Cytomegalovirus pp65 Antigenemia-Guided Early Treatment With Ganciclovir Versus Ganciclovir at Engraftment After Allogeneic Marrow Transplantation: A Randomized Double-Blind Study

By Michael Boeckh, Ted A. Gooley, David Myerson, Terri Cunningham, Gary Schoch, and Raleigh A. Bowden

To determine whether cytomegalovirus (CMV) antigenemia-guided ganciclovir treatment may be as effective, may require less treatment, and thus may cause less marrow toxicity than ganciclovir administered at engraftment, 226 marrow transplant recipients were randomized at engraftment to receive placebo (antigenemia-ganciclovir group) or ganciclovir (ganciclovir group) until day 100 in a double-blind study. In patients with antigenemia of 3 or more positive cells in 2 slides and/or viremia, study drug was discontinued and ganciclovir was started for at least 3 weeks or until negative CMV antigenemia and resumed only if antigenemia recurred. More patients in the antigenemia-ganciclovir group developed CMV disease before day 100 after transplantation compared with the ganciclovir group (14% *v* 2.7%, *P* = .002). Of the 16 patients with CMV disease before day 100 in the antigenemia-ganciclovir group, 10 (8.8%) had disease before or during the first episode of antigenemia and 6 (5.3%) developed disease after discontinuation of ganciclovir. Untreated low-grade antigenemia progressed to CMV disease in 19% of patients with grade 3-4 compared with 0% of patients with grade 0-2 acute graft-versus-host disease (*P* = .04). There was no significant difference in CMV disease by day 180 after transplantation and thereafter. CMV-related death, transplant survival, and neutropenia were not significantly different between the groups. In the ganciclovir group, more invasive fungal infections occurred (*P* = .03) and more ganciclovir was used (*P* < .0001). Thus, delaying the start of ganciclovir until high-grade antigenemia and discontinuing ganciclovir based on negative antigenemia results in more CMV disease by day 100 than ganciclovir administered at engraftment. However, ganciclovir at engraftment is associated with more early invasive fungal infections and more late CMV disease resulting in similar survival rates.

MAJOR ADVANCES have been made in the prevention of cytomegalovirus (CMV) disease after allogeneic marrow transplantation with the availability of ganciclovir. Ganciclovir administered at the time of first excretion from urine, blood, throat, or bronchoalveolar lavage (BAL) has been shown to be effective in preventing CMV disease.[1,2] This strategy results in 60% to 70% of patients receiving ganciclovir and successfully prevents CMV disease in these patients. However, 30% to 35% of patients develop neutropenia[1,2] and a substantial number of patients develop disease without preceding CMV excretion or BAL positivity, ie, 12% to 13% in earlier studies[1,2] and 29% to 32% in recent studies that include high-risk seropositive patients.[3,4] The more aggressive approach of administering ganciclovir at engraftment was shown to be even more effective in preventing CMV disease before day 100 after transplantation in seropositive patients (ie, incidence of disease 0% to 9%).[3,5] However, it requires treatment of 100% of patients, resulting in up to 65% of patients receiving ganciclovir unnecessarily; also, neutropenia is prolonged when compared with the culture-guided strategy (4 *v* 12 days) associated with an increased relative risk for bacterial infections,[1,3] and reconstitution of protective CMV-specific T-cell responses is delayed.[6] Thus, a strategy that focuses ganciclovir treatment to patients at highest risk for disease and reduces the incidence and duration of neutropenia would be of benefit.

Recently, more sensitive detection methods have been developed to identify CMV reactivation before the onset of disease, thereby eliminating the need to treat every patient who is seropositive, some of whom are clearly not at risk for CMV disease. These methods include direct detection of CMV pp65 antigen (antigenemia) in peripheral blood leukocytes (PBL)[7] and detection of CMV DNA by the polymerase chain reaction (PCR) in PBL[8] and in plasma and serum.[9,10] The CMV antigenemia assay detects CMV in marrow transplantation patients with CMV pneumonia on an average of 10 days before the onset of pneumonia and can also be quantified, a feature that could be used to further predict the patients at highest risk for CMV disease, thereby reducing the number of patients treated.[11] The aims of this study were to test the hypotheses that an early treatment strategy based on CMV antigenemia can (1) identify patients at risk before the onset of disease; (2) increase the specificity for disease by taking advantage of the quantitative nature of the test; (3) permit discontinuation of ganciclovir based on a negative antigen test without rebound disease; (4) reduce ganciclovir use, thereby reducing neutropenia and its complications; and (5) lead to a better reconstitution of CMV-specific T-cell responses measured by the incidence of late CMV disease. We chose the antigenemia assay rather than PCR as the primary means to guide ganciclovir treatment because initial data showed PCR to be too sensitive, with a lower positive predictive value and a longer time to cessation after the start of ganciclovir compared with antigenemia.[12,13] However, we evaluated the PCR methodology simultaneously to validate these initial findings.

MATERIALS AND METHODS

Patients. CMV-seropositive patients of all ages undergoing allogeneic marrow transplantation were eligible for this study. Condi-

From the Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, WA.

Submitted April 3, 1996; accepted July 9, 1996.

Supported by American Cancer Society Grant No. RD-361 and National Institutes of Health Grants No. CA 18029, HL 36444, and CA 15704.

Address reprint requests to Michael Boeckh, MD, Fred Hutchinson Cancer Research Center, Program in Infectious Diseases, 1124 Columbia St, Seattle, WA 98104.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

0006-4971/96/8810-0026$3.00/0

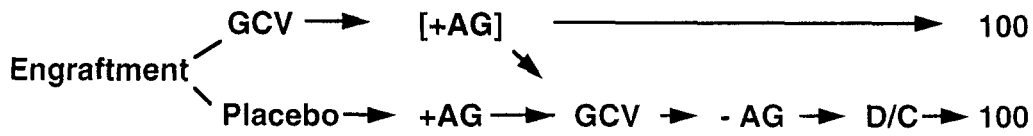

Fig 1. Study design. At engraftment, patients were randomized in a double-blind fashion. If antigenemia at a level of 3 or more positive cells per duplicate slides occurred, patients were started on open label ganciclovir for 3 weeks or until 6 days after cessation of antigenemia, whichever occurred later. Ganciclovir was resumed for 2 weeks or until cessation of antigenemia if antigenemia recurred before day 80 after transplantation. Antigenemia of less than 3 positive cells per duplicate (low-grade) did not result in the start of open-label ganciclovir; however, antigenemia was retested within 2 to 4 days. No treatment decisions were made based on results from PCR testing.

