

## Post-transplant events

# Cytomegalovirus (CMV) infections and CMV-specific cellular immune reconstitution following reduced intensity conditioning allogeneic stem cell transplantation with alemtuzumab

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### Summary:

**We studied the incidence and recurrence of Cytomegalovirus (CMV) infection and reactivation in 38 recipients of Alemtuzumab reduced intensity conditioning-stem cell transplantation, and used CMV-HLA tetramer studies to discover if these events correlated with recovery of circulating CMV-specific CD8<sup>+</sup> T cells (cytotoxic T lymphocyte (CTLs)). The cumulative incidence of CMV infection was 60% at 1 year (95% CI, 45–78%) with a median reactivation time of 24 days (range 5–95 days). All patients with CMV reactivation received Ganciclovir or Foscarnet, and only one developed CMV disease. More strikingly, only 8/21 patients had relapse of CMV antigenemia. Tetramer analysis in 13 patients showed that 11 reconstituted CMV CTLs (7/11 by day 30 and 10/11 by day 90). The development of CMV infection was accompanied by a >5-fold rise of CMV CTLs. Recurrence of CMV infection occurred only in the patients who failed to generate a CTL response to the virus. Hence, recipients of SCT using Alemtuzumab-RIC are initially profoundly immunosuppressed and have a high incidence of early CMV reactivation. However, in the majority of patients, infection is transient, and antiviral T cell reconstitution is rapid. Monitoring with CMV-specific CTLs may help identify the subset of patients at risk from recurrent infection or disease.**

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Stem cell transplantation (SCT) using reduced intensity conditioning (RIC) regimens was developed to reduce transplant-related mortality.<sup>1,2</sup> Fewer infectious complica-

tions were anticipated with RIC regimens because they cause less organ damage, and because immune reconstitution may be faster than after conventional myeloablative transplantation.<sup>3,4</sup> However, high rates of graft rejection and of graft-versus-host disease (GVHD) initially reduced the hoped-for benefits on infection rates and overall survival.<sup>5–8</sup> Interest has therefore grown in incorporating lymphocyte-depleting antibodies in RIC regimens: these antibodies should deplete both host and donor T cells and respectively reduce the risks of rejection and GVHD. Since these antibodies have highly specific cellular targets, this increased immunosuppression should not damage other tissues and thereby subvert the purpose of RIC. Nonetheless, augmented immunosuppression – particularly if long lasting – may lead to a higher morbidity and mortality from infection. Cytomegalovirus (CMV) remains the commonest viral infection after allogeneic SCT, and the CD52 monoclonal antibody (MAb) Alemtuzumab is now one of the most widely used lymphocyte depleting antibodies in RIC. However, the extent and duration of functional immunosuppression produced by Alemtuzumab in this setting have not been fully characterized. We therefore analyzed the effects of CD52 lymphodepletion on recovery of CMV-specific CD8 T cells, and on the incidence and recurrence of CMV antigenemia and disease. Our results confirm that CD52 lymphodepletion produces profound functional immunosuppression in recipients, with a high rate of early reactivation of CMV. However, there is rapid recovery of viral-specific T cells, which expand in response to CMV antigenemia, and in the majority of patients, CMV reactivation is neither persistent nor recurrent, and is not associated with disease.

### Patients and methods

#### Patients

A total of 38 patients underwent Alemtuzumab RIC-SCT for malignant disease between 1999 and 2004 at The Methodist Hospital, Houston, TX, USA. Clinical characteristics of the patients are summarized in Table 1. Median age was 58 years (range 20–75 years). Diagnoses

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**Table 1** Patient characteristics

Number of patients	38
Sex (male/female)	23/15
Median age, years (range)	58 (20–75)
<i>Diagnosis</i>	
Acute leukemias	19
Chronic leukemias	3
Myelodysplastic syndrome	6
Lymphomas	6
Multiple myeloma	4
<i>Preparative regimen</i>	
With Alemtuzumab + CD45Mab	13
With Alemtuzumab only	25
<i>Transplant</i>	
Marrow	5
Blood	33
<i>Donor</i>	
HLA identical related	13
HLA identical unrelated	16
Partially mismatched	9
<i>GVHD prophylaxis</i>	
Tacrolimus	38

were acute leukemia ( $n=19$ ), chronic leukemia ( $n=3$ ), myelodysplastic syndrome ( $n=6$ ), lymphoma ( $n=6$ ), multiple myeloma ( $n=4$ ). All patients were transplanted with unmanipulated peripheral blood stem cells ( $n=33$ ) or bone marrow ( $n=5$ ) either from an HLA identical related donor ( $n=13$ ), an HLA identical unrelated donor ( $n=16$ ) or a one antigen-mismatched donor ( $n=9$ ).

