ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1992, p. 1127–1130
0066-4804/92/051127-04$02.00/0

Vol. 36, No. 5

Ganciclovir Antagonizes the Anti-Human Immunodeficiency Virus Type 1 Activity of Zidovudine and Didanosine In Vitro

D. J. MEDINA,[1] G. D. HSIUNG,[1,2]* AND J. W. MELLORS[3]

Departments of Laboratory Medicine[1] and Internal Medicine,[3] Yale University School of Medicine, New Haven, Connecticut 06510, and the Virology Laboratory, Veterans Affairs Medical Center, West Haven, Connecticut 06516[2]

Received 30 October 1991/Accepted 17 February 1992

In studies examining potential interactions between ganciclovir (GCV) and either zidovudine (AZT) or didanosine (DDI) in H9 cells, GCV was found to consistently reduce the anti-human immunodeficiency virus type 1 potency of both AZT and DDI. In the presence of GCV, the 50% effective doses of AZT and DDI were increased three- to sixfold, depending on the molar ratio of drugs and the measure of human immunodeficiency virus type 1 replication (p24 antigen, reverse transcriptase activity, or infectious virus yield). Multiple dose-effect analysis revealed strong antagonism between GCV and either AZT or DDI (combination indices, 2.2 to 6.7). This antagonistic effect occurred at drug concentrations that were well below the cytotoxic range. At higher drug concentrations, the combination of GCV and AZT was synergistically cytotoxic (combination indices, <1.0), whereas GCV and DDI were only additively cytotoxic (combination indices, ca. 1.0). Thus, the combination of GCV with AZT or DDI may result in antiviral antagonism and either synergistic (AZT-GCV) or additive (DDI-GCV) cytotoxicity.

Human cytomegalovirus (CMV) is the most common opportunistic viral pathogen in patients with human immunodeficiency virus type 1 (HIV-1) infection (1, 19). The nucleoside analogs ganciclovir (9-[1,3-dihydroxy-2-propoxymethyl]guanine [GCV]) and zidovudine (3′-azido-3′-deoxythymidine [AZT]) were the first drugs licensed in the United States for the treatment of CMV and HIV-1 infection, respectively. Didanosine (2′,3′-dideoxyinosine [DDI]), a potent inhibitor of HIV-1, is undergoing widespread clinical evaluation as an alternative to AZT and has been recently approved for limited clinical use. Although GCV is effective for the treatment of CMV retinitis and gastrointestinal disease in patients with AIDS, high recurrence rates necessitate long-term maintenance therapy (2). Because CMV disease usually occurs in patients with advanced HIV-1 infection, clinical indications are usually present for prolonged therapy with both GCV and AZT or DDI. Previous investigators have reported that GCV and AZT are synergistically toxic in vitro (17) and produce severe hematologic toxicity in vivo (11, 12). Nevertheless, combined therapy with AZT and GCV is still advocated for selected patients (3). Preliminary clinical reports suggest that GCV may be better tolerated in patients receiving concomitant therapy with DDI (16).

Despite the frequent use of these antiviral agents in combination, there is no published information on the potential effects of GCV on the antiretroviral activity of AZT or DDI. In the present study, therefore, the effects of GCV on the anti-HIV-1 activity of AZT and DDI were investigated. The concentration ratios of the drug combinations studied were based on peak and trough levels of the drugs achieved in the serum of humans by using standard dosing regimens (8, 11, 15).

H9 target cells (obtained from R. C. Gallo, National Cancer Institute, Bethesda, Md.) were cultured in RPMI 1640 supplemented with 20% heat-inactivated fetal bovine serum, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and antibiotics. Stock virus ($HIV_{HTLV\text{-}IIIB}$ strain; from R. C. Gallo) was harvested from infected H9 cells by the shake-off method as previously described (4, 14, 26). Stock virus infectivity (6.5 $\log_{10}$ 50% tissue culture infective doses [$TCID_{50}$] per ml) was determined by triplicate endpoint dilution in H9 cells with p24 antigen production (day 7) as the indicator of viral replication. The $TCID_{50}$ was calculated by using the Reed and Meunch equation for interpolation (18).

