



Risk factors for treatment failures in patients receiving PCR-based preemptive therapy for CMV infection

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Summary:

PCR-based preemptive therapy with ganciclovir has been shown to reduce the incidence of CMV disease after BMT. Failures of this treatment strategy are CMV disease and secondary non-viral infections. Eighty-six consecutive patients at high risk for CMV disease who received PCR-based preemptive therapy with ganciclovir were assessed for treatment failures and possible risk factors. Ganciclovir was initiated in 57 of 86 patients (66%). Only 28 of 86 (32%) patients received 4 or more weeks of ganciclovir. Recurrence of CMV infection after successful treatment was more frequent among recipients of a BMT from an unrelated compared to a sibling donor ($P = 0.004$). Three (3.5%) patients developed non-fatal early onset CMV disease and seven of 68 (10.3 %) late onset CMV disease (>100 days post transplant). Risk factors for late onset CMV disease were cGVHD ($P = 0.0017$) and duration of prior antiviral therapy >4 weeks ($P = 0.0073$). The incidence of secondary non-viral infections was 28% with the duration of antiviral treatment being a significant risk factor for secondary bacterial ($P = 0.0045$) and invasive fungal infections ($P = 0.006$). Thus, PCR-based preemptive treatment with ganciclovir reduces early onset CMV disease, but the duration of antiviral therapy prior to day +100 is a significant risk factor for late onset CMV disease as well as secondary non-viral infections. *Bone Marrow Transplantation* (2000) 25, 757–763. **Keywords:** CMV; BMT; late onset disease; PCR; secondary infections

when CMV infection was culture-proven – was effective in reducing CMV disease and transplant-related mortality in seropositive allo-BMT recipients.^{4,5} However, 12–13% of patients presented with CMV disease before or coincident with CMV excretion as determined by conventional and shell vial culture assays.^{3–5} Antigenemia⁶ and polymerase chain reaction (PCR) can detect CMV infection earlier.^{7–9} Thus, these more sensitive and rapid techniques are increasingly used for monitoring allogeneic BMT recipients.^{10–13}

In a recently published analysis we showed PCR-based preemptive therapy to be superior to a culture-based one with a significant decrease in the rate of CMV disease and CMV-related mortality.¹³ Here, we present our extended experience with PCR-based preemptive therapy in 86 consecutive patients at high risk of developing CMV disease post BMT. We specifically analyzed risk factors for treatment failures resulting in the recurrence of CMV infection and early or late onset CMV disease as well as side-effects of antiviral therapy.

Patients and methods

Patients

CMV-seropositive marrow transplant recipients and/or those receiving a transplant from a CMV-seropositive donor for a hematological malignancy were eligible for study entry. The CMV serostatus of patients and bone marrow donors as well as of blood donors was determined by an ELISA technique. Exclusion criteria included a serum creatinine of more than 220 $\mu\text{mol/l}$ or severe neutropenia ($<500/\mu\text{l}$) at the time of a second positive PCR signal obtained from blood. For conditioning therapy, total body irradiation (TBI) was performed on 3 successive days (2 Gy twice a day, lung shielding 10 Gy). Cyclophosphamide $2 \times 60 \text{ mg/kg/day}$ was infused following TBI (TBI/Cy). In patients with accelerated chronic myelogenous leukemia (CML) or acute leukemia not in first remission, etoposide (VP-16/Cy/TBI) was added at a dose of 40 mg/kg on day –4. Patients treated with busulfan and cyclophosphamide received busulfan at a dose of $4 \times 4 \text{ mg/kg/day}$ and cyclophosphamide at $2 \times 60 \text{ mg/kg/day}$. Patients with severe aplastic anemia (SAA) received cyclophosphamide $4 \times 50 \text{ mg/kg/day}$ with antilymphocyte globulin (Pasteur-Merieux, MDS GmbH, Leiden, Germany). For GVHD prophylaxis

Cytomegalovirus (CMV) disease, in spite of recent developments in the diagnosis and therapy of CMV infection, is still associated with a high mortality in allogeneic bone marrow transplant (BMT) recipients.¹ In two studies,^{2,3} prophylactic ganciclovir administered to CMV-seropositive allogeneic marrow transplant recipients was shown to reduce incidence and severity of CMV infection, but was found to be frequently associated with neutropenia and non-viral infections. Preemptive therapy – ganciclovir started

laxis, patients undergoing BMT from an unrelated or HLA-mismatched related donor as well as patients age >45 years received CsA and short-course methotrexate. All other patients received CsA alone.

