Control of cytomegalovirus viremia after T cell depleted allogeneic stem cell transplantation

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Chapter 3

Comparable incidence and severity of cytomegalovirus infections following T cell depleted allogeneic stem cell transplantation preceded by reduced intensity or myeloablative conditioning

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Abstract

Reports on infectious complications following reduced intensity conditioning (RIC) before allogeneic stem cell transplantation (alloSCT) are equivocal. This prospective follow-up study compared the impact of cytomegalovirus (CMV) infections following RIC with fludarabine, ATG and busulphan or conventional myeloablative conditioning (MAC). Forty-eight RIC and 59 MAC patients were enrolled. The occurrence and severity of CMV infections within 100 days following alloSCT were assessed, using plasma CMV DNA load kinetics. CMV DNAemia was observed in 21 RIC (60%) and in 19 MAC (44%) patients at risk for CMV. The mean CMV DNAemia free survival time was comparable following RIC and MAC: 70 days (95% (confidence interval) CI: 59–80 days) and 77 days (95% CI: 68–86 days), respectively (P¼0.24). Parameters indicative for the level of CMV reactivation, including the area under the curve of CMV DNA load over time as well as the onset, the peak values and duration of CMV infection episodes, the numbers and duration of CMV treatment episodes and recurrent infections, were not different in both groups. During follow-up, none of the patients developed CMV disease. RIC with fludarabine, ATG and busulphan demonstrated safety comparable to conventional MAC with regard to frequency and severity of CMV infections within 100 days following T cell depleted alloSCT.

Introduction

Allogeneic stem cell transplantation (alloSCT) is increasingly used to treat hematological and non-hematological malignancies. Recently, conditioning regimens have been designed to exploit the graft-versus-tumor effects while reducing the intensity of the conditioning to minimize toxicities[1-3]. Results of studies demonstrate rapid allogeneic engraftment with minimal non-hematological toxicity and a significant antitumor effect. Despite the lower toxicity of the reduced intensity conditioning (RIC), acute and chronic graft-versus-host disease (GvHD) remains a significant cause of morbidity and mortality with a reported incidence of severe GvHD of 30–60%[1].

Recently, an in vitro T cell depleted alloSCT protocol following non-myeloablative conditioning with fludarabine, antithymocyte globulin (ATG), busulphan and Campath-in-the-bag was reported as a suitable platform for subsequent cellular immunotherapy[4]. It was shown that this protocol leads to durable donor engraftment, favorable response of the disease and minimal GvHD. Still, infections remain a prominent cause of transplant-related mortality following RIC[5]. As in myeloablative SCT recipients, risk factors for infections include the degree of myeloablation, GvHD and organ toxicities. However, as the timing and types of infections may differ[5], information regarding infectious risks and outcomes are important to develop preventative strategies in alloSCT recipients following RIC.

Cytomegalovirus (CMV) is one of the major causes of infectious complications following alloSCT[6], and the strategy of viral load guided pre-emptive antiviral therapy has been shown to reduce the risk of CMV disease[7, 8]. Viral load kinetics has been reported to be predictive for the development of CMV disease, with the initial viral load and the initial rate of increase in viral load being independent risk factors[9] and as such this method can also be applied to assess the incidence and severity of CMV reactivation following transplantation. However, in this context, it should be considered that an episode of CMV viremia is characterized not only by its level (for example, peak load), but also by its duration[9, 10]; as a consequence, long-term viremia at lower levels may have the same clinical significance as shorter episodes of high-level viremia. A novel approach has been devised previously to assess both quantities (level and duration of viremia) with a single parameter, which is based on calculating the area under the curve (AUC) of viral load over time[10]. Hence, the AUC approach is a universal means of assessing interrelated determinants, including peak viral load, initial viral load and rate of increase of viral load, parameters that have been described as independent risk factors for CMV disease[9].

In the current prospective follow-up study, viral load kinetics were used to assess the incidence and the level of CMV reactivation in patients receiving in vitro T cell depleted alloSCT following either non-myeloablative conditioning with fludarabine, ATG and busulphan or after myeloablative conditioning (MAC).

