Chapter 10

Prevention of Cytomegalovirus disease in recipients of allogeneic stem cell transplants: a review

Ellen Meijer,
Greet J. Boland,
Leo F. Verdonck

Clinical Microbiology Review 2003; 16: 647-657

Abstract

The main risk factors for cytomegalovirus (CMV) disease in recipients of allogeneic stem cell transplants (SCT) are recipient CMV-seropositivity and acute graft-versus-host disease. Currently, two antiviral strategies -prophylactic or pre-emptive antiviral treatment- are used for prevention of CMV disease, which is most favourable when short-term (14 days) pre-emptive treatment is applied.

Several methods are available for monitoring of CMV reactivations. PCR based CMV DNA detection assays are the most sensitive methods, however, the clinical benefit of this high sensitivity is unclear. Even more, there is lack of clarity whether PCR tests can better be performed in plasma, whole blood or peripheral blood leukocyte samples.

Recovery of a CMV-specific CD8+ cytotoxic T lymphocyte (CTL) response is necessary for preventing CMV reactivation and disease. Reconstitution of absolute CMV-specific CTL counts to values above 10-20 x 10^6 CTLs/L is associated with protection from CMV disease. In the near future, pre-emptive therapy might be withheld in patients with CMV reactivation who show to have adequate CMV-specific cytotoxic T cell levels.

Antiviral therapy with (val)acyclovir has only been studied as prophylactic treatment modality for prevention of CMV infection. High-dose oral valacyclovir is more effective compared to acyclovir, when used in addition to pre-emptive treatment of CMV reactivations with ganciclovir or foscarnet.

Three antiviral drugs have been tested for pre-emptive therapy of CMV reactivations and/or treatment of CMV disease. Although intravenous ganciclovir is considered the drug of choice, foscarnet has similar efficacy and less, especially haematologic, toxicity. Cidofovir has not been tested extensively, so far results are disappointing. Oral valganciclovir for pre-emptive treatment is currently studied among SCT recipients.

In addition to antiviral therapy, adoptive immunotherapy with CMV-specific cytotoxic T cells as prophylactic or pre-emptive therapy is a very elegant strategy, however, generation of these cells is expensive and time-consuming and therefore not available at every transplantation centre. Magnetic selection of CMV-specific CD8+ T cells from peripheral blood using HLA class I-peptide tetramers may be very promising, making this strategy more accessible.

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%[1]. This very serious complication occurred mainly in CMV-seropositive patients, with acute graft-versus-host disease (aGVHD) being the most important risk factor[1]. Treatment of CMV pneumonia with GCV and immunoglobulin decreased mortality to 30-50%[2-3]. 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/grafts[4-7]. Currently, two antiviral strategies, prophylactic or pre-emptive treatment, are used for prevention of CMV disease. Prophylactic treatment usually consists of antiviral therapy started at engraftment until al least day 100 post-transplant. Pre-emptive therapy is defined as antiviral treatment based on the detection of reactivated CMV infection by positive CMV cultures, a positive antigenemia (Ag) assay or positive molecular assays.

In the present report, we review these antiviral strategies. Furthermore, several other aspects of prevention of CMV disease are reviewed, which are: 1) methods available for early detection of CMV reactivations, 2) monitoring of CMV-specific T cell responses, 3) the value of several antiviral drugs and 4) adoptive immunotherapy as prophylaxis or (pre-emptive) treatment of CMV reactivations/CMV disease.

Antiviral strategies: prophylaxis or pre-emptive treatment

In randomised trials[8,9] (Table 1) among CMV-seropositive recipients, long-term (3-4 months) GCV prophylaxis initiated at engraftment showed to be effective in suppressing early CMV disease (< 100 days post-transplant). However, mortality was not influenced due to an increased incidence of bacterial and fungal infections and late CMV disease[8-12]. When studying recovery of CMV-specific cytotoxic T lymphocytes (CMVs-CTLs), it appeared that long-term ganciclovir treatment impaired CMVs-CTL reconstitution causing the increase in late CMV infections[13].

Table 1 Ganciclovir prophylaxis of CMV disease

Study	Randomised trial	Donor	Dose of GCV	Early CMV disease Placebo vs GCV	Overall mortality Placebo vs GCV
Goodrich[8]	Yes	MRD/ MUD	10 mg/kg/d (5d) fb 5 mg/kg/d[a]	29% vs 0% ($p < 0.001$)	26% vs 30% (at d 180, ns)
Winston[9]	Yes	(P)MRD/ (P)MUD	6 mg/kg/d[b]	24% vs 10% ($p = 0.06$)	36% vs 30% (at d 120, ns)

GCV = ganciclovir; (P)MRD = (partially) matched related donor; (P)MUD = (partially) matched unrelated donor; d = day; fb = followed by; ns = not significant; [a] from engraftment until day 100; [b] from engraftment until day 120.

Many studies have been performed using pre-emptive therapy in SCT recipients to prevent CMV disease. The paper by Schmidt et al[14] 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 randomised between pre-emptive GCV treatment or observation. In the GCV group 5 of 20 patients died or had CMV pneumonia before day 120 compared to 14 of 20 in the observation group ($p = 0.01$), while in the group of patients with negative CMV cultures the rate of CMV infection was 12 of 55. Results of reports with a minimum of 30 patients and published after 1995 are summarized in Tables 2-5, according to the CMV monitoring assay used; three of them were randomised trials[10,15,18]. Pre-emptive treatment based on qualitative CMV DNA detection by PCR lowered the incidence of CMV disease and CMV-associated mortality compared with pre-emptive therapy instituted when positive CMV cultures were obtained (Table 5 and 2, respectively; $p = 0.02$)[15]. Boeckh et al[10,11] compared two types of Ag based pre-emptive therapy (Table 4) with prophylactic treatment. Ag based treatment was given for 28 days[10] or until day 100 post-transplant[11]. In both the prophylactically treated group and the group receiving long-term pre-emptive treatment late CMV disease was diagnosed more frequently, while more invasive fungal infections were seen in the prophylactically treated group only. The incidence of CMV disease at day 400 post-transplant and overall survival were similar in the three treatment arms. Humar et al (Table 4 and 3) showed that Ag based pre-emptive treatment reduced the incidence of CMV disease at day 400 post-transplant to 1.7% compared to

Table 2 Pre-emptive therapy of CMV reactivation based on positive CMV cultures

Study	Rando-mised trial	Donor	No.	Dose of GCV	CMV disease % (at day)	CMV-a-mortality % (at day)	Overall Survival % (at day)
Einsele[15]	Yes, see Table 5	(P)MRD/ (P)MUD	34	5 mg/kg bid (14d) fb 5 mg/kg/d until negative PCR	32 (180)	15 (180)	59 (180)
Ljung-man[16]	No	(P)MRD/ MUD	58	5 mg/kg bid (14d)	19 (100)	2 (100)	nd[a]

GCV = ganciclovir; CMV-a = CMV associated; (P)MRD = (partially) matched related donor; (P)MUD = (partially) matched unrelated donor; bid= twice a day; d = day; fb = followed by; nd = not described; [a] overall survival was not specified, however, reported as similar as observed in patients receiving PCR-based pre-emptive treatment, see Table 5.