Anti-infectious prophylaxis

All CMV-seropositive patients as well as those receiving a transplant from a seropositive donor received blood products which were unscreened for CMV. Oral acyclovir was administered to all patients at a dose of 4×400 mg/day for prophylaxis of herpes simplex infections until day 60 after BMT.

All patients received oral antimicrobial prophylaxis with ciprofloxacin 2×500 mg/day and fluconazole 400 mg/day beginning from day -7 until day 60 after BMT.

Blood chemistry

Complete and differential blood cell counts as well as tests for serum creatinine, electrolytes and liver function were undertaken before, daily during, and at least twice weekly following the study period until day 100 to assess patients for treatment-related side-effects.

Virus screening

Blood samples were collected weekly on Wednesday, DNA extraction and amplification were performed on Thursday. When the second CMV-PCR assay on blood collected the subsequent Wednesday following a first positive result again tested positive, the patient was started on antiviral therapy on the Friday of this week.

Virus culture and PCR assay

Virus culture and PCR assay were performed as previously reported.⁷⁻⁹

CMV disease

CMV pneumonia was diagnosed on the basis of dyspnoea, interstitial infiltrates on chest radiograph, and a positive CMV culture of bronchial washings. A diagnosis of CMV enteritis was based on clinical and endoscopic signs of enteritis and colitis plus histologic demonstration of CMV inclusions, positive *in situ* hybridization assays or positive culture for CMV from the tissue samples obtained at biopsy. A CMV-related death was defined as death due to histopathologically and/or immunohistologically proven CMV disease.

CMV-related mortality was defined as death occurring within 6 weeks of the diagnosis of CMV disease being made. A copathogen was defined as any viral, fungal, bacterial or parasitic pathogen that was detected by histopathology, culture or immunofluorescence assay at the same site(s) where CMV was demonstrated.

Graft-versus host disease

Diagnosis and clinical grading of acute and chronic graft-versus host disease (aGVHD and cGVHD) were performed according to current criteria.¹⁴

Protocol design

Patients were followed-up weekly by viral cultures from blood, urine and throat washings and PCR from whole blood, beginning on day 0 and continued until day 100 after BMT. After day 100 patients were only screened by culture assay every other week, and additionally when CMV infection was clinically suspected. The study was approved by the local ethics committee. Informed consent was obtained from all patients or their parents (age <18 years) included in the study. Asymptomatic patients received preemptive antiviral therapy at the time of the second consecutive positive PCR signal. Ganciclovir was given at a dose of 5 mg/kg body weight, administered intravenously twice daily for 14 days. Patients still PCR positive after 14 days of maintenance therapy with ganciclovir received foscarnet (2×60 mg/kg BW) until tested PCR negative.

During the first 100 days after BMT ganciclovir was reinstated for another 14 days (2×5 mg/kg) when two consecutive positive PCR results were obtained after successful antiviral therapy. After day 100 patients were only treated when CMV disease was documented.

Treatment of documented CMV disease

Treatment of CMV-IP and -enteritis included ganciclovir (2×5 mg/kg BW) for 14 days plus CMV hyperimmunoglobulin in a dosage of 0.2 g/kg BW administered on days 1, 3, 5, 7 and 14. Maintenance therapy was administered when signs and symptoms of CMV disease or PCR positivity persisted.

Toxicity

A neutropenic episode was defined as a decrease of the absolute neutrophil count to $<1000/\mu\text{l}$ for 2 consecutive days. If the neutrophil count dropped below $1000/\mu\text{l}$ for 3 consecutive days G-CSF was administered. If the neutrophil count dropped below $500/\mu\text{l}$ for >2 consecutive days in spite of therapy with the hematopoietic growth factor, treatment with ganciclovir was temporarily discontinued until the neutrophil count returned to a level $>1000/\mu\text{l}$ on 2 successive days. Bacterial infection was defined as recovery of an organism from blood culture or other normally sterile sites in a febrile patient. Invasive fungal infection was reported when proven by culture or histopathologically. Afebrile patients were not screened for bacterial or fungal growth.

Statistical analysis

The effects of risk factors were assessed by logistic regression if the target variable was dichotomous, and by univariate and multivariate Cox regression if the target variable was time to the relevant event (eg late onset CMV disease) taking into account censoring. In order to quantify the effects, odds ratios were calculated together with their 95% confidence intervals. Statistical analysis was performed using the statistical package JMP (SAS Institute).

