Journal of Medical Virology 59:323–328 (1999)

Response of Asymptomatic Cytomegalovirus Viraemia to Oral Ganciclovir 3 g/day or 6 g/day in HIV-Infected Patients

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Reactivation of cytomegalovirus (CMV) following immunosuppression may result in the development of CMV disease and is associated with an increased risk of death. CMV viraemia detected by the polymerase chain reaction (PCR) precedes CMV disease in HIV-infected patients and identifies individuals at high risk of disease. Pre-emptive ganciclovir (GCV) therapy in patients who have evidence of CMV viraemia is effective in preventing disease. An open study was conducted to assess the response of CMV viraemia to oral GCV at a dose of 3 or 6 g/day for 28 days. HIV RNA was measured to determine if CMV inhibition affected HIV viral load. Fourteen patients were studied, three of whom entered both phases of the study. None of the patients had evidence of CMV disease at the time of entry into the trial; two patients developed CMV retinitis after completion of the trial. Oral GCV at both 3 and 6 g/day caused a decrease in CMV viral load in individual patients. However, a rebound in CMV viral load occurred in patients receiving the 3-g/day dose. None of the patients receiving oral GCV 3 g/day became PCR negative after 21 days compared with six of eight patients receiving 6 g/day. Five of eight patients (63%) receiving GCV 6 g/day were concurrently taking protease inhibitors compared with two of nine (22%) receiving 3 g/day. Ten patients remained PCR negative throughout follow up. No change was found in HIV viral load during receipt of GCV at either dose. Thus, oral GCV is effective in reducing CMV viral load, but a dose of 3 g/day is insufficiently potent for pre-emptive therapy. ***J. Med. Virol. 59:323–328, 1999.***

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KEY WORDS: viral load; cytomegalovirus; polymerase chain reaction; pre-emptive therapy

INTRODUCTION

Prior human cytomegalovirus (CMV) infection is almost universal in the HIV-infected population [Gallant et al., 1992]. Reactivation of CMV replication following immunosuppression results in a high risk of disease development and an increased risk of death [Bowen et al., 1996]. Before the availability of highly active antiretroviral therapy (HAART) up to 40% of HIV-infected patients developed CMV disease, the most common manifestation of which is retinitis [Gallant et al., 1992; Feinberg et al., 1998]. CMV retinitis is costly both in terms of patient quality of life and economically as current practice dictates life-long maintenance treatment following 3–4 weeks of induction therapy with an intravenous agent [Jacobson, 1997]. CMV load may be determined using a quantitative competitive polymerase chain reaction (QCPCR) assay. Using this method, it has been shown that CMV load increases up to the time of diagnosis of retinitis [Bowen et al., 1997]. Treatment with ganciclovir or foscarnet decreases CMV load and QCPCR can be used to monitor response to therapy [Bowen et al., 1996].

Several laboratories have been attempting to identify markers of imminent CMV disease so that early intervention could potentially be used to limit end organ damage, as has been demonstrated for transplant patients (pre-emptive therapy) [Goodrich et al., 1991; Einsele et al., 1995]. It is now well established that

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Accepted 2 February 1999

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CMV PCR viraemia precedes disease in AIDS patients and identifies a cohort of individuals at high risk of CMV disease [Bowen et al., 1997; Dodt et al., 1997; Shinkai et al., 1997].

Pre-emptive therapy of asymptomatic patients should ideally be based on oral medication. Earlier work showed that oral ganciclovir is an effective alternative to the intravenous route in prevention of relapse of CMV retinitis following induction therapy [Drew et al., 1995] and it has become used widely in the maintenance therapy of retinitis. A randomised placebo-controlled trial of oral ganciclovir conducted in HIV-infected patients at high risk of CMV disease, selected only by CD4 criteria and AIDS-defining diagnosis, showed a reduction in incidence of CMV retinitis from 24% to 12% after a median of 367 days [Spector et al., 1996]. However, the study cohort was not defined virologically at baseline and therefore included patients without CMV viraemia who would have been at lower risk of retinitis as well as those with CMV viraemia at imminent risk of disease. This approach of using continuous untargeted oral ganciclovir in patients with low CD4 counts has not been adopted clinically and the cost effectiveness of such a strategy has been questioned [Moore and Chaisson, 1997; Rose and Sacks, 1997].