Patients and methods

Patients

Forty-eight consecutive patients who received alloSCT following RIC between January 2001 and December 2004 were analyzed for CMV reactivation. Patients eligible for alloSCT were selected to receive RIC either when MAC was contraindicated (due to comorbidity or age) or in patients with an HLA identical donor who failed to respond on conventional treatment for lymphoma, multiple myeloma or chronic lymphocytic leukemia, or in patients with solid tumors such as metastatic renal cell carcinoma or breast carcinoma. Forty-three RIC patients had hematological malignancies, four had renal cell carcinoma and one had breast carcinoma. Additionally, 59 consecutive patients who received alloSCT using conventional MAC regimens between August 2001 and December 2004 were included in this analysis. All conventional MAC patients had hematological malignancies. General institutional policy with respect to patients' informed consent for inclusion into the study, approved by the ethical institutional board, was applied.

Transplantation

T cell depleted transplantation was performed either according to a RIC protocol or a MAC regimen as described previously[4, 11]. The RIC regimen consisted of fludarabine (30mg/m^2, intravenously, day -10 to -6), busulphan (3.2 mg/kg, intravenously, day -6 and -5) and ATG (10mg/kg/day intravenously, day -4 to -1), for both sibling and matched unrelated donor (MUD) grafts. The MAC regimen consisted of cyclophosphamide (60mg/kg/day intravenously for 2 consecutive days) followed by single dose of total body irradiation (TBI, 9 Gy, day -1) in patients receiving sibling donor grafts. Recipients of MUD grafts, in the myeloablative regimen, received additional Campath-1G or -1H (day -8 and -4) and cyclosporine (3 mg/kg intravenously, starting on day -1) and TBI (6 Gy, day -8 and -7). The stem cell product was infused on day 0. In all conditioning regimens, T cell depletion of the graft was performed by in vitro incubation of the graft with Campath-1H (20mg). Prophylaxis for GvHD was not administered. Assessment of acute and chronic GvHD was performed using the Glucksberg and Shulman criteria[12, 13]. In the absence of GvHD or graft failure, patients received donor lymphocyte infusion (DLI) after RIC transplantation or in mixed chimerism or relapsed disease after MAC transplantation. DLI was never administered before 6 months following transplantation.

CMV monitoring and pre-emptive treatment

CMV DNA load was measured at least once a week for up to 100 days following transplantation. The real-time quantitative PCR for detection of CMV DNA in plasma was performed according to the method described previously[14]. The course of CMV DNA load in plasma was documented longitudinally for each patient during follow-up. Individual areas under the CMV DNAemia curve post-transplant were calculated using the trapezoidal rule as described previously[10, 15].

CMV DNA load guided pre-emptive therapy was initiated according to a protocol based on criteria established in a previous study[14]. In short, CMV DNAemia episodes following transplantation treatment was initiated at a CMV DNA load level of $>10^4$ copies/ml or at a level of $>10^3$ copies/ml and more than one $_{10}$log increase as compared to previous measurement, without clinical symptoms of CMV disease[14]. Pre-emptive treatment consisted of 900 mg valganciclovir b.i.d. or intravenous 5 mg/kg ganciclovir b.i.d for an average duration of 2 weeks. CMV disease would be treated with intravenous 5 mg/kg ganciclovir b.i.d. Ganciclovir and valganciclovir dose were adjusted to renal function as described previously[16]. Serum creatinine levels and hematological parameters (that is, hemoglobin, leucocyte and thrombocyte counts) were monitored throughout treatment episodes.

Study end points and statistical analysis

The primary end point for this study was CMV infection, defined as 'detection of two consecutive positive CMV DNA loads (more than $\log_{10}$ 2.7 (=500) copies/ml plasma) within 100 days following alloSCT transplantation'. The level of $\log_{10}$ 2.7 copies/ml plasma as the lower detection limit of the 'real-time' quantitative CMV DNA PCR was established by earlier assessments with respect to the sensitivity and reproducibility of the assay[14]. The number of two consecutive detections of $\log_{10}$ 2.7 copies/ml as the definition of CMV infection was arbitrarily chosen to exclude incidental single positive findings. Secondary end points were CMV DNA load requiring antiviral treatment and recurrent infections. Definitions for CMV infection, CMV disease, CMV detection in blood and recurrent infection were adopted from internationally accepted criteria[17].

