Infections Post Transplant

Cytomegalovirus infections in allogeneic stem cell recipients after reduced-intensity or myeloablative conditioning assessed by quantitative PCR and pp65-antigenemia

J Schetelig¹, O Oswald², N Steuer², A Radonic², S Thulke², TK Held³, J Oertel¹, A Nitsche^{2,4} and W Siegert²

¹Charité Campus Virchow Klinikum, Humboldt-Universität zu Berlin, Medizinische Klinik m.S. Hämatologie und Onkologie, Berlin, Germany; ²Charité Campus Mitte, Humboldt-Universität zu Berlin, Medizinische Klinik II m.S. Onkologie und Hämatologie, Berlin, Germany; ³Charité Campus Buch, Robert-Rössle Klinik, Medizinische Klinik mit Schwerpunkt Onkologie, Hämatologie und Tumormmunologie, Germany; and ⁴Robert Koch-Institut, Berlin, Germany

Summary:

Since the incidence of cytomegalovirus (CMV) infections after hematopoietic stem cell transplantation (HSCT) may depend on the intensity of the pretreatment, we studied the incidence of CMV infections after reduced-intensity compared to myeloablative conditioning. A total of 82 patients with matched related or unrelated donors were prospectively monitored for CMV infections after HSCT by CMV-PCR techniques, CMV-antigenemia and clinical observation. A total of 45 patients received reduced-intensity conditioning consisting of fludarabine, busulfan and ATG and 37 patients received myeloablative conditioning. Leukocyte engraftment occurred after a median of 15 vs 18 days ($P=0.012$) and platelet engraftment after 12 days vs 20 days ($P=0.001$), respectively. Acute graft-versus-host disease (GVHD) grade II–IV was observed in 58 vs 54% patients ($P=0.737$), respectively. The onset and peak values of CMV-antigenemia and DNAemia and the incidence of CMV infections did not differ statistically significantly between the two treatment groups. Multivariate analysis confirmed CMV seropositivity of the recipient ($P=0.035$), acute GVHD II–IV ($P=0.001$) but not the type of conditioning as significant risk factors for CMV-antigenemia. In conclusion, the kinetics of CMV-antigenemia and DNAemia and the incidence of CMV infections were not statistically different in patients who received HSCT after reduced-intensity conditioning with fludarabine, busulfan and ATG compared to myeloablative conditioning.

Bone Marrow Transplantation (2003) 32, 695–701.
doi:10.1038/sj.bmt.1704164

Keywords: allogeneic; blood stem cell transplantation; cytomegalovirus; reduced-intensity; quantitative PCR

Cytomegalovirus (CMV) infection is a frequent complication of allogeneic hematopoietic stem cell transplantation (HSCT) causing significant morbidity and mortality during the first 100 days after transplantation.^{1,2} Important risk factors for CMV infections are the serological status of donor and recipient, graft-versus-host disease (GVHD) and T-cell depletion. Reduced-intensity conditioning is an increasingly applied treatment option for patients with an indication for allogeneic hematopoietic stem cell transplantation, but contraindications against myeloablative treatment. Today, there is good evidence that the early infectious mortality can be reduced with reduced-intensity conditioning regimens.^{3,4} This effect is largely explained by a shorter period of cytopenia and less organ damage due to reduced dosages of cytotoxic agents. However, there have been some alarming reports on a high incidence of CMV infections after reduced-intensity conditioning regimens containing T-cell-depleting agents.^{5,6} Therefore, we studied the impact of reduced-intensity compared to myeloablative conditioning on the incidence and severity of CMV infections. The comparison included weekly clinical status, detection of CMV-antigenemia and a quantitative Taq-Man-based PCR assay.⁷

Patients and methods

Patient selection

Between January 1998 and March 2001 all patients who received allogeneic HSCT at the Charité-Campus Virchow Klinikum, Berlin, were included in the study. Within this period of time, patients with relative contraindications against myeloablative treatment were entered into a prospective study on reduced-intensity conditioning with busulfan, fludarabine and antithymocyte globulin (ATG).⁸ Patients gave written informed consent for inclusion into the study, which was approved by the Ethical Institutional Review board of the Charité hospital, Humboldt

Correspondence: Professor Dr med. W Siegert, Medizinische Klinik II mit Schwerpunkt Onkologie und Hämatologie, Charité-Campus Charité Mitte, Schumannstr. 20-21, 10117 Berlin, Germany
Received 20 December 2002; accepted 20 March 2003

Universität zu Berlin. Standard risk patients received myeloablative conditioning according to standard protocols.

