, 0.08, 0.16	14a-4/87	7	0.84	0.65	0.50	0.42
5	0.125, 0.25, 0.5, 1.0	0.02, 0.04, 0.08, 0.16	14a-6/89	10	0.24	0.23	0.25	0.28
6	0.125, 0.25, 0.5, 1.0	0.02, 0.04, 0.08, 0.16	14a-6/89	7	0.61	0.48	0.38	0.32

^a In all experiments the combination flasks used a fixed ratio of the single-agent concentrations. Using the data generated in each experiment, we obtained median-effect curves through computer estimation for each agent and the combination of agents. For each agent and the combination, median-effect curve parameters m (the slope) and D_m , or IC_{50} (the x intercept) defined the shape of the curve over its entire length. These values were used to determine CIs by using the mutually exclusive CI equation. CIs of <1 , 1 , and >1 indicate synergism, additive effects, and antagonism, respectively (3-5, 14).

sorbent assay (NEN Research Products, Boston, Mass.) as previously described (16).

In each experiment, the dose-effect data for each single agent and the combination were used to plot a median-effect curve by computer estimation (3). These curves, which are log-log plots, are defined by the slope (m) and the x intercept (D_m , or 50% inhibitory concentration [IC_{50}]). These values, m and D_m , are used for a computerized calculation of the combination index (CI) (Table 1) as described previously (3-5, 14). CI of <1 , 1 , and >1 indicate synergism, additive effects, and antagonism, respectively. The data were also analyzed by computer-generated classical isobolograms, which evaluate drug interactions by a dose-oriented geometric method. Data were subjected to quantitative computer analysis only if the linear correlation coefficient of the median-effect plot was high ($r \geq 0.75$).

In experiments 1 and 2 performed on H9 cells, AZT and ddC as single agents inhibited HIV-1_{IIIB} p24 antigen production in a dose-dependent manner (data not shown). The combination of agents demonstrated greater inhibition of p24 antigen production at all concentrations at which the single agents caused at least a 50% reduction of p24 antigen relative to that in the untreated infected control. At concentrations at which ddC had no effect, the effect of the combination in reducing HIV-1_{IIIB} p24 antigen production was variable, and at two such datum points (one in experiment 1 and one in experiment 2), the combination resulted in less inhibition than AZT alone caused (data not shown). Median-effect plots showed good correlation with the dose-effect data, with linear correlation coefficients (r) of ≥ 0.9 for each drug treatment condition except AZT alone and ddC alone in experiment 1, where r values were 0.76 and 0.84, respectively. Combination indices from both experiments, calculated at the 50, 75, 90, and 95% effective doses, all determined synergy of the combination ($CI < 1$) (Table 1).

In experiments 3 and 4, the AZT-sensitive clinical isolate, 14a-4/87, was used to infect HIV-1-seronegative PBMC. In both experiments, each single agent inhibited HIV-1 replication in a dose-dependent manner. The isolate demonstrated an AZT-sensitive phenotype (IC_{50} s of 0.008 and 0.01 μM). At all drug concentrations tested in both experiments, the combination of AZT and ddC more effectively suppressed p24 antigen production than either agent alone. Representative data from experiment 4 are shown in Fig. 1. The median-effect curves generated from the raw dose-effect data had uniformly high correlation coefficients (r values). The combination indices indicated synergy ($CI < 1$) at the 50, 75, 90, and 95% levels of drug effectiveness in both experiments (Table 1).

In experiments 5 and 6, the AZT-resistant clinical isolate,

14a-6/89 (19), was used. IC_{50} s for AZT in these experiments were 0.89 and >1 μM , respectively, i.e., 100- to 1,000-fold higher than in the experiments in which the AZT-sensitive isolate was used. In contrast, ddC showed similar efficacy against both the AZT-sensitive (IC_{50} s = 0.034 and 0.063 μM) and the AZT-resistant (IC_{50} s = 0.063 and 0.028 μM) isolates. As in experiments 3 and 4, the combination suppressed HIV-1 replication more effectively than either agent alone at all concentrations tested. Representative data from experiment 6 are shown in Fig. 2. Computer-generated median-effect curves showed good correlation with the actual dose-effect data for each of the drug treatment conditions. Again, CIs at 50, 75, 90, and 95% effective doses in both experiments were all <1 , indicating synergism (Table 1).

Synergistic inhibition of HIV-1 replication by AZT and ddC was also demonstrated in the six experiments using computer-generated dose-oriented classical isobolograms (data not shown).