tioning regimens and prophylaxis for graft-versus-host disease (GVHD) have been described previously.[14,15] The protocol was approved by the Institutional Review Board and signed informed consent was obtained before transplantation. Acyclovir was administered to herpes simplex virus (HSV)-seropositive individuals at a dose of 250 mg/m^2 intravenously (IV) twice daily from start of conditioning until day 30 after transplantation. Documented HSV infections were treated with acyclovir at a dose of 250 mg/m^2 IV three times daily or 400 mg orally (PO) five times daily for 1 week. All patients received fluconazole at a dose of 400 mg/kg daily from conditioning until day 75 after transplantation.[16]

Protocol design. At engraftment (absolute neutrophil count [ANC] 0.750 $\times$ 10^{-9}/L for 2 days), either placebo (antigenemia-ganciclovir group) or ganciclovir (5 mg/kg body weight, administered twice daily for 5 days and then once daily 6 days per week until day 100 after transplantation; ganciclovir group) was administered in a randomized, double-blind fashion (Fig 1). If CMV antigenemia at a level of 3 or more positive cells in 2 slides (high-grade) and/or CMV viremia was detected, the study drug was stopped and open-label ganciclovir was started at a dose of 5 mg/kg twice daily for 7 days followed by once daily for 3 weeks or until 6 days after cessation of antigenemia, whichever occurred later. Antigenemia of less than 3 positive cells in 2 slides (low-grade) did not result in start of open-label ganciclovir; however, antigenemia was retested within 2 to 4 days. Ganciclovir was resumed 7 days at 5 mg/kg twice a day followed by 7 days of 5 mg/kg or until cessation of antigenemia if high-grade antigenemia recurred before day 80 after transplantation. Patients were excluded from randomization if they had a serum creatinine of $\geq$2.5 mg/L; had CMV disease, viremia, or antigenemia before randomization; or had received ganciclovir or foscarnet in the preceding 7 days or any other investigational antiviral drug at any time.

CMV monitoring. Weekly heparinized blood samples were fractionated by dextran-sedimentation, and PBL were subsequently divided for culture inoculation, CMV antigenemia testing, and testing by PCR.[17] Blood cultures included both shell vial centrifugation and conventional cultures that were maintained for at least 3 weeks.[18,19] CMV antigenemia testing was performed in duplicate as described[17] using cytocentrifugation slides prepared of 1.5 $\times$ 10^5 PBL per slide, fixation initially with acetone[11] and subsequently (after October 14, 1992) with formaldehyde[17,20] (Table 1), and immunofluorescence staining with monoclonal antibodies C10/C11 (Clonab; provided by Biotest Diagnostic Corp, Denville, NJ). For detection of CMV DNA by PCR, 1.5 $\times$ 10^5 PBL were alkaline extracted and DNA was amplified from the 4th exon of the CMV immediate early 1 gene, as described.[21,22] Ten copies of CMV DNA were routinely detectable by this method.[23] PCR results were not used to make treatment decisions.

Definitions. CMV disease was defined as the demonstration of CMV by biopsy specimens from visceral sites by culture or histology[1] or if CMV was detected in culture or direct fluorescent antibody stain in BAL in the presence of new or changing pulmonary infiltrates.[1,3] CMV disease before day 100 after transplantation (early CMV disease) was analyzed separately as disease occurring before or during the first episode of high-grade antigenemia and as disease occurring after discontinuation of ganciclovir based on a negative antigen test. CMV disease after day 100 was defined as late CMV disease.

Statistical design. The primary endpoints of this study were CMV disease and neutropenia. The study was designed to show both equivalence in preventing CMV disease and a reduction in the incidence of neutropenia. The sample size was calculated to assure that the difference in CMV disease rates was no more than 5% between the groups, assuming a projected incidence of CMV disease of 0% to the ganciclovir group[3] and 0% to 6% in the antigenemia-ganciclovir group.[11] This required 108 patients per group (80% power, two-sided .05 significance level).[24] To allow detection of a reduction in rates of neutropenia of 30% in the ganciclovir group to 10% in the antigenemia-ganciclovir group, 80 patients per group were required (90% power, two-sided .05 significance level). Thus, the projected total study population was 216 randomized patients.[24] Patients were stratified for the presence of acute GVHD (which is a major risk factor for CMV disease and invasive fungal infections) and the use of hematopoietic growth factors before engraftment due to a possible influence on the development of neutropenia. An interim analysis performed after half of the projected patients had completed a follow-up of 75 days did not show a difference in CMV pneumonia or survival at a two-sided significance level of P = .001 and the study was continued. Patients who received at least one dose of study drug were analyzed by intent-to-treat. Neutropenia, CMV infection, and disease rates were estimated by the proportion of patients reaching the endpoint. Survival rates were estimated by the Kaplan-Meier method. Comparison of the hazard rates of disease, neutropenia, and survival were made with the log-rank test. Patients who died without reaching the appropriate event were censored at the time of death. Cox proportional hazards models were used to analyze risk factors for secondary CMV disease or antigenemia and to estimate relative risks of disease and infection. No adjustments were made for multiple comparisons in calculating reported P values, and all P values are two-sided.

RESULTS

Study population. From March 1992 to February 1994, 284 patients signed an informed consent agreement before transplantation. Between transplantation and engraftment, 53 patients became ineligible for study before randomization due to death before engraftment (n = 25; including 2 with CMV-related death), renal failure (n = 10), early discharge from the center (n = 4), antigenemia or viremia before engraftment (n = 6), patient refusal (3), marrow boost or additional transplantation (2), ganciclovir (n = 1) or high-dose acyclovir (n = 1) before engraftment by error, and incorrect