Patient characteristics

Patients who received reduced-intensity conditioning were significantly older (median age 43 vs 36 years, $P < 0.001$), more often received peripheral blood stem cells instead of bone marrow (87 vs 65%, $P = 0.020$), and GVHD prophylaxis was more heterogeneous compared to the myeloablative treatment group (Table 1).

Preparative regimens and transplantation

Reduced-intensity conditioning consisted of fludarabine 30 mg/m² once daily on days -10 to -5, busulfan 4 mg/m² in divided doses on days -6 and -5 and ATG (Fresenius) 10 mg/kg once daily by continuous i.v. infusion over 4–8 h on days -4 to -1.⁴ Myeloablative conditioning was carried out according to published protocols.⁹ Patients with unrelated donors and more advanced disease status who were eligible for myeloablative conditioning received an intensive conditioning protocol consisting of cyclophosphamid, busulfan, thiotepa and ATG.¹⁰ G-CSF-mobilized

peripheral blood stem cells (PBSC) were transplanted in 63 patients. The median count of CD34-positive cells in PBSC-products was 6×10^6 CD34+ cells/kg (range 2.2– 18.9×10^6). A total of 19 patients with unrelated donors received bone marrow (BM) with a median of 3.4×10^6 mononuclear cells/kg (range 0.9– 20.1×10^6). No graft was T-cell depleted. GVHD prophylaxis was carried out with cyclosporine A (CSA) alone ($n = 22$), CSA combined with short-course methotrexate (MTX) ($n = 41$) or CSA combined with mycophenolate mofetil (MMF) ($n = 19$). CSA levels were targeted toward the upper therapeutic range in the early post-transplantation period. In standard risk patients, CSA taper started on day +100 in the absence of GVHD and disease progression.

Supportive care was carried out in a standardized manner. For infection prophylaxis, patients received oral quinolones and amphotericin B suspension from the beginning of conditioning treatment on. Empiric antibiotic treatment for neutropenic fever consisted of broad-spectrum antibiotics. Intravenous amphotericin B was added for refractory neutropenic fever or if fungal infection was suspected or documented. For the prophylaxis of herpes simplex and varicella zoster infections, all patients received acyclovir 500 mg intravenously three times daily from day

Table 1 Patient characteristics

	Reduced-intensity conditioning ($n = 45$)	Myeloablative conditioning ($n = 37$)	P-value
Sex (female/male) [N (%)]	18 (40) / 27 (60)	16 (43) / 21 (57)	NS
Disease [N (%)]			
AML/ALL	7 (16)	12 (32)	NS
CML	17 (38)	16 (43)	
HD/NHL	15 (33)	5 (14)	
MDS	1 (2)	4 (11)	
Germ cell cancer	5 (11)	—	
Risk of relapse ^a [N (%)]			
Standard	27 (60)	19 (51)	NS
High	18 (40)	18 (49)	
Median age (range)	43 (20–64)	36 (17–58)	<0.001
CMV serostatus [N (%)]			
D-/R-	19 (42)	14 (38)	NS
D+/R-	5 (11)	5 (13)	
D-/R+	12 (27)	8 (22)	
D+/R+	9 (20)	10 (27)	
Donors [N (%)]			
HLA-ident. sibling	22 (49)	13 (35)	NS
Related nonident.	6 (13)	2 (6)	
Matched unrelated	17 (38)	22 (59)	
Stem cell source [N (%)]			
PBSC	39 (87)	24 (65)	0.020
BM	6 (13)	13 (35)	
Conditioning [N (%)]			
BU/FLU/ATG	45 (100)		NA
BU/CY/thiotepa/ATG		19 (51)	
BU/CY		9 (24)	
CY/TBI ± VP16		6 (16)	
Other		3 (9)	
GVHD prophylaxis [N (%)]			
CSA-mono	21 (47)	1 (3)	<0.001
CSA + MTX	5 (11)	36 (97)	
CSA + MMF	19 (42)		

AML=acute myelogenous leukemia; ALL=acute lymphoblastic leukemia; CML=chronic myelogenous leukemia; HD=Hodgkin's disease; NHL=Non-Hodgkin's lymphoma; MDS=myelodysplastic syndromes; NS=not significant; NA=not applicable; D=donor; R=recipient; PBSC=peripheral blood stem cells; BM=bone marrow; BU=busulfan; FLU=fludarabine; ATG=antithymocyte globulin; CY=cyclophosphamide; TBI=total body irradiation; VP16=etoposide; GVHD=graft-versus-host disease; CSA=cyclosporine A; MTX=methotrexate; MMF=mycophenolate mofetil.