All database entries and statistical analyses were performed with SPSS version 12.0.1. Differences in age at transplantation, time to the first CMV DNA load detection, CMV DNA peak load, the duration of the CMV infection and the area under the DNAemia curve (AUC) were compared between groups using Mann–Whitney U-test and analyses of variance. For all measurements, the median and range or the 25th and 75th percentiles are presented. Differences in the distribution of CMV serostatus, underlying disease, GvHD and gender were tested using χ^2 and Fisher exact-test statistics. Kaplan–Meier analysis was performed to detect differences in CMV DNAemia free survival between groups during the first 100 days following transplantation and a Cox regression analysis was used to adjust for the possible confounders age and donor type. Relative risks for occurrence of CMV disease are presented with 95% confidence interval (95% CI).

Results

Patient characteristics

A total of 107 patients were included in this study. The demographic and disease characteristics for patients in both conditioning groups are shown in Table 1. Distribution of

the characteristics across the two groups was similar with respect to risk for CMV infections (based on donor and recipients CMV serostatus), underlying disease, GvHD and gender. However, significant differences were noted with regard to mean age at transplantation and donor type (**Table 1**). The mean age at transplantation was 54.5 years in the RIC patients compared with 44.0 years in the MAC patient group ($P<0.01$). In the reduced intensity group, 31 patients were transplanted with hematopoietic stem cells from an HLA identical donor and 17 patients had mismatched unrelated donors (in the myeloablative group, 52 and 7, respectively) ($P=0.004$). Further analyses were restricted to 78 patients who were considered to be at risk for CMV infection/reactivation (based on donor and receptor serostatus: 8 D^+R^-, 40 D^+R^+ and 30 D^-R^+). This selection did not introduce significant change in the patients' characteristics.

Table 1. Relevant characteristics of the study population in both conditioning groups. No significant differences were present between the two groups, with the exception of age and donor type. Systemic treatment of GvHD consisted of oral prednisone, intravenous methylprednisolone and/or oral cyclosporine. RIC: reduced intensity conditioning, MAC: myeloablative conditioning, ns: not significant. CLL: chronic lymphocytic leukaemia; CML: chronic myelogenous leukaemia; CMV: cytomegalovirus; GvHD: graft-versus-host disease; MAC: myeloablative conditioning; MM: multiple myeloma; NHL: non-Hodgkin lymphoma; NS: not significant; RIC: reduced intensity conditioning.

Characteristics	RIC (n = 48)	MAC (n = 59)	Statistical relevance
Age (median/range)	54.5 (26-76)	44.0 (21-62)	p<0.01
Male gender (%)	(34) 71	(43) 73	*ns*
Serostatus: (%)			
D^+R^+	20 (42)	20 (34)	*ns*
D^+R^-	4 (8)	4 (7)	
D^-R^+	11 (23)	19 (32)	
D^-R^-	13 (27)	16 (27)	
Donor type (%)			
Related	31 (65)	52 (88)	p<0.01
Unrelated	17 (35)	7 (12)	
Underlying disease (%)			*ns*
Acute leukemia	10 (21)	33 (56)	
CML	5 (10)	10 (17)	
CLL	5 (10)	1 (2)	
MM	5 (10)	7 (12)	
NHL	10 (21)	7 (12)	
Other	13 (27)	1 (2)	
T cell depletion (%)	48 (100)	59 (100)	*ns*
Acute GvHD (%)			p=0.07
Grade I/II	4 (8)	13 (22)	
Grade III/IV	0	0	
Chronic GvHD (%)	0	5 (9)	*p=0.07*
GvHD treatment (%) (systemic)	0	5 (8.5)	*ns*