H9 cells were infected with HIV-1 at a multiplicity of infection of 0.01 $TCID_{50}$ per cell in all experiments. Virus was adsorbed for 2 h at 37°C; this was followed by three washes to remove nonadsorbed virus. Immediately after infection, 5×10^4 cells per well were added in quadruplicate to 24-well tissue culture plates (Costar, Cambridge, Mass.) containing one of the following: AZT alone (Sigma Chemical Co., St. Louis, Mo.), DDI alone (Bristol-Myers Squibb, Wallingford, Conn.), GCV alone (Syntex Laboratories, Palo Alto, Calif.), or AZT or DDI combined with GCV at several fixed molar ratios ranging from 1:1 to 1:20. On day 5 after infection, culture supernatants were collected, clarified by centrifugation (800 × *g* for 15 min), and stored for assay. The amount of supernatant virus was measured by p24 enzyme-linked immunosorbent assay (ELISA; Abbott Laboratories, North Chicago, Ill.), reverse transcriptase (RT) assay (5), and infectious virus yield in MT-2 cells (10).

The cytotoxic effects of drugs alone and in combination were assayed by using uninfected H9 cells. Serial dilutions of drug were added to duplicate wells of a 24-well plate containing 4×10^5 H9 cells per well. Cells cultured without drug were included as controls in each experiment. Cytotoxicity was assessed by determining the number of viable cells (trypan blue exclusion) and total cellular protein (bicinchoninic acid method [23]) after 3 and 5 days of culture.

The drug concentrations that inhibited HIV-1 replication by 50% (ED_{50}) and 90% (ED_{90}) and reduced the viable cell number by 50% (CyD_{50}) were interpolated from linear regression plots of percent inhibition versus drug concentration by using the computer program of Chou and Chou (6).

* Corresponding author.

TABLE 1. Inhibition of HIV-1 replication in H9 cells by AZT and GCV each alone and in combination

Concn (μM) of:		% Inhibition (mean ± SD)[a] by:		
AZT	GCV	p24	RT	Virus yield
1		97 ± 3	93 ± 4	97 ± 2
0.25		86 ± 2	84 ± 3	89 ± 1
0.0625		71 ± 2	67 ± 3	74 ± 3
0.0156		54 ± 4	53 ± 4	63 ± 3
0.0039		43 ± 3	40 ± 4	40 ± 7
0.0009		15 ± 2	9 ± 5	18 ± 5
	160	76 ± 4	76 ± 4	79 ± 7
	40	47 ± 9	44 ± 8	37 ± 4
	10	24 ± 10	17 ± 5	14 ± 6
1[b]	10[b]	89 ± 1	86 ± 3	91 ± 3
0.25	2.5	78 ± 1	74 ± 2	81 ± 3
0.0625	0.625	61 ± 3	54 ± 3	58 ± 4
0.0156	0.156	26 ± 2	25 ± 4	40 ± 6
0.0039	0.039	11 ± 1	13 ± 3	8 ± 5
0.0009	0.009	6 ± 4	6 ± 2	3 ± 2

[a] In no-drug control cultures, the mean p24 antigen level was 860 ± 50 ng/ml, the RT activity was $5 \times 10^6 \pm 2 \times 10^4$ cpm/ml, and the infectious-virus yield titer was 5.3 $\log_{10}$ ± 1.2 $\log_{10}$ $TCID_{50}$/ml. Results are the mean ± standard deviation (SD) of three experiments.

[b] When used in combination, the drugs were present at an AZT-to-GCV ratio of 1:10.