^aRisk of relapse: standard risk defined as patients with CML in first chronic phase, AML in first CR, and patients with HD or NHL; high risk defined as patients with other diagnoses or more advanced disease.

+ 1 on. It was switched to 400 mg orally four times daily as soon as possible and stopped with the taper of GVHD prophylaxis. If both patient and stem cell donor were serologically negative for CMV, CMV-IgG negative blood products were transfused.

Monitoring of CMV infections

All patients were prospectively monitored for CMV infections and CMV disease for at least 1 year. During the first 3 months after HSCT, peripheral blood samples were analyzed weekly for antigenemia or DNAemia. Subsequently, monthly analyses were carried out. CMV hepatitis or enterocolitis was confirmed by tissue biopsy. CMV pneumonia was diagnosed on the basis of radiologic signs and a broncho-alveolar lavage or lung biopsy specimen positive for CMV by culture or immunohistology. Detection of CMV-antigenemia was done with a monoclonal mouse antibody against pp65 Antigen (Clonab, Biotest, Germany) as previously described.¹¹ The number of antigen-positive cells was related to a total of 10 000 cells. Heparinized plasma samples for the detection of CMV-DNA by TaqMan-PCR were stored whenever blood for the detection of CMV antigen was taken. After preparation of the DNA using the Blood & Tissue Kit (Qiagen, Hilden, Germany), samples were stored at -20°C . The elution volume was chosen to obtain a concentration of 1 μl of DNA per μl prepared plasma. Templates of 5 μl were analysed per assay. As a positive control, template serial dilutions of 10^7 – 10^1 plasmids containing the target sequence from the major immediate-early region of CMV were prepared by using plasma from anti-CMV IgG-negative blood donors as a carrier. PCR was performed with a Perkin-Elmer model 7700 Sequence Detection System as described previously.⁷ This method has a detection limit of 10 genome equivalents per assay (2000 ge/ml plasma) with a range of 10^1 – 10^7 genome equivalents per assay. Human DNA or DNA of other herpesviruses are not amplified. Concordance between CMV-antigenemia and the TaqMan assay has been shown in patients after bone marrow transplantation.¹²

Pre-emptive CMV therapy

Pre-emptive therapy was guided by CMV-antigenemia. In patients with a first positive antibody staining result, ganciclovir treatment was started. If a second test confirmed the initial result, 5 mg/kg ganciclovir was given intravenously every 12 h until two consecutive blood samples were negative for CMV antigen. Dose reductions in patients with renal insufficiency were made on the basis of the calculated creatinine clearance. No prophylactic ganciclovir was given. Foscarnet was given as second-line treatment in three patients.

Definitions and statistical analysis

According to previous publications, CMV risk groups were defined as follows: low risk (recipient and donor serologically CMV negative), intermediate risk (recipient negative, donor positive) and high risk (recipient positive, donor

either positive or negative).^{1,2} Comparisons of categorical variables from 2×2 tables were made by means of χ^2 and Fisher exact tests for small numbers. Differences between numerical variables were calculated by means of the Mann–Whitney U-test. Incidences of time-dependent variables were estimated by the method of Kaplan–Meier. Intervals were measured from the day of transplantation until first detection of CMV infection by Taqman-PCR or CMV-antigenemia or until the last day of follow-up or treatment-related death. The number of patients was determined to detect a difference of 30% in the 1-year probability of CMV-antigenemia between treatment and control group with a power of 80%. Multivariate Cox regression model was used to analyze the relative influence of different risk factors on the probability of CMV-antigenemia during the first year after transplantation. Variables with a *P*-value below 0.1 in univariate analysis were selected for multivariate analysis. For Cox regression analysis, acute GVHD and CMV-antigenemia were tested as time-dependent covariates. In order to test for a potential influence of the type of conditioning on the occurrence of CMV-antigenemia, this variable was included into the multivariate model regardless of its significance.