Studies in transplant recipients show that pre-emptive therapy in patients who have evidence of CMV viraemia is highly effective in preventing disease and avoids unnecessary treatment of low risk patients [Goodrich et al., 1991; Einsele et al., 1995]. Prolonged receipt of ganciclovir can result in the development of drug resistance, which may render treatment ineffective if disease develops subsequently [Chou et al., 1997]. A short course of pre-emptive therapy in patients with CMV viraemia identified by PCR may therefore be an efficacious method of preventing disease.

To determine whether oral ganciclovir could be considered for pre-emptive therapy, we conducted an open study to assess the response of asymptomatic CMV viraemia to 3 or 6 g/day of oral ganciclovir for 28 days. Herpesviruses, including CMV, have been implicated in up-regulation of HIV by a variety of mechanisms [Griffiths, 1998] and antiherpes therapy has been associated with decreased mortality in AIDS patients [Stein and Graham, 1996]. Therefore, we also wished to measure HIV RNA to determine if inhibition of CMV had a consequential effect on reducing HIV viral load.

MATERIALS AND METHODS

Patients

All HIV-seropositive individuals attending the Ian Charleson Day Centre at the Royal Free Hospital who had ever had a CD4 lymphocyte count of 100 cells/μl or less were screened for CMV viraemia by qualitative PCR for CMV DNA at each visit using laboratory methods described in detail elsewhere [Kidd et al., 1993]. Patients who became viraemic were screened for retinitis by indirect ophthalmoscopy by an experienced ophthalmologist (PW). Patients with no evidence of retinitis were entered into the trial after consent was obtained. A second sample was obtained at trial entry and used to define the level of baseline CMV viraemia. Monthly screening for retinitis was conducted throughout the trial period by the same ophthalmologist and has been continued subsequently. The plan was for 10 episodes of asymptomatic viraemia to be treated with 3 g/day oral ganciclovir initially followed by 10 episodes treated with 6 g/day once a clinical trials exemption certificate for the use of this dose was obtained.

Sample Preparation

Whole blood (200 μl) was processed by ion-exchange chromatography (Qiagen, UK) and the DNA stored at −70°C. Plasma (100 μl) was also stored at −70°C as part of the HIV-1 RNA quantitation kit (Roche, UK).

Sample Analysis

The PCR assays used to detect HCMV qualitatively and quantitatively have been described previously [Fox et al., 1992; Kidd et al., 1993]. An extract of the processed whole blood (5 μl) was used for the PCR analysis (equivalent to approximately 30 ng cellular DNA). The term "PCR-viraemia" is used to denote the detection of CMV in the blood by PCR. HIV-1 RNA load was measured by HIV Amplicor kits (Roche Molecular Systems, Welwyn Garden City, UK), according to the manufacturer's instructions.

RESULTS

Patients

Seventeen episodes of asymptomatic viraemia were studied. Sixteen patients were recruited to the trial. Two patients were excluded from the final analysis as they were subsequently found to be CMV negative at baseline, despite being PCR positive 3 and 8 weeks previously. Clinical details of the remaining 14 participants at trial entry are shown in Table I. Three individuals (patients 1, 2 and 4) entered both the 3- and 6-g/day phases of the study. These patients experienced recurrent CMV viraemia after completing the lower dose regimen and received the higher dose after a washout period of at least 3 months. None of the patients had evidence of retinitis at time of entry into the trial.

