Prevention of Cytomegalovirus Disease in Recipients of Allogeneic Stem Cell Transplants

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INTRODUCTION

In the era before introduction of ganciclovir (GCV), cytomegalovirus (CMV) infection and pneumonia developed in 38 and 17%, respectively, of recipients of allogeneic stem cell transplants (SCT), while mortality due to CMV pneumonia was 85% (64). This very serious complication occurred mainly in CMV-seropositive patients, with acute graft-versus-host disease being the most important risk factor (64). Treatment of CMV pneumonia with GCV and immunoglobulin decreased mortality to 30 to 50% (22, 81). In CMV-seronegative recipients, primary CMV infection could be prevented by a transfusion and transplantation policy making use of either CMV-seronegative donors or leukocyte-depleted blood products or grafts (11, 17, 95, 96). Currently, two antiviral strategies, prophylactic or preemptive treatment, are used for prevention of CMV disease. Prophylactic treatment usually consists of antiviral therapy started at engraftment and continued until at least day 100 posttransplant. Preemptive therapy is defined as antiviral treatment initiated based on the detection of primary or reactivated CMV infection by positive CMV cultures, a positive antigenemia (Ag) assay, or positive molecular assays.

In this paper, we review these antiviral strategies. Furthermore, several other aspects of prevention of CMV disease are reviewed: (i) methods available for early detection of CMV reactivation, (ii) monitoring of CMV-specific (CMVs) T-cell

responses, (iii) the value of several antiviral drugs, and (iv) adoptive immunotherapy as prophylaxis or preemptive treatment of CMV reactivation or CMV disease.

ANTIVIRAL STRATEGIES: PROPHYLAXIS OR PREEMPTIVE TREATMENT

In randomized trials (31, 100) (Table 1) among CMV-seropositive recipients, long-term (3- to 4-month) GCV prophylaxis initiated at engraftment was shown to be effective in suppressing early CMV disease (occurring <100 days posttransplant). However, mortality was not influenced, due to an increased incidence of bacterial and fungal infections and late CMV disease (5, 8, 20, 31, 100). When the recovery of CMVs cytotoxic T lymphocytes (CTLs) was studied, it appeared that long-term GCV treatment impaired CMVs CTL reconstitution, causing the increase in late CMV infections (50).

Many studies using preemptive therapy in SCT recipients to prevent CMV disease have been performed. A study by Schmidt et al. (88) was the first to evaluate this strategy, based on positive CMV cultures of bronchoalveolar lavage (BAL) fluid. Patients underwent routine BAL on day 35. The 40 patients with positive CMV cultures were randomized between preemptive GCV treatment and observation. In the GCV group 5 of 20 patients died or had CMV pneumonia before day 120, compared to 14 of 20 patients in the observation group ($P = 0.01$). In the group of patients with negative CMV cultures, the rate of CMV infection was 12 of 55. The results of studies with a minimum of 30 patients and published after 1995 are summarized in Tables 2 to 5 according to the CMV mon-

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TABLE 1. GCV prophylaxis of CMV disease

Study authors (reference)	Randomized trial	Donors ^a	Dose of GCV	% with early CMV disease (placebo vs GCV)	% Overall mortality (placebo vs GCV)
Goodrich et al. (31)	Yes	MRD, MUD	10 mg/kg/day (5 days) followed by 5 mg/kg/day ^b	29 vs 0 ($P < 0.001$)	26 vs 30 (at day 180) (NS ^d)
Winston et al. (100)	Yes	(P)MRD, (P)MUD	6 mg/kg/day ^c	24 vs 10 ($P = 0.06$)	36 vs 30 (at day 120) (NS)

^a (P)MRD, (partially) matched related donor; (P)MUD, (partially) matched unrelated donor.

^b From engraftment until day 100.

^c From engraftment until day 120.

^d NS, not significant.

itoring assay used; three of them were randomized trials (5, 19, 42). Preemptive treatment based on qualitative CMV DNA detection by PCR lowered the incidence of CMV disease and CMV-associated mortality compared with preemptive therapy instituted when positive CMV cultures were obtained (Tables 5 and 2, respectively) ($P = 0.02$) (19). Boeckh et al. (5, 8) compared two types of Ag-based preemptive therapy (Table 4) with prophylactic treatment. Ag-based treatment was given for 28 days (5) or until day 100 posttransplant (8). In both the prophylactically treated group and the group receiving long-term preemptive treatment, late CMV disease was diagnosed more frequently, while more invasive fungal infections were seen in the prophylactically treated group only. The incidences of CMV disease at day 400 posttransplant and the overall survival rates were similar in the three treatment arms. Humar et al. (42) (Tables 3 and 4) showed that Ag-based preemptive treatment reduced the incidence of CMV disease at day 400 posttransplant to 1.7%, compared to 12.1% when therapy was instituted at the detection of positive CMV cultures of BAL fluid obtained at day 35 posttransplant ($P = 0.022$). Again, overall survival rates in the two treatment arms were similar.

Prevention of CMV disease with low CMV-associated mortality seemed to be superior in studies using short-term (14-day) Ag- or PCR-based preemptive GCV treatment (19, 20, 42, 45, 52, 58, 59, 73, 86, 97; H. Hebart, W. Brugger, U. Grigoleit, B. Gscheidle, J. Loeffler, H. Schafer, L. Kanz, and H. Einsele, Letter, Blood 97:2183-2185, 2001). In those studies preemptive treatment was extended only when CMV monitoring tests were still positive after the short-term treatment period. When overall survival was considered the end point, the various preemptive treatment strategies all were equally effective.

The introduction of preemptive therapy among CMV-seropositive patients receiving grafts from matched related donors has resulted in transplant-related mortality and survival rates similar to those for CMV-seronegative recipients (41, 63, 70, 73). However, in CMV-seropositive recipients of grafts from matched unrelated donors, transplant-related mortality and

overall survival rates were still inferior to those for CMV-seronegative recipients, despite preemptive antiviral treatment (13, 15, 49, 63).

Overall, the introduction of preemptive antiviral therapy has greatly reduced the incidence and mortality rate of CMV disease. Prophylactic treatment has no advantage over preemptive treatment; in fact, it results in an increased incidence of bacterial and fungal infections and late CMV disease. Preemptive treatment based on the Ag assay or PCR tests is superior to culture or BAL fluid-based strategies. Short-term (14-day) antiviral treatment is the most favorable approach for prevention of CMV disease.

AG ASSAY VERSUS MOLECULAR MONITORING

The Ag assay is widely used to monitor SCT recipients for CMV reactivation. This assay has some drawbacks compared to molecular tests: (i) during neutropenia no monitoring can be performed, which is similar to the case for molecular tests performed with leukocytes; (ii) the test is laborious; and (iii) the test is subject to intra- and interobserver variability. Furthermore, a false-negative Ag test (using C10 and C11 antibodies) was reported for an SCT recipient with CMV disease. Reexamination of the Ag-negative samples with a different pp65 antibody pool (CINA antibodies) revealed a high level of Ag (90). Compared to Ag assays, the work time per sample is reduced from approximately 4 h to less than 2 h when automated DNA isolation and PCR tests are used. The difference in workload is even more obvious when large numbers of samples are processed, since the Ag assay is not automated and every sample has to be processed separately.

With the molecular assays, qualitative or quantitative detection of CMV DNA or RNA in cell-free plasma, peripheral blood leukocytes (PBL), or whole blood (WB) is performed. The technical details of several methods for CMV monitoring have been reviewed by Boeckh et al. (7).

TABLE 2. Preemptive therapy of CMV reactivation based on positive CMV cultures

Study authors (reference)	Randomized trial	Donors ^a	No. of patients	Dose of GCV	% with CMV disease (day)	% CMV-associated mortality (day)	% Overall survival (day)
Einsele et al. (19)	Yes (see Table 5)	(P)MRD, (P)MUD	34	5 mg/kg b.i.d. (14 days) followed by 5 mg/kg/day until negative PCR	32 (180)	15 (180)	59 (180)
Ljungman et al. (52)	No	(P)MRD, MUD	58	5 mg/kg b.i.d. (14 days)	19 (100)	2 (100)	ND ^b

^a (P)MRD, (partially) matched related donor; (P)MUD, (partially) matched unrelated donor.

^b ND, not described. Overall survival was not specified; however, it was reported as being similar to that observed for patients receiving PCR-based preemptive treatment (Table 5).

TABLE 3. Preemptive therapy of CMV reactivation based on CMV-positive BAL fluid

Study authors (reference)	Randomized trial	Donors ^a	No. of patients	Dose of GCV	% with CMV disease (day)	% CMV-associated mortality (day)	% Overall survival (day)
Reddy et al. (80)	No	MRD, MUD	55	5 mg/kg b.i.d. (14–28 days) followed by maintenance (16 wk)	11 (ND ^b)	5 (ND)	57 (3 yr)
Humar et al. (42)	Yes (see Table 4)	(P)MRD, MUD	58	5 mg/kg b.i.d. (14 days) followed by maintenance (8 wk)	12.1 (400)	ND	72.4 (365)

^a (P)MRD, (partially) matched related donor; MUD, matched unrelated donor.

^b ND, not described.

