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Human monoclonal anti-cytomegalovirus (CMV) antibody (MSL 109): enhancement of in vitro foscarnet- and ganciclovir-induced inhibition of CMV replication

Mostafa Nokta[a,*], Mark D. Tolpin[b], Paul I. Nadler[c], Richard B. Pollard[a]

[a]*Department of Internal Medicine, Division of Infectious Diseases, The University of Texas Medical Branch, Galveston, TX 77555, USA*

[b]*Sandoz Research Institute, East Hanover, NJ, USA*

[c]*Protein Design Laboratories, 2375 Garcia Ave, Mountain View, CA 94043, USA*

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Abstract

Human CMV causes a number of diseases that cause considerable morbidity and that can be life-threatening in immunocompromised patients, particularly those with AIDS. Ganciclovir (GCV) and Foscarnet (PFA) are currently the drugs of choice for management of CMV disease. Both are not without side effects and have a relatively narrow margin of safety. In this report the effects of a human IgG_1 neutralizing monoclonal antibody MSL-109 (MSL, Sandoz Pharmaceuticals) on CMV replication was examined both alone or in combination with either GCV or PFA. Human embryonic lung fibroblasts were infected with CMV strain AD169 with a multiplicity of infection of 3 plaque forming units/cell for 1 h. Prior to infection the virus was incubated for 30 min at 37°C with serial concentrations of the MSL Ab (0.1–3.0 μg/ml). Concentrations of GCV (0.3 to 30 μM) or PFA (50–400 μM) were added to CMV-infected cells that had been either previously incubated with MSL or not. Four days after infection CMV replication was measured by DNA/DNA probe hybridization using the Hybriwix system. MSL in combination with GCV had an additive effect that was observed at concentrations of GCV of 3–10 μM and MSL of 1–10 μg/ml. On the other hand, MSL (3–10 μg/ml) together with PFA (100–400 μM) produced a synergistic effect on CMV replication. The data suggest that MSL at doses achievable in humans, enhanced GCV- and PFA-induced antiviral effect in a dose-dependent manner and that the combination might be clinically useful in the treatment of CMV disease.

*Corresponding author.

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1. Introduction

Human cytomegalovirus (CMV) has been recognized as a major cause of morbidity and mortality among individuals with immunosuppressive disorders, as in bone marrow, cardiac and renal transplant patients (Meyers et al., 1986; Neiman et al., 1977; Stinson et al., 1971; Hill et al., 1964) and more recently in those with acquired immunodeficiency syndrome (Stover et al., 1985; Emanuel et al., 1986; Wallace and Hannah, 1985; Quinan et al., 1984).

CMV is a double-stranded DNA virus that belongs to the virus family Herpesviridae. Though it was first isolated more than 30 years ago, an effective non-toxic anti-CMV therapy for patients with CMV disease remains to be developed. Two anti-CMV compounds have recently been approved for treatment of patients with CMV disease, ganciclovir (GCV) (Shepp et al., 1985; DHPG Study Group, 1986) and (PFA) (Ringden et al., 1986; Singer et al., Walmsley et al., 1988), however, both have serious side effects. The former has a narrow margin of safety, producing irreversible testicular damage and transient neutropenia (Felsenstein et al., Erice et al., 1987), and the latter results in nephrotoxicity and hypocalcemia (Walmsley et al., 1988; Youle et al., 1988).

Ganciclovir is a guanine derivative that is phosphorylated intracellularly to the triphosphate derivative, that in turn competitively inhibits binding of deoxyguanosine triphosphate to DNA polymerase, inhibiting DNA synthesis and also terminating DNA elongation. Unlike GCV, the activity of PFA does not depend upon the conversion to an active triphosphate inside the cell. Foscarnet selectively inhibits DNA polymerase induced by CMV in infected cells, preventing the elongation of the viral DNA chain. It binds directly to a receptor site on the enzyme that is different from that of the pyrophosphate (Derse et al., 1982). Thus cleavage of pyrophosphates from deoxynucleoside triphosphates is blocked and the formation of an inactive PFA-DNA polymerase complex prevents the enzyme's translocation along the template primer (Chrisp and Clissol, 1991).