Table 1. Characteristics of Study Groups

Characteristic	Antigenemia-Ganciclovir (n = 114)	Ganciclovir (n = 112)
Median age in years (range)	37 (0.8-60.0)	33 (1.4-59.7)
Sex (M/F)	63/51	62/50
Serostatus before transplant		
Recipient CMV-positive	114 (100)	112 (100)
Donor CMV-positive	69 (61)	76 (68)
Underlying disease		
Acute lymphocytic leukemia	10 (9)	16 (14)
Acute nonlymphocytic leukemia	23 (20)	28 (25)
Chronic myelogenous leukemia	50 (44)	39 (35)
Myelodysplastic syndrome	11 (10)	9 (8)
Multiple myeloma	6 (5)	8 (7)
Non-Hodgkin's lymphoma	5 (4)	2 (2)
Hodgkin's disease	1 (1)	4 (4)
Aplastic anemia	7 (6)	5 (4)
Other	1 (1)	1 (1)
Disease stage		
Remission or chronic phase	45 (40)	51 (45)
Advanced disease	17 (15)	11 (10)
Relapse	31 (27)	31 (28)
Not applicable	21 (18)	19 (17)
No. of transplants		
First transplant	111 (97)	109 (97)
Second transplant	3 (3)	3 (3)
HLA donor matching		
Matched-related	56 (49)	55 (49)
Mismatched-related	21 (18)	18 (16)
Unrelated	37 (33)	39 (35)
Conditioning therapy		
Cyclophosphamide + TBI	65 (57)	56 (50)
Busulfan + cyclophosphamide	23 (20)	24 (21)
Busulfan + cyclophosphamide + FTBI	11 (10)	15 (13)
Other	15 (13)	17 (15)
Any TBI	86 (75)	80 (71)
GVHD prophylaxis		
CSA plus methotrexate	77 (68)	73 (65)
Methotrexate	5 (4)	5 (5)
CSA	10 (9)	5 (5)
Other	22 (19)	29 (25)
Intravenous Ig*		
Weekly administration	52 (46)	48 (43)
Substitution if hypogammaglobulinemic	62 (54)	64 (57)
GVHD		
Grades 0-1	29 (25)	23 (22)
Grades 2-4	85 (75)	87 (78)
Grades 3-4	40 (35)	37 (33)
Start of study drug (day after transplant)		
Median (range)	21.5 (12-41)	20.5 (9-41)
Fixative in CMV antigenemia assay		
Acetone	34 (29)	32 (29)
Formaldehyde	80 (71)	80 (71)

Abbreviations: TBI, total body irradiation; FTBI, fractionated total body irradiation; CSA, cyclosporine A.

Unless otherwise indicated, the values shown are the no. of patients, with the percentage in parentheses.

* Weekly administration as described[38]; after January 1, 1993, hypogammaglobulinemic patients (IgG < 400 mg/dL) were substituted to keep serum IgG levels between 400 and 600 mg/dL.

classification of transplant type (ie, syngeneic transplant; n = 1). Five patients met the criteria of engraftment and were randomized but were not allowed to receive any study drug by request of the attending physician. Two of these 5 patients developed CMV pneumonia; both were randomized to ganciclovir. The remaining 226 patients were randomized and received at least one dose of study drug (Table 1).

CMV disease. The incidence of CMV disease is shown in Table 2. In the antigenemia-ganciclovir group, 4 of 10 patients with disease before or during the first course of ganciclovir (all pneumonia) had low-grade antigenemia detected 9.5 days (range, 3 to 14 days) before the onset of disease or first high-grade antigenemia; 4 patients (including the 3 with gastrointestinal [GI] disease) did not have antigenemia detected at the onset of disease. Before the onset of disease after discontinuation of antigenemia-guided treatment, 2 of 8 patients had low-grade antigenemia (detected at 2 and 7 days, respectively); 4 patients did not have antigenemia detected at the onset of disease.

Late CMV disease occurred in 8 of 96 patients (8.3%) in the antigenemia-ganciclovir group who were alive at day 100 compared with 16 episodes in 97 patients (16.5%) in the ganciclovir group (Table 2; $P = .2$). Fourteen of 18 cases of late CMV pneumonia (78%) were fatal, compared with none of 6 cases of GI disease.

No statistically significant risk factors (ie, total body irradiation, acute GVHD 2-4 and 3-4, unrelated donor status, or positive CMV donor serology) for CMV disease or antigenemia after completion of the first course of antigenemia-guided ganciclovir could be identified.

The impact of the fixative (ie, acetone *v* formaldehyde; Table 1) was analyzed separately because previous reports had suggested a higher sensitivity with formaldehyde fixation.[17,20] There appeared to be a somewhat higher incidence of disease by day 100 in the antigenemia-ganciclovir group when acetone fixation was used (ie, 7 of 34 [20.6%]) compared with the period when formaldehyde fixation was used (ie, 9 of 80 [11.3%]). All 7 cases of disease with acetone fixation occured before or during ganciclovir early treatment. In contrast, of the 9 cases that occured during the period when formaldehyde fixation was used, 6 were diagnosed shortly after discontinuation of ganciclovir based on a negative antigen test. In the ganciclovir group, there was only 1 case of disease in 32 patients (0.9%) before day 100 when acetone fixation was used, compared with 2 in 80 patients (3%) when formaldehyde was used. However, the incidence of disease by day 100 between both study groups remained statistically different (acetone: 20.6% *v* 0.9%, $P = .02$; formaldehyde: 11.3% *v* 3%, $P = .03$). Also, there was no significant difference in CMV disease by day 400 between both study groups (acetone: 26.5% *v* 15.6%, $P = .22$; formaldehyde: 17.5% *v* 16.3%, $P = .89$).

CMV pp65 antigenemia. The probabilities of developing CMV antigenemia in both groups are shown in Fig 2. In the antigenemia-ganciclovir group, 90 patients (79%) developed antigenemia at any level and high-grade antigenemia occurred in 80 patients (70%). Of 46 patients in the antigenemia-ganciclovir group who presented with low-grade antigenemia, 38 (82.3%) progressed to high-grade antigenemia after a median of 8 days (range, 2 to 47 days); 4 (8.7%) progressed to CMV pneumonia, 2 of whom had developed high-grade antigenemia and were started on ganciclovir before the onset of disease. Progression from low-grade antigenemia to CMV pneumonia was more likely in the presence of grade 3-4 GVHD, because 4 of 21 patients with low-grade antigenemia/grade 3-4 GVHD (19%) progressed to CMV disease compared with none of 25 patients with low-grade antigenemia/no or grade 1-2 acute GVHD ($P = .037$).

In the ganciclovir group, 46 patients (41%) developed anti-

Table 2. Occurrence of CMV Disease

Incidence of CMV disease	No. of Patients (%) Antigenemia-Ganciclovir (n = 114)	Ganciclovir (n = 112)	*P* Value*	RR	(95% CI)†
Between study entry and day 100					
Before or during ganciclovir	10 (8.8)	1 (0.9)	.01		
Pneumonia	7‡	0			
GI disease	3	1			
After stop of ganciclovir§	6 (5.3)	2‖ (1.8)			
Pneumonia	6	2‖			
Total CMV disease	16 (14.1)	3 (2.7)	.002	5.7	(1.7-19.5)
Between study entry and day 400					
Pneumonia	19	10¶			
GI disease	4	7			
Other	0	2			
Total CMV disease	23 (20.2)	18 (16.1)	.42	1.3	(0.7-2.4)

* By log-rank test.

† Relative risk (RR) and 95% confidence interval (CI) of event for antigenemia-ganciclovir group relative to ganciclovir group.

‡ In 1 patient, ganciclovir treatment was delayed for 7 days after detection of high-grade antigenemia; 2 patients were not retested after initial low-grade antigenemia and developed pneumonia simultaneously with the next scheduled test.