Table 3 Pre-emptive therapy of CMV reactivation based on CMV positive BAL

Study	Rando-mised trial	Donor	No.	Dose of GCV	CMV disease % (at day)	CMV-a-mortality % (at day)	Overall Survival % (at day)
Reddy[17]	No	MRD/ MUD	55	5 mg/kg bid (14-28 d) fb maintenance (16 w)	11 (nd)	5 (nd)	57 (3yr)
Humar[18]	Yes, see Table 4	(P)MRD/ MUD	58	5 mg/kg bid (14d) fb maintenance (8 w)	12.1 (400)	nd	72.4% (365)

TCD = T cell depletion; GCV = ganciclovir; CMV-a = CMV associated; (P)MRD = (partially) matched related donor; (P)MUD = (partially) matched unrelated donor; bid= twice a day; d = day; fb = followed by; w= weeks; nd = not described.

Table 4 Pre-emptive therapy of CMV reactivation based on antigenemia

Study	Rando-mised trial	Donor	No.	No. of positive cells	Dose of GCV	CMV disease % (at day)	CMV-a-mortality %	Overall Survival % (at day)
Boeckh[10]	Yes	(P)MRD/ MUD	114	≥ 3 cells/ 2 slides	5 mg/kg bid (7d) fb maintenance (21d)	22 (400)	nd	73 (180) 61 (400)
Verdonck[19]	No	MRD	41	any antigenemia	2.5 mg/kg bid (14d)[a] fb 5 mg/kg bid until negative test	0 (180)	0	76 (180)
Manteiga[20]	No	MRD	52	any antigenemia	5 mg/kg bid (7d) fb maintenance (21d)	13 (100)	2	nd
Boeckh[11]	No	(P)MRD/ MUD	102	any antigenemia	5 mg/kg bid (7d) fb maintenance (100d)	17 (400)	nd	77 (180) 66 (400)
Machado[21,22]	No	MRD	76	≥ 2 cells/ 300.000	5 mg/kg bid (14-21d) or until negative test	4 (200) 14.4 (365)	0 0	nd

Table 4 continued

Study	Randomised trial	Donor	No.	No. of positive cells	Dose of GCV	CMV disease % (at day)	CMV-a-mortality %	Overall Survival % (at day)
Mori[23]	No	MRD/ MUD	50	≥ 10 cells/ 2 slides or [b] any antigenemia	5 mg/kg bid (14d) fb 5 mg/kg/d until negative test	16 (100)	0	nd
Kanda[24]	No	(P)MRD/ (P)MUD	77	≥ 5-10/ 50.000	5 mg/kg bid (7d) fb 5 mg/kg/d until negative test	3 (365)	0	86 (100) 73 (365)
Small[25]	No	MRD	61	≥ 2 cells/ slide	nd	7 (nd)	7	nd
Humar[18]	Yes, see Table 3	(P)MRD/ MUD	60	any antigenemia	5 mg/kg bid (14d) or until negative test	1.7 (400)	0	80 (365)

GCV = ganciclovir; CMV-a = CMV associated; (P)MRD = (partially) matched related donor; (P)MUD = (partially) matched unrelated donor; bid= twice a day; d = day; fb = followed by; nd = not described; [a] patients treated with high-dose corticosteroids for aGVHD grade II-IV received prophylactic GCV similar to the pre-emptive dose; [b] during aGVHD grade II-IV.

Table 5 Pre-emptive therapy of CMV reactivation based on CMV DNA PCR

Study	Rando-mised trial	Donor	No.	Therapy started at	Dose of GCV	CMV disease % (at day)	CMV-a-mortality %	Overall Survival % (at day)
Einsele[15]	Yes, see Table 2	(P)MRD/ (P)MUD	37	2nd positive PCR (Qual)	5 mg/kg bid (14d) fb 5 mg/kg/d until negative PCR	8 (180)	0 (180)	84 (180)
Ljungman[16]	No	(P)MRD/ MUD	58	2nd positive PCR (Semi-Q)	5 mg/kg bid (14d) or until negative PCR	6 (100) 10 (180)	0 (180)	nd[a]
Einsele[12]	No	(P)MRD/ (P)MUD	86	2nd positive PCR (Qual)	5 mg/kg bid (14d) fb foscarnet 60 mg/kg bid until negative PCR	12 (>100)	1 (>100)	nd
Peggs[26]	No	MRD/ MUD	52	1st or 2nd positive PCR (Qual)	5 mg/kg bid (14d) fb foscarnet 60 mg/kg bid until negative PCR	6 (100)	2 (100)	nd

Table 5 continued

Study	Rando-mised trial	Donor	No.	Therapy started at	Dose of GCV	CMV disease % (at day)	CMV-a-mortality %	Overall Survival % (at day)
Hebart[27]	No	(P)MRD/ (P)MUD	52	2nd positive PCR (Qual)	5 mg/kg bid (14d) fb 5 mg/kg/d until negative PCR	12 (?)	6 (?)	nd
Mori[28]	No	MRD/ MUD	39	1st positive PCR (real-time)	5 mg/kg/d (14d) until negative test 10 mg/kg/d when VL increased	8 (120)	0	nd

GCV = ganciclovir; CMV-a = CMV associated; (P)MRD = (partially) matched related donor; (P)MUD = (partially) matched unrelated donor; Qual = qualitative; Semi-Q = semi-quantitative; bid = twice a day; d = day; fb = followed by; nd = not described; VL= viral load; [a] overall survival was similar as observed in a historical control group receiving pre-emptive treatment based on positive conventional cultures.

12.1% when therapy was instituted at the detection of positive CMV cultures of BAL fluid obtained at day 35 post-transplant ($p = 0.022$)[18]. Again overall survival was similar in the two treatment arms.

Prevention of CMV disease with low CMV-associated mortality seemed to be superior in studies using a short-term (14 days) Ag or PCR based pre-emptive GCV treatment[12,15,16,18,19,21,22,24,26,27]. In these studies pre-emptive treatment was only extended when CMV monitoring tests were still positive after the short-term treatment period. When 'overall survival' was considered the endpoint, the varying pre-emptive treatment strategies all were equally effective.

The introduction of pre-emptive therapy among CMV-seropositive patients receiving grafts from matched related donors has resulted in similar transplant-related mortality and survival rates compared to CMV-seronegative recipients[26,29-31]. However, in CMV-seropositive recipients of grafts from matched unrelated donors transplant-related mortality and overall survival rates were still inferior compared to CMV-seronegative recipients, despite pre-emptive antiviral treatment[31-34].