Patients were placed in CMV risk groups based on donor and recipient CMV serologies:<sup>9</sup> low risk (seronegative recipient and donor), intermediate risk (recipient negative and donor seropositive) and high risk (recipient seropositive). Patients were considered 'at risk' for CMV reactivation if they were in the intermediate or high-risk groups.

#### Conditioning regimen

In protocol 1 ( $n=25$ ), patients received total body radiation 450 cGy (day -6), fludarabine 30 mg/m<sup>2</sup> daily (days -5 to -2) and Alemtuzumab 10 mg/daily (days -5 to -2). In protocol 2 ( $n=13$ ), patients received total body radiation 450 cGy (day -1), fludarabine 30 mg/m<sup>2</sup> daily (days -8 to -5), Alemtuzumab 10 mg daily (days -8 to -6) and CD 45 MAb (manufactured in the GMP facility at our own institution and by the Therapeutic Antibody Center at the University of Oxford (Professor Geoffrey Hule) 0.4 mg/kg daily (days -5 to -2). CD45 MAb was added in an effort to further intensify recipient immunosuppression: the 8 h half life of these MAb prevented any effect on incoming donor T cells.<sup>10</sup>

#### GVHD prophylaxis

All patients received tacrolimus starting on day -2, at 0.03 mg/kg/day as an intravenous infusion, continued

until engraftment or until oral intake was possible. Doses were adjusted to maintain the blood level between 5 and 15 ng/ml.

#### Antiinfections prophylaxis

We gave all patients prophylactic antibiotics (levofloxacin 500 mg/day) until neutrophil engraftment. We used fluconazole (200 mg/day to day 100) as antifungal prophylaxis and trimethoprim-sulfamethoxazole or pentamidine for prophylaxis of pneumocystis carinii. All patients received acyclovir 5 mg/kg intravenously every 8 h until engraftment and commencement of ganciclovir. Immunglobulins were given once a month through day +100.

#### CMV prophylaxis and treatment

To prevent CMV disease, we intended to give ganciclovir prophylaxis to all at-risk patients after neutrophil engraftment, at 5 mg/kg/twice a day for 2 weeks, and then 5 mg/kg/day for 5 days a week until day +100. Of the patients, 13 received ganciclovir prophylaxis as planned. In all, 22 patients could not be started on prophylaxis due to reactivation before engraftment ( $n=12$ ) or marrow suppression ( $n=10$ ). Three patients were at low risk for CMV infection ( $n=3$ ) and did not get prophylaxis. Treatment for CMV infection as manifested by positive blood CMV antigen or PCR was ganciclovir at 5 mg/kg twice a day for 2 weeks or until negative blood CMV antigen or PCR, then 5 mg/kg/day 5 days a week until day 100. Valganciclovir or foscarnet were substituted for ganciclovir at the discretion of the treating physician.

#### CMV surveillance

Patients were monitored for CMV infection and disease using peripheral blood mononuclear cells (PBMNC) obtained weekly until day +100 and then on each clinic visit. We measured viral antigenemia by immunofluorescence (CMV Brite immunofluorescence kit Biotest Diagnostics, Denville, NJ, USA) and viral DNA by quantitative real-time polymerase chain reaction (PCR) amplification as previously described.<sup>11</sup> Patients with pneumonia were evaluated by bronchoalveolar lavage and lung biopsies. Immunohistochemical staining and shell vial cultures were performed on the samples for CMV. Similarly, immunohistochemical staining and shell vial cultures were performed on all tissue biopsy specimens.

#### CMV-specific CD8<sup>+</sup> CTL analysis

The CMV-specific PE-tetramers were constructed by the Tetramer and Protein Core Facilities at Baylor College of Medicine, Houston, TX, USA. We used the HLA A\*2 restricted CMV pp65 peptide NLVPMVATV (NLV) and the HLA B\*7 restricted pp65 peptide-TPRVTGGGAM (TPR). PBMNC were washed and pelleted and tetramers added at 1:100 final dilutions together with CD3-PerCP and CD8-FITC or isotype control antibodies (BD, Becton Dickinson, Mountain View, CA, USA) in saturating quantities. After incubation for 30 min at room tempera-

ture in the dark, the cells were washed twice with phosphate-buffered saline (PBS; Sigma, St Louis, MO, USA) with 2% FBS and 0.1% sodium azide (Sigma), and then fixed in PBS with 0.5% paraformaldehyde (Sigma) and analyzed within 24 h using a FACSCalibur instrument (BD, Becton Dickinson, Mountain View, CA, USA) using CellQuest software (BD). Antibodies were purchased from Becton Dickinson. The isotype control for antibody staining was IgG1-PE.