All patients completed 28 days of oral ganciclovir. There were no adverse events associated with therapy at either dose. Clinical follow-up data complete to February 1998 are shown in Table II. Two patients developed CMV disease. Patient 2 developed CMV retinitis 1 month after completing the 6-g schedule of oral ganciclovir. He received induction intravenous ganciclovir followed by oral ganciclovir as maintenance. At this time he received protease inhibitors for the first time as part of triple combination antiretroviral therapy. Progression of the retinitis occurred 2 months later and was again treated with intravenous ganciclovir. This treatment was followed by intravitreal foscarnet injections until he had a ganciclovir implant inserted 7

TABLE I. Clinical and Demographic Details of Patients at Time of Entry Into Trial

Patient	Dose of oral ganciclovir (g/day)	Sex	Age (years)	Previous AIDS diagnosis	Baseline	
					Antiretroviral therapy	CD4 (cells/μl)
1	3	M	50	Yes	AZT, SQV	10
1	6	—	—	—	3TC, d4T, IDV	10
2	3	M	45	No	AZT, 3TC	10
2	6	—	—	—	AZT, 3TC	50
3	3	M	44	Yes	3TC, IDV	10
4	3	M	34	Yes	d4T, 3TC, Nev	10
4	6	—	—	—	d4T, 3TC, IDV	59
5	3	M	27	Yes	AZT, 3TC	120
6	3	M	44	No	AZT, 3TC	120
7	3	M	37	No	d4T, ddI, Nev	60
8	3	M	30	Yes	AZT, 3TC	20
9	3	M	36	Yes	d4T, ddI	70
10	6	M	38	Yes	AZT, 3TC, ddI	20
11	6	M	44	Yes	d4T, 3TC, SQV	31
12	6	M	26	Yes	None	10
13	6	M	40	Yes	AZT, 3TC, RTV	91
14	6	F	35	Yes	d4T, RTV, SQV	63

Non-nucleoside reverse transcriptase inhibitors (protease inhibitors): AZT, zidovudine; 3TC, lamivudine; ddI, didanosine; d4T, stavudine; IDV, indinavir; RTV, ritonavir; SQV, saquinavir. Non-nucleoside reverse transcriptase inhibitor: Nev, nevirapine.

TABLE II. Clinical Follow-up Data as of February 1998

Patient	Time since completion of trial (months)	CMV disease after last dose oral GCV	Death	CMV PCR	Antiretroviral therapy
1	10	No	No	Pos	ddI, d4T, RTV, IDV, DMP
2	12	Retinitis at 1 month	No	Pos	ddI, d4T, RTV
3	15	No	No	Neg	d4T, 3TC, IDV
4	9	No	Suicide at 9 months	Neg	—
5	15	No	No	Neg	d4T, ddI, RTV, SQV
6	15	No	No	Neg	d4T, 3TC, Nev
7	14	No	No	Neg	d4T, ddI, Nev
8	14	No	No	Neg	d4T, RTV, SQV, Nev
9	14	No	No	Neg	AZT, 3TC, IDV
10	13	No	No	Neg	d4T, 3TC, RTV
11	13	Retinitis at 11 months	Death at 13 months	Pos	—
12	11	No	No	Pos	d4T, 3TC, NFV
13	10	No	No	Neg	RTV, IDV, DMP
14	3	No	No	Neg	d4T, RTV, SQV

For patients 1, 2, and 4, time since completion of trial is calculated from second course of treatment. CMV, cytomegalovirus; GCV, ganciclovir; PCR, polymerase chain reaction. For CMV PCR, Neg indicates patients who have remained CMV PCR negative from time of completion of trial, Pos indicates patients who have had at least one positive CMV PCR result. Nucleoside reverse transcriptase inhibitors (protease inhibitors): AZT, zidovudine; 3TC, lamivudine; ddI, didanosine; d4T, stavudine; IDV, indinavir; RTV, ritonavir; SQV, saquinavir; NFV, Nelfinavir. Non-nucleoside reverse transcriptase inhibitors: Nev, Nevirapine; DMP, DMP 266; efavirenz.

months after the initial diagnosis. The retinitis has since remained quiescent.

Patient 11 developed CMV retinitis 11 months after completing the trial. The retinitis responded to intravenous induction therapy with ganciclovir. Having recently stopped combination antiretroviral therapy with d4T, ritonavir, and saquinavir, he was recommenced on d4T, ddI, ritonavir, indinavir, and efavirenz (obtained on a named-patient basis). However, he became increasingly ill with the development of disseminated *Mycobacterium avium intracellulare* infection, *Pseudomonas aeruginosa* septicaemia, *Pneumocystis carinii* pneumonia, cardiomegaly, and intractable diarrhoea. Progressive renal and respiratory failure developed despite intensive treatment and he died 2 months after presenting with CMV retinitis. The other death in this cohort was that of patient 4, who committed suicide 9 months after completion of the trial.