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

Antigenemia vs molecular monitoring

The Ag assay is widely used to monitor SCT recipients for CMV reactivations. This assay has some drawbacks compared to molecular tests: 1) during neutropenia no monitoring can be performed, which is similar for molecular tests performed in leucocytes, 2) the test is laborious and 3) liable to intra/interobserver variability. Furthermore, a false negative Ag test (using C10/C11 antibodies) was reported in a SCT recipient with CMV disease. Re-examination of the Ag negative samples with a different pp65 antibody pool (CINA antibodies) revealed a high level of Ag[35]. Compared to Ag assays the workload per sample has been

reduced from approximately 4 to less than 2 hours when automated DNA isolation and PCR tests are used. The difference in workload is even more obvious when large amounts of samples are processed, since the Ag assay is not automated and every sample has to be processed separately.

With the molecular assays a qualitative or quantitative detection of CMV DNA or RNA is performed in cell-free plasma, peripheral blood leucocytes (PBL) or whole blood (WB). Technical details of several methods for CMV monitoring have been reviewed by Boeckh et al[36].

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 in cell-free plasma, PBL or WB. Five studies found CMV DNA PCR monitoring (qualitative or quantitative) to be more sensitive compared to Ag, irrespective of performance in plasma, PBL or WB[37-41]. In only one report leucocyte-based assays (Ag and PBL-PCR) were more sensitive compared to a plasma PCR, showing a higher number of patients with a CMV reactivation, earlier positivity and a more rapid decrease of viral load after the start of pre-emptive antiviral therapy[42]. In one other study the gold standard to calculate sensitivity and specificity of the Ag assay and PCR test was defined as "CMV reactivation based on positive results of the Ag assay or PCR test"[37]. This method of calculation is incorrect and results in the exclusion of false positive results, giving a specificity and positive predictive value of 100%. The value of a higher sensitivity of molecular assays in a clinical setting is not clear. Solano et al[40] reported 9 of 43 SCT recipients with positive plasma PCR, while Ag was negative. None of these 9 patients progressed to CMV disease, although they did not receive pre-emptive treatment. Furthermore, none of the patients with an initial positive Ag and PCR result who remained PCR positive after conversion of the Ag assay to a negative result, did develop CMV disease. The authors concluded that the Ag assay appeared to be most suitable for guiding initiation of pre-emptive therapy and monitoring response to antiviral therapy.

Real-time automated CMV DNA PCR test using a TaqMan™ probe With the real-time automated CMV DNA PCR assay using a TaqMan™ probe, a 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 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 compared to a positive Ag test. CMV DNA detection by real-time PCR often preceded Ag and yielded more positive samples[43-45].

Qualitative and quantitative in house CMV DNA PCR assay In (partly) retrospective studies, CMV DNA monitoring by in house PCR assays in plasma, WB or PBL yielded similar results as described above, with higher sensitivity for molecular tests compared to the Ag assay[38,46,47]. Hebart et al[48] prospectively monitored CMV reactivation by Ag and in house semiquantitative PCR in plasma and WB. WB-PCR showed the lowest sensitivity, however, overall a good correlation was seen between Ag, WB-PCR and plasma PCR. All three assays were negative after 14 days of GCV treatment in 12 of 13 patients. In contrast, 2 studies[49,50] reported the in house plasma PCR to be less sensitive compared to PBL-PCR or Ag.

Murex™ CMV DNA Hybrid Capture assay This assay is a commercially available solution hybridisation antibody capture assay (HCA) for the quantitative detection of CMV DNA in leukocytes. It was less sensitive in diagnosing CMV infection compared to an in house qualitative PCR[38,51]. When CMV disease was used as the gold standard for comparison, however, the positive predictive value of the HCA and PCR assay was 33% and 22%, respectively[51].

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 compared to DNA based assays and the Ag assay[38,52,53]. Detection of immediate-early mRNA[54], the $beta_{2.7}$ transcript[55] or spliced late CMV genes[56] all were shown to be more useful, however, these results have not been validated by other groups.

Summary In Table 6 results of studies comparing commercially available surveillance methods with the Ag assay or in house PCR tests are summarized. Not all studies described above are included, due to varying study designs and endpoints or lack of clinical data[37,41,42,55,56]. In several papers sensitivity, specificity, positive predictive value and negative predictive value of the experimental assay(s) were calculated. In these studies the gold standard to calculate these values was defined as CMV reactivations based on positive results from Ag and/or PCR assays[37,55,56]. In our view, a surveillance method for CMV reactivation should be judged on its clinical merits, the incidence of CMV disease and transplant related mortality being the most significant endpoints.

Table 6 Surveillance methods

Study	CMV reactivations (n)	No. of days of earlier positivity of experimental assay compared to standard assay	CMV disease (no. of patients/total)
Schulenberg[39] Quantitative PCR Cobas (plasma and blood) vs Ag	10 PCR+/Ag+ 7 PCR+/Ag- 0 PCR-/Ag-	11	2/74
Solano[40] Qualitative PCR Cobas (plasma) vs Ag	14 PCR+/Ag+ 18 PCR+/Ag- 5 PCR-/Ag+	7	2/43 (PCR+/Ag-: no disease)
Griscelli[44] Realtime Taqman (PBL) vs Ag	20 PCR+/Ag+ 2 PCR+/Ag- 1 PCR-/Ag-	15	2/16
Yakushiji[45] Realtime Taqman (plasma) vs Ag	39 PCR+/Ag+ 4 PCR+/Ag- 0 PCR-/Ag+ 8 PCR-/Ag-	PCR before Ag: 20 (n) Ag before PCR: 5 (n) Simultaneously:14 (n)	2/51
Hebart[51] Hybrid Capture assay (PBL) vs ih PCR	12 HCA+/PCR+ 6 HCA-/PCR+	HCA after or simultaneously with PCR: 18 (n)	4/18 (HCA-/PCR+: no disease)
Gerna[52] Qualitative pp67 NASBA vs Ag	9 NASBA+/Ag+ 6 NASBA-/Ag+ 9 NASBA-/Ag-	-3	2?/24
Hebart[53] Qualitative pp67 NASBA vs ih PCR	16 NASBA+/PCR+ 1 NASBA+/PCR- 3 NASBA-/PCR+	0	3/33 (NASBA+/PCR-: no disease)
Gerna[54] Qualitative IE NASBA vs Ag	32 NASBA+/Ag+ 6 NASBA+/Ag- 0 NASBA-/Ag+ 13 NASBA-/Ag-	2	0/51 (NASBA+/Ag-: no disease)

PBL = peripheral blood leukocytes; ih = in house.

Overall, one can conclude that PCR based CMV DNA monitoring is more sensitive compared to Ag based monitoring. However, the clinical benefit is unclear. Even more, there is lack of clarity whether PCR tests can better be performed in plasma, WB or PBL samples, although molecular monitoring in plasma has the advantage of performance irrespective of neutropenia. Currently, there is no evidence that qualitative CMV detection assays have a better or worse predictive value for the occurrence of CMV disease after SCT as compared to quantitative assays. To answer this question, randomised controlled trials should be performed, monitoring patients prospectively with either detection assay without applying pre-emptive treatment of CMV reactivation. Such trials will never be done. Theoretically, a quantitative method enables monitoring of response to therapy. In case of an increasing viral load after the start of pre-emptive therapy, a dose or drug modification may be applied. This was implemented by Mori et al[28] but did not significantly change the incidence of CMV disease.