In this report the combination effects of a novel neutralizing human monoclonal antibody against gH glycoprotein of CMV, together with either ganciclovir or PFA on CMV replication were examined.

2. Materials and methods

2.1. Cells and virus

Human foreskin fibroblasts (HFF) (passages 5–8) were used in these experiments. The cells were grown in EMEM containing 10% heat-inactivated fetal calf serum (FCS), 0.22% $NaHCO_3$ and maintained in the same medium but with 2% FCS. CMV strain AD-169 was used in this study and was prepared and titrated as previously described (Albrecht and Weller 1980).

2.2. DNA-DNA hybridization assay for CMV replication

Confluent monolayers of HFF cells grown in 24 well tissue culture plates were infected in duplicate with CMV for 1 h at 37°C. The virus was then aspirated, and the monolayers washed twice with PBS. The cultures were then grown in maintenance media containing the appropriate treatments. The virus was incubated with MSL and/or PFA or GCV for 30 min prior to infection. Control virus (used to infect cells without prior MSL incubation, or further incubation with MSL alone and/or GCV or PFA) was treated during the same 30 min incubation with CMV seronegative pooled human sera. The latter per se had no effect on CMV replication.

At 96 h post-infection CMV replication was assayed by a DNA-DNA probe hybridization assay (hybriwix) developed by Diagnostic Hybrids Inc. (Athens, Ohio). Briefly, on the day of harvest CMV-infected wells were lysed by 2 drops of Lysis buffer after complete removal of culture medium. Hybridization filters (hybriwix) were then placed into the wells and the cell lysates were allowed to absorb by capillary attraction. After the wicking was completed the filters were put in a vial containing a CMV probe (*Pst*I N fragment of CMV strain AD169 map) Hybridization buffer. The CMV probe had been previously labeled with 125 iodine tracer by the manufacturer. The hybridization was carried out for 2 h at 60°C, then the hybridization solution was aspirated, the hybriwix were washed with distilled water, and incubated in a washing buffer at 73°C for 30 min in a water bath. At the end of the incubation the hybriwix were then measured for relative determination of CMV replication by a gamma counter (Dankner et al., 1990).

2.3. Compounds

GCV was obtained from Syntex, Palo Alto, CA. Foscarnet was purchased from Sigma Chemical Co, St. Louis, MO. MSL 109 was developed by and provided to us by Sandoz Research Institute. This antibody recognize the 86 kDa gH glycoprotein of CMV.

2.4. Calculation of drug interactions

Drug interactions were evaluated using the median effect principle and isobalogram technique by a computer generated program by calculating the combination index (*CI*) (Chou and Talalay, 1984; Chou and Chou, 1986; Eriksson and Schinazi, 1989). The (*CI*), is a method for evaluation of multiple drug effects. It involves the plotting of dose-effect curves for each drug alone and for serially diluted fixed ratio combinations of the agents using the median effect equation. The slope of the plot, which indicate the shape of the dose-effect curve, and the x intercept which signifies the potency of each compound or combination were then used to calculate the *CI*. The *CI* were calculated assuming non exclusive conditions for GCV/MSL and PFA/MSL mixtures. Values of *CI* of <1, 1, or >1 indicated synergistic, additive or antagonistic effect of the tested combinations, respectively.