Results

A total of 82 consecutive patients were analyzed. Data were analyzed as of 01.12.2001. For all patients, data on CMV infections, status at last follow-up and causes of death were available. The minimum follow-up of living patients was 1 year. The total number of analyzed blood samples was 1222 with a median of 14 samples (range 3–41) per patient.

Hematopoietic regeneration and incidence of GVHD

Regeneration of leukocytes $> 1 \times 10^9/\text{l}$ took a median of 15 days (range 0–33) and 18 days (range 11–34) after reduced-intensity and myeloablative conditioning, respectively ($P=0.012$). Platelet counts $> 20 \times 10^9/\text{l}$ were reached after 12 days (0–91) and 20 days (12–46), respectively ($P=0.002$). Two patients after reduced-intensity and four patients after myeloablative conditioning died before hematologic regeneration. Acute GVHD II–IV was observed in 26 patients (58%) after reduced-intensity and in 20 patients (54%) after myeloablative conditioning ($P=0.737$). The median onset of acute GVHD was on day +22 (range 12–78) and day +18 (range 9–88) after HSCT ($P=0.180$), respectively. Chronic GVHD occurred in 56% of patients after reduced-intensity and in 57% of patients after myeloablative conditioning with a minimum follow-up of 100 days.

Incidence of CMV infections and disease

In 14 of 45 patients (31%) after reduced-intensity and in seven of 37 patients (19%) after myeloablative conditioning, pp65-positive leukocytes were detected (see Table 2). The probability of CMV-antigenemia during the first year

Table 2 Incidence of CMV-antigenemia according to type of conditioning and CMV serostatus of donor and recipient

CMV serostatus	Reduced-intensity conditioning (n=45)	Myeloablative conditioning (n=37)
D-/R- (%)	1/19 (5)	0/14 (0)
D+/R-	0/5 (0)	1/5 (20)
D-/R+	6/12 (50)	4/8 (50)
D+/R+	7/9 (78)	2/10 (20)

D = donor; R = recipient; + = seropositive; - = seronegative.

was 33% (95% CI 19–47%) and 23% (95% CI 8–38%) ($P=0.344$; log-rank test), respectively. CMV-DNA in the peripheral blood as detected by PCR was present in 26 of 45 patients (58%) in the reduced-intensity conditioning group and in 15 of 37 patients (41%) in the myeloablative conditioning group. The probability to detect CMV-DNA during the first year in the two treatment groups of patients was 65% (95% CI 49–81%) and 45% (95% CI 27–73%) ($P=0.300$; log-rank test), respectively. CMV disease occurred in three out of 45 patients after reduced-intensity conditioning (on days +41, +65 and +80) and in two out of 37 patients (on days +60 and +156) after myeloablative treatment ($P=0.978$; log-rank test).

Kinetics of CMV-antigenemia and DNAemia

In order to compare the kinetics of CMV infections, we analyzed onset, peak level and duration of CMV-antigenemia and CMV-DNAemia in the two treatment groups. Median onset of CMV-antigenemia was on day +49 (range 6–75) after reduced-intensity and on day +49 (range 28–65) after myeloablative conditioning ($P=0.913$). Maximum levels were 7.5 positive cells/slide (range 1–200) after reduced-intensity and two positive cells/slide (range 2–16) after myeloablative treatment, respectively ($P=0.218$). The interval between the begin and the end of CMV-antigenemia was 10 days (range 1–14) in patients after reduced-intensity conditioning and 11.5 days (range 1–33) after myeloablative conditioning ($P=0.887$). Pre-emptive therapy was introduced in 18 of 82 patients with at least two positive results for CMV-antigenemia. The median duration of pre-emptive therapy was 18.5 days (range 13–33 days) after reduced-intensity conditioning and 15 days (range 10–36) after myeloablative conditioning ($P=0.479$). CMV infections relapsed after successful pre-emptive therapy with ganciclovir in two patients after reduced-intensity transplantation and one patient after myeloablative conditioning.