No cytotoxicity of the combination was seen; in each pair

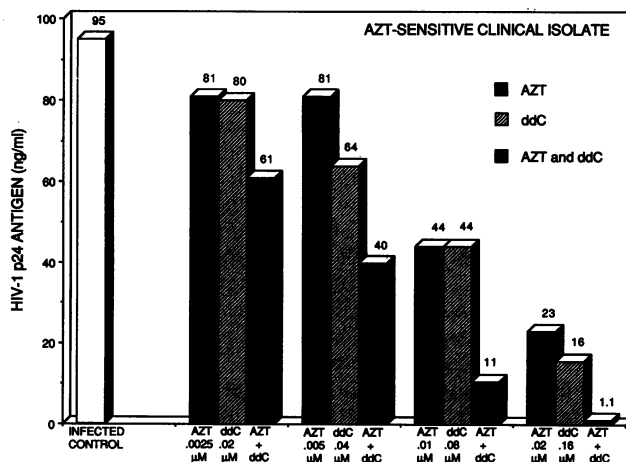


FIG. 1. Inhibition of p24 antigen production by the AZT-sensitive isolate in acutely infected PBMC treated with AZT, ddC, or the two combined. The results are from experiment 4, day 7 in culture, and are presented with results for the untreated infected control. Dose-effect data shown in this figure for AZT, ddC, and the combination were subjected to median-effect curve computer analysis. The linear correlation coefficients of these curves were 0.95, 0.99, and 0.98, respectively. Similar dose-effect data were obtained in experiment 3, and r values were 0.98, 0.99, and 0.97 for the median-effect curves for AZT, ddC, and the combination, respectively. CIs for both experiments are shown in Table 1. Similar results were obtained when the data were adjusted for viable cell numbers.

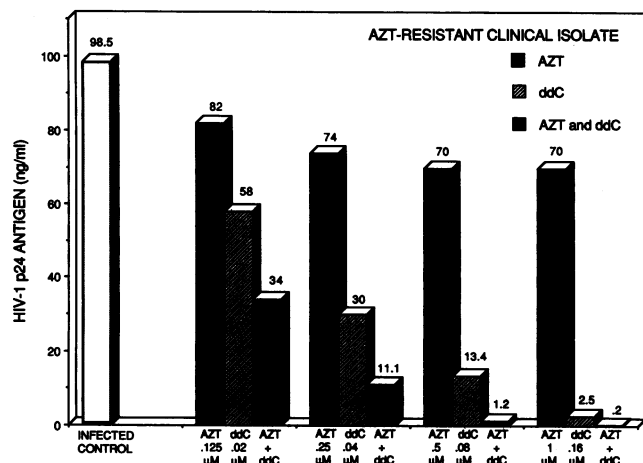


FIG. 2. Inhibition of HIV-1 p24 antigen production by the AZT-resistant isolate in acutely infected PBMC from experiment 6, day 7 in culture, along with results for the untreated infected control. Dose-effect data subjected to the median-effect curve computer analysis gave r values of 0.90, 0.91, and 0.99 for AZT, ddC, and the combination, respectively. Similar dose-effect data were obtained in experiment 5, and r values were 0.75, 0.90, and 0.96, respectively. CIs are shown in Table 1. Similar results were obtained when the data were adjusted for viable cell numbers.

of experiments, mean viable cell numbers in uninfected drug-treated flasks were at least 87% of the mean viable cell numbers of the uninfected untreated controls.

The combination of ddC and AZT in this study synergistically inhibited HIV-1 replication of both HIV-1_{IIIB} and clinically derived AZT-sensitive and AZT-resistant HIV-1 paired isolates, as determined by the multiple-drug-effect analysis of Chou et al. (3-5). At all drug concentrations tested against the clinical isolates (as low as 0.0025 μM AZT and 0.02 μM ddC), more-effective suppression of HIV-1 replication was seen with the two agents together than with either agent alone. Synergy was seen against replication of the AZT-resistant isolate despite a highly resistant genotype and phenotype. Similar synergistic interactions were reported previously for AZT combined with ddI against these clinical isolates (19). The mechanism of synergy by AZT-containing regimens against phenotypically AZT-resistant virus may involve inhibition of AZT-sensitive viruses in the mixture of viruses in a clinical isolate (2, 19). An alternative mechanism of synergy is that one chain-terminating nucleoside analog may potentiate the effect of another by stereochemically altering the agents' binding to reverse transcriptase, resulting in more-complete viral inhibition within infected cells.