Results

Patients

Eighty-eight consecutive CMV-seropositive recipients of an allogeneic marrow graft or those receiving a transplant from a CMV-seropositive donor between May 1993 and January 1996 at the bone marrow transplant unit in Tübingen were eligible for study entry. Two patients were excluded from the study, both for not being willing to participate in the study. Thus, 86 patients were eligible for analysis. No technical problems with the PCR assay were documented during the study. Four of the 86 screened patients developed fatal complications (two bacterial septicemia, one invasive Aspergillosis, one refractory severe acute GVHD) early (<35 days) after BMT post transplant. These patients were included in the analysis.

The characteristics of the patients (median age 41, range 5–57 years) analyzed in this study are shown in Table 1. A high percentage of patients (29/86, 34%) received their transplant from an unrelated donor representing a population at very high risk of developing CMV disease.¹⁵

Virus detection and antiviral therapy

Fifty-seven of 86 patients (66%) were found to be PCR positive on 2 consecutive blood samples and started on ganciclovir a median of 45 days post transplant (Table 2). None of the PCR-negative patients were culture positive during the screening period. Onset of PCR positivity preceded a positive culture assay by 15 days (median). Seven of 57 patients received PCR-based preemptive therapy before and 20 without testing culture positive.

Fifteen of 57 (26%) patients required maintenance therapy for another 14 days, four (7%) additionally received foscarnet for 2–4 weeks. Ganciclovir resistance was not demonstrated in any of the isolates. All patients were PCR and culture negative at the time of cessation of antiviral therapy.

Nineteen (33%) patients required retreatment with ganciclovir for reactivation of CMV infection as detected by PCR assay.

Thus, 57 (66%) of the patients at risk received PCR-based antiviral therapy, 29 of them (51%) for only 2 and 23% for more than 4 weeks (Figure 1).

Early onset CMV disease

Three (3%) patients developed early onset CMV disease (CMV-IP) at the time of the second positive PCR signal. A copathogen was identified only in one BAL, and the patient improved with combined antiviral and antibacterial treatment. All three patients were still alive >180 days after BMT. One patient died 1 year after transplant due to invasive aspergillosis when suffering from extensive chronic GVHD.

Late onset CMV infection and disease

Seven out of 68 (10%) patients surviving day 100, developed late onset CMV disease (five CMV-IP, one

Table 1 Patient characteristics

	Patients receiving PCR-based preemptive therapy (n = 57)	All patients at risk of developing CMV disease (n = 86)
Age (in years)		
Median	39	41
Range	5–56	5–57
Sex		
Male	27	45
Female	30	41
Disease		
ALL in 1st or 2nd CR	3	5
AML in 1st CR	10	16
acute leukemia in >2nd CR or in relapse	2	3
CML in 1st chronic phase	27	39
CML in >1st chronic phase/AP	4	6
MDS	4	8
CLL	2	2
Multiple myeloma	3	4
SAA	2	2
PNH	0	1
Conditioning therapy		
Cyclophosphamide plus TBI	21	31
Busulfan + cyclophosphamide	25	40
TBI/Cyclophosphamide/VP-16	9	12
Cyclophosphamide + ALG	2	3
Source of marrow transplant		
HLA-identical sibling	36	57
Unrelated donor	21	29
GVHD prophylaxis		
CsA	18	28
CsA/MTX	39	58
Graft-versus host disease		
Acute		
grades 0–1	14	18
grade II	29	46
grade III/IV	14	22
Chronic		
no	25	40
limited	13	15
extensive	13	13
death prior to day +100	6	18
CMV serostatus		
R+/D+	29	43
R+/D–	21	32
R–/D+	7	11

TBI = total body irradiation; VP-16 = etoposide 40 mg/kg BW; ALL = acute lymphocytic leukemia; AML = acute myelogenous leukemia; MDS = myelodysplastic syndrome; GVHD = graft-versus-host disease; CR = complete remission; CML = chronic myelogenous leukemia; CLL = chronic lymphocytic leukemia.

CMV enteritis, one CMV retinitis). Copathogens were identified in three of these seven patients and included bacteria (two patients) and Candida species (one patient) in low colony numbers. Only one patient died within 6 weeks after the diagnosis of CMV-IP, and another five during the first 2–6 months following late onset CMV disease (four invasive aspergillosis, one bacterial endocarditis). Only one patient is still alive more than 2 years after late onset CMV enteritis.