Cobas Amplicor CMV DNA (Monitor) Test

The Cobas Amplicor CMV DNA test is a commercially available qualitative CMV DNA PCR assay for plasma, while the quantitative Cobas Amplicor CMV DNA monitor test can be performed with cell-free plasma, PBL, or WB. Five studies found CMV DNA PCR monitoring (qualitative or quantitative) to be more sensitive than Ag assay, irrespective of performance with plasma, PBL, or WB (26, 40, 76, 89, 93). In only one report were leukocyte-based assays (Ag and PBL PCR assays) more sensitive than a plasma PCR, showing a higher number of patients with CMV reactivation, earlier positivity, and a more rapid decrease of viral load after the start of preemptive antiviral therapy (9). In one other study, the “gold standard” to calculate the sensitivities and specificities of the Ag assay and PCR test was defined as “CMV reactivation based on positive results of the Ag assay or PCR test” (40). This method of calculation is incorrect and results in the exclusion of false-positive results, giving a specificity and a positive predictive value of 100%. The value of a higher sensitivity of molecular assays in a clinical setting is not clear. Solano et al. (93) reported that 9 of 43 SCT recipients had a positive plasma PCR, while Ag was negative. None of these nine patients progressed to CMV disease, although they did not receive preemptive treatment. Furthermore, none of the patients with initially positive Ag and PCR results who remained PCR positive after conversion of the Ag assay to a negative result developed CMV disease. The authors concluded that the Ag assay appeared to be most suitable for guiding initiation of preemptive therapy and monitoring the response to antiviral therapy.

Real-Time Automated CMV DNA PCR Test with a TaqMan Probe

With the real-time automated CMV DNA PCR with a TaqMan probe, quantitative CMV monitoring in plasma, WB, and PBL can be performed. With this method, PCR products are detected as they accumulate during the PCR, in contrast to the case for other quantitative PCR techniques such as the Cobas Amplicor CMV DNA monitor test. This results in a greater linear dynamic detection range of the real-time TaqMan PCR compared to the Cobas Amplicor CMV DNA monitor test. Analogous to the Cobas Amplicor tests, this assay also proved to be more sensitive than the Ag assay. CMV DNA detection by real-time PCR often preceded a positive Ag test and yielded more positive samples (33, 60, 102).

Qualitative and Quantitative In-House CMV DNA PCR Assay

In partly retrospective studies, CMV DNA monitoring by in-house PCR assays with plasma, WB, or PBL yielded results similar to those described above, with higher sensitivity for molecular tests than for the Ag assay (27, 44, 76). Hebart et al. (35) prospectively monitored CMV reactivation in plasma and WB by Ag and in-house semiquantitative PCR assays. WB PCR showed the lowest sensitivity; however, overall a good correlation was seen between Ag, WB PCR, and plasma PCR assays. All three assays were negative after 14 days of GCV treatment in 12 of 13 patients. In contrast, two studies (6, 62) reported the in-house plasma PCR assay to be less sensitive than PBL PCR or Ag assay.

Murex CMV DNA Hybrid Capture Assay

The Murex CMV DNA hybrid capture assay is a commercially available solution hybridization antibody capture assay for the quantitative detection of CMV DNA in leukocytes. It was less sensitive in diagnosing CMV infection than an in-house qualitative PCR (36, 76). When CMV disease was used as the gold standard for comparison, however, the positive predictive values of the hybridization antibody capture and PCR assays were 33 and 22%, respectively (36).

CMV mRNA-Based Monitoring

The qualitative determination of CMV pp67 mRNA by nucleic acid sequence-based amplification proved to be the least sensitive technique to assess CMV reactivation when compared to DNA-based assays and the Ag assay (28, 39, 76). Detection of immediate-early mRNA (29), the beta_{2.7} transcript (1), or spliced late CMV genes (10) all were shown to be more useful; however, these results have not been validated by other groups.

Summary

In Table 6 the results of studies comparing commercially available surveillance methods with the Ag assay or in-house PCR tests are summarized. Not all of the studies described above are included, due to varying study designs and end points or lack of clinical data (1, 9, 10, 26, 40). In several papers the sensitivity, specificity, positive predictive value, and negative predictive value of the experimental assay(s) were calculated. In those studies the gold standard to calculate these values was defined as CMV reactivation based on positive

TABLE 4. Preemptive therapy of CMV reactivation based on Ag

Study authors (reference)	Randomized trial	Donors ^a	No. of patients	No. of positive cells	Dose of GCV ^b	% with CMV disease (day)	CMV-associated mortality	% Overall survival (day)
Boeckh et al. (5)	Yes	(P)MRD, MUD	114	≥3/2 slides	5 mg/kg b.i.d. (7 days) fb maintenance (21 days)	22 (400)	ND ^c	73 (180), 61 (400)
Verdonek et al. (97)	No	MRD	41	Any Ag	2.5 mg/kg b.i.d. (14 days) ^d fb 5 mg/kg b.i.d. until negative test	0 (180)	0	76 (180)
Manteiga et al. (61)	No	MRD	52	Any Ag	5 mg/kg b.i.d. (7 days) fb maintenance (21 days)	13 (100)	2	ND
Boeckh et al. (8)	No	(P)MRD, MUD	102	Any Ag	5 mg/kg b.i.d. (7 days) fb maintenance (100 days)	17 (400)	ND	77 (180), 66 (400)
Machado et al. (58, 59)	No	MRD	76	≥2/300,000	5 mg/kg b.i.d. (14–21 days) or until negative test	4 (200), 14.4 (365)	0	ND
Mori et al. (67)	No	MRD, MUD	50	≥10/2 slides or any Ag ^e	5 mg/kg b.i.d. (14 days) fb 5 mg/kg/day until negative test	16 (100)	0	ND
Kanda et al. (45)	No	(P)MRD, (P)MUD	77	≥5–10/50,000	5 mg/kg b.i.d. (7 days) fb 5 mg/kg/day until negative test	3 (365)	0	86 (100), 73 (365)
Small et al. (92)	No	MRD	61	≥2/slide	ND	7 (ND)	7	ND
Humar et al. (42)	Yes (see Table 3)	(P)MRD, MUD	60	Any Ag	5 mg/kg b.i.d. (14 days) or until negative test	1.7 (400)	0	80 (365)

^a (P)MRD, (partially) matched related donor; (P)MUD, (partially) matched unrelated donor.^b fb, followed by.^c ND, not described.^d Patients treated with high-dose corticosteroids for acute graft-versus-host disease grades II to IV received prophylactic GCV in a dose similar to the preemptive dose.^e During acute graft-versus-host disease grades II to IV.

results from Ag and/or PCR assays (1, 10, 40). In our view, a surveillance method for CMV reactivation should be judged on its clinical merits, with the incidences of CMV disease and transplant-related mortality being the most significant end points.

Overall, one can conclude that PCR-based CMV DNA monitoring is more sensitive than Ag-based monitoring. However, the clinical benefit is unclear. Even more, there is lack of clarity whether PCR tests can better be performed with plasma, WB, or PBL samples, although molecular monitoring in plasma has the advantage of performance irrespective of neutropenia. Presently, there is no evidence that qualitative CMV detection assays have a better or worse predictive value for the occurrence of CMV disease after SCT than quantitative assays. To answer this question, randomized controlled trials should be performed, with patients monitored prospectively with either detection assay without application of preemptive antiviral treatment of CMV reactivation. Such trials will never be done. Theoretically, a quantitative method enables monitoring of the response to therapy. If the viral load increases after the start of preemptive therapy, a dose or drug modification may be applied. This was implemented by Mori et al. (68) but did not significantly change the incidence of CMV disease.

MONITORING OF CMVs T-CELL RESPONSES

Studies of immune recovery after allogeneic SCT have shown a temporal delay in the recovery of CMVs T-cell responses and have identified a decisive role for the recovery of CD8⁺ CTL responses in preventing the development of CMV disease (79, 82, 87). The generation of CD8⁺ CMVs CTLs was associated with recovery of CD4⁺ CMVs T-helper (Th) cells (82). Li et al. (50) analyzed the kinetics of endogenous reconstitution of CD4⁺ and CD8⁺ CMVs T-cell responses, by lymphoproliferation and cytotoxicity assays, in 47 allogeneic SCT recipients who were randomized to GCV prophylaxis or placebo after recovery of peripheral neutrophil counts. Between days 40 and 90 posttransplant, recovery of CD8⁺ and CD4⁺ CMVs T-cell responses occurred in the majority of individuals receiving the placebo but in a minority of patients receiving GCV. Thus, long-term prophylactic GCV treatment can delay posttransplant reconstitution of CMVs CTL responses. Reports of several studies using screening assays for CMVs T-cell reconstitution to identify patients at risk of developing CMV disease have been published (2, 16, 32, 38, 48, 72). Krause et al. (48) performed lymphoproliferation assays to assess the CD4⁺ CMVs Th response at regular monthly intervals. None of the patients with CMVs T-cell proliferation on day 120 developed CMV disease after day 120. In contrast, of the patients lacking such a response at day 120, 30.8% developed late CMV disease (after day 120). Hebart et al. (38) quantified CD8⁺ CMVs CTLs and CD4⁺ CMVs Th cells by intracellular gamma interferon staining with flow cytometry after CMVs stimulation. Reconstitution of both cell types was associated with rapid clearance of CMV infection. In addition to cytotoxicity and gamma interferon staining assays, the use of HLA-peptide tetramers to quantify CMVs CD8⁺ T-cell reconstitution might enable prediction of the development of CMV disease (2, 16, 32). Reconstitution of absolute CMVs CTL counts to values above 10×10^6 to 20×10^6 /liter was associated with protection