Monitoring of CMV-specific 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[57-59]. Generation of CD8+ CMVs-CTLs was associated with recovery of CD4+ CMVs T helper cells[58]. Li et al[13] analysed 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 randomised to GCV prophylaxis or placebo after recovery of peripheral neutrophil counts. Between day 40 and 90 post-transplant recovery of CD8+ and CD4+ CMVs T cell responses occurred in the majority of individuals receiving placebo, but in a minority of patients receiving GCV. Thus, long-term prophylactic GCV treatment can delay post-transplant reconstitution of CMVs-CTL responses. Today, several reports have been published using screening assays for CMVs T cell reconstitution to identify patients at risk of developing CMV disease[60-65]. Krause et al[60] performed lymphoproliferation assays, to assess CD4+ CMVs T helper (Th) response, at regular monthly intervals. None of the patients with a 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[65] quantified CD8+ CMVs-CTLs and CD4+ CMVs Th cells by intracellular interferon-γ staining with flow cytometry after CMV-specific stimulation. Reconstitution of both cell types was associated with rapid clearance of CMV infection. Next to cytotoxicity and interferon-γ staining assays, the use of HLA-peptide tetramers to quantify CMVs CD8+ T cell reconstitution might enable prediction of the development of CMV disease[61-63]. Reconstitution of absolute CMVs-CTL counts to values above 10-20 x 10^6/L was associated with protection from CMV disease[62,63]. In contrast, Ozdemir et al[64] recently reported that frequencies and absolute numbers of CMVs CD8+ T cells were greater in subjects who experienced CMV Ag following SCT. They conclude that recovery of CMVs-CTLs, as measured by HLA-peptide tetramer staining, is insufficient to control CMV Ag. However, it is not described whether these patients with Ag did develop CMV disease. 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[61]. Patients with Ag who did not develop CMV disease, demonstrated higher levels of CMVs CD8+ T cells compared to CMV-seropositive recipients without Ag. It should be noted that most 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 have to be performed to evaluate CMVs CD8+ T cell reconstitution after allogeneic SCT. When the abovementioned results will be validated, pre-emptive antiviral therapy might be withheld in patients with CMV reactivation who show to have adequate CMVs-CTL levels. At this moment, 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[65,66,67] which will make tetramer based or interferon-γ staining based quantification of CMVs CD8+ T cell recovery accessible for more SCT recipients.

Antiviral therapy

Intravenous GCV is generally considered the drug of choice for pre-emptive therapy of CMV reactivations or treatment of CMV disease[68]. Several other antiviral drugs have in vitro or in vivo activity against CMV (acyclovir -ACV-, valacyclovir -VACV-, foscarnet, cidofovir -CDV-, valganciclovir -VGCV-). (V)ACV and (V)GCV are nucleoside analogues. VACV and VGCV are

oral prodrugs of ACV and GCV, respectively, and converted to ACV and GCV, respectively, after cleavage of the valine moiety by the liver and intestine. The nucleosides first have to be converted to monophosphate by a viral protein kinase (which is the gene product of UL97 in 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[69]. Furthermore GCV triphosphate is a competitive inhibitor of the CMV DNA polymerase. VGCV has a ten-fold greater bio-availability than oral GCV. Pharmacokinetic studies of VGCV have been performed in HIV infected individuals and recipients of liver transplants[70]. Among SCT recipients a randomised cross-over trial using intravenous GCV or oral VGCV as pre-emptive treatment will be conducted at European Group for Blood and Marrow Transplantion (EBMT) centres[70]. The nucleotide analogue CDV already is a monophosphate, 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 is inhibiting viral DNA polymerase activity.

Acyclovir/Valacyclovir (V)ACV has only been studied as prophylactic therapy for prevention of CMV reactivations/disease and not as (pre-emptive) treatment. ACV has only limited activity against CMV when tested in vivo. Two studies using ACV prophylaxis were performed in SCT recipients, however, before the strategy of pre-emptive GCV therapy based on Ag or PCR was introduced[71,72]. Intravenous ACV followed by high-dose oral ACV maintenance therapy was not effective as prevention of CMV disease, but resulted in a decreased CMV-associated mortality and increased survival. Vusirikala et al[73] compared data of 31 SCT recipients who were prophylactically treated with VACV 1 gram 3 times a day with a group receiving only low-dose oral ACV. Primary and secondary CMV reactivations were observed in 3/12 and 5/19 VACV treated patients, respectively, compared to 24/31 and 16/24 in the control group, respectively. Since this was a retrospective report with small patient numbers combining primary and secondary reactivations, it just suggested a potential benefit of VACV as prevention for CMV reactivation. In a large randomised multi-centre study oral VACV showed to be more effective in preventing CMV viremia in SCT recipients compared to oral ACV, although overall survival and the incidence of CMV disease did not differ between the two groups (75% vs 76% and 5.5% vs 3.5% for the ACV and VACV groups, respectively, ns). All patients included were initially treated with intravenous acy-

clovir until day 28 after transplantation or until discharge[74]. In these two studies a PCR or Ag based pre-emptive treatment with GCV or foscarnet was used as well[73,74].

Foscarnet Intravenous foscarnet is considered second line therapy for CMV reactivations or disease, however, for patients developing dose-limiting neutropenia or CMV strains resistant to GCV it is the drug of choice[68]. In a survey of herpesvirus resistance to antiviral drugs, GCV was replaced by foscarnet in 15 patients with suspected or proven GCV resistance and this resulted in a better clinical or virological outcome in 13 of these 15 patients[75].

Four non-randomised studies were published using foscarnet prophylactically[76-79]. In all four studies patient numbers were very small (≤ 21), therefore, no firm conclusions can be drawn regarding effectiveness of foscarnet prophylaxis.

Preemptive treatment consisting of foscarnet has also been reported in four studies, showing similar efficacy of foscarnet compared to GCV[80-83]. Only two were randomised trials[82,83], one with a low patient number[82]. Reusser et al[83] treated 110 patients with CMV reactivation (Ag or PCR diagnosed) with foscarnet 60 mg/kg bid and 103 patients with GCV 5 mg/kg bid. When test results were still positive after 14 days of treatment both drugs were continued in a reduced dose (90 mg/kg/day:foscarnet; 6mg/kg/day:GCV) for again 2 weeks (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 regarding 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 haematotoxicity than GCV. In the GCV group neutropenia was more often observed, despite the use of growth factors.