3. Results

3.1. Effect of PFA and MSL on CMV replication

HFF cells were infected with an MOI of 2–3 PFU/cell with CMV that had been either exposed or not exposed to CMV specific neutralizing monoclonal antibody, MSL. After 1 h of adsorption the cells were incubated in the presence or absence of PFA and/or MSL. As shown in Table 1, a dose-dependent inhibition of CMV replication was observed with each drug alone or in combination. The median effective concentration (EC_{50}) that reduced the replication of CMV by 50% was 107 μM for PFA and 1.4 μg/ml for MSL. The MSL mAb enhanced the PFA-induced inhibition of CMV replication. At doses of 0.3 μg and 1 μg/ml of MSL the observed effects in combination with PFA were essentially additive as determined from their *CI*. At doses of 3 μg and 10 μg/ml of MSL, the combination with PFA at doses between 100 and 400 μM had a synergistic effect. The level of synergy increased as the dose of PFA increased. Based on the range of activity observed from Table 1, two combination ratios of PFA/MSL (40:1 and 20:1) were chosen for further analysis. The top portion of Table 3, shows the slope, correlation coefficient and EC_{50}s generated from median effect plot shown in Fig. 1. The slopes M of each of the plots was greater than 1 which is frequently observed in biological systems, indicating a sigmoidal pattern of the inhibitors and their combinations. The EC_{50} of PFA was 129 μM and MSL 2.5 μg/ml. Since the median effect plots were not parallel, and both compounds have different sites of action, the data were analyzed considering non exclusivity of the combinations. The *CI* at 70, 90 and 95% inhibition of the PFA:MSL combinations were less than 1 and indicated synergy. Fig. 2 show the computer generated representation of *CI* at fractions of inhibition between 0.05 to 0.95. The level of synergy increased with an increase in concentration. The drug combinations had no cytotoxic effects above that was observed by either drug alone (data not shown).

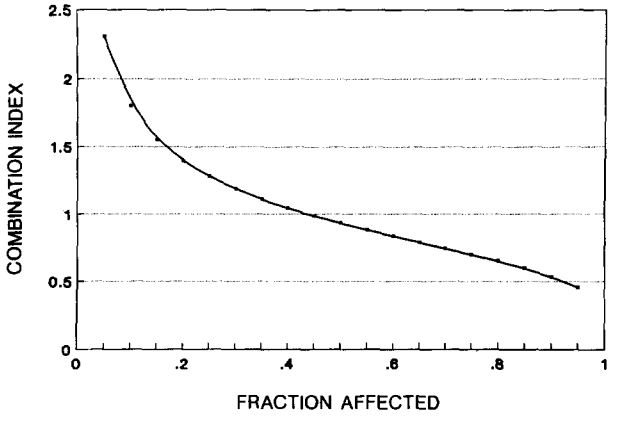

Fig. 1. Computer generated *CI* for PFA/MSL (40:1) under mutually non exclusive assumptions. *CI* values were plotted against fractions affected (FA) between 0.05 and 1.

Table 1
Effect of MSL and PFA combinations on CMV replication

Treatment	Conc.	Mean CPM values* (cpm)	% Inhibition	CI
PFA (μM)	0	11615 ± 264		
	50	9594 ± 152	17.4	
	100	6779 ± 142	41.6	
	200	2858 ± 100	75.4	
	400	457 ± 50	96.1	
	800	25 ± 12	99.8	
MSL (μg/ml)	0	11615 ± 264		
	0.3	8071 ± 300	30.5	
	1.0	6256 ± 154	46.1	
	3.0	5636 ± 263	51.5	
	10.0	2090 ± 112	82.0	
PFA/MSL	50/0.3	8123 ± 164	30.0	1.48
	100/0.3	5285 ± 200	54.5	1.19
	200/0.3	1979 ± 156	83.0	1.08
	400/0.3	292 ± 36	97.5	0.99
	50/1.0	6257 ± 102	46.1	1.91
	100/1.0	4449 ± 112	61.7	1.39
	200/1.0	2055 ± 98	82.3	1.19
	400/1.0	209 ± 28	98.2	0.86
	50/3	3920 ± 122	66.2	1.36
	100/3	2140 ± 130	81.6	0.86
	200/3	892 ± 82	92.3	0.82
	400/3	96 ± 24	99.2	0.65
	50/10	2249 ± 102	80.6	1.29
	100/10	1384 ± 82	88.1	0.88
	200/10	457 ± 46	96.1	0.65
	400/10	49 ± 12	99.6	0.50

*Determined at day 4 PI ± S.D. The data representative of 3 independent experiments.