TABLE 5. Preemptive therapy of CMV reactivation based on CMV DNA PCR

Study authors (reference)	Randomized trial	Donors	No. of patients	No. of positive PCRs ^d	Dose of GCV ^e	% with CMV disease (day)	% CMV-associated mortality (day)	% Survival (day)
Einsele et al. (19)	Yes (see Table 2)	(P)MRD, (P)MUD	37	Second positive PCR (Qual)	5 mg/kg b.i.d. (14 days) to 5 mg/kg/day until negative PCR	8 (180)	0 (180)	84 (180)
Ljungman et al. (52)	No	(P)MRD, MUD	58	Second positive PCR (Semi-Q)	5 mg/kg b.i.d. (14 days) or until negative PCR	6 (100), 10 (180)	0 (180)	ND ^{f,g}
Einsele et al. (20)	No	(P)MRD, (P)MUD	86	Second positive PCR (Qual)	5 mg/kg b.i.d. (14 days) to foscarnet (60 mg/kg b.i.d.) until negative PCR	12 (>100)	1 (>100)	ND
Peggs et al. (73)	No	MRD, MUD	52	First or second positive PCR (Qual)	5 mg/kg b.i.d. (14 days) to foscarnet (60 mg/kg b.i.d.) until negative PCR	6 (100)	2 (100)	ND
Hebart et al., letter	No	(P)MRD, (P)MUD	52	Second positive PCR (Qual)	5 mg/kg b.i.d. (14 days) to 5 mg/kg/day until negative PCR	12 (?)	6 (?)	ND
Mori et al. (68)	No	MRD, MUD	39	First positive PCR (real time)	5 mg/kg/day (14 days) until negative test, 10 mg/kg/day when VL increased	8 (120)	0	ND
Reusser et al. (86)	Yes ^f	MRD, MUD	213	Second positive PCR or any Ag	5 mg/kg b.i.d. or foscarnet (60 mg/kg b.i.d.) (14 days) ^g	5 (180)	1	76 (180)

^a(P)MRD, (partially) matched related donor; (P)MUD, (partially) matched unrelated donor.

^bQual, qualitative; Semi-Q, semiquantitative.

^cFb, followed by; VL, viral load.

^dOverall survival was similar to that observed in a historical control group receiving preemptive treatment based on positive conventional cultures.

^eND, not described.

^fData from a randomized multicenter trial comparing foscarnet and GCV for preemptive treatment.

^gWhen CMV tests were still positive after 14 days, patients were treated for another 14 days with a 50% dose reduction.

from CMV disease (2, 16). In contrast, Ozdemir et al. (72) recently reported that frequencies and absolute numbers of CMVs CD8⁺ T cells were greater in subjects who experienced CMV Ag following SCT. They concluded that recovery of CMVs CTLs, as measured by HLA-peptide tetramer staining, is insufficient to control CMV Ag. However, whether these patients with Ag did develop CMV disease was not reported. This might be important, since only patients with Ag and decreased recovery of CMVs CTLs progressed to CMV disease in the study of Gratama et al. (32). Patients with Ag who did not develop CMV disease demonstrated higher levels of CMVs CD8⁺ T cells than CMV-seropositive recipients without Ag. It should be noted that most of the CMVs CTL studies discussed in this review were performed retrospectively and used tetrameric complexes of HLA A*0201 and/or B*0702 molecules. Larger prospective studies must be performed to evaluate CMVs CD8⁺ T-cell reconstitution after allogeneic SCT. When the above-mentioned results are validated, preemptive antiviral therapy might be withheld in patients with CMV reactivation who are shown to have adequate CMVs CTL levels. At present, several other HLA class I (A*0101, A*0301, A*1101, A*2401, A*6801/2, B*3502, B*3801/2, B*44XX)-restricted pp65- and pp150-derived epitopes have been identified (38, 51, 57), which will make tetramer-based or gamma interferon staining-based quantification of CMVs CD8⁺ T-cell recovery possible for more SCT recipients.

ANTIVIRAL THERAPY

Intravenous GCV is generally considered the drug of choice for preemptive therapy of CMV reactivation or treatment of CMV disease (69). Several other antiviral drugs (acyclovir [ACV], valacyclovir [VACV], foscarnet, cidofovir [CDV], and valganciclovir [VGCV]) have in vitro or in vivo activity against CMV. (V)ACV and (V)GCV are nucleoside analogues. VACV and VGCV are oral prodrugs of ACV and GCV, respectively, and are converted to ACV and GCV, respectively, after cleavage of the valine moiety by enzymes from the liver and intestine. The nucleosides first have to be converted to monophosphates by a viral protein kinase (which is the gene product of UL97 in the case of CMV). Second and third phosphorylations are performed by cellular kinases. ACV or GCV triphosphate is then incorporated in viral DNA and acts as an obligate chain terminator (54). Furthermore, GCV triphosphate is a competitive inhibitor of the CMV DNA polymerase. VGCV has a 10-fold-greater bioavailability than oral GCV. Pharmacokinetic studies of VGCV have been performed with human immunodeficiency virus-infected individuals and recipients of liver transplants (85). Among SCT recipients, a randomized crossover trial using intravenous GCV or oral VGCV as a preemptive treatment will be conducted at European Group for Blood and Marrow Transplantation centers (85). The nucleotide analogue CDV already is a monophosphate, and therefore no phosphorylation by viral enzymes is necessary. Foscarnet is a pyrophosphate analogue forming a complex with the pyrophosphate binding site of viral DNA polymerase. This is an essential site during incorporation of nucleotides in DNA in which a pyrophosphate group has to be spliced from the nucleotide. Thereby, foscarnet inhibits viral DNA polymerase activity.

TABLE 6. Surveillance methods

Study authors (reference), study type	No. of CMV reactivations	No. of days of earlier positivity of exptl assay compared to standard assay	No. of patients with CMV disease/total
Schulenburg et al. (89), quantitative Cobas PCR (plasma and blood) vs Ag	10 PCR ⁺ /Ag ⁺ , 7 PCR ⁺ /Ag ⁻ , 0 PCR ⁻ /Ag ⁻	11	2/74
Solano et al. (93), qualitative Cobas PCR (plasma) vs Ag	14 PCR ⁺ /Ag ⁺ , 18 PCR ⁺ /Ag ⁻ , 5 PCR ⁻ /Ag ⁺	7	2/43 (PCR ⁺ /Ag ⁻ , no disease)
Griscelli et al. (33), real-time TaqMan (PBL) vs Ag	20 PCR ⁺ /Ag ⁺ , 2 PCR ⁺ /Ag ⁻ , 1 PCR ⁻ /Ag ⁻	15	2/16
Yakushiji et al. (102), real-time TaqMan (plasma) vs Ag	39 PCR ⁺ /Ag ⁺ , 4 PCR ⁺ /Ag ⁻ , 0 PCR ⁻ /Ag ⁺ , 8 PCR ⁻ /Ag ⁻	PCR before Ag, 20; Ag before PCR, 5; simultaneously, 14	2/51
Hebart et al. (36), HCA ^a (PBL) vs in-house PCR	12 HCA ⁺ /PCR ⁺ , 6 HCA ⁻ /PCR ⁺	HCA after or simultaneously with PCR, 18	4/18 (HCA ⁻ /PCR ⁺ , no disease)
Gerna et al. (28), qualitative pp67 NASBA ^b vs Ag	9 NASBA ⁺ /Ag ⁺ , 6 NASBA ⁻ /Ag ⁺ , 9 NASBA ⁻ /Ag ⁻	-3	2?/24
Hebart et al. (39), qualitative pp67 NASBA vs in-house PCR	16 NASBA ⁺ /PCR ⁺ , 1 NASBA ⁺ /PCR ⁻ , 3 NASBA ⁻ /PCR ⁺	0	3/33 (NASBA ⁺ /PCR ⁻ , no disease)
Gerna et al. (29), qualitative immediate-early NASBA vs Ag	32 NASBA ⁺ /Ag ⁺ , 6 NASBA ⁺ /Ag ⁻ , 0 NASBA ⁻ /Ag ⁺ , 13 NASBA ⁻ /Ag ⁻	2	0/51 (NASBA ⁺ /Ag ⁻ , no disease)

^a HCA, hybrid capture assay.^b NASBA, nucleic acid sequence-based amplification.