Cidofovir Ljungman et al[84] performed a retrospective survey among 17 BMT centres and enrolled 82 patients who were treated with CDV for CMV disease (n = 20) or for CMV reactivation (primary pre-emptive treatment n = 24; secondary pre-emptive treatment in patients who had failed or relapsed after previous pre-emptive treatment with another antiviral drug n = 38). The dosage was 1-5 mg/kg per week followed by maintenance treatment and all patients received probenicid and prehydration. Overall 62% showed a response to CDV therapy; the response being defined as disease regression without addition of other specific therapy or, for pre-emptive therapy, conversion of a positive Ag or PCR test. Twenty-one patients developed renal toxicity, which persisted after cessation of therapy in 9 patients. No

toxicity was seen in 45 patients, while 15 developed other side effects potentially associated with CDV therapy (nausea, vomiting, thrombocytopenia, rash, ophthalmologic and neurologic toxicity). Kiehl et al[85] published prospective data on 21 patients receiving first-line pre-emptive treatment with CDV 5 mg/kg once a week for 2 weeks, thereafter fortnightly. Treatment was Ag and/or DNA PCR based and continued until test results were negative for at least three weeks. Only one patient showed a complete response. In 15 patients PCR became negative, however, 2-3 weeks later a positive PCR was again observed in all 15 patients. In 5 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[86] treated 4 patients pre-emptively with CDV 5 mg/kg/week for 4 weeks. Two responded but developed severe nausea/vomitus and uveitis. Two were non responders of which one died from CMV pneumonia and one developed CMV pneumonia eventually responding to foscarnet. The less favourable outcome of the last two reports are in concordance with recent prospective results from Platzbecker et al[87]. Here, only one of seven SCT recipients showed a transient clearance of pp65 Ag after treatment with CDV 5 mg/kg/week for two weeks, thereafter fortnightly. In contrast, these authors[87] showed that pre-emptive treatment with CDV was very successful in 10 SCT recipients treated with a non-myeloablative conditioning regimen. Toxicity was moderate and consisted of reversible renal impairment (n = 4), proteinuria (n = 1) and nausea/vomiting (n = 3).

Antiviral drug resistance When prolonged (> 100 days) antiviral therapy is given, drug resistance may develop[88]. Data about antiviral drug resistance have largely been obtained in AIDS patients and very little information is available about drug resistance in the SCT setting. Resistance of CMV to GCV is associated with lack of therapeutic response and progression of CMV disease[89,90]. 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 (BMT) centres. CMV resistance to GCV was proven in 2 patients and suspected in 23 patients[75]. In patients with CMV pneumonia, the virus often persists for a long time despite GCV treatment. GCV resistance was determined in CMV isolates obtained from BAL fluid or from autopsy lung tissue by DNA hybridisation. In only 1 of 12 patients a GCV resistant isolate was detected[91]. In a study among 50 allogeneic SCT recipients, 10 patient exhibited sustained or recurrent antigenemia despite GCV treatment. Samples from these 10 patients were screened for the pres-

ence of the most frequent CMV UL97 mutations by restriction enzyme analysis and none of these mutations were detected[92]. Altogether, antiviral drug resistance has only sporadically been reported in adult SCT recipients[75,91-93]. There is some evidence that it might be more frequent in paediatric SCT recipients, especially in patients with primary immunodeficiencies[90,94,95].

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[89]. The varying laboratory methods used for drug susceptibility testing of CMV isolates have been reviewed by Erice[89] 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 genome 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[96] 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 analysed 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 3 viral variants (one wild type and two UL97 mutants) present in vivo.

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

Adoptive immunotherapy with CMVs T cells

Adoptive transfer of CD8+ CMVs-CTLs for prevention of CMV reactivation/disease has been shown effective[59,97]. When no CMVs Th response developed, CMVs-CTLs declined progressively. However, none of the 17 patients treated with CD8+ CMVs-CTLs developed CMV infection[59,97]. Einsele et al[98] treated 8 patients with antiviral resistant CMV reactivation, 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 6 of 7 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 this study 3 patients subsequently developed invasive aspergillosis or respiratory syncytial virus interstitial pneumonitis. In a commentary it is stated that more efficient culture systems are needed to make this therapy more accessible. Furthermore, since eventually 3 patients died from fungal or viral infections a more comprehensive approach to reconstitute immunity in SCT recipients is required[99]. Peggs et al[100] 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 pre-emptively to 13 allogeneic transplant recipients, of which 10 received a non-myeloablative conditioning regimen. Within 23 days following infusion CD8+ CMVs-CTLs reached absolute counts as high as 540 x 10^6/L and were detectable for up to 6 months post-transfusion. 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 post-transplant, when Campath-1H (the IgG1 humanized monoclonal antibody against CD52) probably was still circulating, which might have induced lysis of these T cells.

Adoptive immunotherapy with CMV-specific cytotoxic T cells as pre-emptive therapy is a very elegant strategy, however, generation of these cells is expensive and time-consuming and therefore not available at every transplantation centre. Magnetic selection of CMVs CD8+ T cells from peripheral blood of CMV-seropositive donors using HLA class I-peptide

tetramers as decribed by Keenan et al[101] may be very promising, making adoptive immunotherapy more accessible.

Conclusion

This review focused on prevention of CMV disease in recipients of allogeneic SCT. The introduction of pre-emptive antiviral therapy has greatly reduced the incidence and mortality rate of CMV disease, especially when Ag or PCR based CMV monitoring is performed. A lot of questions still remain to be answered. We currently 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 in plasma, whole blood or peripheral blood leukocyte samples. At which viral load or Ag level should antiviral therapy be instituted and for how long should it be continued? The current conclusion is that prevention of CMV disease with low CMV-associated mortality seems to be superior in studies using a short-term (14 days) Ag or PCR based pre-emptive treatment. In these studies pre-emptive treatment was only extended when CMV monitoring tests were still positive after the short-term treatment period. When overall survival was considered the endpoint, the varying pre-emptive treatment strategies all were equally effective.

In the near future, monitoring CMVs T cell recovery may change our current pre-emptive 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.

Lastly, efforts should focus on immune reconstitution. Once adoptive immunotherapy will become more accessible, controlled trials should be designed to study the effectivity of immunotherapy regarding prevention of CMV disease.

References

1 Meyers JD, Flournoy N, Thomas ED. Risk factors for cytomegalovirus infection after human marrow transplantation. J Infect Dis 1986; 153: 478-488.

2 Emanuel D, Cunningham I, Jules-Elysee K et al. Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. Ann Intern Med 1988; 109: 777-782.

3 Reed EC, Bowden RA, Dandliker PS et al. Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. Ann Intern Med 1988; 109: 783-788.

4 Bowden RA, Slichter SJ, Sayers MH et al. Use of leukocyte-depleted platelets and cytomegalovirus-seronegative red blood cells for prevention of primary cytomegalovirus infection after marrow transplant. Blood 1991; 78: 246-250.

5 Verdonck LF, de Graan-Hentzen YCE, Dekker AW et al. Cytomegalovirus seronegative platelets and leukocyte-poor red blood cells from random donors can prevent primary cytomegalovirus infection after bone marrow transplantation. Bone Marrow Transplant 1987; 2: 73-78.

6 van Prooyen HC, Visser JJ, van Oostendorp WR et al. 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 1994; 87: 144-147.