3.2. *Effect of GCV and MSL on CMV replication*

To determine whether MSL had a similar enhancing effect on GCV-induced inhibition of CMV replication, three GCV/MSL ratios were examined for their effects on CMV replication. Table 2 shows the effect of either drug alone and in combination ratios of GCV/MSL of 1:1, 3:1 and 10:1. A dose-dependent inhibition of CMV replication was observed with either drug alone or with the combinations. The EC_{50}s of GCV and MSL in this experiment were 2.6 μM and 0.3 μg/ml, respectively. The lower portion of Table 3 shows the slopes and EC_{50}s of the combination mixtures. The EC_{50}s appeared to decrease as the ratio of the drugs to each other decreased. MSL had an enhancing effect on GCV inhibitory activity. The enhancing effect was of an additive nature at doses between 3 and 10 μM of GCV and 1 and 10 μg/ml of MSL. Table 3 shows the *CI* of the 3 combination mixtures that reduced CMV replication by 70, 90 and 95%. The three mixtures showed a similar pattern

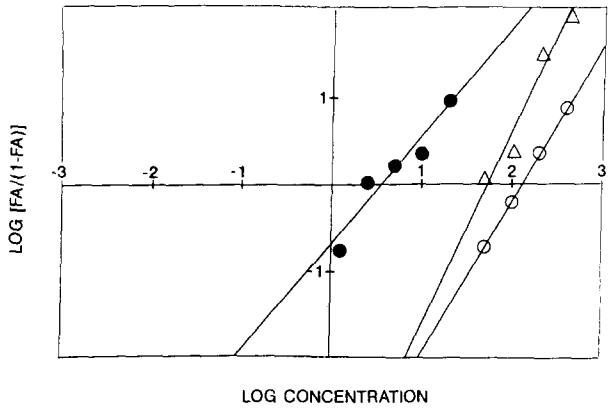

Fig. 2. Median effect plots for the inhibition of CMV replication by PFA (○), MSL (●) and a representative combination of PFA and MSL at a ratio of 40:1 (△). Slope values ± standard error of the mean are, PFA 1.75 ± 0.03, MSL 1.21 ± 0.21 and the combination 2.2 ± 0.33. FA, is the fraction of inhibition produced by a given drug concentration.

Table 2
Effect of MSL and/or GCV on CMV replication

Treatment	Conc.	Mean CPM values (CMV)	Inhibition of CMV Replication (%)
GCV (μM)	0	6988 ± 254	
	0.3	6527 ± 302	6
	1.0	4626 ± 158	33
	3.0	3231 ± 116	53
	10.0	1800 ± 112	74
	30.0	290 ± 45	96
MSL (μg)	0	6988 ± 254	
	0.1	4123 ± 198	41
	0.3	4163 ± 156	40
	1.0	2367 ± 160	66
	3.0	889 ± 78	87
	10.0	144 ± 45	98
GCV MSL			
1:1	0.3:0.3	4336 ± 202	38
	1.0:1.0	2162 ± 128	69
	3.0:3.0	510 ± 78	93
	10.0:10.0	123 ± 28	98
3:1	0.3:0.1	5384 ± 400	22
	1.0:0.3	4294 ± 188	38
	3.0:1.0	1848 ± 98	73
	10.0:3.0	422 ± 58	94
	30.0:10.0	59 ± 12	99
10:1	1.0:0.1	4942 ± 156	29
	3.0:0.3	2214 ± 116	68
	10.0:1.0	856 ± 82	88
	30.0:3.0	132 ± 40	98

*Determined at day 4 PI ± S.D. A representative of 3 independent experiments.

Table 3
CI values further effect of MSL and either GCV or PFA on CMV replication

Drug Ratio	Parameter			CI at different degrees of inhibition		
	M ± S.E.	EC_{50}	R*	EC_{70}	EC_{90}	EC_{95}
Foscarnet	1.64 ± 0.37	129	0.99			
MSL	1.21 ± 0.2	2.5	0.96			
PFA/MSL						
40:1	2.02 ± 0.14	53.2	0.98	0.71	0.43	0.34
20:1	1.32 ± 0.33	24.1	0.99	0.57	0.46	0.44
GCV	1.18 ± 0.12	2.6	0.98			
MSL 109	1.0 ± 0.18	0.3	0.95			
1:1 GCV/MSL	1.2 ± 0.08	0.9	0.99	1.61	1.13	0.93
3:1 GCV/MSL	1.3 ± 0.12	1.4	0.99	1.73	1.21	1.00
10:1 GCV/MSL	1.4 ± 0.09	2.1	0.99	1.48	1.06	0.90

*Linear correlation coefficient.

with additivity observed with increasing the concentration of both compounds. The suboptimal concentrations used in these combinations had no combined toxicity on untreated cells above what was observed with either drug alone (data not shown).