(V)ACV

(V)ACV has been studied only as prophylactic therapy for prevention of CMV reactivation or disease and not as a (pre-emptive) treatment. ACV has only limited activity against CMV when tested in vivo. Two studies using ACV prophylaxis were performed with SCT recipients; however, these studies were done before the strategy of preemptive GCV therapy based on Ag or PCR assay was introduced (65, 77). Intravenous ACV followed by high-dose oral ACV maintenance therapy was not effective for prevention of CMV disease but resulted in decreased CMV-associated mortality and increased survival. Vusirikala et al. (98) compared data from 31 SCT recipients who were prophylactically treated with VACV at 1 g three times a day with those from a group receiving only low-dose oral ACV. Primary and secondary CMV reactivations were observed in 3 of 12 and 5 of 19 VACV-treated patients, respectively, compared to 24 of 31 and 16 of 24 in the control group, respectively. Since this was a retrospective report with small numbers of patient combining primary and secondary reactivations, it only suggested a potential benefit of VACV as prevention for CMV reactivation. In a large randomized multicenter study, oral VACV was shown to be more effective in preventing CMV viremia in SCT recipients than oral ACV, although the overall survival and the incidence of CMV disease did not differ between the two groups (75 versus 76% and 5.5 versus 3.5% for the ACV and VACV groups, respectively [no significant difference]). All patients included were initially treated with intravenous ACV until day 28 after transplantation or until discharge (56). In these two studies, PCR- or Ag-based preemptive treatment with GCV or foscarnet was used as well (56, 98).

Foscarnet

Intravenous foscarnet is considered second-line therapy for CMV reactivation or disease; however, for patients developing dose-limiting neutropenia or CMV strains resistant to GCV, it is the drug of choice (69). In a survey of herpesvirus resistance to antiviral drugs, GCV was replaced by foscarnet in 15 pa-

tients with suspected or proven GCV resistance, and this resulted in a better clinical or virologic outcome in 13 of these 15 patients (84).

Four nonrandomized studies using foscarnet prophylactically were published (3, 12, 71, 83). In all four studies the patient numbers were very small (≤ 21); therefore, no firm conclusions regarding the effectiveness of foscarnet prophylaxis can be drawn.

Preemptive treatment with foscarnet has also been reported in four studies, which showed similar efficacies of foscarnet and GCV (4, 53, 66, 86). Only two were randomized trials (66, 86), one of which had a low patient number (66). Reusser et al. (86) treated 110 patients with CMV reactivation (Ag or PCR diagnosed) with foscarnet (60 mg/kg twice a day [b.i.d.]) and treated 103 patients with CMV reactivation with GCV (5 mg/kg b.i.d.). When test results were still positive after 14 days of treatment, both drugs were continued at a reduced dose (90 mg/kg/day for foscarnet and 6 mg/kg/day for GCV) for 2 weeks longer (5 days a week). When CMV was still detectable after this second treatment period, treatment was considered a failure, and patients were treated at the discretion of the investigator. Event-free survival and overall survival at day 180 were similar in both groups, as was the occurrence of CMV disease and treatment failures. No difference was observed in regard to other herpesvirus infections or major nonviral infections. Preemptive treatment with foscarnet did not raise safety concerns (when appropriate hydration was used) and was associated with significantly less serious hematotoxicity than GCV. In the GCV group, neutropenia was more often observed, despite the use of growth factors.

CDV

Ljungman et al. (55) performed a retrospective survey among 17 bone marrow transplantation centers and enrolled 82 patients who were treated with CDV for CMV disease ($n = 20$) or for CMV reactivation (primary preemptive treatment, $n = 24$; secondary preemptive treatment in patients who had failed or relapsed after previous preemptive treatment with

another antiviral drug, $n = 38$). The dosage was 1 to 5 mg/kg per week followed by maintenance treatment, and all patients received probenecid and prehydration. Overall, 62% showed a response to CDV therapy, with a response being defined as disease regression without addition of other specific therapy or, for preemptive therapy, conversion of a positive Ag or PCR test. Twenty-one patients developed renal toxicity, which persisted after cessation of therapy in nine patients. No toxicity was seen in 45 patients, while 15 developed other side effects potentially associated with CDV therapy (nausea, vomiting, thrombocytopenia, rash, or ophthalmologic or neurologic toxicity). Kiehl and Basara (M. G. Kiehl and N. Basara, Letter, Blood 98:1626, 2001) published prospective data on 21 patients receiving first-line preemptive treatment with CDV at 5 mg/kg once a week for 2 weeks and thereafter every 2 weeks. Treatment was Ag and/or DNA PCR based and continued until test results were negative for at least 3 weeks. Only one patient showed a complete response. In 15 patients the PCR became negative; however, 2 to 3 weeks later a positive PCR was again observed for all 15 patients. In five patients CMV reactivation was not cleared. The authors stated that it might be more effective to give CDV once a week for a longer period, which is supported by the low toxicity rate observed in this study: only one patient developed renal toxicity. Chakrabarti et al. (14) treated four patients preemptively with CDV at 5 mg/kg/week for 4 weeks. Two responded but developed severe nausea, vomiting, and uveitis. Two were nonresponders; one died from CMV pneumonia, and one developed CMV pneumonia that eventually responded to foscarnet. The less favorable outcome in the last two reports is in concordance with recent prospective results described by Platzbecker et al. (75). In that study, only one of seven SCT recipients showed a transient clearance of pp65 Ag after treatment with CDV at 5 mg/kg/week for 2 weeks and thereafter every 2 weeks. In contrast, those authors (75) showed that preemptive treatment with CDV was very successful in 10 SCT recipients treated with a nonmyeloablative conditioning regimen. Toxicity was moderate and consisted of reversible renal impairment ($n = 4$), proteinuria ($n = 1$), and nausea or vomiting ($n = 3$).

Antiviral Drug Resistance

When prolonged antiviral therapy (>100 days) is given, drug resistance may develop (23). Data about antiviral drug resistance have largely been obtained with AIDS patients, and very little information about drug resistance in the SCT setting is available. Resistance of CMV to GCV is associated with lack of a therapeutic response and progression of CMV disease (25, 101). The clinical outcome of infections caused by foscarnet- and CDV-resistant CMV strains is unknown. In 1996 a survey of herpesvirus resistance to antiviral drugs was performed in 68 bone marrow transplantation centers. CMV resistance to GCV was proven in 2 patients and suspected in 23 patients (84). In patients with CMV pneumonia, the virus often persists for a long time despite GCV treatment. GCV resistance was determined for CMV isolates obtained from BAL fluid or from autopsy lung tissue by DNA hybridization. In only 1 of 12 patients was a GCV-resistant isolate detected (91). In a study of 50 allogeneic SCT recipients, 10 patients exhibited sustained or recurrent Ag despite GCV treatment. Samples from these

10 patients were screened for the presence of the most frequent CMV UL97 mutations by restriction enzyme analysis, and none of these mutations were detected (30). Overall, antiviral drug resistance in adult SCT recipients has been reported only sporadically (24, 30, 84, 91). There is some evidence that it might be more frequent in pediatric SCT recipients, especially in patients with primary immunodeficiencies (18, 78, 101).

In clinical CMV strains, resistance to antiviral agents has been associated with mutations in the viral protein kinase UL97 (for GCV only) and viral DNA polymerase UL54 (for GCV, foscarnet, and CDV) genes (25). The various laboratory methods used for drug susceptibility testing of CMV isolates have been reviewed by Erice (25) and may be classified as phenotypic or genotypic. Phenotypic methods generally are culture based and designed to determine the concentration of an antiviral agent that would inhibit the virus in culture. Genotypic methods are designed to determine known UL97 or UL54 mutations present in the genomes of the viruses being studied, using restriction enzyme analysis and/or sequencing, and do not require viral cultures. A drawback of phenotypic methods is the possible in vitro selection of specific virus isolates by several culture passages. This was recently proven by Hamprecht et al. (34), who performed only one culture passage of CMV isolates before phenotypic drug susceptibility assays were performed. Virus strains isolated from these cultures were also genotypically analyzed by UL97 restriction assays and sequencing and were compared with primary DNA extracts of the same specimens. This resulted in the molecular proof of the in vitro selection of one UL97 mutant strain from three viral variants (one wild-type strain and two UL97 mutants) present in vivo.

Summary

Overall, in the era before the introduction of preemptive antiviral therapy, high-dose prophylactic ACV was shown to be effective in reducing the CMV-associated mortality rate. When preemptive treatment with GCV or foscarnet was used, VACV proved to be more effective as prophylaxis against CMV viremia than ACV, but without significantly affecting overall survival and the incidence of CMV disease. Currently it is not clear whether VACV prophylaxis combined with a preemptive antiviral strategy is better than preemptive therapy alone, which needs to be tested in a randomized controlled trial. Although intravenous GCV is considered the drug of choice for (preemptive) treatment of CMV reactivation or disease, foscarnet has similar efficacy and less hematologic toxicity. The third agent used for preemptive treatment, CDV, has been tested prospectively in only a few studies, with all of them showing disappointing results.