Incidence of CMV DNAemia

CMV DNAemia occurred in 40 patients within 100 days following transplantation, which accounts for 37% of all 107 patients and 51% of patients at risk for CMV (n=78). The first signs of CMV DNAemia were observed at a median of 27 days (range: 8–81) and all first episodes occurred within 90 days following transplantation. None of the patients developed CMV disease during the follow-up of 100 days following alloSCT. Among the 78 patients at risk for CMV DNAemia, the highest incidence of CMV DNAemia was observed in R^+ cases; 21 (53%) D^+R^+ and 18 (60%) D^+R^+ compared with 1 (12.5%) D^+R^- patients within 100 days following transplantation. Within the group of patients at risk for CMV (35 and 43 receiving RIC and MAC, respectively), CMV DNAemia was observed in 21 (60%) patients receiving RIC and in 19 (44%) patients receiving MAC. Although the mean CMV DNAemia free survival time was shorter in RIC patients (70 days, 95% CI: 59–80) then in MAC patients (77 days, 95% CI: 68–86), this difference was not statistically significant (P=0.24; **Figure 1**). This was not different when a multivariate Cox regression analysis was performed to control for the possible confounders age, GvHD and donor type.

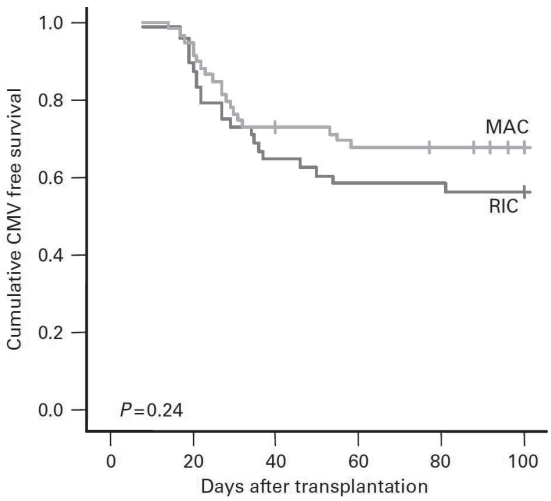

Figure 1.
Pattern of CMV-free survival (Kaplan-Meier) during the first 100 days following allo-SCT in patients receiving reduced intensity (RIC) or myeloablative conditioning (MAC). CMV viraemia was observed in 21 (60%) and 19 (44%) of the RIC and MAC patients respectively. The mean CMV free survival time in RIC patients was 70 days, (95% CI: 59-80 days) compared to 77 days (95% CI: 68-86 days) in MAC patients (p= 0.24).

Level of CMV reactivation following RIC and MAC

To assess the level of CMV reactivation, the onset of the first positive CMV PCR following transplantation, the peak load of the first episodes following alloSCT and the duration of the first CMV DNAemia episodes were evaluated in patients receiving RIC or MAC. There was no difference in the onset of the first CMV DNAemia episodes following RIC or MAC; median of 27 days (range: 8–81) and 27 days (range: 14–58) following transplant in recipients of RIC and MAC, respectively (P=0.36). Also the median peak loads of the first CMV episodes following alloSCT were comparable between the RIC and MAC patients: $\log_{10}$ 4.7 copies/ml (range: $\log_{10}$ 3.2–$\log_{10}$ 5.6) and $\log_{10}$ 4.7 copies/ml (range: $\log_{10}$ 3.5–$\log_{10}$ 6.2), respectively (P=0.74). The median duration of the first CMV DNAemia episode was longer in RIC patients (42 days (range: 7–73)) compared with MAC patients (28 days (range: 2–83)). However, this difference was not statistically different (P=0.72). These findings did not change after correcting for the possible confounders age, GvHD and donor type. Alternatively, the level of CMV reactivation was evaluated by calculating the time-adjusted area under the DNAemia curve (assessing both, the level and the duration of CMV DNAemia in mentioned time period). Although the median area under the DNAemia curve over time during the first 100 days following alloSCT was higher in RIC patients (0.61 (range: 0.08–1.68)) compared with MAC patients (0.49 [range: 0.10–1.42]), this difference was not statistically significant (P=0.41). These findings did not change after correcting for differences in age, GvHD and donor type between the two induction groups. Another approach to assess the level of CMV reactivation in both groups was to evaluate CMV load episodes requiring antiviral treatment. (Val)ganciclovir was administered to an equal amount of RIC and MAC patients with CMV DNAemia: 17 out of 21 (81%) and 16 out of 19 (84%), respectively (P=0.45). The total duration of CMV treatment was also comparable in both groups: median duration of 14 days (range: 7–53) in RIC patients and 14 days (range: 11–29) in MAC patients (P=0.279). Multiple treatment episodes (with a maximum of 2) within 100 days following alloSCT were seen in 7 patients (41%) following RIC and in 4 patients (25%) following MAC. This difference did not reach statistical significance (P=0.458), also not after correction for the possible confounders age, GvHD and donor type. Foscarnet was never administered within 100 days following alloSCT. These findings also indicate equal levels of CMV reactivation in both conditioning groups.