7 de Gast GC, Boland GJ, Vlieger AM et al. Abortive human cytomegalovirus infection in patients after allogeneic bone marrow transplantation. Bone Marrow Transplant 1992; 9: 221-225.

8 Goodrich JM, Bowden RA, Fisher L et al. Ganciclovir prophylaxis to prevent cytomegalovirus disease after allogeneic marrow transplant. Ann Intern Med 1993; 118: 173-178.

9 Winston DJ, Ho WG, Bartoni K et al. Ganciclovir prophylaxis of cytomegalovirus infection in allogeneic bone marrow transplant recipients. Ann Intern Med 1993; 118: 179-184.

10 Boeckh M, Gooley TA, Myerson D et al. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood 1996; 88: 4063-4071.

11 Boeckh M, Bowden RA, Gooley TA, Myerson D, Corey L. Successful modification of a pp65 antigenemia-based early treatment strategy for prevention of cytomegalovirus disease in allogeneic marrow transplant recipients. Blood 1999; 93: 1781-1782.

12 Einsele H, Hebart H, Kauffman-Schneider C et al. Risk factors for treatment failures in patients receiving PCR-based preemptive therapy for CMV infection. Bone Marrow Transplant 2000; 25: 757-763.

13 Li CR, Greenberg PD, Gilbert MJ et al. Recovery of HLA-restricted cytomegalovirus (CVM)-specific T-cell responses after allogeneic bone marrow transplant: correlation with CMV disease and effect of ganciclovir prophylaxis. Blood 1994; 83: 1971-1979.

14 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; The City of Hope-Stanford-Syntex CMV Study Group. N Engl J Med 1991; 324: 1005-1011.

15 Einsele H, Ehninger G, Hebart H et al. Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. Blood 1995; 86: 2815-2820.

16 Ljungman P, Lore K, Aschan J, Klaesson S, Lewensohn-Fuchs I, Lonnqvist B, Ringden O, Winiarski J, Ehrnst A. Use of a semiquantitative PCR for cytomegalovirus DNA as a basis for pre-emptive antiviral therapy in allogeneic bone marrow transplant recipients. Bone Marrow Transplant 1996; 17: 583-587.

17 Reddy V, Hao Y, Lipton J, Meharchand J, Minden M, Mazzulli T, Chan C, Messner HA. 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 1999;13 : 149-159.

18 Humar A, Lipton J, Welsh S, Moussa G, Messner H, Mazzulli T. 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 2001; 28: 485-490.

19 Verdonck LF, Dekker AW, Rozenberg-Arska M, van den Hoek MR. 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 1997; 24: 901-907.

20 Manteiga R, Martino R, Sureda A, Labeaga R, Brunet S, Sierra J, Rabella N. Cytomegalovirus pp65 antigenemia-guided pre-emptive treatment with ganciclovir after allogeneic stem cell transplantation: a single-center experience. Bone Marrow Transplant 1998; 22: 899-904.

21 Machado CM, Dulley FL, Vilas Boas LS, Castelli JB, Macedo MCA, Silva RL, Pallota R, Saboya RS, Pannuti CS. CMV pneumonia in allogeneic recipients undergoing early treatment or pre-emptive ganciclovir therapy. Bone Marrow Transplant 2000; 26: 413-417.

22 Machado CM, Menezes RX, Macedo MCA, Mendes AVA, Vilas Boas LS, Castelli JB, Dulley FL, Pannuti CS. Extended antigenemia surveillance and late cytomegalovirus infection after allogeneic BMT. Bone Marrow Transplant 2001; 28: 1053-1059.

23 Mori T, Okamoto S, Matsuoka S et al. Risk-adapted pre-emptive therapy for cytomegalovirus disease in patients undergoing allogeneic bone marrow transplantation. Bone Marrow Transplant 2000; 25: 765-769.

24 Kanda Y, Mineishi S, Saito T et al. 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 2001; 27: 437-444.

25. Small TN, Papadopoulos EP, Boulad F, Kiehn T, Stiles J, Sepkowitz K, O'Reilly RJ. Outcome of pre-emptive or prophylactic anti-viral therapy to prevent CMV disease following T cell depleted related or unrelated BMT. Blood 2001; 98: S394a. Abstract 1659.

26 Peggs KS, Preiser W, Kottaridis PD et al. Extended routine polymerase chain reaction surveillance and pre-emptive antiviral therapy for cytomegalovirus after allogeneic transplantation. Br J Haematol 2000; 111: 782-790.

27 Hebart H, Brugger W, Grigoleit U et al. Risk for cytomegalovirus disease in patients receiving polymerase chain reaction-based pre-emptive antiviral therapy after allogeneic stem cell transplantation depends on transplantation modality. Blood 2001; 97: 2183-2185. Letter.

28 Mori T, Okamoto S, Watanabe R, Yajima T, Iwao Y, Yamazaki R, Nakazato T, Sato N, Iguchi T, Nagayama H, Takayama N, Hibi T, Ikeda Y. Dose-adjusted pre-emptive therapy for cytomegalovirus disease based on real-time polymerase chain reaction after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2002; 29: 777-782.

29 Humar A, Wood S, Lipton J et al. Effect of cytomegalovirus infection on 1-year mortality rates among recipients of allogeneic bone marrow transplants. Clin Infect Dis 1998; 26: 606-610.

30 Nichols WG, Corey L, Gooley T et al. 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 2002; 185: 273-282.

31 Meijer E, Dekker AW, Rozenberg-Arska M, Weersink AJL, Verdonck LF. 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 2002; 35: 703-712.

32 Craddock C, Szydlo RM, Dazzi F et al. 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 2001; 112: 228-236.

33 Kröger N, Zabelina T, Krüger W et al. 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 2001; 113: 1060-1071.

34 Castro-Malaspina H, Harris RE, Gajewski J et al. Unrelated marrow transplantation for myelodysplastic syndromes: outcome analysis in 510 transplants facilitated by the national marrow donor program. Blood 2002; 99: 1943-1951.

35 Seropian S, Ferguson D, Salloum E, Cooper D, Landry ML. Lack of reactivity to CMV pp65 antigenemia testing in a patient with CMV disease following allogeneic bone marrow transplant. Bone Marrow Transplant 1998; 22: 507-509.

36 Boekch M, Boivan G. Quantitation of cytomegalovirus: methodologic aspects and clinical applications. Clin Microbiol Rev 1998; 11: 533-554.

37 Hiyoshi M, Tagawa S, Takubo T et al. Evaluation of the Amplicor CMV test for direct detection of cytomegalovirus in plasma specimens. J Clin Microbiol 1997; 10: 2692-2694.

38 Preiser W, Brauninger S, Schwerdtfeger R, Ayliffe U, Garson JA, Brink NS, Franck S, Doerr HW, Rabenau HF. Evaluation of diagnostic methods for the detection of cytomegalovirus in recipients of allogeneic stem cell transplants. J Clin Virol 2001; 20: 59-70.