TABLE 2. Analysis of the anti-HIV-1 effects of AZT and GCV each alone and in combination

Assay and compounds	Parameters[a]			CI values at[b]:	
	ED_{50} (μM)	*m*	*r*	ED_{50}	ED_{90}
p24 ELISA					
AZT	0.01	0.685	0.984		
GCV	41.97	0.831	0.998		
AZT + GCV (1:10)	0.58	0.772	0.993	4.83 (4.89)	3.36 (3.41)
RT assay					
AZT	0.02	0.647	0.980		
GCV	50.22	0.988	0.999		
AZT + GCV (1:10)	0.69	0.693	0.997	3.64 (3.68)	2.93 (3.03)
Virus yield					
AZT	0.01	0.682	0.993		
GCV	54.05	1.133	0.994		
AZT + GCV (1:10)	0.51	0.822	0.982	5.29 (5.34)	3.07 (3.13)

[a] *m* (slope) signifies the shape of the dose-effect curve; *r* is the linear correlation coefficient.

[b] CI values were calculated from the experimental data in Table 1 by using a computer simulation of the classical isobologram equation. The CI values in parentheses were calculated from the conservative equation assuming independent modes of action. CI values of <1, 1, and >1 indicate synergism, additivism, and antagonism, respectively.

Combination indices (CI) for the antiviral and cytotoxic effects of the drugs in combination were also computed by using this program (6). This program has been used by other investigators to analyze antiviral interactions (13, 21). These computations are based on the multiple drug-effect equations of Chou and Talalay (7). The CI values were calculated by using both mutually exclusive (similar modes of action) and mutually nonexclusive (different modes of action) equations. CI values of <1, 1, and >1 indicate synergistic, additive, and antagonistic drug effects, respectively.

Initial experiments examined the effect of GCV on the antiviral activity of AZT at fixed molar ratios of AZT to GCV ranging from 1:1 to 1:20 as measured by p24 antigen production. These experiments (n = 3) demonstrated that GCV consistently reduced the antiviral potency of AZT at all molar ratios studied. This effect of GCV was more pronounced at higher ratios, reaching a maximum at an AZT-to-GCV ratio of 1:10. At this ratio, the ED_{50} of AZT was increased ca. 11-fold, from 0.015 μM (AZT alone) to 0.169 μM (AZT-GCV combination).

In more detailed experiments, serial fourfold dilutions of drugs at a fixed AZT-to-GCV ratio of 1:10 were examined for antiviral activity by using three different measures of HIV-1 replication: p24 antigen production, RT activity, and infectious virus yield. Table 1 shows that in the presence of GCV the HIV-1 inhibitory potency of AZT was reduced regardless of the measure of HIV-1 replication. Further analysis of the data from these experiments (n = 3) is shown in Table 2. GCV increased the ED_{50}s of AZT four- to sixfold, depending on the measure of HIV-1 replication. The CI values for the AZT-GCV combination ranged from 3.64 to 5.29 at the ED_{50} and from 2.93 to 3.36 at the ED_{90} levels, indicating strong antagonistic interaction (CI > 1.0). GCV exerted this antagonistic effect at concentrations of 0.04 to 10 μM (Table 1), which is ca. 8 to 2,000 times below the 50% cytotoxic concentration of GCV (81 μM) for H9 cells (see below). This indicates that GCV-induced cytotoxicity is an unlikely explanation for the observed antiviral antagonism.

In similar experiments (n = 3), GCV was also noted to reduce the antiviral potency of DDI (Table 3). Initial experiments at several DDI-to-GCV molar ratios (1:5, 1:10, and 1:20) demonstrated maximal antagonism at ratios of 1:10 and 1:20. At a DDI-to-GCV ratio of 1:10, the ED_{50}s of DDI were two- to threefold higher than those of DDI alone (Table 4). The CI values ranged from 2.23 to 2.83 at the ED_{50} and 2.49 to 6.73 at the ED_{90} levels, indicating substantial antagonism. GCV exerted this antagonistic effect at 0.09 to 25 μM (Table 3), which is 3- to 500-fold lower than the 50% cytotoxic concentration of GCV (81 μM).