§ CMV disease that occurred after discontinuation of open-labeled ganciclovir based on a negative antigen test.

‖ Both patients developed secondary disease after switch to open label ganciclovir for breakthrough antigenemia and subsequent discontinuation based on a negative antigen test.

¶ One patient had two episodes of CMV pneumonia after day 100 at day 137 and day 382 after transplantation, respectively.

genemia at any level and 27 patients (24%) developed high-grade antigenemia. Of those 27 patients, 22 became positive while receiving ganciclovir a median of 14 days (range, 1 to 43 days) after the start of ganciclovir, and 5 became positive after discontinuation of ganciclovir due to neutropenia.

In the antigenemia-ganciclovir group, antigenemia was detectable for more than 3, 5, and 8 weeks after start of ganciclovir in 39.2%, 18.3%, and 4.5% of patients, respectively. After the start of ganciclovir, an intermittent increase in quantitative antigenemia for up to 2 weeks occurred in 33% of patients without an increased risk of subsequent disease. A second episode of high-grade antigenemia occurred in 30% of patients after a median of 12 days (range, 1 to 25 days) after discontinuation of the first ganciclovir course. A third episode occurred in 3 of 23 (13%) patients who completed a second treatment course after a median of 7 days (range, 1 to 8 days).

Detection of CMV DNA by PCR. The probabilities of becoming positive by PCR in the two groups are shown in Fig 2. In the antigenemia-ganciclovir group, the median times of onset were 42 days (range, 21 to 94 days) for any antigenemia, 43 days (range, 20 to 97 days) for high-grade antigenemia, 33 days (range, 16 to 98 days) for single PCR, and 41 days (range, 18 to 99 days) for the second of two consecutive positive tests. Eighty-four percent of antigenemia-ganciclovir patients became PCR-positive between engraftment and day 100, compared with 50% of ganciclovir recipients. Two consecutive positive PCR results occurred in 84 (73%) patients in the antigenemia-ganciclovir group and in 33 (29%) patients in the ganciclovir group. After the start of ganciclovir for high-grade antigenemia, PCR and antigenemia became negative after a median of 18 days (range, 3 to 90 days) and 18 days (range, 3 to 62 days), respectively ($P = .46$).

In the antigenemia-ganciclovir group, CMV was detected by PCR in all 10 patients with disease that occurred before or during the first episode of antigenemia a mean of 14.3 days (range, 2 to 34 days) before the onset of disease or first high-grade antigenemia; 8 of the 10 patients (including 5 with CMV pneumonia) were positive by PCR at least 1 week before onset. PCR positivity for 2 consecutive weeks was detectable in 5 of 10 patients a mean of 12.6 days (range, 2 to 21 days; for the second of 2 consecutive tests) before onset of disease or first high-grade antigenemia. In all 8 patients in both groups who developed disease after discontinuation of ganciclovir, PCR was also negative at the time when ganciclovir was discontinued based on a negative antigen test. PCR positivity preceded the onset of early relapse disease in only 3 of 8 patients by 5, 9, and 12 days.

CMV viremia. Nine antigenemia-ganciclovir patients (7.9%) developed viremia and antigenemia on the same day compared with no ganciclovir patients ($P = .003$). Viremia without concomitant antigenemia occurred in 1 patient in the antigenemia-ganciclovir group and in no patients in the ganciclovir group. Thus, viremia detected by shell viral cultures was the first sign of infection in only 1 of 90 patients (1.1%) with antigenemia and/or viremia.

Adverse events. The incidence of neutropenia for all randomized patients in both groups and subset analyses are shown in Table 3. Although significantly less ganciclovir was used in the antigenemia-ganciclovir group (Table 3), the overall incidence of neutropenia between randomization and day 100 after transplantation was not reduced. The incidence of more severe neutropenia was also not significantly different between the groups (antigenemia-ganciclovir *v* ganciclovir): ANC less than 0.5×10^{-9}/L, 26% versus 20%; ANC less than 0.2×10^{-9}/L, 9% versus 4%. The median duration of various levels of neutropenia in patients with

neutropenia also was not different between the groups (ANC $<0.75 \times 10^{-9}$/L, 6 days in both groups; ANC $<0.5 \times 10^{-9}$/L, 3 *v* 3.5 days; ANC $<0.2 \times 10^{-9}$/L, 2.5 *v* 1 days).

The overall use of growth factors was not different between the groups, and growth factor use did not change the incidence of neutropenia (Table 3). In the antigenemia-ganciclovir group, 9% of patients developed neutropenia between engraftment and first antigenemia and 23% after treatment with ganciclovir for antigenemia.

Discontinuation of study drug due to unclear coma, ataxia, or seizures was more common in the ganciclovir group (antigenemia-ganciclovir, 0; ganciclovir, 5; $P = .04$). Serum creatinine, platelet requirement, and days of hospitalization were not statistically significantly different between the groups (Table 3).

Bacterial, fungal, and other viral infections. The probability of developing an invasive fungal infection in the ganciclovir group was significantly increased ($P = .03$; Table 4). There was significantly more HSV-related disease in the antigenemia-ganciclovir group ($P < .001$). There were no significant differences in the incidence of bacterial and varicella zoster virus infections (Table 4).

Survival. There was no statistically significant difference in survival and nonrelapse mortality between both groups. Survival rates for the antigenemia-ganciclovir and ganciclovir groups were 84% and 87%, respectively, at day 100 ($P = .51$); 73% and 71%, respectively, at day 180 ($P = .91$); 61% and 59%, respectively, at day 400 ($P = .80$); and 54% and 47%, respectively at last contact (January 11, 1996, $P = .48$). Therefore, the competing risk of death (without CMV disease) had no discernable effect on the incidence of CMV disease by day 400 after transplantation. The incidence of CMV-related deaths (ie, within 6 weeks of diagnosis of disease) was not statistically different between the groups (day 180: antigenemia-ganciclovir 8, ganciclovir 4, $P = .21$; day 400: 13 in both groups), although the study was not powered to detect potential differences. By day 180, there were more deaths related to gram-negative bacterial or invasive fungal infections in patients randomized to ganciclovir at engraftment (antigenemia-ganciclovir 6; ganciclovir 15; $P = .035$).