ADOPTIVE IMMUNOTHERAPY WITH CMVS T CELLS

Adoptive transfer of CD8⁺ CMVs CTLs for prevention of CMV reactivation or disease has been shown to be effective (87, 99). When no CMVs Th response developed, CMVs CTLs declined progressively. However, none of the 17 patients treated with CD8⁺ CMVs CTLs developed CMV infection (87, 99). Einsele et al. (21) treated eight patients with antiviral-resistant

CMV reactivation, and who had a CMV-seropositive donor, with CMVs T cells (10^7 CMVs T cells/ m^2). These cells consisted of CD4⁺ CMVs Th cells and CD8⁺ CMVs CTLs. Only patients lacking a CMVs lymphoproliferative response in vitro, indicating a deficient CMVs Th response, were enrolled. A response was seen in six of seven evaluable patients, in which CMV reactivation was cleared. Once CMVs T cells emerged in the peripheral blood, they persisted at numbers comparable to those in healthy individuals. The authors hypothesize that adoptive immunotherapy is more effective when CD4⁺ CMVs Th cells are given together with CD8⁺ CMVs CTLs, by inducing expansion of CD8⁺ CMVs CTLs from precursors that without T-cell help would not have been activated. In that study three patients subsequently developed invasive aspergillosis or respiratory syncytial virus interstitial pneumonitis. In a commentary it was stated that more efficient culture systems are needed to make this therapy more accessible. Furthermore, since eventually three patients died from fungal or viral infections, a more comprehensive approach to reconstitute immunity in SCT recipients is required (C. H. June, Editorial, *Blood* 99:3883, 2002.). Peggs et al. (74) used monocyte-derived dendritic cells to process and present CMV antigen to generate donor-derived CMVs cell lines containing both CD4⁺ and CD8⁺ T cells with a simple and rapid 21-day culture. These cells were administered preemptively to 13 allogeneic transplant recipients, 10 of whom received a nonmyeloablative conditioning regimen. Within 23 days following infusion, CD8⁺ CMVs CTLs reached absolute counts of as high as 540×10^6 /liter and were detectable for up to 6 months posttransfusion. Six patients cleared CMV without antiviral drugs, and no cases of CMV disease were diagnosed. Only 1 of 12 evaluable patients showed subsequent CMV reactivation. This patient had CMVs T cells administered at day 14 posttransplant, when Campath-1H (the immunoglobulin G1 humanized monoclonal antibody against CD52) probably was still circulating, which might have induced lysis of these T cells.

Adoptive immunotherapy with CMVs cytotoxic T cells as preemptive therapy is a very elegant strategy; however, generation of these cells is expensive and time-consuming, and therefore the therapy is not available at every transplantation center. Magnetic selection of CMVs CD8⁺ T cells from peripheral blood of CMV-seropositive donors by using HLA class I-peptide tetramers, as described by Keenan et al. (46), may be very promising, making adoptive immunotherapy more accessible.

CONCLUSION

This review has focused on prevention of CMV disease in recipients of allogeneic SCT. The introduction of preemptive antiviral therapy has greatly reduced the incidence and mortality rate of CMV disease, especially when Ag- or PCR-based CMV monitoring is performed. Many questions still remain to be answered. We presently do not know whether the increased sensitivity of PCR-based CMV DNA assays has any clinical benefit. Furthermore, it is not clear whether PCR tests can better be performed with plasma, WB, or PBL samples. At which viral load or Ag level should antiviral therapy be instituted, and for how long should it be continued? The present conclusion is that prevention of CMV disease with low CMV-

associated mortality seems to be superior in studies using a short-term (14-day) Ag- or PCR-based preemptive treatment. In those studies preemptive treatment was extended only when CMV monitoring tests were still positive after the short-term treatment period. When overall survival was considered the end point, the various preemptive treatment strategies all were equally effective.

In the near future, monitoring of CMVs T-cell recovery may change our present preemptive treatment strategy. The presence of CMVs T cells in patients with a documented CMV reactivation might protect these patients from developing CMV disease. Prospective studies are needed to confirm the results derived from retrospectively performed analyses.

Finally, efforts should focus on immune reconstitution. Once adoptive immunotherapy becomes more accessible, controlled trials should be designed to study the effectiveness of immunotherapy in prevention of CMV disease.

REFERENCES

1. Aono, T., K. Kondo, H. Miyoshi, K. Tanaka-Taya, M. Kondo, Y. Osugi, J. Hara, S. Okada, and K. Yamanishi. 1998. Monitoring of human cytomegalovirus infections in pediatric bone marrow transplant recipients by nucleic acid sequence-based amplification. *J. Infect. Dis.* 178:1244-1249.
2. Aubert, G., A. F. Hassan-Walker, J. A. Madrigal, V. C. Emery, C. Morte, S. Grace, M. B. C. Koh, M. Potter, H. G. Prentice, A. Dodi, and P. J. Travers. 2001. Cytomegalovirus-specific cellular immune responses and viremia in recipients of allogeneic stem cell transplants. *J. Infect. Dis.* 184:955-963.
3. Bacigalupo, A., E. Tedone, M. T. Van Lint, G. Trespi, M. Lonngren, M. A. Sanna, F. Moro, F. Frassoni, D. Occhini, F. Gualandi, T. Lamparelli, G. Hale, and A. M. Marmont. 1994. CMV prophylaxis with foscarnet in allogeneic bone marrow transplant recipients at high risk of developing CMV infections. *Bone Marrow Transplant.* 13:783-788.
4. Bacigalupo, A., M. T. Van Lint, E. Tedone, F. Moro, M. A. Sanna, M. Lonngren, G. Trespi, F. Frassoni, D. Occhini, F. Gualandi, T. Lamparelli, and A. M. Marmont. 1994. Early treatment of CMV infections in allogeneic bone marrow transplant recipients with foscarnet or ganciclovir. *Bone Marrow Transplant.* 13:753-758.
5. Boeckh, M., T. A. Gooley, D. Myerson, T. Cunningham, G. Schoch, and R. A. Bowden. 1996. Cytomegalovirus pp65 Ag-guided early treatment with ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. *Blood* 88:4063-4071.
6. Boeckh, M., G. M. Gallez-Hawkins, D. Myerson, J. A. Zaia, and R. A. Bowden. 1997. Plasma polymerase chain reaction for cytomegalovirus DNA after allogeneic marrow transplantation. *Transplantation* 64:108-113.
7. Boeckh, M., and G. Boivan. 1998. Quantitation of cytomegalovirus: methodologic aspects and clinical applications. *Clin. Microbiol. Rev.* 11:533-554.
8. Boeckh, M., R. A. Bowden, T. A. Gooley, D. Myerson, and L. Corey. 1999. Successful modification of a pp65 antigenemia-based early treatment strategy for prevention of cytomegalovirus disease in allogeneic marrow transplant recipients. *Blood* 93:1781-1782.
9. Boivin, G., R. Belanger, R. Delage, C. Beliveau, C. Demers, N. Goyette, and J. Roy. 2002. Quantitative analysis of cytomegalovirus (CMV) viremia using the pp65 antigenemia assay and the Cobas Amplicor CMV monitor test after blood and marrow allogeneic transplantation. *J. Clin. Microbiol.* 38:4356-4360.
10. Boriskin, Y. S., K. Fuller, R. L. Powles, I. B. Vipond, P. S. Rice, J. C. Booth, E. O. Caul, and P. D. Butcher. 2002. Early detection of cytomegalovirus (CMV) infection in bone marrow transplant patients by reverse transcription-PCR for CMV spliced late gene UL21.5: a two site evaluation. *J. Clin. Virol.* 24:13-23.
11. Bowden, R. A., S. J. Slichter, M. H. Sayers, M. Mori, M. J. Cays, and J. D. Meyers. 1991. Use of leukocyte-depleted platelets and cytomegalovirus-seronegative red blood cells for prevention of primary cytomegalovirus infection after marrow transplant. *Blood* 78:246-250.
12. Bregante, S., S. Bertilsson, E. Tedone, M. T. Van Lint, G. Trespi, N. Mordini, G. Berisso, F. Gualandi, T. Lamparelli, O. Figari, F. Benvenuto, A. M. Raiola, and A. Bacigalupo. 2000. Foscarnet prophylaxis of cytomegalovirus infections in patients undergoing allogeneic bone marrow transplantation (BMT): a dose-finding study. *Bone Marrow Transplant.* 26:23-29.
13. Castro-Malaspina, H., R. E. Harris, J. Gajewski, N. Ramsay, R. Collins, B. Dharan, R. King, and H. J. Deeg. 2002. Unrelated marrow transplantation for myelodysplastic syndromes: outcome analysis in 510 transplants facilitated by the national marrow donor program. *Blood* 99:1943-1951.
14. Chakrabarti, S., K. E. Collingham, H. Osman, C. C. Fegan, and D. W.