All seven patients suffered from chronic GVHD. In the multivariate analysis, risk of late onset CMV disease increased with the occurrence of cGVHD ($P = 0.0017$) and

Table 2 CMV infection and disease

CMV detection at first reactivation ^a (n = 57)	
PCR (blood)	57
culture assay	37
from urine	26
from throat washing	27
from blood	19
Time of detection of first CMV reactivation ^a	
by PCR:	
median	34
range	8–91
by culture	
median	49
range	23–88
CMV detection at second reactivation ^b (n = 19)	
PCR (blood)	19
culture assay	9
from urine	6
from throat washing	3
from blood	4
CMV disease	
prior to day 100 after BMT	3
interstitial pneumonia	3
CMV-related mortality	0
Late CMV infection (after day 100 after BMT)	
interstitial pneumonia	7
gastrointestinal disease	5
retinitis	1
CMV-related mortality	1

^aCMV reactivation was defined as the time point of first detection of CMV after transplant using PCR assay.

^bSecond CMV reactivation was defined as the detection of CMV by PCR or culture assay after the patient had responded to the first therapy. Response to therapy was documented by a blood sample tested PCR negative.

86 patients at risk, first treatment (n = 57), retreatment (n = 19)

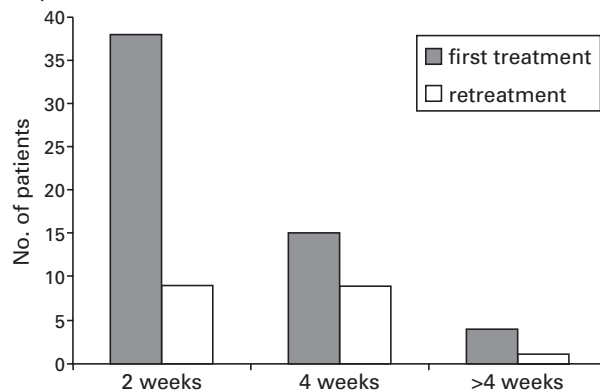


Figure 1 Duration of antiviral chemotherapy among 86 patients receiving PCR-based preemptive antiviral therapy. The number of patients receiving the first course of PCR-based preemptive therapy for 2 weeks, 4 weeks or >4 weeks is shown in dark bars. In addition, for each of these groups the number of patients is presented who required retreatment for PCR-documented recurrence of CMV infection. The number of patients (not the duration of retreatment) is shown by white bars.

duration of antiviral therapy >4 weeks ($P = 0.0073$) (Table 3). Transplant modality (MUD vs identical sibling donor) and conditioning therapy with TBI were not associated with an increased risk of late onset CMV disease ($P = 0.9$ and $P = 0.2$, respectively). The annual risk of late onset CMV disease was 22.9% for patients receiving >4 weeks of antiviral therapy (95% CI: 7.5–54%) vs 2% (95% CI: 0.2–7.7%) for those receiving ≤ 4 weeks of ganciclovir treatment. In patients with cGVHD the incidence of late onset CMV disease was calculated to be 16.4% per year (95% CI: 6.6–34%), in those without cGVHD 0% (95% CI: 0–5%).

Risk factors for recurrence of CMV infection

Among the patients receiving a transplant from an HLA-identical sibling, seven of 36 (19%) showed recurrence of CMV infection following successful antiviral therapy, in contrast to 12 of 21 (57%) MUD transplant recipients (odds ratio: 8.9, 95% CI: 1.2–19.7, $P = 0.03$). Additional significant risk factors in the multivariate analysis for the recurrence of CMV infection were duration of antiviral therapy (odds ratio 13.8, 95% CI: 1.5–121 $P = 0.02$), and cumulative dose of prednisolone (odds ratio 14.6, 95% CI 0.9–510 $P = 0.017$). Culture positivity at the time of first reactivation ($P = 0.13$) and the occurrence of aGVHD ($P = 0.051$) were not found to be significant risk factors for reactivation of CMV infection on multivariate analysis.