Screening for CMV viraemia by PCR has continued since completion of the trial (Table II). Data were censored on 1 February 1998, follow-up times range from 3 to 15 months (median 13.5). Ten patients remained PCR negative throughout follow up. Four patients experienced recurrent viraemia, 2 of whom subsequently developed retinitis (patients 2 and 11). Patients 1 and 12 had a positive PCR result on one occasion only. Subsequently, both patients became PCR negative again and neither has developed CMV disease.

The median response of CMV load to oral ganciclovir either 3 or 6 g/day and the corresponding HIV-1 loads is shown in Figure 1. Previous work from our labora-

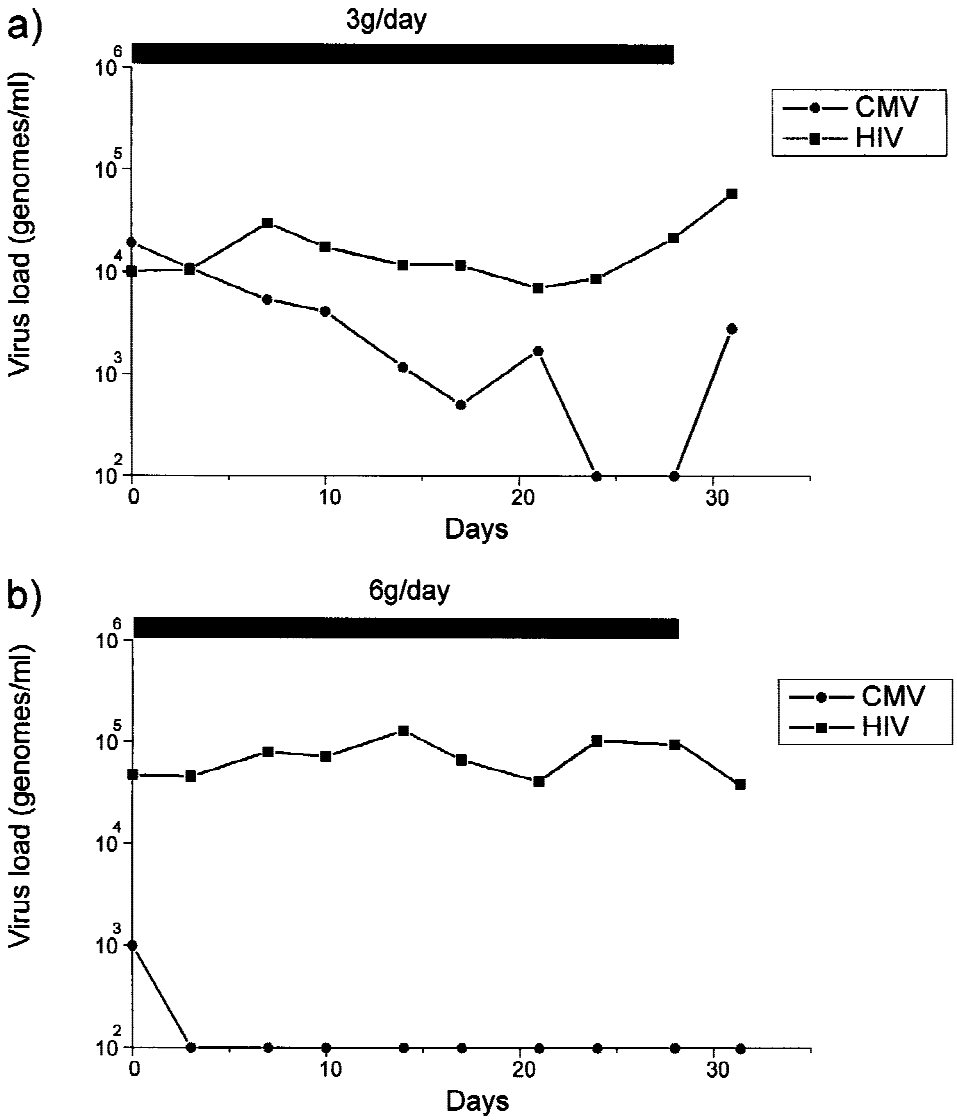

Fig. 1. Median response of cytomegalovirus (CMV) load in patients taking oral ganciclovir at either **(a)** 3 g/day or **(b)** 6 g/day together with their corresponding HIV-1 RNA loads.