The CyD_{50}s were 87 μM for AZT alone, 106 μM for DDI

TABLE 3. Inhibition of HIV-1 replication in H9 cells by DDI and GCV each alone and in combination

Concn (μM) of:		% Inhibition (mean ± SD)[a] by:		
DDI	GCV	p24	RT	Virus yield
10		97 ± 3	95 ± 3	97 ± 2
2.5		90 ± 2	88 ± 2	91 ± 1
0.625		75 ± 3	75 ± 7	77 ± 4
0.156		46 ± 5	52 ± 4	55 ± 3
0.039		20 ± 3	32 ± 6	30 ± 2
0.009		7 ± 2	13 ± 9	3 ± 1
	160	77 ± 5	78 ± 6	78 ± 6
	40	62 ± 4	63 ± 5	54 ± 5
	10	13 ± 2	18 ± 11	14 ± 3
10[b]	100[b]	96 ± 4	92 ± 2	91 ± 4
2.5	25	79 ± 6	71 ± 2	71 ± 3
0.625	6.25	48 ± 6	55 ± 2	59 ± 7
0.156	1.56	26 ± 2	36 ± 4	30 ± 8
0.039	0.39	19 ± 8	22 ± 3	20 ± 5
0.009	0.09	4 ± 2	9 ± 1	11 ± 5

[a] In no-drug control cultures, the mean p24 antigen level was 642 ± 63 ng/ml, the RT activity was $3 \times 10^6 \pm 3 \times 10^4$ cpm/ml, and the infectious-virus yield titer was 5.5 $\log_{10}$ ± 1.5 $\log_{10}$ $TCID_{50}$/ml. Results are the mean ± SD of three experiments.

[b] When used in combination, the drugs were present at a DDI-to-GCV ratio of 1:10.

TABLE 4. Analysis of the anti-HIV-1 effects of DDI and GCV each alone and in combination

Assay and compounds	Parameters[a]			CI values at[b]:	
	ED_{50} (μM)	m	r	ED_{50}	ED_{90}
p24 ELISA					
DDI	0.19	0.865	0.999		
GCV	42.49	1.121	0.955		
DDI + GCV (1:10)	4.36	0.839	0.984	2.23 (2.43)	2.49 (2.91)
RT assay					
DDI	0.13	0.684	0.999		
GCV	36.43	1.003	0.965		
DDI + GCV (1:10)	3.99	0.634	0.990	2.83 (3.10)	3.88 (5.13)
Virus yield					
DDI	0.18	0.926	0.983		
GCV	44.96	1.111	0.987		
DDI + GCV (1:10)	4.37	0.637	0.992	2.25 (2.44)	6.73 (9.18)

[a] See Table 2, footnote *a*.
[b] CI values were calculated from the experimental data in Table 3. See Table 2, footnote *b*.

alone, and 81 μM for GCV alone (mean values from three experiments). Similar CyD_{50}s were obtained when total cellular protein was measured as the indicator of cytotoxicity (not shown). The drugs in combination (AZT-GCV and DDI-GCV, both at a ratio of 1:10) were more cytotoxic than the drugs alone. Figure 1 shows that the CI values for the AZT-GCV combination were consistently <1.0, indicating synergistic cytotoxicity. CI values for the DDI-GCV combination approximated 1.0, which is consistent with additive rather than synergistic cytotoxicity.

These studies demonstrate that GCV reduces the anti-HIV-1 potency of both AZT and DDI in H9 cells by using p24 antigen production, RT activity, and infectious virus yield as measures of viral replication. Although the magnitude of this antagonism varied depending on the drug concentration, the molar drug ratio, and the assay of viral replication, a severalfold reduction in the antiviral potency of AZT and DDI was consistently observed. This degree of antagonism of AZT by GCV is similar to that previously reported for the guanosine analog ribavirin, which decreases the anti-HIV-1 activity of AZT by inhibiting the phosporylation of AZT to the active 5'-triphosphate form (25). The