39 Schulenburg A, Watkins-Riedel T, Greinix HT, Rabitsch W, Loidolt H, Keil F, Mitterbauer M, Kalhs P. CMV monitoring after peripheral blood stem cell and bone marrow transplantation by pp65 antigen and quantitative PCR. Bone Marrow Transplant 2001; 28: 765-768.

40 Solano C, Munoz I, Gutierrez A, Farga A, Prosper F, Garcia-Conde J, Navarro D, Gimeno C. Qualitative plasma PCR assay (Amplicor CMV test) versus pp65 antigenemia assay for monitoring cytomegalovirus viremia and guiding pre-emptive ganciclovir therapy in allogeneic stem cell transplantation. J Clin Microbiol 2001; 39: 3938-3941.

41 Flexman J, Kay I, Fonte R, Herrmann R, Gabbay E, Palladino S. 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 2001; 64: 275-282.

42. Boivin G, Belanger R, Delage R, Beliveau C, Demers C, Goyette N, Roy J. 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 2002; 38: 4356-4360.

43 Machida U, Kami M, Fukui T et al. Real-time automated PCR for early diagnosis and monitoring of cytomegalovirus infection after bone marrow transplantation. J Clin Microbiol 2000; 38: 2536-2542.

44 Griscelli F, Barrois M, Chauvin S, Lastere S, Bellet D, Bourhis JH. Quantification of human cytomegalovirus DNA in bone marrow transplant recipients by real-time PCR. J Clin Microbiol 2001; 39: 4362-4369.

45 Yakushiji K, Gondo H, Kamezaki K et al. Monitoring of cytomegalovirus reactivation after allogeneic stem cell transplantation: comparison of an antigenemia assay and quantitative real-time polymerase chain reaction. Bone Marrow Transplant 2002; 29: 599-606.

46 Gerna G, Furione M, Baldanti F, Percivalle E, Comoli P, Locatelli F. Quantitation of human cytomegalovirus DNA in bone marrow transplant recipients. Br J Haematol 1995; 91: 674-683.

47 Kanda Y, Chiba S, Suzuki T, Kami M, Yazaki Y, Hirai H. Time course analysis of semi-quantitative PCR and antigenemia assay for prevention of cytomegalovirus disease after bone marrow transplantation. Br J Haematol 1998; 100: 222-225.

48. Hebart H, Muller C, Loffler J, Jahn G, Einsele H. 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 1996; 17: 861-868.

49 Boeckh M, Gallez-Hawkins GM, Myerson D, Zaia JA, Bowden RA. Plasma polymerase chain reaction for cytomegalovirus DNA after allogeneic marrow transplantation. Transplantation 1997; 64: 108-113.

50 Matsunaga T, Sakamaki S, Ishigaki S et al. Use of PCR serum in diagnosing and monitoring cytomegalovirus reactivation in bone marrow transplant recipients. Int J Hematol 1999; 69: 105-111.

51 Hebart H, Gamer D, Loeffler J et al. 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 1998; 36: 1333-337.

52 Gerna G, Baldanti F, Middeldorp JM, Furione M, Zavattoni M, Lilleri D, Revello MG. 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 1999; 37: 902-911.

53 Hebart H, Rudolph T, Loeffler J, Middeldorp J, Ljubicic T, Jahn G, Einsele H. Evaluation of the NucliSens CMV pp67 assay for detection and monitoring of human cytomegalovirus infection after allogeneic stem cell transplantation. Bone Marrow Transplant 2002; 30: 181-187.

54 Gerna G, Baldanti F, Lilleri D et al. Human cytomegalovirus immediate-early mRNA detection by nucleic acid sequence-based amplification as a new parameter for pre-emptive therapy in bone marrow transplant recipients. J Clin Microbiol 2000; 38: 1845-1853.

55 Aono T, Kondo K, Miyoshi H, Tanaka-Taya K, Kondo M, Osugi Y, Hara J, Okada S, Yamanishi K. Monitoring of human cytomegalovirus infections in pediatric bone marrow transplant recipients by nucleic acid sequence-based amplification. J Infect Dis 1998; 178: 1244-1249.

56 Boriskin YS, Fuller K, Powles RL, Vipond IB, Rice PS, Booth JC, Caul EO, Butcher PD. 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 2002; 24: 13-23.

57 Quinnan GV, Kirmani N, Rook AH et al. 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 1982; 307: 6-13.

58 Reusser P, Riddell S, Meyers JD, Greenberg PD. Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease. Blood 1991; 78: 1373-1380.

59 Riddell ST, 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 1992; 257: 238-241.

60 Krause H, Hebart H, Jahn G, Muller CA, Einsele H. Screening for CMV-specific T cell proliferation to identify patients at risk of developing late onset CMV disease. Bone Marrow Transplant 1997; 19: 1111-1116.

61 Gratama JW, van Esser JWJ, Lamers CHJ, Tournay C, Lowenberg B, Bolhuis RLH, Cornelissen JJ. 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 2001; 98: 1358-1364.

62 Cwynarski K, Ainsworth J, Cobbold M et al. Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation. Blood 2001; 97: 1232-1240.

63 Aubert G, Hassan-Walker AF, Madrigal JA et al. Cytomegalovirus-specific cellular immune responses and viremia in recipients of allogeneic stem cell transplants. J Infect Dis 2001; 184: 955-963.

64 Ozdemir E, St. John LS, Gillespie G et al. Cytomegalovirus reactivation following allogeneic stem cell transplantation is associated with the presence of dysfunctional antigen-specific CD8+ T cells. Blood 2002; 100: 3690-3697.

65 Hebart H, Daginik S, Stevanovic S et al. 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 2002; 99: 3830-3837.

66 Longmate J, York J, La Rosa C, Krishan R, Zhang M, Senitzer D, Diamond DJ. Population coverage by HLA class-I restricted cytotoxic T-lymphocyte epitopes. Immunogenetics 2001; 52: 165-173.

67 Lim JB, Provenzano M, Bettinotti M, Stroncek DF. Identification of a new candidate HLA-A3 restricted CMV epitope as a target antigen to induce specific CMV cytotoxic lymphocytes. Blood 2001; 98: S780a. Abstract 3244.

68 Nichols WG, Boeckh M. Recent advances in the therapy and prevention of CMV infections. J Clin Virol 2000; 16: 25-40.

69 Ljungman P. Prophylaxis against herpesvirus infections in transplant recipients. Drugs 2001; 61: 187-196.

70 Reusser P. Oral valganciclovir: a new option for treatment of cytomegalovirus infection and disease in immunocompromised hosts. Expert Opin Investig Drugs 2001; 10: 1745-1753.

71 Meyers JD, Reed EC, Shepp DH et al. Acyclovir for prevention of cytomegalovirus infection and disease after allogeneic marrow transplantation. N Engl J Med 1988; 318: 70-75.

72 Prentice HG, Gluckman E, Powles RL et al. Long-term survival in allogeneic bone marrow transplant recipients following acyclovir prophylaxis for CMV infection. Bone Marrow Transplant 1997; 19: 129-133.