tory [Bowen et al., 1997] has shown that after 21 days of intravenous GCV (5 mg/kg bid), 26 of 30 patients became PCR negative compared with 0 of 9 patients given oral GCV 3 g/day and 6 of 8 given 6 g/day (P = .744). However, it should be noted that in the present study the initial CMV load was lowest in patients receiving oral GCV 6 g/day. In seven of the treatment episodes the patient received a regimen including a protease inhibitor before commencement of oral ganciclovir. A greater proportion, five of eight patients (63%), were taking protease inhibitors at the time of participation in the 6-g/day phase of the study compared with two of nine (22%) in the 3-g/day phase (P = .1). All other patients commenced antiretroviral regimens inclusive of a protease inhibitor after completion of the trial except patient 7, who received a combination containing nevirapine. Space precludes showing all the individual curves but the three patients entered into both phases of the study are shown in Figure 2. Patient 2 did not receive HAART between entry into the two phases of the study and his CMV load and HIV load remained high at the time of entry to the 6-g/day study. Patient 4 received HAART in the intervening period and his CMV and HIV loads were lower at entry to the 6-g/day study. However, despite receiving HAART and showing a reduced HIV load, patient 1 had a higher CMV load at entry to the 6-g/day study.

In summary, oral GCV at both 3 and 6 g/day caused a decrease in viral load in individual patients, with a rebound in viral load in patients receiving oral GCV 3 g/day, suggesting that the response to oral GCV is more durable when given at 6 g/day than at 3 g/day. No significant change was seen in HIV viral load during receipt of ganciclovir at either dose.