Toxicity

Ten out of 57 (18%) patients developed neutropenia ($<1000/\mu\text{l}$) but only two – all receiving ganciclovir treatment for at least 4 weeks – required therapy with G-CSF. Ganciclovir therapy had to be stopped for 2 days in one patient due to persistent neutropenia in spite of G-CSF treatment. The risk of secondary neutropenia increased with each additional week of ganciclovir treatment by a factor of 1.3 ($P = 0.15$). Secondary thrombocytopenia occurring in 21 patients was associated with duration of ganciclovir treatment for >4 weeks (odds ratio: 72, 95% CI: 4.1–33, $P = 0.0079$) as well as cGVHD (odds ratio: 17.3, 95% CI: 2.8–162, $P = 0.0011$). In addition, the risk of secondary thrombocytopenia increased with the cumulative dose of prednisolone (100 mg/kg body weight) by a factor of 10.2 (95% CI: 2.6–166.4, $P = 0.0003$).

Incidence of non-viral infection during and after antiviral therapy was 28% (16/57). Eight patients were documented as bacteriemic (*Staphylococcus epidermidis* (one) and

Table 3 Risk factors for late onset CMV disease

Risk factor	Odds ratio (95% CI)	P value
cGVHD	1100 (1.8– ∞)	0.0017
Antiviral therapy >4 weeks	8.5 (3.5–22.1)	0.0073

95% CI = confidence interval.

Risk factors were assessed by Cox regression taking into account censoring.

Results of a multivariate analysis are presented.

aureus (two), *Streptococcus mitis* (three), *E coli* (one) and *Pseudomonas aeruginosa* (one)). Duration of antiviral therapy increased the risk of secondary bacterial infections ($P = 0.0045$) (Table 4).

Eight patients suffered and died from proven invasive aspergillosis. In three neutropenic patients nodular pulmonary infiltrates were documented and *Aspergillus fumigatus* isolated from the bronchoalveolar lavage. The other five patients developed histopathologically proven invasive aspergillosis (two pulmonary and three pulmonary plus cerebral manifestations). Duration of antiviral therapy was associated with an increased risk (odds ratio: 30, 95% CI: 2.7–411, $P = 0.006$) of invasive aspergillosis (Table 4).

The median overall survival of patients receiving 2 weeks of antiviral therapy was >720 days, for patients receiving 4 weeks and more than 4 weeks of antiviral therapy 643 and 456 days, respectively.

Discussion

Two strategies to prevent CMV disease in BMT recipients at risk have been applied over the last few years. Prophylactic ganciclovir, administered from the time of hematopoietic engraftment until day 100^{2,3} and preemptive ganciclovir given to patients with a documented CMV infection up to day 100.^{4,5} Both approaches have two major problems: considerable toxicity – neutropenia and secondary infections – and a high rate of late onset CMV disease caused by a delay in the recovery of CMV-specific T cell responses that are required for complete protection from CMV disease in the later post-transplant period.¹⁶

By reducing the total dose of ganciclovir given, several groups have attempted to decrease toxicity. Unfortunately, reducing the dosage of prophylactic ganciclovir was shown to decrease treatment-related side-effects at the expense of a much higher rate of CMV infection and disease.^{17–19}

Preemptive therapy limits the number of patients exposed to unnecessary treatment. If introduced only after documentation of CMV infection, the use of ganciclovir can be restricted to 50% of high risk patients by culture²⁰ or as shown in our study to 68% by PCR screening. However, culture-based preemptive therapy was hampered by the failure to detect CMV prior to the onset of CMV disease in 12–13% of patients in spite of weekly monitoring by culture

Table 4 Duration of antiviral therapy >4 weeks as a risk factor for secondary non-viral infections

Secondary infections	Odds ratio (95% CI)	P value
Bacterial infections	53 (35–1300)	0.001
Invasive fungal infections	84 (4.1–3800)	0.006

Patients receiving >4 weeks of ganciclovir treatment for CMV infection were compared to those receiving less than 4 weeks of ganciclovir for the occurrence of secondary non-viral infections.

The risk factor (>4 week antiviral therapy) for secondary non-viral infections was assessed by logistic regression taking into account censoring. Results of a multivariate analysis are presented.

95% CI = confidence interval.

assays,^{4,5,20} leading to a 10% CMV-related mortality.²⁰ Earlier therapeutic intervention based on more sensitive assays (PCR, antigenemia) resulted in a significant reduction in the incidence of CMV disease.¹³

Here, PCR-based preemptive therapy prevented fatal CMV disease in the first 100 days post BMT, even in recipients of a transplant from an HLA-mismatched family or unrelated marrow donor. Only three out of 86 (4%) of patients at risk developed CMV disease prior to day 100 and all three were still alive >6 months after the onset of CMV disease.