4. Discussion

The data presented in this report indicated an enhancing effect, for the human neutralizing monoclonal MSL antibody, on the GCV- and PFA-induced inhibition of CMV replication. While the effect of combining MSL with GCV was additive, it was synergistic with PFA. The additive effect observed with MSL/GCV combination was more evident when higher concentrations of both drugs were examined. The moderate synergy between PFA and MSL also increased as the concentration of both compounds increased. Though the mechanisms of anti CMV activity of GCV and PFA are different, this cannot account for the difference in the antiviral response observed in the combination mixtures of each drug with MSL. Particularly, since the effect of the latter is extracellular, while GCV and PFA targets the CMV-induced polymerase. The effect of MSL is possibly mediated through prevention of viral entry leading to a smaller intracellular virus load that is targeted by either agent more efficiently.

CMV is an opportunistic pathogen that tends to produce a life-long persistent infection. The diseases caused by CMV after reactivation in immunocompromised patients, are usually serious and life-threatening, if not treated. The existing anti-CMV therapeutics, GCV and PFA, have narrow margins of safety and have serious side effects (Felsenstein et al., 1985; Youle et al., 1988), that require frequent treatment interruptions and dose reductions. This may interfere with drug efficacy and in

addition have been reported to emerge CMV resistant to these drugs (Erice et al., 1989).

Combination therapy might minimize the risks and side effects of single agents, by using suboptimal non-toxic concentrations of two agents. Immunoglobulins from hyperimmune sera have been used previously for prophylaxis against CMV disease in organ transplant patients (Snydman et al., 1988). They have also been used in combination as therapy for CMV disease when combined with ganciclovir (Reed et al., 1988; Emanuel et al., 1988; Bratanow et al., 1987; Schmidt et al., 1988; Verdonck et al., 1989). In the previous studies there was an improved outcome in patients given combination therapy with immunoglobulin and ganciclovir than for historical controls that received either drug alone. The disadvantage of many immunoglobulins, is that they have been prepared from pooled human sera that make them expensive and potentially hazardous. Thus the availability of a human neutralizing monoclonal antibody to CMV could prove advantageous and more efficacious in combination therapy than the existing methods of treatment. The data from the present study suggest that MSL might be a potential useful anti CMV therapeutic that could be administered with either GCV or PFA. It might also be useful in combination therapy with other neutralizing monoclonal antibodies (directed against a different epitope). Recently, it was reported that a combination of HCMV specific murine monoclonal antibodies synergistically inhibited CMV replication in vitro (Gehrz et al., 1992). It is noteworthy to mention that the MSL doses used in this study are achievable in vivo in AIDS patients who received 0.5 mg/kg of MSL every 2 weeks without noticeable side effects (Pollard et al., 1992).

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References

Albrecht, T. and Weller, T.H. (1980) Heterogeneous morphologic features of plaques induced by five strains of human cytomegalovirus. Am. J. Clin. Pathol. 73, 648–654.

Bratanow, N.C., Ash, R.C., Turner, P.A., Smith, R., Haasler, G., Chitamber, C., Kansen, R. and Casper, J. (1987) Successful treatment of serious cytomegalovirus (CMV) disease with 9 (1,3-dihydroxy-2-propoxymethyl)guanine (ganciclovir, DHPG) and intravenous immunoglobulin (IVIG) in bone marrow transplant (BMT) patients [abstract no 254]. Exp. Hematol. 15, 541.

Chou, T-C. and Talalay, P. (1984) Quantitative analysis of dose-effect relationships: The combined effects of multiple drugs or enzyme inhibitors. Adv. Enzyme Regul. 22, 27–55.