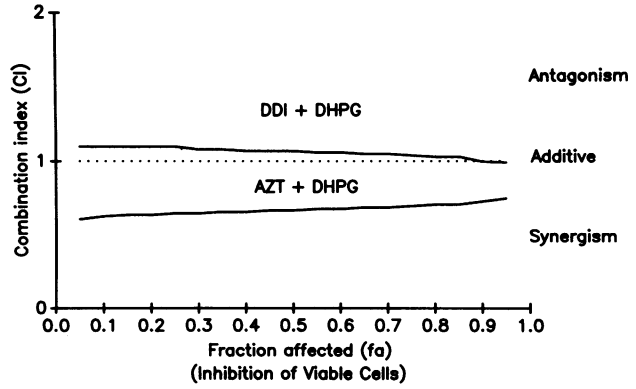

FIG. 1. Computer-generated presentation of the CI values for the cytotoxic effects of AZT-GCV (DHPG) and DDI-GCV combinations at molar ratios of 1:10. Conservative, mutually nonexclusive equations were used for CI calculations.

mechanisms responsible for the antagonism of AZT and DDI by GCV observed in our studies remain to be elucidated.

The concentration ratios at which GCV was antagonistic are within the range of levels achieved in the serum of humans by standard dosing (8, 11, 15). For example, in patients receiving 100 mg of oral AZT every 4 hours and 5 mg of intravenous GCV per kg twice daily, average peak and trough levels of GCV in plasma are ca. 28 and ca. 2.0 μM, respectively (11). The average peak and trough levels of AZT in plasma in these patients are 1.5 and 0.4 μM, respectively (11). Therefore the peak and trough concentration ratios of AZT to GCV in serum range from 1:5 to 1:20, which are ratios at which antagonism was observed in our experiments. DDI levels in the plasma of patients receiving 6 mg of oral DDI per kg twice daily average 6.3 to 9.6 μM at peak and <0.2 μM at trough (8). When compared with average peak and trough GCV levels achieved by standard dosing (11), DDI-to-GCV ratios of 1:3 to >1:10 are likely to be present in humans. Antagonism in our studies was observed at DDI-to-GCV ratios of 1:5 to 1:20.

In addition to these observations with HIV-1, separate studies in our laboratory have shown that AZT antagonizes the anti-CMV activity of GCV in vitro (24, 27) and in vivo (9). Studies by Yang et al. (27) demonstrated that AZT reduced the potency of GCV ca. twofold against guinea pig CMV at GCV-to-AZT ratios of 1:0.8, 1:1.6, and 1:3.2. Similarly, Tian et al. (24) demonstrated that AZT reduced the potency of GCV against human CMV two- to fivefold at GCV-to-AZT ratios of 1:0.2, 1:0.4, and 1:1. These prior observations, taken together with the results of the present study, suggest the possibility of bidirectional antiviral antagonism between AZT and GCV.

Both AZT and GCV have significant bone marrow toxicity when administered as single agents (20, 22). Clinical experience with concomitant AZT and GCV therapy has suggested additive or synergistic hematologic toxicity (11, 12). In our study, GCV and AZT in combination were synergistically toxic to H9 cells. Multiple dose-effect analysis revealed synergistic interactions (CI values of <1.0) over a broad range of drug concentrations and at all affected fractions (Fig. 1). These observations are consistent with a recent report of synergistic GCV-AZT toxicity in nonlymphoid cell lines (17). In contrast, the combination of GCV and DDI was

only additively toxic to H9 cells in our study. The lack of synergistic cytotoxicity in vitro is consistent with initial reports that GCV is better tolerated in patients receiving concomitant therapy with DDI than in those receiving AZT (16).

In summary, potentially important antagonistic antiviral interactions between GCV, AZT, and DDI have been demonstrated in cultured $CD4^+$ T cells. Whether similar interactions occur in vivo is not known. This question can be addressed by using appropriate animal models of retroviral disease or in trials of GCV therapy in patients receiving concomitant therapy with AZT or DDI.

This study was supported by U.S. Public Health Service research grant AI-28241 from the National Institute of Allergy and Infectious Diseases (to G.D.H.) and by the Research Service of the Department of Veterans Affairs. J. W. Mellors is the recipient of a Career Development Award from the Department of Veterans Affairs.