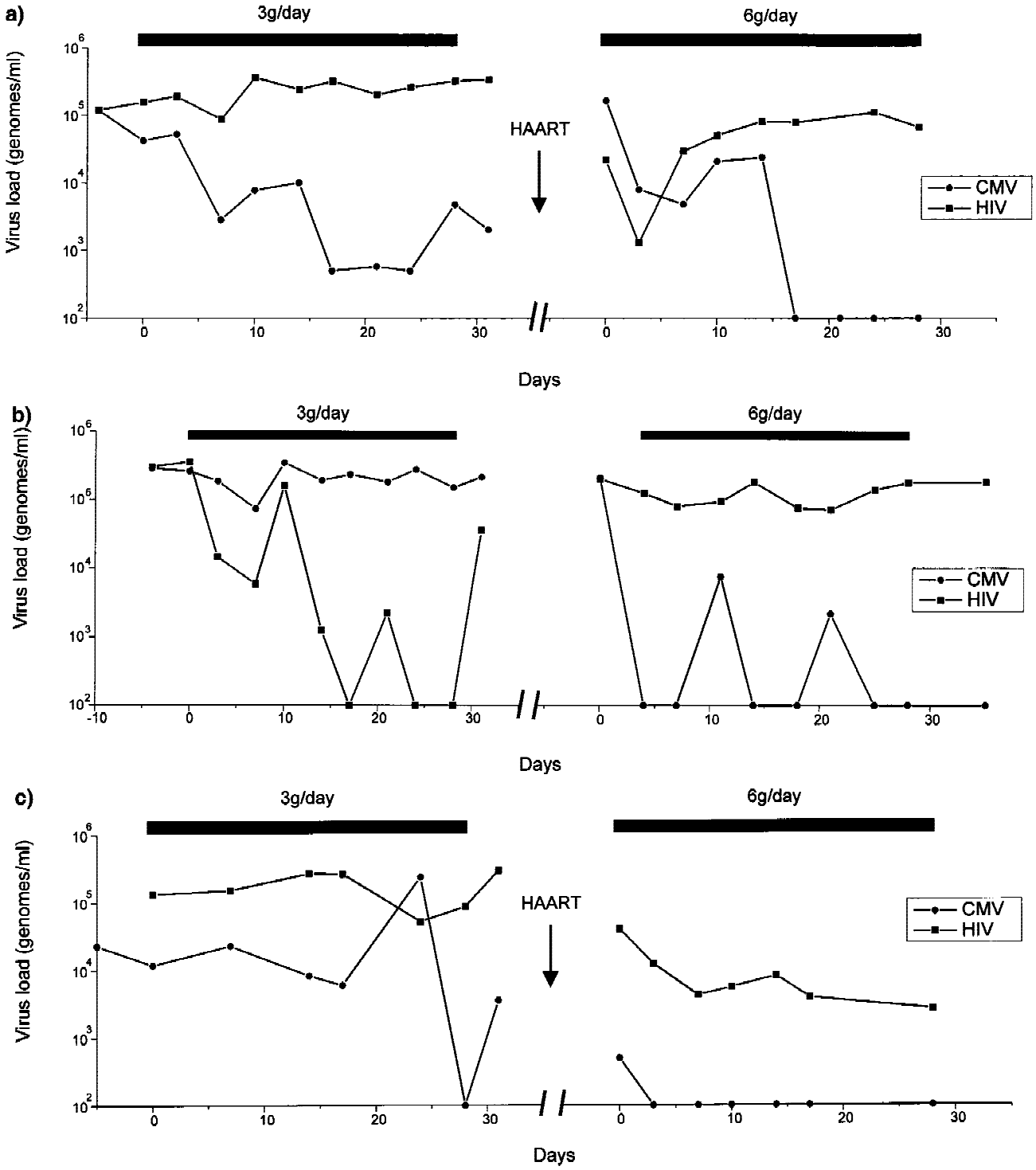

Fig. 2. Cytomegalovirus (CMV) and HIV-1 loads for patient 1 **(a)**, patient 2 **(b)**, and patient 4 **(c)** treated with oral ganciclovir 3 g/day followed by 6 g/day, with a wash-out period between treatments.

DISCUSSION

A 28-day course of oral ganciclovir was effective in reducing CMV viral load in asymptomatic viraemia. The rate of decline appeared more rapid with 6 g/day of oral ganciclovir and the results suggest that the response to this dose may be more durable. Both the 3- and 6-g/day doses were well tolerated with no serious adverse events observed. However, the numbers in our unrandomised study were small and the results may be confounded by the use of HAART in these patients. Recruitment to the study declined once HAART had

become used widely in our clinic, implying that HAART may affect the prevalence of asymptomatic CMV viraemia, an issue that is under investigation. A higher proportion of patients receiving 6 g/day of oral ganciclovir was taking combination antiretroviral therapy including a protease inhibitor, although this difference was not statistically significant. This might account for the lower median CMV loads at baseline in this group. However, the median HIV RNA level was actually higher at baseline in those receiving 6 g/day. Rebound of CMV viraemia was observed in some cases after completion of oral ganciclovir but suppression of CMV viraemia has been sustained in most of these patients and there has been a lower incidence of CMV disease than would have been expected. Whether these long-term effects on viraemia and disease are due to ganciclovir or the high rate of protease inhibitor usage following completion of the trial cannot be defined.

In conclusion, oral ganciclovir is effective in reducing CMV viral load. It is a suitable agent for a controlled trial of pre-emptive therapy of asymptomatic viraemia and we suggest the dose of 6 g/day be considered for this purpose. Evidence that 3 g/day is insufficiently potent for pre-emptive therapy is also provided by analysis of the placebo-controlled trial now that baseline PCR data are available [Spector et al., 1998]. Alternative doses include 4.5 g/day or higher levels obtained through the use of valganciclovir. Because protease inhibitor therapy may be effective in suppressing CMV viraemia in the absence of ganciclovir, we suggest that a trial of pre-emptive therapy could be conducted in patients unable or unwilling to take HAART or those experiencing recurrent CMV viraemia, which may be an early manifestation of HAART failure.

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