Synergy between AZT and ddC against replication of the laboratory strain, HIV-1_{IIIB}, in H9 cells was also seen. Additive to subsynergistic inhibition of HIV-1_{IIIB} replication by the combination had been reported previously, when different cell types, methods of virus quantitation, and data analysis were used (1). Our current study demonstrated synergistic interactions against the replication of HIV-1_{IIIB} at moderate to high levels of drug effectiveness. However, at suboptimal levels of effectiveness of the single agents in H9 cells (specifically when ddC alone had no effect), the combination's benefit was variable compared with that of AZT alone. This phenomenon was not seen with either of the clinical isolates tested in PBMC, which may be a more clinically relevant cell type than the continuous H9 cell line.

The combination of AZT and ddC offers several advantages over monotherapy if the synergistic effect demonstrated here also occurs in vivo. Patients on long-term AZT monotherapy, particularly those with advanced disease, are likely to have isolates that exhibit some degree of AZT resistance (30). Our studies suggest that the addition of ddC would not only offer the antiretroviral effect of ddC, as cross-resistant isolates have yet to be identified, but would also potentiate the antiretroviral effect of AZT. More-complete suppression of sensitive virus would be expected as well. Patients who initiate therapy with a combination of agents may develop resistance more slowly, although this concept remains to be proven. Fischl et al. (12) offered preliminary evidence that resistance may develop more slowly in a cohort treated with AZT and a second synergistic agent, alpha interferon (14), than in patients treated with AZT alone (30).

Finally, the combination of AZT and ddC may be particularly useful for therapy of HIV-1 infection because the toxicities of the agents do not appear to overlap substantially (23). The concentrations used in our study are achieved in human plasma after oral administration (33).

One preliminary trial suggested that the combination of AZT and ddC given to patients with advanced HIV-1 disease produced a greater and more sustained increase in mean CD4+ lymphocyte counts than that observed in historical controls treated with AZT alone (23). Large controlled clinical trials to further define the role of this combination in the management of HIV-1 infections are currently under way.

This work was supported by NIH grants T32AIO7061, CA12464, and CA35020 and by the Elsa U. Pardee Foundation.

REFERENCES

- Baba, M., R. Pauwels, J. Balzarini, P. Herdewijn, E. De Clercq, and J. Desmyter. 1987. Ribavirin antagonizes inhibitory effects of pyrimidine 2',3'-dideoxynucleosides but enhances inhibitory effects of purine 2',3'-dideoxynucleosides on replication of human immunodeficiency virus in vitro. *Antimicrob. Agents Chemother.* 31:1613-1617.
- Boucher, C. A. B., E. O'Sullivan, J. W. Mulder, C. Ramautarsing, P. Kellam, G. Darby, J. M. A. Lange, J. Goudsmit, and B. A. Larder. 1992. Ordered appearance of zidovudine resistance mutations during treatment of 18 human immunodeficiency virus-positive subjects. *J. Infect. Dis.* 165:105-110.
- Chou, J., and T.-C. Chou. 1987. Dose-effect analysis with microcomputers: quantitation of ED50, LD50, synergism, antagonism, low-dose risk, receptor-ligand binding and enzyme kinetics. A computer software for IBM-PC and manual. Biosoft, Cambridge.
- Chou, T.-C. 1991. The median-effect principle and the combination index for the quantitation of synergism and antagonism, p. 61-102. *In* T.-C. Chou and D. Rideout (ed.), *Synergism and antagonism in chemotherapy*. Academic Press, Inc., New York.
- Chou, T.-C., and P. Talalay. 1984. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.* 22:27-55.
- Cooley, T. P., L. M. Kunches, C. A. Saunders, J. K. Ritter, C. J. Perkins, C. McLaren, R. P. McCaffrey, and H. A. Liebman. 1990. Once-daily administration of 2',3'-dideoxyinosine (ddI) in patients with the acquired immunodeficiency syndrome or AIDS-related complex: results of a phase I trial. *N. Engl. J. Med.* 322:1340-1345.
- Eron, J. J., and R. T. D'Aquila. Unpublished data.
- Fischl, M. A., C. B. Parker, C. Pettinelli, M. Wulfsohn, M. S. Hirsch, A. C. Collier, D. Antoniskis, M. Ho, D. D. Richman, E. Fuchs, T. C. Merrigan, R. C. Reichman, J. Gold, N. Steigbigel, G. S. Leoung, S. Rasheed, A. Tsiatis, and the AIDS Clinical Trials Group. 1990. A randomized controlled trial of a reduced daily dose of zidovudine in patients with the acquired immuno-