- Milligan. 2001. Cidofovir as primary pre-emptive therapy for post-transplant cytomegalovirus infections. *Bone Marrow Transplant.* **28**:879–881.
15. Craddock, C., R. M. Szydlo, F. Dazzi, E. Olavarria, K. Cwynarski, A. Yong, P. Brookes, J. de la Fuente, E. Kanfer, J. Apperley, and J. M. Goldman. 2001. Cytomegalovirus seropositivity adversely influences outcome after T-depleted unrelated donor transplant in patients with chronic myeloid leukaemia: the case for tailored graft-versus-host disease prophylaxis. *Br. J. Haematol.* **112**:228–236.
 16. Cwynarski, K., J. Ainsworth, M. Cobbald, S. Wagner, P. Mahendra, J. Apperley, J. Goldman, C. Craddock, and P. A. H. Moss. 2001. Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation. *Blood* **97**:1232–1240.
 17. de Gast, G. C., G. J. Boland, A. M. Vlieger, R. A. de Weger, L. F. Verdonck, F. E. Zwann, and N. M. Jiwa. 1992. Abortive human cytomegalovirus infection in patients after allogeneic bone marrow transplantation. *Bone Marrow Transplant.* **9**:221–225.
 18. Eckle, T., L. Prix, G. Jahn, T. Klingebiel, R. Handgretinger, B. Selle, and K. Hamprecht. 2000. Drug-resistant human cytomegalovirus infection in children after allogeneic stem cell transplantation may have different clinical outcomes. *Blood* **96**:3286–3289.
 19. Einsele, H., G. Ehninger, H. Hebart, K. M. Wittkowski, U. Schuler, G. Jahn, P. Mackes, M. Herter, T. Klingebiel, J. Loeffler, S. Wagner, and C. A. Muller. 1995. 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**:2815–2820.
 20. Einsele, H., H. Hebart, C. Kauffman-Schneider, C. Sinzger, G. Jahn, P. Bader, T. Klingebiel, K. Dietz, J. Loeffler, C. Bokemeyer, C. A. Muller, and L. Kanz. 2000. Risk factors for treatment failures in patients receiving PCR-based pre-emptive therapy for CMV infection. *Bone Marrow Transplant.* **25**:757–763.
 21. Einsele, H., E. Roosnek, N. Rufer, C. Sinzger, S. Riegler, J. Loeffler, U. Grigoleit, A. Moris, H. G. Rammensee, L. Kanz, A. Kleihauer, F. Frank, G. Jahn, and H. Hebart. 2002. Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. *Blood* **99**:3916–3922.
 22. Emanuel, D., I. Cunningham, K. Jules-Elysee, J. A. Brochstein, N. Kernan, J. Laver, D. Stover, D. A. White, A. Fels, B. Polsky, H. Castro-Malaspina, J. R. Peppard, P. Bartus, U. Hammerling, and R. J. O'Reilly. 1988. Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. *Ann. Intern. Med.* **109**:777–782.
 23. Emery, V. C., and P. D. Griffiths. 2000. Prediction of cytomegalovirus load and resistance patterns after antiviral chemotherapy. *Proc. Natl. Acad. Sci. USA* **97**:8039–8044.
 24. Erice, A., N. Borrell, W. Li, W. J. Miller, and H. H. Balfour, Jr. 1998. Ganciclovir susceptibilities and analysis of UL97 region in cytomegalovirus (CMV) isolates from bone marrow recipients with CMV disease after antiviral prophylaxis. *J. Infect. Dis.* **178**:531–534.
 25. Erice, A. 1999. Resistance of human cytomegalovirus to antiviral drugs. *Clin. Microbiol. Rev.* **12**:286–297.
 26. Flexman, J., I. Kay, R. Fonte, R. Herrmann, E. Gabbay, and S. Palladino. 2001. Differences between the quantitative antigenemia assay and the Cobas Amplicor monitor quantitative PCR assay for detecting CMV viremia in bone marrow and solid organ recipients. *J. Med. Virol.* **64**:275–282.
 27. Gerna, G., M. Furione, F. Baldanti, E. Percivalle, P. Comoli, and F. Locatelli. 1995. Quantitation of human cytomegalovirus DNA in bone marrow transplant recipients. *Br. J. Haematol.* **91**:674–683.
 28. Gerna, G., F. Baldanti, J. M. Middeldorp, M. Furione, M. Zavattoni, D. Lilleri, and M. G. Revello. 1999. Clinical significance of expression of human cytomegalovirus pp67 late transcript in heart, lung, and bone marrow transplant recipients as determined by nucleic acid sequence-based amplification. *J. Clin. Microbiol.* **37**:902–911.
 29. Gerna, G., F. Baldanti, D. Lilleri, M. Parea, E. Alessandrino, A. Pagani, F. Locatelli, J. M. Middeldorp, and M. G. Revello. 2000. Human cytomegalovirus immediate-early mRNA detection by nucleic acid sequence-based amplification as a new parameter for preemptive therapy in bone marrow transplant recipients. *J. Clin. Microbiol.* **38**:1845–1853.
 30. Gilbert, C., J. Roy, R. Belanger, R. Delage, C. Beliveau, C. Demers, and G. Boivin. 2001. Lack of emergence of cytomegalovirus UL97 mutations conferring ganciclovir (GCV) resistance following preemptive GCV therapy in allogeneic stem cell transplant recipients. *Antimicrob. Agents Chemother.* **45**:3669–3671.
 31. Goodrich, J. M., R. A. Bowden, L. Fisher, C. Keller, G. Schoch, and J. D. Meyers. 1993. Ganciclovir prophylaxis to prevent cytomegalovirus disease after allogeneic marrow transplant. *Ann. Intern. Med.* **118**:173–178.
 32. Gratama, J. W., J. W. J. van Esser, C. H. J. Lamers, C. Tournay, B. Lowenberg, R. L. H. Bolhuis, and J. J. Cornelissen. 2001. Tetramer-based quantification of cytomegalovirus (CMV)-specific CD8+ T lymphocytes in T-cell depleted stem cell grafts and after transplantation may identify patients at risk for progressive CMV infection. *Blood* **98**:1358–1364.
 33. Griscelli, F., M. Barrois, S. Chauvin, S. Lastere, D. Bellet, and J. H. Bourhis. 2001. Quantification of human cytomegalovirus DNA in bone marrow transplant recipients by real-time PCR. *J. Clin. Microbiol.* **39**:4362–4369.
 34. Hamprecht, K., T. Eckle, L. Prix, C. Faul, H. Einsele, and G. Jahn. 2003. Ganciclovir-resistant cytomegalovirus disease after allogeneic stem cell transplantation: pitfalls of phenotypic diagnosis by in vitro selection of an UL97 mutant strain. *J. Infect. Dis.* **187**:139–143.
 35. Hebart, H., C. Muller, J. Loeffler, G. Jahn, and H. Einsele. 1996. Monitoring of CMV infection: a comparison of PCR from whole blood, plasma-PCR, pp65-antigenemia and virus culture in patients after bone marrow transplantation. *Bone Marrow Transplant.* **17**:861–868.
 36. Hebart, H., D. Gamer, J. Loeffler, C. Mueller, C. Sinzger, G. Jahn, P. Bader, T. Klingebiel, L. Kanz, and H. Einsele. 1998. Evaluation of Murex CMV DNA hybrid capture assay for detection and quantitation of cytomegalovirus infection in patients following allogeneic stem cell transplantation. *J. Clin. Microbiol.* **36**:1333–1337.
 37. Reference deleted.
 38. Hebart, H., S. Daginik, S. Stevanovic, U. Grigoleit, A. Dobler, M. Baur, G. Rauser, C. Sinzger, G. Jahn, J. Loeffler, L. Kanz, H. G. Rammensee, and H. Einsele. 2002. Sensitive detection of human cytomegalovirus peptide-specific cytotoxic T-lymphocyte responses by interferon-gamma-enzyme-linked immunospot assay and flow cytometry in healthy individuals and in patients after allogeneic stem cell transplantation. *Blood* **99**:3830–3837.
 39. Hebart, H., T. Rudolph, J. Loeffler, J. Middeldorp, T. Ljubicic, G. Jahn, and H. Einsele. 2002. Evaluation of the NucliSens CMV pp67 assay for detection and monitoring of human cytomegalovirus infection after allogeneic stem cell transplantation. *Bone Marrow Transplant.* **30**:181–187.
 40. Hiyoshi, M., S. Tagawa, T. Takubo, K. Tanaka, T. Nakao, Y. Higano, K. Tamura, M. Shimaoka, A. Fujii, M. Higashihata, Y. Yasui, T. Kim, A. Hiraoka, and N. Tatsumi. 1997. Evaluation of the Amplicor CMV test for direct detection of cytomegalovirus in plasma specimens. *J. Clin. Microbiol.* **10**:2692–2694.
 41. Humar, A., S. Wood, J. Lipton, H. Messner, J. Meharchand, A. McGeer, K. MacDonald, and T. Mazzulli. 1998. Effect of cytomegalovirus infection on 1-year mortality rates among recipients of allogeneic bone marrow transplants. *Clin. Infect. Dis.* **26**:606–610.
 42. Humar, A., J. Lipton, S. Welsh, G. Moussa, H. Messner, and T. Mazzulli. 2001. A randomised trial comparing cytomegalovirus antigenemia assay vs screening bronchoscopy for the early detection and prevention of disease in allogeneic bone marrow and peripheral blood stem cell transplant recipients. *Bone Marrow Transplant.* **28**:485–490.
 43. Reference deleted.
 44. Kanda, Y., S. Chiba, T. Suzuki, M. Kami, Y. Yazaki, and H. Hirai. 1998. Time course analysis of semi-quantitative PCR and antigenemia assay for prevention of cytomegalovirus disease after bone marrow transplantation. *Br. J. Haematol.* **100**:222–225.
 45. Kanda, Y., S. Mineishi, T. Saito, S. Seo, A. Saito, M. Ohnishi, K. Suenaga, H. Niiya, K. Nakai, T. Takeuchi, and Y. Takaue. 2001. Pre-emptive therapy against cytomegalovirus (CMV) disease guided by CMV antigenemia assay after allogeneic hematopoietic stem cell transplantation: a single center experience in Japan. *Bone Marrow Transplant.* **27**:437–444.
 46. Keenan, R. D., J. Ainsworth, N. Khan, R. Bruton, M. Cobbald, M. Assenmacher, D. W. Milligan, and P. A. Moss. 2001. Purification of cytomegalovirus-specific CD8 T cells from peripheral blood using HLA-peptide tetramers. *Br. J. Haematol.* **115**:428–434.
 47. Reference deleted.
 48. Krause, H., H. Hebart, G. Jahn, C. A. Muller, and H. Einsele. 1997. Screening for CMV-specific T cell proliferation to identify patients at risk of developing late onset CMV disease. *Bone Marrow Transplant.* **19**:1111–1116.
 49. Kröger, N., T. Zabelina, W. Krüger, H. Renges, N. Stute, J. Schrum, H. Kabisch, P. Schafhausen, N. Jaburg, C. Loliger, P. Schafer, A. Hinke, and A. R. Zander. 2001. Patient cytomegalovirus seropositivity with or without reactivation is the most important prognostic factor for survival and treatment-related mortality in stem cell transplantation from unrelated donors using pretransplant in vivo T-cell depletion with antithymocyte globulin. *Br. J. Haematol.* **113**:1060–1071.
 50. Li, C. R., P. D. Greenberg, M. J. Gilbert, J. M. Goodrich, and S. R. Riddell. 1994. 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**:1971–1979.
 51. Lim, J. B., M. Provenzano, M. Bettinotti, and D. F. Stroncek. 2001. Identification of a new candidate HLA-A3 restricted CMV epitope as a target antigen to induce specific CMV cytotoxic lymphocytes. *Blood* **98**:S780a. (Abstract 3244.)
 52. Ljungman, P., K. Lore, J. Aschan, S. Klaesson, I. Lewensohn-Fuchs, B. Lonnqvist, O. Ringden, J. Winiarski, and A. Ehrnst. 1996. Use of a semi-quantitative PCR for cytomegalovirus DNA as a basis for pre-emptive antiviral therapy in allogeneic bone marrow transplant recipients. *Bone Marrow Transplant.* **17**:583–587.
 53. Ljungman, P., G. Oberg, J. Aschan, A. Ehrnst, B. Lonnqvist, K. Pauksen, and P. Sulila. 1996. Foscarnet for pre-emptive therapy of CMV infection