We thank T. C. Chou for reviewing the manuscript and for helping with the data analysis.

REFERENCES

1. **Armstrong, D., J. W. M. Gold, J. Dryjanski, E. Whimby, B. Polsky, C. Hawkins, A. E. Brown, E. Bernard, and T. E. Kiehn.** 1985. Treatment of infections in patients with the acquired immunodeficiency syndrome. Ann. Intern. Med. **103**:738–743.
2. **Buhles, W. C., B. J. Mastre, A. J. Tinker, V. Strand, S. H. Koretz, and the Syntex Collaborative Ganciclovir Treatment Study Group.** 1988. Ganciclovir treatment of life- or sight-threatening cytomegalovirus infection: experience in 314 immunocompromised patients. Rev. Infect. Dis. **10**(Suppl. 3):S495–S506.
3. **Causey, D.** 1991. Concomitant ganciclovir and zidovudine treatment for cytomegalovirus retinitis in patients with HIV infection: an approach to treatment. J. Acquired Immune. Defic. Syndr. **4**:S16–S21.
4. **Chambers, R. F., M. Terada, D. Kufe, and T. Ohno.** 1991. Shaking HIV-1 cells indicates novel behavior of MN strain. AIDS Res. Human Retroviruses **7**:459–463.
5. **Cheng, Y. C., G. E. Dutschman, K. F. Bastow, M. G. Sarngadharan, and R. Y. C. Ting.** 1987. Human immunodeficiency virus reverse transcriptase: general properties and its interaction with nucleoside triphosphate analogs. J. Biol. Chem. **262**:2187–2189.
6. **Chou, J., and T. C. Chou.** 1987. Dose-effect analysis with microcomputers: quantitation of ED_{50}, LD_{50}, synergism, antagonism, low dose risk, receptor-ligand binding and enzyme kinetic. Computer software for IBM PC series. Elsevier-Biosoft, Cambridge.
7. **Chou, T. C., and P. Talalay.** 1984. Quantitative analysis of dose-effect relationships: the combined effect of multiple drugs or enzyme inhibitors. Adv. Enzyme Regul. **22**:27–55.
8. **Dolin, D., J. S. Lambert, G. D. Morse, R. C. Reichman, C. S. Plank, J. Reed, C. Knupp, C. McLaren, and C. Perrinelli.** 1990. 2′,3′-Dideoxyinosine in patients with AIDS or AIDS-related complex. Rev. Infect. Dis. **12**(Suppl. 5):S540–S551.
9. **Feng, J. S., J. Y. Crouch, P. Y. Tian, H. L. Lucia, and G. D. Hsiung.** Zidovudine antagonizes the antiviral effects of ganciclovir against cytomegalovirus infection in cultured cells and in guinea pigs. Antiviral Chem. Chemother., in press.
10. **Haertle, T., C. J. Carrera, D. B. Wasson, L. C. Sowers, D. D. Richman, and D. A. Carson.** 1988. Metabolism and anti-human immunodeficiency virus-1 activity of 2-halo-2′,3′-dideoxyadenosine derivatives. J. Biol. Chem. **263**:5870–5875.
11. **Hochster, H., D. Dieterich, S. Bozzette, R. C. Reichman, J. D. Connor, L. Liebes, R. L. Sonke, S. A. Spector, F. Valentine, C. Pettinelli, and D. Richman.** 1990. Toxicity of combined ganciclovir and zidovudine for cytomegalovirus disease associated with AIDS. Ann. Intern. Med. **113**:111–117.
12. **Jacobson, M. A., P. de Miranda, S. M. Gordon, M. R. Blum, P. Volberding, and J. Mills.** 1988. Prolonged pancytopenia due to combined ganciclovir and zidovudine therapy. J. Infect. Dis. **158**:489–490.
13. **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-α inhibit replication of zidovudine-resistant human immunodeficiency virus type-1 synergistically in vitro. J. Infect. Dis. **164**:646–655.
14. **Kim, S., R. Byrn, J. Groopman, and D. Baltimore.** 1989. Temporal aspects of DNA and RNA synthesis during human immunodeficiency virus infection: evidence for differential gene expression. J. Virol. **63**:2361–2365.