- deficiency syndrome. *N. Engl. J. Med.* 323:1009-1014.
9. Fischl, M. A., D. D. Richman, D. M. Causey, M. H. Grieco, Y. Bryson, D. Mildvan, O. L. Laskin, J. E. Groopman, P. A. Volberding, R. T. Schooley, G. G. Jackson, D. T. Durack, J. C. Andrews, S. Nusinoff-Lehrman, D. W. Barry, and the AZT Collaborative Working Group. 1989. Prolonged zidovudine therapy in patients with AIDS and advanced AIDS-related complex. *JAMA* 262:2405-2410.
 10. Fischl, M. A., D. D. Richman, M. H. Grieco, M. S. Gottlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, R. T. Schooley, G. G. Jackson, D. T. Durack, D. King, and the AZT Collaborative Working Group. 1987. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. *N. Engl. J. Med.* 317:185-191.
 11. Fischl, M. A., D. D. Richman, N. Hansen, A. C. Collier, J. T. Carey, M. F. Para, W. D. Hardy, R. Dolin, W. G. Powderly, J. D. Allan, B. Wong, T. C. Merigan, V. J. McAuliffe, N. E. Hyslop, F. S. Rhame, H. H. Balfour, Jr., S. A. Spector, P. Volberding, C. Pettinelli, J. Anderson, and the AIDS Clinical Trials Group. 1990. The safety and efficacy of zidovudine (AZT) in the treatment of subjects with mildly symptomatic human immunodeficiency virus type I (HIV) infection: a double-blind, placebo-controlled trial. *Ann. Intern. Med.* 112:727-737.
 12. Fischl, M. A., R. B. Uttamchandani, L. Resnick, R. Agarwal, M. A. Fletcher, J. Patrone-Reese, L. Dearmas, J. Chidekel, M. McCann, and M. Myers. 1991. A phase I study of recombinant human interferon-alpha or human lymphoblastoid interferon-alpha and concomitant zidovudine in patients with AIDS-related Kaposi's sarcoma. *J. Acquired Immune Defic. Syndr.* 4:1-10.
 13. 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.
 14. Hartshorn, K. L., M. W. Vogt, T.-C. Chou, R. S. Blumberg, R. Byington, R. T. Schooley, and M. S. Hirsch. 1987. Synergistic inhibition of human immunodeficiency virus in vitro by azidothymidine and recombinant alpha A interferon. *Antimicrob. Agents Chemother.* 31:168-172.
 15. Ho, D. D., T. Moudgil, and M. Alam. 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N. Engl. J. Med.* 321:1621-1625.
 16. Johnson, V. A., and R. E. Byington. 1990. Modified HIV-1 p24 antigen ELISA. Quantitative assays for virus detection, p. 95-97. *In* A. Aldovini and B. D. Walker (ed.), *Techniques in HIV research*. Stockton Press, New York.
 17. Johnson, V. A., and R. E. Byington. 1990. Quantitative assays for virus infectivity, p. 71-76. *In* A. Aldovini and B. D. Walker (ed.), *Techniques in HIV research*. Stockton Press, New York.
 18. Johnson, V. A., and M. S. Hirsch. 1989. Combination therapy for HIV infection, p. 275-302. *In* J. Mills and L. Corey (ed.), *Antiviral chemotherapy: new directions for clinical application and research*. Elsevier, New York.
 19. Johnson, V. A., D. P. Merrill, J. A. Videler, T.-C. Chou, R. E. Byington, J. J. Eron, R. T. D'Aquila, and M. S. Hirsch. 1991. Two-drug combinations of zidovudine, didanosine, and recombinant interferon-alpha A inhibit replication of zidovudine-resistant human immunodeficiency virus type 1 synergistically in vitro. *J. Infect. Dis.* 164:646-655.
 20. Lambert, J. S., M. Seidlin, R. C. Reichman, C. S. Plank, M. Laverly, G. D. Morse, C. Knupp, C. McLaren, C. Pettinelli, F. T. Valentine, and R. Dolin. 1990. 2',3'-Dideoxyinosine (ddI) in patients with the acquired immunodeficiency syndrome or AIDS-related complex: a phase I trial. *N. Engl. J. Med.* 322:1333-1340.
 21. 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.
 22. Larder, B. A., and S. D. Kemp. 1989. Multiple mutations in the HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). *Science* 246:1155-1158.