Recurrent CMV infections following RIC and MAC

CMV infection recurred within 100 days following transplantation in 3 out of 21 patients (14.3%) receiving RIC and also in 3 out of 19 (15.8%) with MAC. None of the six patients with recurrent CMV infections developed more than 2 CMV DNAemia episodes within 100 days following transplant.

Influence of donor and recipient CMV serostatus on CMV infections

In a univariate analysis, serological status of recipient and donor appeared to be associated with the occurrence of CMV infection within 100 days following alloSCT,

when D^-R^- patients were included (P=0.071). Among patients at risk for CMV (donor and/or recipient seropositive), seropositive recipients were at higher risk for CMV infections compared with seronegative recipients, whereas no significant difference was observed between seropositive and seronegative donors (**Table 2**). Within the high-risk CMV patients (seropositive recipients), the relative risk for CMV reactivation was 1.1 for D^-R^+ patients compared with D^+R^+ patients; this difference was not statistically significant (P=0.65; **Figure 2a**). Also, the level of CMV reactivation was comparable (**Figure 2b**). These findings did not change after stratification for conditioning therapy (**Figure 2c–f**). Donor type and recipients' age did not have significant impact on the occurrence of CMV within 100 days following transplantation.

Table 2. Univariate analysis of risk factors for CMV within 100 days following alloSCT in patients at risk for CMV infection (n=78)

Risk factors		Crude RR (95% CI)	P-value
Conditioning (RIC vs MAC)		1.50 (0.81-2.79)	0.20
Recipient age (years) (>45 vs <45)		1.40 (0.69-2.78)	0.35
CMV serostatus	D^- vs D^+	1.40 (0.75-2.62)	0.29
	R^+ vs R^-	6.10 (0.84-45.50)	0.07
Donor type unrelated vs related		1.40 (0.67-2.80)	0.39