By means of PCR technique, CMV-DNA was first detected after a median of +36 days (range 0–252) in the reduced-intensity and +25 days (range 0–65) in the myeloablative conditioning group ($P=0.261$). Maximum levels were 7213 copies/ml (range 2370–497 743) in the reduced-intensity group and 8103 copies/ml (range 2023–149 074) in the myeloablative group ($P=0.499$).

Survival analysis

At 1 year after HSCT, 38 patients were alive and 44 patients had died. Causes of death were disease progression in 17

Figure 1 Probabilities of treatment-related mortality in 82 patients were 5 vs 31% ($P=0.002$) at day +100 and 30 vs 44% ($P=0.134$) at 1-year after reduced-intensity compared to myeloablative conditioning, respectively.

patients and treatment-related complications in 27 patients. The probabilities of day +100 and 1-year treatment-related mortality (TRM) were 5% and 31% after reduced-intensity compared to 30% and 44% after myeloablative conditioning ($P=0.002$ and 0.134; log-rank), respectively (Figure 1). One patient in each group died of CMV disease, whereas the other patients died of septic complications related to GVHD. Patients at intermediate or high of CMV infection did not have an increased day +100 TRM in either group, whereas CMV-antigenemia was a significant predictor of day +100 TRM after myeloablative relative risk ((RR) 8.4, 95% CI 2–34; $P=0.003$), but not after reduced-intensity conditioning.

Risk factor analysis for CMV-antigenemia

Univariate analysis identified serological status of recipient and donor ($P=0.008$), recipient age ($P=0.002$) and the occurrence of acute GVHD ($P=0.020$) to be associated with the incidence of CMV-antigenemia (Table 3). The probabilities of CMV-antigenemia for these variables are shown in Figure 2a–c. In order to control disbalanced risk factors in the two treatment groups, we performed multivariate Cox regression analysis in patients at intermediate or high risk of CMV infection (Table 4). We confirmed CMV serostatus of the patient (RR 9.1, 95% CI 1.2–71.1; $P=0.035$) and acute GVHD II–IV (RR 6.9, 95% CI 2.3–20.7; $P=0.001$) as independent risk factors for CMV-antigenemia. The type of conditioning regimen did not have a significant impact (RR 1.0 (95% CI 0.4–2.6); $P=0.974$).

Discussion

The combination of busulfan, fludarabine and ATG was introduced as a nonmyeloablative conditioning protocol in allogeneic HSCT in 1998.⁴ Since then, the excellent

Table 3 Univariate analysis of risk factors for CMV-antigenemia (82 patients)

Risk factors	Univariate RR (95% CI)	P-value
Reduced-intensity <i>vs</i> myeloablative	1.8 (0.7–4.4)	NS
Recipient age (RR for a change of 10 years)	1.6 (1.2–10.1)	0.002
Standard <i>vs</i> poor prognosis	0.5 (0.2–1.4)	NS
Serological CMV status		0.008
D+/R– <i>vs</i> D–/R–	3.3 (0.2–52.7)	NS
D+/R+ <i>vs</i> D–/R–	21.2 (2.7–167.8)	0.004
D–/R+ <i>vs</i> D–/R–	22.7 (2.9–177.5)	0.003
Related <i>vs</i> unrelated donor	1.5 (0.6–3.7)	NS
ATG <i>vs</i> no ATG	2.3 (0.5–10.0)	NS
BM <i>vs</i> PBSC	1.4 (0.5–3.7)	NS
MMF <i>vs</i> other	1.6 (0.7–4.0)	NS
MTX <i>vs</i> other	0.6 (0.3–1.6)	NS
CSA mono <i>vs</i> other	1.1 (0.4–2.8)	NS
Acute GVHD II–IV <i>vs</i> 0–I	3.6 (1.1–12.3)	0.020

RR=relative risk; CI=confidence interval; NS=not significant; *vs*=versus; D=donor; R=recipient; + =seropositive; – =seronegative.

tolerability of this regimen and its capacity to enable engraftment of BM or PBSC from matched related or unrelated donors has been confirmed several times.¹³ However, largely due to late infections related to acute and chronic GVHD, the 1-year nonrelapse mortality approaches 30% in high-risk patients.⁸ We therefore analyzed the incidence and severity of CMV infections after conditioning with busulfan, fludarabine and ATG as compared to myeloablative pretreatment.