Compliance with the protocol. The following protocol violations were recorded: failure to repeat antigenemia testing after a low positive test result within 4 days (16 of 152 [10.5%] occasions that resulted in the development of pneumonia in 2 patients in the antigenemia-ganciclovir

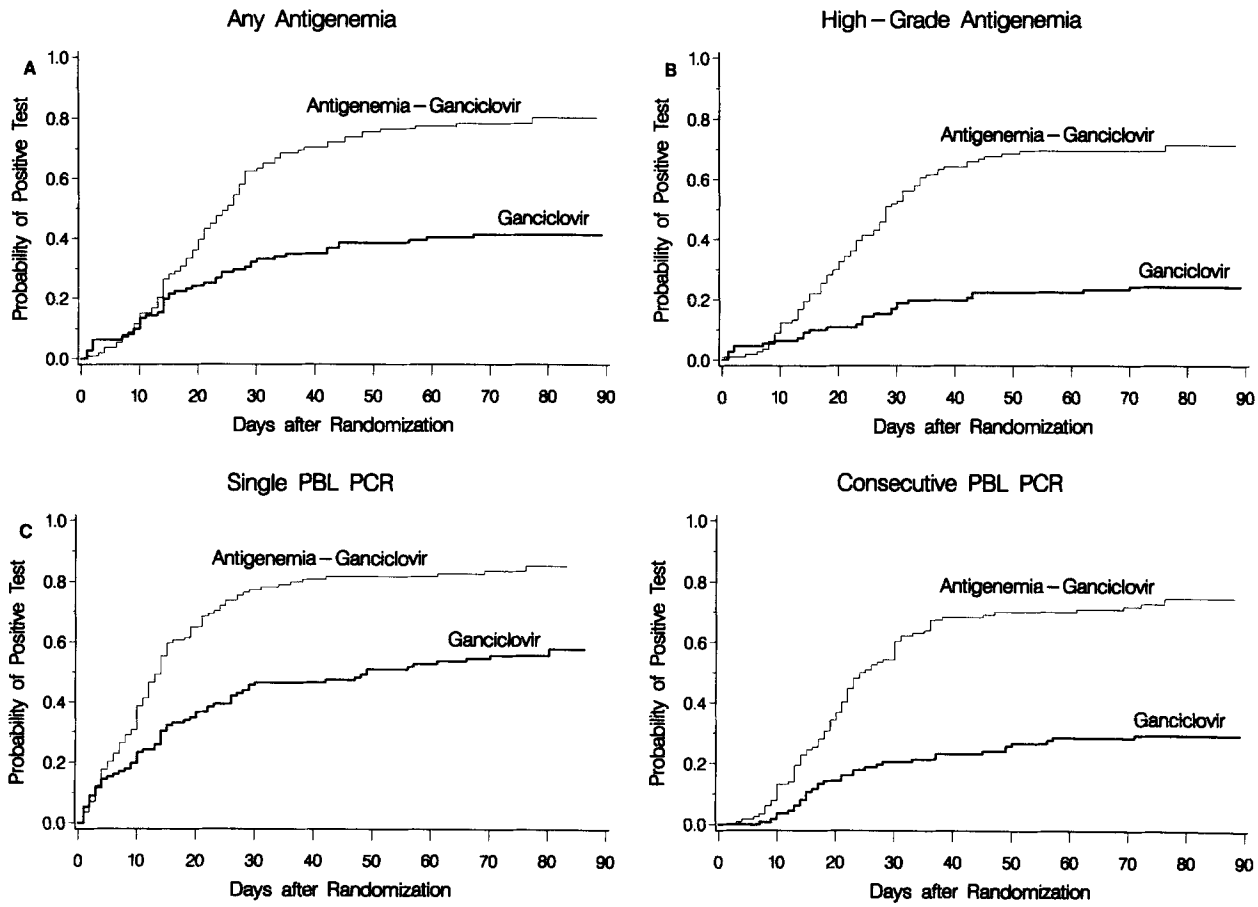

Fig 2. Time to PCR in PBL and antigenemia. Cumulative incidence estimates[37] in patients randomized to placebo (antigenemia-ganciclovir group) and ganciclovir at engraftment (ganciclovir group) for any antigenemia (any AG$^+$; A), high-grade antigenemia (ie, >3 positive cells per duplicate slides [high-grade AG$^+$]; B), a single positive PCR result (single PBL PCR$^+$; C), and two consecutive positive PCR results (consecutive PBL PCR$^+$; D). For patients with two consecutive positive PCR results, the time of the second test is shown.

Table 3. Adverse Events, Use of Growth Factors and Ganciclovir, and Days of Hospitalization

Endpoint*	Antigenemia-Ganciclovir	Ganciclovir	*P* Value
Incidence of neutropenia†			
All randomized patients	36/114 (32)	28/112 (25)	.19‡
With growth factors§	8/16 (50)	8/25 (32)	.13‡
Without growth factors	28/98 (29)	20/87 (23)	.33‡
Patients receiving assigned treatment‖	36/114 (32)	27/86 (31)	.87‡
With growth factors§	8/16 (50)	7/16 (44)	.50‡
Without growth factors	28/98 (29)	20/70 (29)	.97‡
Platelet requirement in median units (range)	18 (0-34)	15 (0-438)	.86¶
Use of ganciclovir in median days (range)	22 (0-73)	56 (1-83)	<.0001¶
Use of growth factors in mean days (range)	6 (0-42)	6 (0-58)	.68¶
Serum creatinine			
>2.0 mg/L	40 (35)	50 (45)	.14#
>2.5 mg/L	16 (14)	22 (20)	.26#
Hospitalization in median days (range)	18 (2-71)	16 (1-84)	.68¶

Unless otherwise indicated, the values shown are the no. of patients, with the percentage in parentheses.

* Figures indicate events occurring between randomization and day 100 after transplantation.

† Neutropenia was defined as an ANC of less than 0.750×10^{-9}/L for 2 consecutive days.[3]

‡ By log-rank test.

§ Hematopoietic growth factors administered before reaching the endpoint of neutropenia (ANC $<0.75 \times 10^{-9}$/L for 2 consecutive days).

‖ Excluding patients in the ganciclovir group who were taken off ganciclovir early due to negative antigenemia after breakthrough antigenemia or due to discontinuation of study drug due to reasons other than neutropenia or antigenemia or CMV disease.

¶ Wilcoxon rank sum test.

χ^2 test.

group); and failure to administer open-label ganciclovir for 7 days after CMV antigenemia was detected in 1 patient in the antigenemia-ganciclovir group who subsequently progressed to pneumonia.