Chou, J. and Chou, T.-C. (1986) Dose-effect analysis with microcomputers: Quantitation of ED_{50}, LD_{50}, synergism and antagonism, low-dose risk, receptor binding and enzyme kinetics: In: A computer for Apple II series or IBM PC and manual. Cambridge: Elsevier-Biosoft.

Chrisp, P. and Clissol, S.P. (1991) Foscarnet: A review of its antiviral activity, Pharmacokinetics properties and therapeutic use in immuno compromised patients with cytomegalovirus retinitis. Drugs

41, 104–129.
Collaborative DHPG Treatment Study Group (1986) Treatment of serious cytomegalovirus infection with 9-(1,3dihydroxy-2-propoxymethyl)guanine in patients with AIDS and other immunodeficiencies. N. Eng. J. Med. 314, 801–805.
Dankner, W.N., Scholl, D., Stanat, S.C., Xartin, M., Sonke, R.L. and Spectar, S.A. (1990) Rapid antiviral DNA-DNA hybridization assay for human cytomegalovirus. J. Virol. Methods 28, 293–298.
Derse, D., Bastow, K.P. and Cheng, Y.-C. (1982) Characterization of the DNA polymerases induced by a group of herpes simplex virus type I variants selected for growth in the presence of phosphonoformic acid. J. Biol. Chem. 257, 10251–10260.
Emanuel, D., Peppard, J., Stover, D., et al. (1986) Rapid immunodiagnosis of cytomegalovirus pneumonia by bronchoalveolar lavage using human and murine monoclonal antibodies. Ann. Int. Med. 104, 476–481.
Emanuel, D., Cunningham, I., Jules-Elysee, K., Brochstein, J.A., Kernan, N.A., Laver, J., Stover, D., White, D.A., Pels, A., Polsky, B., Castro-Xalaspina, H., Peppard, J.R., Bartus, P., Hammering, U. and O'Reilly, R.J. (1988) Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. Ann. Inter. Med. 109, 777–782.
Erice, A., Jordan, C., Chace, B., et al. (1987) Ganciclovir treatment of cytomegalovirus disease in transplant recipients and other immunocompromised hosts. JAMA 257, 3082–3087.
Erice, A., Chou, S., Biron, K.K., et al. (1989) Progressive disease due to Ganciclovir-resistant cytomegalovirus in immuno compromised patients. N. Engl. J. Med. 320, 289–293.
Eriksson, B.P. and Schinazi, R.P. (1989) Combinations of 3'-aido-3'-deoxythymidine (Zidovudine) and Phosphonoformate (Foscarnet) against human immunodeficiency virus type 1 and cytomegalovirus replication in vitro. Antimicrob. Agents Chemother. 33, 663–669.
Felsenstein, D., Dimico, D., Hirsch, N., et al. (1985) Treatment of cytomegalovirus retinitis with 9-(2-hydroxy-1-(hydroxymethyl) propoxymethyl)guanine. Ann. Intern. Med. 103, 377–380.
Gehrz, R.C., Nelson, C.N. and Kari, B.E. (1992) A combination of human cytomegalovirus (HCMV)-specific murine monoclonal antibodies exhibits synergistic antiviral activity in vitro. Antiviral Res. 17, 115–131.
Hill, R.B., Jr., Rowlands, D.T. and Rifkind, D. (1964) Infectious pulmonary disease in patients receiving immunosuppressive therapy for organ transplantation. N. Eng. J. Med. 271, 1021–1027.
Meyers, J.D., Flournoy, N. and Thomas, E.D. (1982) Nonbacterial pneumonia of the allogenic marrow transplantation: A review of ten years experience. Rev. Infect. Dis. 4, 1119–1132.
Neiman, P.E., Reeves, W., Ray, G., et al. (1977) A prospective analysis of interstitial pneumonia and opportunistic viral infection among recipients of allogenic bone marrow grafts. J. Infect. Dis. 136, 754–767.
Pollard, R.B., Nokta, M.A., Pappas, P., Holloway, M., Borucki, M.J., Wood, D.L., Zitelli, A.M., Tolpin, M.D., Nadler, P.I. and Whitley, R.J. (1992) A phase 1/11A study of a human monoclonal anti-cytomegalovirus antibody in patients with AIDS. Antiviral Res. 175(1), 111.
Quinan, G.V., Jr., Masur, H., Rook, A.H., et al. (1984) Herpes Virus infections in the acquired immune deficiency syndrome. JAMA 252, 72–76.
Reed, E.C., Bowden, R.A., Dandliker, P.S., Lilleby, K.E. and Meyers, J.D. (1988) Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. Ann. Intern. Med., 109, 783–788.
Ringden, O., Lonnqvist, B., Paulin, T., Ahlmen, J., Klintmalm, G., Wahren, B. and Lernestedt, J.O. (1986) Pharmacokinetics, safety and preliminary clinical experiences using foscarnet in the treatment of cytomegalovirus infections in bone marrow and renal transplant recipients. J. Antimicrob. Chemother. 17, 373–387.
Schmidt, G.M., Kovacs, A., Zaia, J.A., Horak, D.A., Blume, K.G., Nademanee, A.P., M.D. O'Dollell, Snyder, D.S. and Forman, S.J. (1988) Ganciclovir/immunoglobulin combination therapy for the treatment of human cytomegalovirus-associated interstitial pneumonia in bone marrow allograft recipients. Transplantation 46, 905–907.
Shepp, D.H., Dandliker, P.S., de Miranda, P., et al. (1985) Activity of 9-(2 hydroxy-1-(hydroxymethy-lethoxymethyl)guanine in the treatment of cytomegalovirus pneumonia. Ann. Intern. Med. 103, 368–