In univariate analysis we observed a trend to a higher incidence of CMV-antigenemia after reduced-intensity compared to myeloablative conditioning (33 *vs* 23% 1-year probability; $P=0.211$; log-rank test). In order to control disbalanced risk factors in the two treatment groups, we performed a multivariate analysis among patients at intermediate or high risk of CMV infection. A seropositive CMV status of the recipient ($P=0.035$) and the occurrence of acute GVHD II–IV ($P=0.001$) were found to predict CMV-antigenemia independently, while the type of the conditioning regimen had no influence. With the use of a quantitative PCR, no significant difference in the 1-year probability of viremia could be discovered between the two groups, either. The peak level of CMV-antigenemia and CMV-DNA load are both associated with the severity of CMV infections.¹⁴ However, neither the peak levels of CMV-antigenemia nor the DNA-load were statistically different between the two treatment groups. Furthermore, the onset of CMV-antigenemia and CMV-DNAemia did not differ after reduced-intensity and myeloablative conditioning and therefore significant differences in the CMV immunity between the two treatment groups are unlikely. Although the study had not enough power to rule out small differences between the two treatment groups, considering the high risk of CMV infection attributed to acute GVHD (RR 6.9; 95% CI 2.3–20.8), the small impact of the dose intensity of the conditioning regimen can hardly be considered relevant.

Recent data of a matched pair analysis published by Junghanss *et al*¹⁵ comparing CMV infections after

Figure 2 The 1-year probabilities of CMV-antigenemia in 82 patients according to CMV serostatus were 3% in seronegative patients and donors, 12.5% in seronegative patients with positive donors, 54% in seropositive patients and donors, and 58% in seropositive patients with negative donors (a), 6% in patients with an age below 40 years and 50% in patients above 40 years (b) and 17% in patients with GVHD 0–I compared to 37% in patients with acute GVHD II–IV (c).

Table 4 Univariate and multivariate analysis of risk factors for CMV infection in patients at intermediate or high risk of CMV infections (48 patients)

Risk factors	Univariate RR (95% CI)	P-value	Multivariate RR (95% CI)	P-value
Reduced-intens. vs myeloablative	1.5 (0.6–3.9)	NS	1.0 (0.4–2.6)	NS
Recipient age	1.3 (0.9–1.7) ^a	NS		
Standard vs poor prognosis	0.5 (0.2–1.3)	NS		
CMV serostatus D+ vs D–	1.6 (0.7–4.0)	NS		
CMV serostatus R+ vs R–	6.7 (0.9–50.4)	0.064	9.1 (1.2–71.1)	0.035
Related vs unrelated donor	1.8 (0.7–4.4)	NS		
ATG vs no ATG ^b	1.9 (0.4–8.3)	NS		
BM vs PBSC	1.6 (0.6–4.1)	NS		
MMF vs other	1.6 (0.6–4.1)	NS		
MTX vs other	0.6 (0.2–1.5)	NS		
CSA mono vs other	1.2 (0.5–3.1)	NS		
Acute GVHD II–IV vs 0–I	5.8 (2.0–17.1)	0.001	6.9 (2.3–20.8)	0.001

D = donor; R = recipient.

^aRR for a change of 10 years.^bOnly eight out of 48 patients received no ATG.

nonmyeloablative (200 cGy total body irradiation (TBI)) vs myeloablative pretreatment showed a trend to less pronounced and delayed CMV-antigenemia and viremia after nonmyeloablative transplants. Despite a comparable 1-year probability of 53% CMV-antigenemia among high-risk patients in their and 61% in our study, the reduction of early CMV disease after nonmyeloablative conditioning contrasts with our results. Junghanss *et al* hypothesized that persisting immune-competent cells of the host might have protected against early CMV infections. The fact that we observed the same kinetics of CMV infections in terms of onset, peak levels and duration of antigenemia could reflect a similar CMV immunity in the two treatment groups. A possible reason for this could be that the reduced-intensity conditioning regimen we used still produces significant hematotoxicity and is known to induce full donor chimerism within 1 month, whereas the 200 cGy TBI-based regimen is truly nonmyeloablative and enables mixed chimerism for a period of 3 months.^{8,16} Therefore, immune-competent cells of the host might not have persisted in sufficient numbers after reduced-intensity conditioning in our study. Martino *et al*¹⁷ reported a very low incidence of CMV infections in patients after a reduced-intensity conditioning regimen which was similar to our protocol, but did not contain ATG. In a retrospective multicenter study they analyzed the risk of severe infections after reduced-intensity compared to myeloablative conditioning. The incidence of CMV infections in patients at intermediate or high risk of reactivation was lower after reduced-intensity compared to myeloablative conditioning (21 vs 43%, $P=0.01$). Therefore, the use of ATG in our protocol could be responsible for the high incidence of CMV infections. This question cannot be answered from our data, because most patients with myeloablative conditioning received ATG, too. Generally, the use of T-cell-depleting agents increases the risk of delayed recovery of CMV immunity. Mohty *et al*⁶ reported a high incidence of 65% CMV-antigenemia in 21 patients at intermediate or high risk of CMV infection after reduced-intensity conditioning with busulfan, fludarabine and ATG (Merieux). On the other hand, in a randomized study that compared GVHD prophylaxis with or without ATG the