DISCUSSION

It was our hypothesis that the quantitative CMV antigenemia assay could be used to initiate ganciclovir treatment in patients at highest risk for disease early enough to prevent CMV disease and to use it to guide safe discontinuation of ganciclovir, thereby limiting the duration of ganciclovir and thus preventing ganciclovir-related neutropenia and its complications. This study showed that ganciclovir administered at engraftment was more effective in prevention of CMV disease before day 100 than was intermittent antigenemia-guided ganciclovir. However, there was no statistically sig-

Table 4. Bacterial, Fungal, and Other Viral Infections

Time to First Infection*	No. of Patients (%) Antigenemia-Ganciclovir (n = 114)	No. of Patients (%) Ganciclovir (n = 112)	*P* Value†	RR	(95% CI)‡
Bacterial infections					
Gram-positive bacteremia	32 (28)	27 (24)	.53	1.2	(0.7-2.0)
Gram-negative bacteremia	12 (11)	17 (15)	.35	0.7	(0.3-1.5)
Organ-site infection§	2 (2)	6 (5)	.16	0.3	(0.1-1.7)
Any bacterial infection	46 (40)	50 (45)	.86		
Fungal infections					
Fungemia	1 (1)	4 (4)	.18	0.3	(0.03-2.2)
Invasive aspergillosis	6 (5)	14 (12)	.11	0.5	(0.2-1.2)
Any invasive fungal infection‖	7 (6)	18 (16)	.03	0.4	(0.2-0.9)
Viral infections					
HSV	38 (33)	14 (13)	<.001	0.3	(0.2-0.6)
VZV	3 (3)	0 (0)	.08	NA	

Abbreviation: NA, not assessed, estimates not calculable because no events in the ganciclovir group.

* Between randomization and day 100 after transplantation. Bacterial and fungal infections were defined as previously described.[39,40]

† By log-rank test.

‡ Relative risk (RR) and 95% CI for antigenemia-ganciclovir group relative to ganciclovir group.

§ Bacterial infections of organ sites were defined as infections from normally sterile sites (ie, sinuses) or results from biopsies in association with symptoms.

‖ Including 2 patients in whom the diagnostic biopsy was performed at day 110 after transplantation.

nificant difference in the hazard of CMV disease by day 180 and thereafter. Moreover, survival was similar at all times after transplantation. It appeared that this lack of difference was related to more invasive fungal infections and more fatal late CMV disease in recipients of ganciclovir at engraftment. When compared with shell vial culture-based early treatment in patients with comparable transplant characteristics in recent randomized trials, the incidence of early CMV disease appeared to be substantially lower with antigenemia-guided early treatment (ie, 8.8% *v* 29-32%)[3,4] and similar to recently published results on a PCR-based strategy (ie, 8.8% *v* 7.7%).[4]

There seem to be two major reasons for the lack of overall success of the antigenemia-guided strategy in preventing CMV disease before day 100 compared with ganciclovir at engraftment: (1) the study design of not instituting therapy for low-grade antigenemia and (2) discontinuation of ganciclovir if antigenemia became negative. Our initial study suggested that patients with low viral load indicated by low-level antigenemia are less likely to develop CMV disease and that patients with disease had increasing levels of antigenemia before the onset of disease.[11] Because a major goal of the present study was to target ganciclovir to patients at highest risk rather than treating the majority of patients, we took advantage of the quantitative nature of the antigenemia assay[11,25,26] and did not treat all patients with low-grade antigenemia but repeated antigenemia testing after 2 to 4 days to identify patients with increasing levels of antigenemia. Although this strategy saved 7% of patients from receiving early ganciclovir treatment and delayed the start of treatment by a median of 8 days in 40% of patients with antigenemia, resulting in significantly less ganciclovir use, it led to CMV disease in 9% of patients who presented with low-grade antigenemia or 4% of all randomized patients. Subset analysis of these data showed that patients with grade 0-2 acute GVHD were significantly less likely to progress from low-grade antigenemia to disease than patients with grade 3-4 GVHD. Therefore, delaying treatment in patients with low viral load may be possible in patients who have no or low-grade acute GVHD. In contrast, immediate treatment of low-grade antigenemia may be required in the presence of grade 3-4 acute GVHD due to the rapid progression of infection. These findings are consistent with data in less immunosuppressed patients (eg, recipients of solid organ transplants) in whom viral load is predictive for CMV disease and the tempo of progression of CMV infection appears to be slower.[27-29]

Because neutropenia occurs after a median of 35 days after start of ganciclovir,[1,3] we hypothesized that a shorter treatment course of ganciclovir might prevent the development of neutropenia. The results of our strategy that consisted of discontinuation of ganciclovir based on a negative antigen test were unexpected. Our data suggest that such an approach is not safe, because 7.7% of patients who received antigenemia-guided treatment developed CMV disease shortly after discontinuation of ganciclovir, which contributed substantially to the higher incidence of CMV disease before day 100 in the antigenemia-guided group. Of note, all patients who developed disease after discontinuation of ganciclovir were also negative by PCR when ganciclovir was discontinued, and only 25% and 37.5%, respectively, of these patients had low-grade antigenemia or were positive by PCR before the onset of secondary disease. Early relapsing disease after antigenemia-guided early treatment has also been reported by Vlieger et al.[13] In contrast, these results are different from a recent study by Einsele et al,[4] who did not report any CMV disease early after discontinuation of ganciclovir based on a negative PCR test. However, because the incidence is only 7.7%, this phenomenon might have been missed in that study due to the small sample size (ie, 22 treated patients). We were unable to identify any statistically significant risk factors for the development of CMV disease after the discontinuation of ganciclovir.

Early discontinuation of ganciclovir based on negative antigenemia testing also did not result in the projected reduction in the overall incidence of neutropenia. Despite no differences in the hazard and duration of neutropenia, there were more early invasive fungal infections in the ganciclovir group, and more patients died of nonviral infections. The mechanism remains unexplained. It is conceivable that there is an interference between prolonged ganciclovir administration and host defenses in marrow transplant recipients. Although the effect of ganciclovir on neutrophil function has not been studied, a suppressive effect of ganciclovir on T lymphocyte function has been shown.[6,30,31]

An important result of this study is that it highlights the importance of the CMV-specific immunologic recovery and its interaction with antiviral prophylaxis. This study suggests that ganciclovir administered at engraftment is associated with a higher incidence of late CMV disease. Sixteen percent of recipients of ganciclovir at engraftment who were alive at day 100 developed CMV disease between day 100 and 400 after transplantation, compared with 8% in the antigenemia-ganciclovir group; however, this difference did not reach statistical significance. With either of the strategies we tested, the incidence of CMV disease during the first 400 days after transplantation approached 20% and CMV-related mortality was 12%. Late CMV disease most likely occurs in patients who lack protective $CD8^+$ cytotoxic T-cell responses,[32,33] and ganciclovir administered at engraftment may contribute to the delay of reconstitution of CMV-specific T-cell immunity.[6] Thus, there appears to be a fine balance between in vivo antigen exposure, use of ganciclovir, and the tempo of CMV-specific immunologic reconstitution that provides long-term protection against CMV disease; ganciclovir administered at engraftment prevents CMV disease almost entirely but also requires maximum treatment and suppresses CMV reactivation at a level that does not seem sufficient to allow immunologic recovery. In contrast, antigenemia-guided ganciclovir early treatment is somewhat less effective in prevention of early disease but permits more CMV reactivation and is thus associated with less late CMV disease.