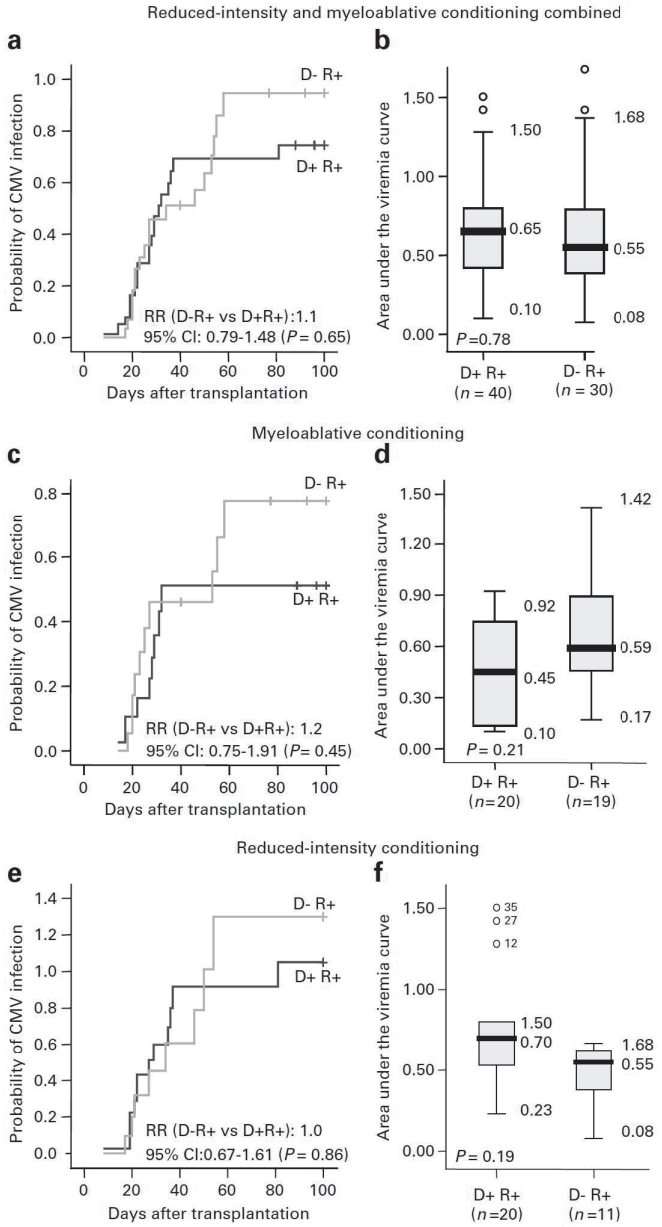

Figure 2.
The probability (left panels) and severity (right panels) of CMV infection in high-risk patients (i.e. CMV seropositive recipients) within 100 days following alloSCT, according to CMV serostatus of the donor and conditioning regimens. The probability and severity of CMV infection were comparable between seropositive and seronegative donors (panels **A** and **B**). This did not change after stratification for induction therapy (**C** and **D**, and **E** and **F**). The box plots display the median (horizontal bars), the 25th and 75th percentiles (box), and the smallest and largest values (whiskers). Open circles depict the outliers (values between 1.5 and 3 box lengths from the upper or lower edge of the box).

Discussion

It has been established that allogeneic transplantation with RIC can be successfully performed in individuals with a wide variety of different diseases and with reduced risk of transplant-related mortality[5, 18]. Previously, an in vitro T cell depleted alloSCT protocol following RIC with fludarabine, ATG, busulphan and Campath-in-the-bag was reported to lead to durable donor engraftment and favorable response of the disease with no GvHD[4]. The current analysis demonstrates that there was no significant difference in incidence and severity of CMV infections within 100 days following alloSCT preceded by RIC compared to a conventional MAC. A limitation in the current study concerns its non-randomized nature. Patients were allocated to the RIC or MAC group on clinical grounds, rather than by random selection. Therefore the possibility of confounding by indication could not be entirely excluded.

Although there was a trend towards a shorter CMV DNAemia free survival following RIC, this difference was not statistically significant. Furthermore, various parameters related to the severity of CMV infections (that is, the onset of CMV DNA detection in plasma following alloSCT, the duration of a CMV infection, the peak load, the area under the DNAemia curve, the number and duration of pre-emptive CMV treatment episodes as well as the number of recurrent infections within 100 days following alloSCT) were not different after RIC and MAC, supporting the conclusion of comparable severity of CMV infections in both groups.

In this study, both patient groups received T cell depleted grafts. By itself, T cell depletion of the graft is associated with an increased risk for CMV infections[19], which seems to be reflected by the high overall incidence of CMV infections (51%) within 100 days following alloSCT in this study.

Previous studies have reported variable outcomes with regard to CMV infections following RIC[20-22]. Such differences can be explained by the variable immune suppressive potentials of the RIC regimens investigated at different centers, presumably reflecting a balance between more residual immunity in the host and a higher risk for opportunistic infections either due to more persisting intracellular pathogens or an increased incidence of GvHD following RIC. A high rate of CMV infections was observed in alemtuzumab-based RIC regimen[20]. Recent reports with respect to CMV infections following fludarabine, busulphan and ATG-based RIC regimens compared to MAC have either reported no influence of conditioning protocols[23] or a significant increase of CMV infection following RIC[22]. However, limitations in these studies included analysis of CMV infections mainly using CMV antigenemia detection rather than the more sensitive and accurate CMV DNA PCR in plasma[14]. Another difference is the use of GvHD prophylaxis in these previous studies, which may be of major importance with respect to CMV infections.