 23. Meng, T.-C., M. A. Fischl, A. M. Boota, S. A. Spector, D. Bennett, Y. Bassiakos, S. Lai, B. Wright, and D. D. Richman. 1992. Combination therapy with zidovudine and dideoxycytidine in patients with advanced human immunodeficiency virus infection: a phase I/II study. *Ann. Intern. Med.* 116:13-20.
 24. Merigan, T. C., G. Skowron, S. A. Bozzette, D. D. Richman, R. Uttamchandani, M. Fischl, R. Schooley, M. Hirsch, W. Soo, C. Pettinelli, H. Schaumburg, and the ddC Study Group of the AIDS Clinical Trials Group. 1989. Circulating p24 antigen levels and responses to dideoxycytidine in human immunodeficiency virus (HIV) infections: a phase I and II study. *Ann. Intern. Med.* 110:189-194.
 25. Mitsuya, H., and S. Broder. 1986. Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2',3'-dideoxynucleosides. *Proc. Natl. Acad. Sci. USA* 83:1911-1915.
 26. Mitsuya, H., R. F. Jarrett, M. Matsukura, F. D. M. Veronese, A. L. DeVico, M. G. Sarngadharan, D. G. Johns, M. S. Reitz, and S. Broder. 1987. Long-term inhibition of human T-lymphotropic virus type III/lymphadenopathy-associated virus (human immunodeficiency virus) DNA synthesis and RNA expression in T cells protected by 2',3'-dideoxynucleosides in vitro. *Proc. Natl. Acad. Sci. USA* 84:2033-2037.
 27. Mitsuya, H., R. Yarchoan, and S. Broder. 1990. Molecular targets for AIDS therapy. *Science* 249:1533-1544.
 28. Perno, C., R. Yarchoan, D. Cooney, N. Hartman, S. Gartner, M. Popovic, Z. Hao, T. Gerrard, Y. Wilson, D. Johns, and S. Broder. 1988. Inhibition of human immunodeficiency virus (HIV-1/HTLV-IIb) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2',3'-dideoxynucleosides. *J. Exp. Med.* 168:1111-1125.
 29. Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo. 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 224:497-500.
 30. Richman, D. D., J. M. Grimes, and S. W. Lagakos. 1990. Effect of stage of disease and drug dose on zidovudine susceptibilities of isolates of human immunodeficiency virus. *J. Acquired Immune Defic. Syndr.* 3:743-746.
 31. 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.
 32. Volberding, P. A., S. W. Lagakos, M. A. Koch, C. Pettinelli, M. W. Myers, D. K. Booth, H. H. Balfour, R. C. Reichman, J. A. Bartlett, M. S. Hirsch, R. L. Murphy, W. D. Hardy, R. Soeiro, M. A. Fischl, J. G. Bartlett, T. C. Merigan, N. E. Hyslop, D. D. Richman, F. T. Valentine, L. Corey, and the AIDS Clinical Trials Group of the National Institute of Allergy and Infectious Diseases. 1990. Zidovudine in asymptomatic human immunodeficiency virus infection: a controlled trial in persons with fewer than 500 CD4-positive cells per cubic millimeter. *N. Engl. J. Med.* 322:941-949.
 33. Yarchoan, R., H. Mitsuya, C. Myers, and S. Broder. 1989. Clinical pharmacology of 3'-azido-2',3'-dideoxythymidine (zidovudine) and related dideoxynucleosides. *N. Engl. J. Med.* 321:726-738.
 34. Yarchoan, R., C. F. Perno, R. V. Thomas, R. W. Klecker, J.-P. Allain, R. J. Wills, N. McAtee, M. A. Fischl, R. Dubinsky, M. C. McNeely, H. Mitsuya, J. M. Pluda, T. J. Lawley, M. Leuther, B. Safai, J. M. Collins, C. E. Myers, and S. Broder. 1988. Phase I studies of 2',3'-dideoxycytidine in severe human immunodeficiency virus infection as a single agent and alternating with zidovudine. *Lancet* i:76-81.
 35. Yarchoan, R., J. M. Pluda, C.-F. Perno, H. Mitsuya, and S. Broder. 1991. Anti-retroviral therapy of human immunodeficiency virus infection: current strategies and challenges for the future. *Blood* 78:859-884.
 36. Yarchoan, R., J. M. Pluda, R. V. Thomas, H. Mitsuya, P. Brouwers, K. M. Wyvill, N. Hartman, D. G. Johns, and S. Broder. 1990. Long-term toxicity/activity profile of 2',3'-dideoxyinosine in AIDS or AIDS-related complex. *Lancet* 336:526-529.