In conclusion, this study has shown a trade-off of CMV-related morbidity for toxicity. Intermittent antigenemia-based ganciclovir was less effective in preventing CMV disease before day 100 than ganciclovir at engraftment, but more fatal invasive fungal infections and more late CMV disease occurred in patients who received ganciclovir at engraftment, resulting in no apparent difference in survival and

CMV-related mortality between the groups. As one develops early treatment strategies aimed at further reduction of CMV disease early after transplantation using either modified antigenemia- or PCR-based strategies, more ganciclovir than that used in the antigenemia stategy in this study will undoubtedly be required. This may lead to decreased early CMV disease but also to increased late CMV disease, invasive fungal infections, toxicity (eg, central nervous system), and cost. Our data suggest that antigenemia-guided antiviral treatment should prevent most cases of CMV disease before day 100 with the following modifications of the strategy used in this study: (1) the start of antiviral treatment based on any positive antigenemia test results, in particular in patients with previously diagnosed grade 3-4 acute GVHD; (2) the use of formaldehyde fixation for the antigenemia assay; and (3) continuation of ganciclovir until day 100 regardless of antigenemia test results. Institution of antiviral treatment based in detection of CMV in two consecutive weekly PCR results, which has recently been shown to be superior to ganciclovir administered for CMV excretion,[4] appears to be equivalent to antigenemia-guided early treatment with our test methods, whereas treatment of the first positive PCR result will increase the use of ganciclovir further (Fig 2). Because effective prevention of CMV disease before day 100 is associated with an increased risk for late CMV disease regardless of which early prophylaxis strategy is used, prophylaxis for late CMV disease will be needed. Options for long-term prophylaxis include continued suppression of viral replication with antiviral agents or immunologic strategies that may be the optimal way of preventing late disease after marrow transplantation. Such strategies may consist of restoration of CMV-specific T-cell immunity by adoptive transfer of CMV-specific T-cell clones[34] or boosting donor or patient immunity (eg, by using subunit vaccines such as gB, pp65).[35,36]

ACKNOWLEDGMENT

We appreciate the expert technical support of T. Stevens-Ayers and P. Woogerd in performing the antigenemia assay and thank P. Lingenfelter, N. Cochran, and C. Bevan for performing the PCR assay. We are indebted to Dr L. Fisher for assistance in developing the statistical design, to Dr J.M. Goodrich for his contribution in the planning phase of the study, to Dr S.R. Riddell for review of the manuscript, and to C. Keller, RN, who was the research nurse in the initial part of the study. We appreciate the technicians of virology laboratory and the Seattle Marrow Transplant team, without whose support this study would not have been possible.

REFERENCES

1. Goodrich JM, Mori M, Gleaves CA, DuMond C, Cays M, Ebeling DF, Buhles WC, DeArmond B, Meyers JD: Early treatment with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplantation. N Engl J Med 325:1601, 1991

2. Schmidt GM, Horak DA, Niland JC, Duncan SR, Forman SJ, Zaia JA: A randomized, controlled trial of prophylactic ganciclovir for cytomegalovirus pulmonary infection in recipients of allogeneic bone marrow transplants. N Engl J Med 324:1005, 1991

3. Goodrich JM, Bowden RA, Fisher L, Keller C, Schoch G, Meyers JD: Ganciclovir prophylaxis to prevent cytomegalovirus disease after allogeneic marrow transplant. Ann Intern Med 118:173, 1993

4. Einsele H, Ehninger G, Hebart H, Wittkowski KM, Schuler U, Jahn G, Mackes P, Hertes M, Klingebiel T, Löffler J, Wagner S, Müller CA: Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. Blood 86:2815, 1995

5. Winston DJ, Ho WG, Bartoni K, Du Mond C, Ebeling DF, Buhles W, Champlin RE: Ganciclovir prophylaxis of cytomegalovirus infection and disease in allogeneic bone marrow transplant recipients. Ann Intern Med 118:179, 1993

6. Li C-R, Greenberg PD, Gilbert MJ, Goodrich JM, Riddell SR: Recovery of HLA-restricted cytomegalovirus (CMV)-specific T-cell responses after allogeneic bone marrow transplant: Correlation with CMV disease and effect of ganciclovir prophylaxis. Blood 83:1971, 1994

7. van der Bij W, Torensma R, van Son WJ, Anema J, Schirm J, Tegzess AM, The TH: Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leucocytes. J Med Virol 25:179, 1988

8. Einsele H, Steidle M, Vallbracht A, Saal JG, Ehninger G, Müller CA: Early occurrence of human cytomegalovirus infection after bone marrow transplantation as demonstrated by the polymerase chain reaction technique. Blood 77:1104, 1991

9. Wolf DG, Spector SA: Early diagnosis of human cytomegalovirus disease in transplant recipients by DNA amplification in plasma. Transplantation 56:330, 1993

10. Aspin MM, Gallez-Hawkins GM, Giugni TD, Tegtmeier B, Lang DJ, Schmidt GM, Forman SJ, Zaia JA: Comparison of plasma PCR and bronchoalveolar lavage fluid culture for detection of cytomegalovirus infection in adult bone marrow transplant recipients. J Clin Microbiol 32:2266, 1994

11. Boeckh M, Bowden RA, Goodrich JM, Pettinger M, Meyers JD: Cytomegalovirus antigen detection in peripheral blood leukocytes after allogeneic marrow transplantation. Blood 80:1358, 1992

12. Boeckh M, Myerson D, Bowden RA: Early detection and treatment of cytomegalovirus infections in marrow transplant—Methodological aspects and implications for therapeutic interventions. Bone Marrow Transplant 14:S66, 1994

13. Vlieger AM, Boland GJ, Jiwa NM, de Weger RA, Willemze R, de Gast GC, Falkenburg JHF: Cytomegalovirus antigenemia assay or PCR can be used to monitor ganciclovir treatment in bone marrow transplant recipients. Bone Marrow Transplant 9:247, 1992

14. Buckner CD, Clift RA, Appelbaum FR, Storb R, Petersen FB, Sanders JE, Thomas ED, Hansen JA: Effects of treatment regimens in patients allografted for acute and chronic myelogenous leukemia. Bone Marrow Transplant 7:6, 1991