15. **Lambert, J. S., M. Seidlin, R. C. Reichman, C. S. Plank, M. Laverty, G. D. Morse, C. Knupp, C. McLaren, C. Pettinelli, F. T. Valentine, and R. Dolin.** 1990. 2′,3′-dideoxyinosine (ddI) in patients with acquired immunodeficiency syndrome or AIDS-related complex. N. Engl. J. Med. **322**:1333–1340.
16. **Liebman, H. A., L. M. Kunches, C. A. Saunders, S. L. Kelley, R. P. McCaffrey, and T. P. Cooley.** 1990. Treatment with 2′-3′ dideoxyinosine (ddI) given twice daily to patients with cytomegalovirus retinitis receiving maintenance DHPG (ganciclovir). Abstr. S.B.474. Abstr. 6th Int. Conf. AIDS. 1990.
17. **Prichard, M. M., L. E. Prichard, W. A. Baguly, M. R. Nassiri, and C. Shipman.** 1991. Three-dimensional analysis of the synergistic cytotoxicity of ganciclovir and zidovudine. Antimicrob. Agents Chemother. **35**:1060–1065.
18. **Reed, L. J., and H. Muench.** 1938. A simple method for estimating fifty percent endpoints. Am. J. Hyg. **27**:493–496.
19. **Reichart, C. M., T. J. O'Leary, D. L. Levens, C. R. Simrell, and A. M. Macher.** 1983. Autopsy pathology in the acquired immune deficiency syndrome. Am. J. Pathol. **112**:357–382.
20. **Richman, D. D., M. A. Fischl, M. H. Grieco, M. S. Gottlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, M. S. Hirsch, G. G. Jackson, D. T. Durack, S. Nusinoff-Lehrman, and the AZT Collaborative Working Group.** 1988. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. N. Engl. J. Med. **317**:192–197.
21. **Schinazi, R. F., T. C. Chou, R. T. Scott, X. Yas, and A. J. Nakmias.** 1986. Delayed treatment with combinations of antiviral drugs in mice infected with herpes simplex virus and application of the median-effect method of analysis. Antimicrob. Agents Chemother. **30**:491–498.
22. **Shepp, D. H., P. S. Dandliker, P. de Miranda, T. C. Burnette, D. M. Cederburg, L. E. Kirk, and J. D. Meyers.** 1985. Activity of 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethy]guanine in the treatment of cytomegalovirus pneumonia. Ann. Intern. Med. **103**:368–373.
23. **Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartney, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk.** 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. **150**:76–85.
24. **Tian, P. Y., J. Y. Crouch, and G. D. Hsiung.** 1991. Combined antiviral effect and cytotoxicity of ganciclovir and azidothymidine against cytomegalovirus infection in cultured cells, abstr. 134. Abstr. Fourth Int. Conf. Antiviral Res. 1991.
25. **Vogt, W. M., K. L. Hartshorn, P. A. Furman, T. C. Chou, J. A. Fyfe, L. A. Colman, C. Crumpacker, R. T. Schooley, and M. S. Hirsch.** 1987. Ribavarin antagonizes the effect of azidothymidine on HIV replication. Science **235**:1376–1379.
26. **Vujcic, L. K., D. H. Shepp, M. Klutch, M. A. Wells, R. M. Hendry, A. E. Wittek, L. Krilou, and G. V. Quinnar.** 1988. Use of a sensitive neutralization assay to measure the prevalence of antibodies to the human immunodeficiency virus. J. Infect. Dis. **157**:1047–1050.
27. **Yang, Z. H., J. Y. Crouch, J. S. Feng, T. C. Chou, and G. D. Hsiung.** 1990. Combined antiviral effects of paired nucleosides against guinea pig cytomegalovirus replication in vitro. Antiviral Res. **14**:249–266.