The association of CMV positive serostatus of the recipients (R^+) and an increased risk for CMV infections following alloSCT is well established[24]. Recently, it has been demonstrated that a CMV seronegative donor for a seropositive patient (D^-R^+) in particular was found to be a risk factor for CMV infections following alloSCT in an study including both reduced

intensity as well as MAC regimens. Although the previous report did not show a difference between conditioning regimens[25], we observed increased frequency and severity of CMV infections in seropositive patients receiving a graft from seronegative donors (D^-R^+) compared to seropositive donor and recipient combination (D^+R^+) only following MAC, presumably reflecting residual immunity following RIC. However, this difference was not statistically significant and the clinical relevance of this observation is questionable.

Another relevant conclusion resulting from the current study was that irrespective of the conditioning regimen, monitoring of CMV DNA in plasma and pre-emptive therapy proved highly effective in preventing CMV disease following alloSCT, as CMV disease was not seen in any patient.

In conclusion, RIC with busulphan, fludarabine and ATG demonstrated comparable safety to conventional MAC with regard to the frequency and severity of CMV infections within 100 days following T cell depleted alloSCT. Moreover, with RIC, pre-emptive CMV treatment guided by CMV DNA load monitoring in plasma is highly effective in preventing CMV disease following T cell depleted alloSCT.

References

1. Giralt S, Estey E, Albitar M, van Besien K, Rondon G, Anderlini P *et al.* Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997; **89**(12)**:** 4531-4536.
2. Khouri IF, Keating M, Korbling M, Przepiorka D, Anderlini P, O'Brien S *et al.* Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. *J Clin Oncol* 1998; **16**(8)**:** 2817-2824. doi: 10.1200/JCO.1998.16.8.2817
3. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G *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**(3)**:** 756-763.
4. Barge RM, Osanto S, Marijt WA, Starrenburg CW, Fibbe WE, Nortier JW *et al.* Minimal GVHD following in-vitro T cell depleted allogeneic stem cell transplantation with reduced-intensity conditioning allowing subsequent infusions of donor lymphocytes in patients with hematological malignancies and solid tumors. *Exp Hematol* 2003; **31**(10)**:** 865-872.
5. Junghanss C, Marr KA. Infectious risks and outcomes after stem cell transplantation: are nonmyeloablative transplants changing the picture? *Curr Opin Infect Dis* 2002; **15**(4)**:** 347-353.
6. Ljungman P, Reusser P, de la Camara R, Einsele H, Engelhard D, Ribaud P *et al.* Management of CMV infections: recommendations from the infectious diseases working party of the EBMT. *Bone Marrow Transplant* 2004; **33**(11)**:** 1075-1081. doi: 10.1038/sj.bmt.1704505
7. Crumpacker CS. Ganciclovir. *N Engl J Med* 1996; **335**(10)**:** 721-729. doi: 10.1056/NEJM199609053351007
8. Ljungman P. Beta-herpesvirus challenges in the transplant recipient. *J Infect Dis* 2002; **186 Suppl 1:** S99-S109. doi: 10.1086/342962
9. Emery VC, Sabin CA, Cope AV, Gor D, Hassan-Walker AF, Griffiths PD. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. *Lancet* 2000; **355**(9220)**:** 2032-2036. doi: 10.1016/S0140-6736(00)02350-3
10. Schafer P, Tenschert W, Cremaschi L, Schroter M, Zollner B, Laufs R. Area under the viraemia curve versus absolute viral load: utility for predicting symptomatic cytomegalovirus infections in kidney transplant patients. *J Med Virol* 2001; **65**(1)**:** 85-89.
11. Barge RM, Brouwer RE, Beersma MF, Starrenburg CW, Zwinderman AH, Hale G *et al.* Comparison of allogeneic T cell depleted peripheral blood stem cell and bone marrow transplantation: effect of stem cell source on short- and long-term outcome. *Bone Marrow Transplant* 2001; **27**(10)**:** 1053-1058. doi: 10.1038/sj.bmt.1703024
12. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA *et al.* Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation* 1974; **18**(4)**:** 295-304.

13. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE *et al.* Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 1980; **69**(2)**:** 204-217.
14. Kalpoe JS, Kroes AC, de Jong MD, Schinkel J, de Brouwer CS, Beersma MF *et al.* Validation of clinical application of cytomegalovirus plasma DNA load measurement and definition of treatment criteria by analysis of correlation to antigen detection. *J Clin Microbiol* 2004; **42**(4)**:** 1498-1504.
15. Journot V, Chene G, Joly P, Saves M, Jacqmin-Gadda H, Molina JM *et al.* Viral load as a primary outcome in human immunodeficiency virus trials: a review of statistical analysis methods. *Control Clin Trials* 2001; **22**(6)**:** 639-658.
16. Paya C, Humar A, Dominguez E, Washburn K, Blumberg E, Alexander B *et al.* Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant* 2004; **4**(4)**:** 611-620. doi: 10.1111/j.1600-6143.2004.00382.x
17. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. *Clin Infect Dis* 2002; **34**(8)**:** 1094-1097. doi: 10.1086/339329
18. Mackinnon S, Thomson K, Morris E, Kottaridis PD, Peggs KS. Reduced intensity transplantation: where are we now? *Hematol J* 2004; **5 Suppl 3:** S34-38. doi: 10.1038/sj.thj.6200419
19. Boeckh M, Nichols WG, Papanicolaou G, Rubin R, Wingard JR, Zaia J. Cytomegalovirus in hematopoietic stem cell transplant recipients: Current status, known challenges, and future strategies. *Biol Blood Marrow Transplant* 2003; **9**(9)**:** 543-558.
20. Chakrabarti S, Mackinnon S, Chopra R, Kottaridis PD, Peggs K, O'Gorman P *et al.* High incidence of cytomegalovirus infection after nonmyeloablative stem cell transplantation: potential role of Campath-1H in delaying immune reconstitution. *Blood* 2002; **99**(12)**:** 4357-4363.
21. Junghanss C, Boeckh M, Carter RA, Sandmaier BM, Maris MB, Maloney DG *et al.* Incidence and outcome of cytomegalovirus infections following nonmyeloablative compared with myeloablative allogeneic stem cell transplantation, a matched control study. *Blood* 2002; **99**(6)**:** 1978-1985.
22. Nachbaur D, Larcher C, Kircher B, Eibl G, Nussbaumer W, Gunsilius E *et al.* Risk for cytomegalovirus infection following reduced intensity allogeneic stem cell transplantation. *Ann Hematol* 2003; **82**(10)**:** 621-627. doi: 10.1007/s00277-003-0706-1
23. Schetelig J, Oswald O, Steuer N, Radonic A, Thulke S, Held TK *et al.* Cytomegalovirus infections in allogeneic stem cell recipients after reduced-intensity or myeloablative conditioning assessed by quantitative PCR and pp65-antigenemia. *Bone Marrow Transplant* 2003; **32**(7)**:** 695-701. doi: 10.1038/sj.bmt.1704164
24. Boeckh M, Nichols WG. The impact of cytomegalovirus serostatus of donor and recipient before hematopoietic stem cell transplantation in the era of antiviral prophylaxis and preemptive therapy. *Blood* 2004; **103**(6)**:** 2003-2008. doi: 10.1182/blood-2003-10-3616
25. Ljungman P, Perez-Bercoff L, Jonsson J, Avetisyan G, Sparrelid E, Aschan J *et al.* Risk factors for the development of cytomegalovirus disease after allogeneic stem cell transplantation. *Haematologica* 2006; **91**(1)**:** 78-83.