incidence of CMV infections was not increased in the ATG group.¹⁸ One reason for this observation could be that while ATG increases host immunosuppression, its capacity to reduce the frequency and severity of GVHD might have been beneficial in terms of CMV infections. Alemtuzumab is another T-cell-depleting agent that is increasingly used in reduced-intensity conditioning. Chakrabarti *et al*⁵ recently reported data on a high incidence of CMV infections after an Alemtuzumab-containing reduced-intensity regimen. CMV infections assessed by PCR were observed in 85% of the seropositive patients and 71% suffered from recurrent infections. Pérez-Simón *et al* compared retrospectively the incidence of GVHD between two prospective studies on reduced-intensity conditioning in the United Kingdom and Spain.^{17,19,20} Both study groups used the combination of fludarabine and melphalan, but in the United Kingdom GVHD prophylaxis consisted of CSA and Alemtuzumab pretransplant, whereas in Spain CSA was combined with post-transplant MTX. They were able to demonstrate that despite a lower incidence of acute GVHD in patients who had received Alemtuzumab, the incidence of CMV infections was higher after conditioning with Alemtuzumab compared to MTX (47 vs 22.7%, $P=0.018$). However, in patients who had received Alemtuzumab the diagnosis was based on a PCR assay, while it was based on CMV-antigenemia in the other patients. The use of different techniques could have influenced the outcome. In our study the incidence of CMV infections was higher defined by CMV-DNAemia detected by PCR (58%) compared to CMV-antigenemia (31%). However, the effect of any T-cell-depleting agent given as GVHD prophylaxis in terms of CMV infection can only be determined in prospective randomized trials. This is particularly important, because of the strong association between acute GVHD and CMV infections. Owing to widely differing incidences of CMV infections, a stratification for low, intermediate and high risk will be mandatory.²

As shown before, day +100 TRM was significantly lower (5 vs 30%, $P=0.002$) after reduced-intensity compared to myeloablative conditioning.⁴ Only one patient in each group died of CMV disease. Therefore, the high incidence of CMV infections in patients after reduced-intensity

conditioning did not translate into high day +100 TRM. However, CMV-antigenemia predicted day +100 TRM after myeloablative conditioning. Presumably in this group the combination of organ damage caused by myeloablation, significantly longer periods of cytopenia, acute GVHD and side effects of ganciclovir therapy exerted deleterious consequences despite successful treatment of CMV infection.

The significance of our study is limited by the use of different protocols for myeloablative conditioning and different types of GVHD prophylaxis. As a result of the great variability among reduced-intensity regimens, our results pertain only to the combination of busulfan, fludarabine and ATG applied in our study. However, we were able to demonstrate that in patients who almost exclusively received ATG as GVHD prophylaxis, the incidence of CMV infections depends largely on the serostatus of donor and recipient and on the occurrence of acute GVHD, whereas the dose intensity of the conditioning protocol is less important. Like others, we were able to show that seronegative patients with a seropositive donor are at much lower risk of CMV infection compared to seropositive patients.

In conclusion, CMV-antigenemia is a frequent complication after reduced-intensity conditioning with busulfan, fludarabine and ATG and allogeneic HSCT, especially in seropositive patients suffering from acute GVHD. Weekly monitoring of CMV-antigenemia or CMV-DNAemia and pre-emptive therapy during the first 100 days after transplantation should therefore be applied in patients at intermediate or high risk of CMV infection irrespective of the reduced dose intensity of busulfan, fludarabine and ATG.