373.

Singer, D.R.J., Fallon, T.J., Schulenburg, W.E., Williams, G. and Cohen, J. (1985) Foscarnet for cytomegalovirus retinitis. Ann. Intern. Med. 103, 962.

Snydman, D.R., Werner, B.G., Tilney, N.L., Kirkman, R.L., Milford, E.L., Cho, 5.1., Bush, H.L. Jr., Levey, A.S., Strom, T.B., Carpenter, C.B., Berardi, V.P., Levey, R.H., Harmon, W.E., Zimmerman, C.E. II., Tenny, A., Heinze-Lacey, B., Shapiro, M.E., Steimnan, T., LoGerfo, F., Idelson, B., Mclver, J., Leszczynski, J. and Grady, G.F. (1988) A further analysis of primary cytomegalovirus disease prevention in renal transplant recipients with a cytomegalovirus immune globulin: interim comparison of a randomized and an open-label trial. Transplant Proc. 20 (Suppl 8), 24–30.

Stinson, E.B., Biebu, C.P., Griepp, R.B., et al. (1971) Infectious complication after cardiac transplantation in man. Ann. Intern. Med. 74, 22–36.

Stover, D.E., White, D.A., Romano, P.A., et al. (1985) Spectrum of pulmonary diseases associated with the acquired immunodeficiency syndrome. Am. J. Med. 78, 429–437.

Verdonck, L.F., de Gast, D.C., Dekker, A.W., de Weger, R.A., Schuurman, H.-J. and Rozenberg-Arska, M. (1989) Treatment of cytomegalovirus pneumonia after bone marrow transplantation with cytomegalovirus immunoglobulin combined with ganciclovir. None Marrow Transplant 4, 187–189.

Wallace, J.M. and Hannah, J. (1985) Cytomegalovirus pneumonitis in patients with AIDS. Chest 9212, 198–203.

Walmsley, L.L., Chew, E., Read, S.E., Vellend, H., Salit, I., Rachlis, A. and Fanning, M.M. (1988) Treatment of cytomegalovirus retinitis with trisodium phosphonoformate hexahydrate (Foscarnet). J. Infect. Dis. 157, 569–572.

Youle, M.S., Clarbour, J., Gazzard, B. and Chanas, A. (1988) Severe hypocalcaemia in AIDS patients treated with PFA and pentamidine. Lancet i, 1455–1456.