Thus, in contrast to the antigenemia assay,^{21,22} PCR monitoring allows reliable detection of CMV infection early post transplant during severe neutropenia. Early CMV disease occurred in patients suffering from severe acute GVHD only. Thus, PCR screening at shorter intervals, perhaps twice a week, might further reduce the incidence of CMV disease, especially in patients at very high risk of developing CMV disease, eg recipients of a T cell-depleted or MUD stem cell transplant and patients with severe acute GVHD.^{16–19}

Some allogeneic BMT recipients ($n = 13$, 15%) had only a single positive PCR assay and none of these developed CMV disease in spite of not receiving antiviral treatment. This is similar to the experience of others,²³ and we still feel the indication for a potentially highly toxic treatment should not be based on a single positive PCR assay, even in high risk patients.

On multivariate analysis, several risk factors were identified for the occurrence of late CMV disease as well as antiviral treatment-related toxicity. Due to the small number of patients developing late onset CMV disease as well as secondary non-viral infections in the analysis, the confidence intervals are rather large. Thus, interpretation of the multivariate analysis has to be performed with caution.

Late onset CMV disease, occurring in seven (10%) patients surviving day 100 post transplant, was the main CMV-related problem in this study. All these seven patients were only PCR screened until day 100. Late onset CMV disease, which involved a high fatality rate,^{24–25} only occurred in patients with cGVHD. Therefore, this subgroup of patients might benefit from PCR screening and PCR-based preemptive therapy after day 100 post BMT. Monitoring of viral load might also help to define a high risk group for late onset CMV disease.²⁶

A further major risk factor of late onset CMV disease was antiviral therapy for >4 weeks. Thus, to further reduce the complications (late onset CMV disease, myelosuppression, bacterial and fungal infections, nephrotoxicity) several groups have tried to shorten the duration of ganciclovir administration. Three weeks of ganciclovir were reported to be sufficient to control CMV infection in 78% of BMT recipients,²⁷ similar to 51% of patients responding to only 14 days of antiviral therapy in our analysis.

It is important to identify those patients that need maintenance therapy after 14 days of antiviral therapy. Culture assays become negative in 85–93% of patients during antiviral treatment^{9,23} and are thus not suitable for monitoring antiviral therapy. Stopping treatment with ganciclovir in a PCR-negative patient was found to be safe with no patient developing CMV disease. PCR-based preemptive therapy

allowed restriction of antiviral therapy to 2 weeks in 51% (29/57) and to ≤ 4 weeks in 77% (44/57) of patients.

In our study, side-effects of antiviral therapy occurred predominantly in patients receiving ganciclovir for >4 weeks. Only mild neutropenia and no increase in non-viral infections was observed in patients receiving only 3 weeks of ganciclovir.²⁷ In our study, only 18% (10/57) of patients experienced neutropenia ($<1000/\mu\text{l}$), in contrast to the 41% previously reported for culture-based preemptive therapy until day +100 post transplant.⁵ Only two patients required hematopoietic growth factors, further indicating the reduced myelotoxicity of short-term ganciclovir.

Duration of ganciclovir therapy was also a significant risk factor for secondary thrombocytopenia, invasive aspergillosis and bacterial infections. This might be due to the combined CMV- and ganciclovir-mediated immuno- and myelosuppression.²⁸ With each week of ganciclovir treatment the risk of invasive aspergillosis increased by the factor 1.4 and consequently, invasive aspergillosis was the most common cause of death in patients surviving CMV disease after having received long-term ganciclovir treatment.

Shortening of ganciclovir therapy might also help to reduce the delay in CMV-specific immune reconstitution.¹⁶ In our study, late onset CMV disease – indicating delayed CMV-specific immune reconstitution^{16,29} – was only observed in patients who had received at least 4 weeks of antiviral therapy.

In conclusion, PCR-based preemptive therapy was safe and as shown before, reduced CMV-related morbidity and mortality early post transplant. Patients with cGVHD and those requiring long-term ganciclovir treatment post transplant, probably due to a markedly delayed CMV-specific immune reconstitution, are at high risk of developing late onset CMV disease. Thus, monitoring with sensitive assays and preemptive therapy beyond day 100 should be assessed in future trials as should means of improving and hastening CMV-specific immune reconstitution in these patients.^{30,31}

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