Acknowledgements

We would like to thank Delia Barz for excellent technical assistance.

References

- Boeckh M. Current antiviral strategies for controlling cytomegalovirus in hematopoietic stem cell transplant recipients: prevention and therapy. *Transplant Infect Dis* 1999; **1**: 165–178.
- 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.
- McSweeney PA, Niederwieser D, Shizuru JA et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 2001; **97**: 3390–3400.
- Slavin S, Nagler A, Naparstek E et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998; **91**: 756–763.
- Chakrabarti S, Mackinnon S, Chopra R et al. High incidence of cytomegalovirus infection after nonmyeloablative stem cell transplantation: potential role of Campath-1H in delaying immune reconstitution. *Blood* 2002; **99**: 4357–4363.
- Mohty M, Faucher C, Vey N et al. High rate of secondary viral and bacterial infections in patients undergoing allogeneic bone marrow mini-transplantation. *Bone Marrow Transplant* 2000; **26**: 251–255.
- Nitsche A, Steuer N, Schmidt CA et al. Different real-time PCR formats compared for the quantitative detection of human cytomegalovirus DNA. *Clin Chem* 1999; **45**: 1932–1937.
- Schetelig J, Kroger N, Held TK et al. Allogeneic transplantation after reduced conditioning in high risk patients is complicated by a high incidence of acute and chronic graft-versus-host disease. *Haematologica* 2002; **87**: 299–305.
- Clift RA, Radich J, Appelbaum FR et al. Long-term follow-up of a randomized study comparing cyclophosphamide and total body irradiation with busulfan and cyclophosphamide for patients receiving allogeneic marrow transplants during chronic phase of chronic myeloid leukemia. *Blood* 1999; **94**: 3960–3962.
- Przepiorka D, Khouri I, Thall P et al. Thiotepa, busulfan and cyclophosphamide as a preparative regimen for allogeneic transplantation for advanced chronic myelogenous leukemia. *Bone Marrow Transplant* 1999; **23**: 977–981.
- Schmidt CA, Oettle H, Peng R et al. Comparison of polymerase chain reaction from plasma and buffy coat with antigen detection and occurrence of immunoglobulin M for the demonstration of cytomegalovirus infection after liver transplantation. *Transplantation* 1995; **59**: 1133–1138.
- Nitsche A, Steuer N, Schmidt CA et al. Detection of human cytomegalovirus DNA by real-time quantitative PCR. *J Clin Microbiol* 2000; **38**: 2734–2737.
- Bornhauser M, Thiede C, Platzbecker U et al. Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. *Clin Cancer Res* 2001; **7**: 2254–2262.
- Nichols WG, Corey L, Gooley T et al. Rising pp65 antigenemia during preemptive anticytomegalovirus therapy after allogeneic hematopoietic stem cell transplantation: risk factors, correlation with DNA load, and outcomes. *Blood* 2001; **97**: 867–874.
- Junghanss C, Boeckh M, Carter RA et al. Incidence and outcome of cytomegalovirus infections following nonmyeloablative compared with myeloablative allogeneic stem cell transplantation, a matched control study. *Blood* 2002; **99**: 1978–1985.
- Faucher C, Mohty M, Chabannon C et al. Allogeneic transplantation with ATG base reduced intensity regimen and bone marrow (BM) graft is associated with rapid donor chimerism, low GVHD rate and rapid CD8+ T cells recovery. *Bone Marrow Transplant* 2002; **29** (Suppl 2): 157 (abstract 616).
- Martino R, Caballero MD, Canals C et al. Reduced-intensity conditioning reduces the risk of severe infections after allogeneic peripheral blood stem cell transplantation. *Bone Marrow Transplant* 2001; **28**: 341–347.
- Bacigalupo A, Lamparelli T, Bruzzi P et al. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood* 2001; **98**: 2942–2947.
- Perez-Simon JA, Kottaridis PD, Martino R et al. Nonmyeloablative transplantation with or without alemtuzumab: comparison between 2 prospective studies in patients with lymphoproliferative disorders. *Blood* 2002; **100**: 3121–3127.
- Kottaridis PD, Milligan DW, Chopra R et al. In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. *Blood* 2000; **96**: 2419–2425.