- detected by a leukocyte-based nested PCR in allogeneic bone marrow transplant patients. *Bone Marrow Transplant*. **18**:565–568.
54. Ljungman, P. 2001. Prophylaxis against herpesvirus infections in transplant recipients. *Drugs* **61**:187–196.
 55. Ljungman, P., G. Lambertenghi Deliliers, U. Platzbecker, S. Matthes-Martin, A. Bacigalupo, H. Einsele, J. Ullman, M. Musso, R. Trensche, P. Ribaud, M. Bornhauser, and C. Cordonnier. 2001. Cidofovir for cytomegalovirus infection and disease in allogeneic stem cell transplant recipients. *Blood* **97**:388–392.
 56. Ljungman, P., R. de la Camara, N. Milpied, L. Volin, C. A. Russell, A. Crisp, and A. Webster. 2002. Randomized study of valganciclovir as prophylaxis against cytomegalovirus reactivation in recipients of allogeneic bone marrow transplants. *Blood* **99**:3050–3056.
 57. Longmate, J., J. York, C. La Rosa, R. Krishan, M. Zhang, D. Senitzer, and D. J. Diamond. 2001. Population coverage by HLA class-I restricted cytotoxic T-lymphocyte epitopes. *Immunogenetics* **52**:165–173.
 58. Machado, C. M., F. L. Dulle, L. S. Vilas Boas, J. B. Castelli, M. C. A. Macedo, R. L. Silva, R. Pallota, R. S. Saboya, and C. S. Pannuti. 2000. CMV pneumonia in allogeneic recipients undergoing early treatment or pre-emptive ganciclovir therapy. *Bone Marrow Transplant*. **26**:413–417.
 59. Machado, C. M., R. X. Menezes, M. C. A. Macedo, A. V. A. Mendes, L. S. Vilas Boas, J. B. Castelli, F. L. Dulle, and C. S. Pannuti. 2001. Extended antigenemia surveillance and late cytomegalovirus infection after allogeneic BMT. *Bone Marrow Transplant*. **28**:1053–1059.
 60. Machida, U., M. Kami, T. Fukui, Y. Kazuyama, M. Kinoshita, Y. Tanaka, Y. Kanda, S. Ogawa, H. Honda, and H. Hirai. 2000. Real-time automated PCR for early diagnosis and monitoring of cytomegalovirus infection after bone marrow transplantation. *J. Clin. Microbiol.* **38**:2536–2542.
 61. Manteiga, R., R. Martino, A. Sureda, R. Labeaga, S. Brunet, J. Sierra, and N. Rabella. 1998. Cytomegalovirus pp65 antigenemia-guided pre-emptive treatment with ganciclovir after allogeneic stem cell transplantation: a single-center experience. *Bone Marrow Transplant*. **22**:899–904.
 62. Matsunaga, T., S. Sakamaki, S. Ishigaki, K. Kohda, M. Takeda, J. Katoh, H. Kuroda, Y. Hirayama, T. Kusakabe, T. Akiyama, T. Kuga, Y. Niitsu, T. Masaoka, T. Sagawa, and Y. Matsumoto. 1999. Use of PCR serum in diagnosing and monitoring cytomegalovirus reactivation in bone marrow transplant recipients. *Int. J. Hematol.* **69**:105–111.
 63. Meijer, E., A. W. Dekker, M. Rozenberg-Arska, A. J. L. Weersink, and L. F. Verdonck. 2002. Influence of cytomegalovirus-seropositivity on outcome after T cell depleted bone marrow transplantation: contrasting results between recipients of grafts from related and unrelated donors. *Clin. Infect. Dis.* **35**:703–712.
 64. Meyers, J. D., N. Flournoy, and E. D. Thomas. 1986. Risk factors for cytomegalovirus infection after human marrow transplantation. *J. Infect. Dis.* **153**:478–488.
 65. Meyers, J. D., E. C. Reed, D. H. Shepp, M. Thornquist, P. S. Dandliker, C. A. Vicary, N. Flournoy, L. E. Kirk, J. H. Kersey, D. E. Thomas, and H. H. Balfour. 1988. Acyclovir for prevention of cytomegalovirus infection and disease after allogeneic marrow transplantation. *N. Engl. J. Med.* **318**:70–75.
 66. Moretti, S., P. Zikos, M. T. Van Lint, E. Tedone, D. Occhini, F. Gualandi, T. Lamparelli, N. Mordini, G. Berisso, S. Bregante, B. Bruno, and A. Bacigalupo. 1998. Foscarnet vs ganciclovir for cytomegalovirus (CMV) antigenemia after allogeneic hemopoietic stem cell transplantation (HSCT): a randomised study. *Bone Marrow Transplant*. **22**:175–180.
 67. Mori, T., S. Okamoto, S. Matsuo, T. Yajima, M. Wakui, R. Watanabe, A. Ishida, Y. Iwao, M. Mukai, T. Hibi, and Y. Ikeda. 2000. Risk-adapted pre-emptive therapy for cytomegalovirus disease in patients undergoing allogeneic bone marrow transplantation. *Bone Marrow Transplant*. **25**:765–769.
 68. Mori, T., S. Okamoto, R. Watanabe, T. Yajima, Y. Iwao, R. Yamazaki, T. Nakazato, N. Sato, T. Iguchi, H. Nagayama, N. Takayama, T. Hibi, and Y. Ikeda. 2002. Dose-adjusted pre-emptive therapy for cytomegalovirus disease based on real-time polymerase chain reaction after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. **29**:777–782.
 69. Nichols, W. G., and M. Boeckh. 2000. Recent advances in the therapy and prevention of CMV infections. *J. Clin. Virol.* **16**:25–40.
 70. Nichols, W. G., L. Corey, T. Gooley, C. Davis, and M. Boeckh. 2002. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. *J. Infect. Dis.* **185**:273–282.
 71. Ordemann, R., R. Naumann, G. Geissler, F. Kroschinsky, M. Bornhauser, R. Schwerdtfeger, and G. Ehninger. 2000. Foscarnet—an alternative for cytomegalovirus prophylaxis after allogeneic stem cell transplantation. *Ann. Hematol.* **79**:432–436.
 72. Ozdemir, E., L. S. St. John, G. Gillespie, S. Rowland-Jones, R. E. Champlin, J. J. Mollndrem, and K. V. Komanduri. 2001. Cytomegalovirus-specific CD8+ T cell recovery, as measured by HLA-peptide tetramer staining, is insufficient to control viremia following allogeneic stem cell transplantation. *Blood* **98**:S389a. (Abstract 1638.)
 73. Peggs, K. S., W. Preiser, P. D. Kottaridis, N. McKeag, N. S. Brink, R. S. Tedder, A. H. Goldstone, D. C. Linch, and S. Mackinnon. 2000. Extended routine polymerase chain reaction surveillance and pre-emptive antiviral therapy for cytomegalovirus after allogeneic transplantation. *Br. J. Haematol.* **111**:782–790.
 74. Peggs, K. S., S. Verfuere, A. Pizzey, N. Khan, P. Moss, and S. Mackinnon. 2001. Cytomegalovirus-specific adoptive cellular therapy results in massive and persistent expansions of CMV-specific T-cells and significantly reduces the incidence of CMV reactivation following allogeneic transplantation. *Blood* **98**:S779a. (Abstract 3240.)
 75. Platzbecker, U., D. Bandt, C. Thiede, A. Helwig, J. Freiberg-Richter, U. Schuler, R. Plettig, G. Geissler, A. Rethwilm, G. Ehninger, and M. Bornhauser. 2001. Successful pre-emptive cidofovir treatment for CMV antigenemia after dose-reduced conditioning and allogeneic blood stem cell transplantation. *Transplantation* **71**:880–885.
 76. Preiser, W., S. Brauninger, R. Schwerdtfeger, U. Ayliffe, J. A. Garson, N. S. Brink, S. Franck, H. W. Doerr, and H. F. Rabenau. 2001. Evaluation of diagnostic methods for the detection of cytomegalovirus in recipients of allogeneic stem cell transplants. *J. Clin. Virol.* **20**:59–70.
 77. Prentice, H. G., E. Gluckman, R. L. Powles, P. Ljungman, N. J. Milpied, R. Camara, F. Mandelli, P. Kho, L. Kennedy, and A. R. Bell. 1997. Long-term survival in allogeneic bone marrow transplant recipients following acyclovir prophylaxis for CMV infection. *Bone Marrow Transplant*. **19**:129–133.
 78. Prix, L., K. Hamprecht, B. Holzhter, R. Handgretinger, T. Klingebiel, and J. Jahn. 1999. Comprehensive restriction analysis of the UL97 region allows early detection of ganciclovir-resistant human cytomegalovirus in an immunocompromised child. *J. Infect. Dis.* **180**:491–495.
 79. Quinnan, G. V., N. Kirmani, A. H. Rook, J. F. Manischewitz, L. Jackson, G. Moreschi, G. W. Santos, R. Saral, and W. H. Burns. 1982. Cytotoxic T cells in cytomegalovirus infection: HLA-restricted T-lymphocyte and non-T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone marrow transplant recipients. *N. Engl. J. Med.* **307**:7–13.
 80. Reddy, V., Y. Hao, J. Lipton, J. Meharchand, M. Minden, T. Mazzulli, C. Chan, and H. A. Messner. 1999. Management of allogeneic bone marrow transplant recipients at risk for cytomegalovirus disease using a surveillance bronchoscopy and prolonged pre-emptive ganciclovir therapy. *J. Clin. Virol.* **13**:149–159.
 81. Reed, E. C., R. A. Bowden, P. S. Dandliker, K. E. Lilleby, and J. D. Meyers. 1988. Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. *Ann. Intern. Med.* **109**:783–788.
 82. Reusser, P., S. Riddell, J. D. Meyers, and P. D. Greenberg. 1991. Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease. *Blood* **78**:1373–1380.
 83. Reusser, P., J. G. Gambertoglio, K. Lilleby, and J. D. Meyers. 1992. Phase I-II trial of foscarnet for prevention of cytomegalovirus infection in autologous and allogeneic marrow transplant recipients. *J. Infect. Dis.* **166**:473–479.
 84. Reusser, P., C. Cordonnier, H. Einsele, D. Engelhard, H. Link, A. Locasciulli, and P. Ljungman. 1996. European survey of herpesvirus resistance to antiviral drugs in bone marrow transplant recipients. *Bone Marrow Transplant*. **17**:813–817.
 85. Reusser, P. 2001. Oral valganciclovir: a new option for treatment of cytomegalovirus infection and disease in immunocompromised hosts. *Expert Opin. Investig. Drugs* **10**:1745–1753. (Erratum, **11**:733, 2002.)
 86. Reusser, P., H. Einsele, J. Lee, L. Volin, M. Rovira, D. Engelhard, J. Finke, C. Cordonnier, H. Link, and P. Ljungman. 2002. Randomized multicenter trial of foscarnet versus ganciclovir for pre-emptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation. *Blood* **99**:1159–1164.
 87. Riddell, S. T., K. S. Watanabe, J. M. Goodrich, C. R. Li, M. E. Agha, and P. D. Greenberg. 1992. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. *Science* **257**:238–241.
 88. Schmidt, G. M., D. A. Horak, J. C. Niland, S. R. Duncan, S. J. Forman, and J. A. Zaia. 1991. A randomized, controlled trial of prophylactic ganciclovir for cytomegalovirus pulmonary infection in recipients of allogeneic bone marrow transplants. *N. Engl. J. Med.* **324**:1005–1011.
 89. Schulenburg, A., T. Watkins-Riedel, H. T. Greinix, W. Rabitsch, H. Loidolt, F. Keil, M. Mitterbauer, and P. Kalls. 2001. CMV monitoring after peripheral blood stem cell and bone marrow transplantation by pp65 antigen and quantitative PCR. *Bone Marrow Transplant*. **28**:765–768.
 90. Seropian, S., D. Ferguson, E. Salloom, D. Cooper, and M. L. Landry. 1998. Lack of reactivity to CMV pp65 antigenemia testing in a patient with CMV disease following allogeneic bone marrow transplant. *Bone Marrow Transplant*. **22**:507–509.
 91. Slavin, M. A., R. R. Bindra, C. A. Gleaves, M. B. Pettinger, and R. A. Bowden. 1993. Ganciclovir sensitivity of cytomegalovirus at diagnosis and during treatment of cytomegalovirus pneumonia in marrow transplant recipients. *Antimicrob. Agents Chemother.* **37**:1360–1363.
 92. Small, T. N., E. P. Papadopoulos, F. Boulad, T. Kiehn, J. Stiles, K. Sepkowitz, and R. J. O'Reilly. 2001. Outcome of pre-emptive or prophylactic