73 Vusirikala M, Wolff SN, Stein RS, Brandt SJ, Morgan DS, Greer JP, Schuening FG, Dummer JS, Goodman SA. Valacyclovir for the prevention of cytomegalovirus infection after allogeneic stem cell transplantation: a single institution retrospective cohort analysis. Bone Marrow Transplant 2001; 28: 265-270.

74 Ljungman P, de la Camara R, Milpied N, Volin L, Russell CA, Crisp A, Webster A. Randomized study of valacyclovir as prophylaxis against cytomegalovirus reactivation in recipients of allogeneic bone marrow transplants. Blood 2002; 99: 3050-3056.

75 Reusser P, Cordonnier C, Einsele H, Engelhard D, Link H, Locasciulli A, Ljungman P. European survey of herpesvirus resistance to antiviral drugs in bone marrow transplant recipients. Bone Marrow Transplant 1996; 17: 813-817.

76 Reusser P, Gambertoglio JG, Lilleby K, Meyers JD. Phase I-II trial of foscarnet for prevention of cytomegalovirus infection in autologous and allogeneic marrow transplant recipients. J Infect Dis 1992; 166: 473-479.

77 Bacigalupo A, Tedone E, Van Lint MT et al. CMV prophylaxis with foscarnet in allogeneic bone marrow transplant recipients at high risk of developing CMV infections. Bone Marrow Transplant 1994; 13: 783-788.

78 Bregante S, Bertilson S, Tedone E et al. Foscarnet prophylaxis of cytomegalovirus infections in patients undergoing allogeneic bone marrow transplantation (BMT): a dose-finding study. Bone Marrow Transplant 2000; 26: 23-29.

79 Ordemann R, Naumann R, Geissler G, Kroschinsky F, Bornhauser M, Schwerdtfeger R, Ehninger G. Foscarnet-an alternative for cytomegalovirus prophylaxis after allogeneic stem cell transplantation. Ann Hemat 2000; 79: 432-436.

80 Bacigalupo A, Van Lint MT, Tedone E et al. Early treatment of CMV infections in allogeneic bone marrow transplant recipients with foscarnet or ganciclovir. Bone Marrow Transplant 1994; 13: 753-758.

81 Ljungman P, Oberg G, Aschan J, Ehrnst A, Lonnqvist B, Pauksen K, Sulila P. Foscarnet for pre-emptive therapy of CMV infection detected by a leukocyte-based nested PCR in allogeneic bone marrow transplant patients. Bone Marrow Transplant 1996; 18: 565-568.

82 Moretti S, Zikos P, Van Lint MT et al. Foscarnet vs ganciclovir for cytomegalovirus (CMV) antigenemia after allogeneic hemopoietic stem cell transplantation (HSCT): a randomised study. Bone Marrow Transplant 1998; 22: 175-180.

83 Reusser P, Einsele H, Lee J, Volin L, Rovira M, Engelhard D, Finke J, Cordonnier C, Link H, Ljungman P. Randomized multicenter trial of foscarnet versus ganciclovir for pre-emptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation. Blood 2002; 99: 1159-1164.

84 Ljungman P, Lambertenghi Deliliers G, Platzbecker U et al. Cidofovir for cytomegalovirus infection and disease in allogeneic stem cell transplant recipients. Blood 2001; 97: 388-392.

85 Kiehl MG, Basara N. Cidofovir for cytomegalovirus pre-emptive therapy in stem cell transplant recipients. Blood 2001; 98: 1626. Letter.

86 Chakrabarti S, Collingham KE, Osman H, Fegan CD, Milligan DW. Cidofovir as primary pre-emptive therapy for post-transplant cytomegalovirus infections. Bone Marrow Transplant 2001; 28: 879-881.

87 Platzbecker U, Bandt D, Thiede C et al. Successful pre-emptive cidofovir treatment for CMV antigenemia after dose-reduced conditioning and allogeneic blood stem cell transplantation. Transplantation 2001; 71: 880-885.

88 Emery VC, Griffiths PD. Prediction of cytomegalovirus load and resistance patterns after antiviral chemotherapy. Proc Natl Acad Sci U S A. 2000; 97: 8039-8044.

89 Erice A. Resistance of human cytomegalovirus to antiviral drugs. Clin Microbiol Rev 1999; 12: 286-297.

90 Wolf DG, Yaniv L, Honigman A, Kassis L, Schonfeld T, Ashkenazi S. Early emergence of ganciclovir-resistant human cytomegalovirus strains in children with primary combined immunodeficiency. J Infect Dis 1998; 178: 535-538.

91 Slavin MA, Bindra RR, Gleaves CA, Pettinger MB, Bowden RA. Ganciclovir sensitivity of cytomegalovirus at diagnosis and during treatment of cytomegalovirus pneumonia in marrow transplant recipients. Antimicrob Agents Chemother 1993; 37: 1360-1363.

92 Gilbert C, Roy J, Belanger R, Delage R, Beliveau C, Demers C, Boivin G. Lack of emergence of cytomegalovirus UL97 mutations conferring ganciclovir (GCV) resistance following preemptive GCV therapy in allogeneic stem cell transplant recipients. Antimicrob Agents Chemother 2001; 45: 3669-3671.

93 Erice A, Borrell N, Li W, Miller WJ, Balfour HH. Ganciclovir susceptibilities and analysis of UL97 region in cytomegalovirus (CMV) isolates from bone marrow recipients with CMV disease after antiviral prophylaxis. J Infect Dis 1998; 178: 531-534.

94 Eckle T, Prix L, Jahn G, Klingebiel T, Handgretinger R, Selle B, Hamprecht K. Drug-resistant human cytomegalovirus infection in children after allogeneic stem cell transplantation may have different clinical outcomes. Blood 2000; 96: 3286-3289.

95 Prix L, Hamprecht K, Holzhuter B, Handgretinger R, Klingebiel T, Jahn J. Comprehensive restriction analysis of the UL97 region allows early detection of ganciclovir-resistant human cytomegalovirus in an immunocompromised child. J Infect Dis 1999; 180: 491-495.

96 Hamprecht K, Eckle T, Prix L, Faul C, Einsele H, Jahn G. 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 2002; 187: 139-143.

97 Walter EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED, Riddell SR. 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 1995; 333: 1038-1044.

98 Einsele H, Roosnek E, Rufer N et al. Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. Blood 2002;99:3916-3922.

99 June CH. CD4 T cells to kiss off chemotherapy-resistant CMV? Blood 2002; 99: 3883. Editorial.

100 Peggs K, Verfuerth S, Pizzey A, Khan N, Moss P, Mackinnon S. 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 2001; 98: S779a. Abstract 3240.

101 Keenan RD, Ainsworth J, Khan N, Bruton R, Cobbold M, Assenmacher M, Milligan DW, Moss PA. Purification of cytomegalovirus-specific CD8 T cells from peripheral blood using HLA-peptide tetramers. Br J Haematol 2001; 115: 428-434.