15. Storb R, Leisenring W, Deeg HJ, Anasetti C, Appelbaum F, Bensinger W, Buckner CD, Clift R, Doney K, Hansen J, Martin P, Sanders J, Stewart P, Sullivan K, Thomas ED, Witherspoon R: Long-term follow-up of a randomized trial of graft-versus-host disease prevention by methotrexate/cyclosporine versus methotrexate alone in patients given marrow grafts for severe aplastic anemia (letter). Blood 83:2749, 1994

16. Slavin MA, Osborne B, Adams R, Levenstein MJ, Schoch HG, Feldman AR, Meyers JD, Bowden RA: Efficacy and safety of fluconazole for fungal infections after marrow transplant—A prospective, randomized, double-blind study. J Infect Dis 171:1545, 1995

17. Boeckh M, Woogerd PM, Stevens-Ayers T, Ray CG, Bowden RA: Factors influencing detection of quantitative cytomegalovirus antigenemia. J Clin Microbiol 32:832, 1994

18. Gleaves CA, Smith TF, Shuster EA, Pearson GR: Comparison of standard tube and shell vial culture techniques for the detection of cytomegalovirus in clinical specimens. J Clin Microbiol 21:217, 1985

19. Boeckh M, Gleaves CA, Bindra R, Meyers JD: Comparison of MRC-5 and U-373MG astrocytoma cells for detection of cytomegalovirus in shell vial centrifugation cultures. Eur J Clin Microbiol Infect Dis 10:569, 1991

20. Gerna G, Revello MG, Percivalle E, Morini F: Comparison of different immunostaining techniques and monoclonal antibodies to the lower matrix phosphoprotein (pp65) for optimal quantitation of human cytomegalovirus antigenemia. J Clin Microbiol 30:1232, 1992

21. Finckh U, Lingenfelter PA, Myerson D: Producing single stranded DNA probes with the Taq DNA polymerase: A high yield protocol. Biotechniques 10:35, 1991

22. Bauer HM, Ting Y, Greer CE, Chambers JC, Tashiro CJ, Chimera J, Reingold A, Manos MM: Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA 265:472, 1991

23. Myerson D, Lingenfelter PA, Gleaves CA, Meyers JD, Bowden RA: Diagnosis of cytomegalovirus pneumonia by the polymerase chain reaction with archived frozen lung tissue and bronchoalveolar lavage fluid. Am J Clin Pathol 100:407, 1993

24. Makuch R: Sample size requirements for evaluating a conservative therapy. Cancer Treat Rep 62:1037, 1978

25. The TH, van der Ploeg M, van den Berg AP, Vlieger AM, van der Giessen M, van Son WJ: Direct detection of cytomegalovirus in peripheral blood leukocytes—A review of the antigenemia assay and polymerase chain reaction. Transplantation 54:193, 1992

26. Gerna G, Revello MG, Percivalle E, Zavattoni M, Parea M, Battaglia M: Quantification of human cytomegalovirus viremia by using monoclonal antibodies to different viral proteins. J Clin Microbiol 28:2681, 1990

27. van der Berg AP, van der Bij W, van der Son WJ, Anema J, van der Giessen M, Schirm J, Tegzess AM, The TH: Cytomegalovirus antigenemia as a useful marker of symptomatic cytomegalovirus infection after renal transplantation—A report of 130 consecutive cases. Transplantation 48:991, 1989

28. van den Berg AP, Klompmaker IJ, Haagsma EB, Scholten-Sampson A, Bijleveld CMA, Schirm J, van der Giessen M, Slooff MJH, The TH: Antigenemia in the diagnosis and monitoring of active cytomegalovirus infection after liver transplantation. J Infect Dis 164:265, 1991

29. Gerna G, Zipeto D, Parea M, Grazia Revello M, Silini E, Percivalle E, Zavattoni M, Grossi P, Milanesi G: Monitoring of human cytomegalovirus infections and ganciclovir treatment in heart transplant patients by determination of viremia, antigenemia, and DNAemia. J Infect Dis 164:488, 1991

30. Bowden RA, Digel J, Reed EC, Meyers JD: Immunosuppressive effects of ganciclovir on in vitro lymphocyte responses. J Infect Dis 156:899, 1987

31. Heagy W, Crumpacker C, Lopez PA, Finberg RW: Inhibition of immune functions by antiviral drugs. J Clin Invest 87:1916, 1991

32. Quinnan GV, Kirmani N, Rook AH, Manischewitz JF, Jackson L, Moreschi G, Santos GW, Saral R, Burns WH: Cytotoxic T cells in cytomegalovirus infection. N Engl J Med 307:7, 1982

33. Reusser P, Riddell SR, Meyers JD, Greenberg PD: Cytotoxic T-lymphocyte response to cytomegalovirus following human allogeneic bone marrow transplantation: Pattern of recovery and correlation with cytomegalovirus infection and disease. Blood 78:1373, 1991

34. Riddell SR, Watanabe KS, Goodrich JM, Li CR, Agha ME, Greenberg PD: Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. Science 257:238, 1992

35. Gonczol E, Ianacone J, Ho W, Starr S, Meignier B, Plotkin S: Isolated gA/gB glycoprotein complex of human cytomegalovirus envelope induces humoral and cellular immune-responses in human volunteers. Vaccine 8:130, 1990

36. McLaughlin-Taylor E, Pande H, Forman SJ, Tanamachi B, Li CR, Zaia JA, Greenberg PD, Riddell SR: Identification of the major late human cytomegalovirus matrix protein pp65 as a target antigen for CD8+ virus-specific CTL. J Med Virol 43:103, 1994

37. Pepe MS, Longton G, Pettinger M, Mori M, Fisher LD, Storb R: Summarizing data on survival, relapse, and chronic graft-versus-host disease after bone marrow transplantation: Motivation for and description of new methods. Br J Haematol 83:602, 1993

38. Sullivan KM, Kopecky KJ, Jocom J, Fisher L, Buckner CD, Meyers JD, Counts GW, Bowden RA, Peterson FB, Witherspoon RP, Budinger MD, Schwartz RS, Appelbaum FR, Clift RA, Hansen JA, Sanders JE, Thomas ED, Storb R: Immunomodulatory and antimicrobial efficacy of intravenous immunoglobulin in bone marrow transplantation. N Engl J Med 323:705, 1990

39. Sayer HG, Longton G, Bowden R, Pepe M, Storb R: Increased risk of infection in marrow transplant patients given methylprednisolone for graft-versus-host disease prevention. Blood 84:1328, 1994

40. Bowden RA, Cays M, Gooley T, Mamelok RD, van Burik JA: Phase I study of amphotercin B colloidal dispersion for the treatment of invasive fungal infections after marrow transplant. J Infect Dis 173:1208, 1996