- anti-viral therapy to prevent CMV disease following T cell depleted related or unrelated BMT. *Blood* **98**:S394a. (Abstract 1659.)
93. **Solano, C., I. Munoz, A. Gutierrez, A. Farga, F. Prosper, J. Garcia-Conde, D. Navarro, and C. Gimeno.** 2001. Qualitative plasma PCR assay (Amplicor CMV test) versus pp65 antigenemia assay for monitoring cytomegalovirus viremia and guiding preemptive ganciclovir therapy in allogeneic stem cell transplantation. *J. Clin. Microbiol.* **39**:3938–3941.
 94. Reference deleted.
 95. **van Prooyen, H. C., J. J. Visser, W. R. van Oostendorp, G. C. de Gast, and L. F. Verdonck.** 1994. Prevention of primary transfusion-associated cytomegalovirus infection in bone marrow transplant recipients by the removal of white cells from blood components with high-affinity filters. *Br. J. Haematol.* **87**:144–147.
 96. **Verdonck, L. F., Y. C. E. de Graan-Hentzen, A. W. Dekker, G. C. Mudde, and G. C. de Gast.** 1987. Cytomegalovirus seronegative platelets and leukocyte-poor red blood cells from random donors can prevent primary cytomegalovirus infection after bone marrow transplantation. *Bone Marrow Transplant.* **2**:73–78.
 97. **Verdonck, L. F., A. W. Dekker, M. Rozenberg-Arska, and M. R. van den Hoek.** 1997. A risk adapted approach with a short course of ganciclovir to prevent cytomegalovirus (CMV) pneumonia in CMV-seropositive recipients of allogeneic bone marrow transplantation. *Clin. Infect. Dis.* **24**:901–907.
 98. **Vusirikala, M., S. N. Wolff, R. S. Stein, S. J. Brandt, D. S. Morgan, J. P. Greer, F. G. Schuening, J. S. Dummer, and S. A. Goodman.** 2001. Valacyclovir for the prevention of cytomegalovirus infection after allogeneic stem cell transplantation: a single institution retrospective cohort analysis. *Bone Marrow Transplant.* **28**:265–270.
 99. **Walter, E. A., P. D. Greenberg, M. J. Gilbert, R. J. Finch, K. S. Watanabe, E. D. Thomas, and S. R. Riddell.** 1995. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N. Engl. J. Med.* **333**:1038–1044.
 100. **Winston, D. J., W. G. Ho, K. Bartoni, C. Du Mond, D. F. Ebeling, W. C. Buhles, and R. E. Champlin.** 1993. Ganciclovir prophylaxis of cytomegalovirus infection in allogeneic bone marrow transplant recipients. *Ann. Intern. Med.* **118**:179–184.
 101. **Wolf, D. G., I. Yaniv, A. Honigman, I. Kassis, T. Schonfeld, and S. Ashkenazi.** 1998. Early emergence of ganciclovir-resistant human cytomegalovirus strains in children with primary combined immunodeficiency. *J. Infect. Dis.* **178**:535–538.
 102. **Yakushiji, K., H. Gondo, K. Kamezaki, K. Shigematsu, S. Hayashi, M. Kuroiwa, S. Taniguchi, Y. Ohno, K. Takase, A. Numata, K. Aoki, K. Kato, K. Nagafuji, and M. Harada.** 2002. Monitoring of cytomegalovirus reactivation after allogeneic stem cell transplantation: comparison of an antigenemia assay and quantitative real-time polymerase chain reaction. *Bone Marrow Transplant.* **29**:599–606.