### Definitions

CMV infection/reactivation was defined as asymptomatic CMV antigenemia or PCR positivity in peripheral blood. Recurrence of CMV infection was defined as CMV infection occurring after two or more negative antigenemia assays following treatment of the initial episode of infection. Pulmonary CMV disease was defined as positive CMV cultures or antigen detection in a bronchoalveolar lavage specimen in the presence of respiratory signs and symptoms with either pulmonary infiltrates radiologically or histological evidence on lung biopsies. CMV gastrointestinal disease was defined as evidence of CMV by culture, immunohistochemistry or *in situ* hybridization from biopsy specimens.

### Statistical analysis

We used the SPSS software version 10 for statistical analysis. Univariate and multivariate analysis used the Cox regression model to determine the relationship between CMV infection and clinical parameters. Cumulative incidence curves for CMV infection were obtained up to 365 days post transplant. We estimated the overall survival, and time to CMV infection by Kaplan–Meier product limit method.

## Results

### CMV infection and disease

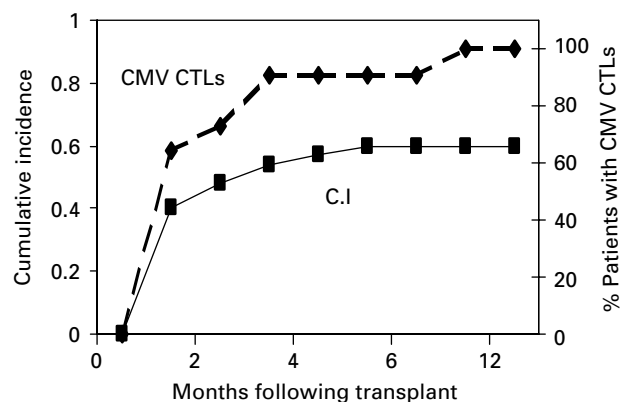
All CMV infections occurred in the intermediate or high-risk groups, which accounted for 35/38 of the patients studied. Of the patients at risk, the cumulative incidence of CMV infection at 1 year post transplant was 60% (95% CI, 46–80%). The first CMV infections occurred early post transplant, at a median time of 24 days (range 5–95 days). In all, 22 patients could not be started on ganciclovir prophylaxis due to reactivation before engraftment ( $n = 12$ ) or marrow suppression ( $n = 10$ ), and 21 of these patients developed CMV infection. All initial episodes of CMV infection were before day 100. Only one patient developed CMV disease (colitis), which was ganciclovir and foscarnet responsive. There was no CMV-related mortality. The incidence of CMV infection in the subset of patients who received anti-CD45 MAb in addition to Alemtuzumab was no different from those who received only Alemtuzumab, 54% (7/13) vs 60% (14/23), respectively,  $P = 1.0$ . Also, there was no difference in the incidence of CMV infection in patients who received allografts from a related vs unrelated donor,  $P = 0.8$ .

### Treatment and recurrence of CMV infection

Ganciclovir was the first-line therapy in 62% (13/21) of patients, valganciclovir in 33% (7/21) and foscarnet in 5% (1/21). In all, 19% (4/21) of patients required a change in therapy either due to nonresponsiveness ( $n = 1$ ) or marrow suppression ( $n = 3$ ). The median time for clearance of infection measured by CMV antigenemia and PCR assays was 14 days (range, 6–42 days). Of patients at risk, 23% (8/35) developed recurrent CMV infection. The median number of recurrences was 2 (range, 1–6). In these recurrent episodes, CMV clearance occurred at a median interval of 9 days (range 6–15 days) after initiation of treatment. All recurrences occurred when patients were off ganciclovir maintenance treatment due to marrow suppression.

### Recovery of CMV-specific CTLs

Recovery of CMV-specific CTLs was serially monitored in 13 patients post transplant who had informative HLA types (Table 2). Seven of these patients developed CMV infection and one patient developed CMV colitis. One patient died in the early post transplant period and could not be monitored beyond day 50. He did not reconstitute in this short monitoring period. One patient (number 12), did not reconstitute CMV CTLs. He developed graft rejection and was serologically CMV negative with a graft from CMV-positive donor. Of the 11 evaluable patients, seven developed a CMV CTL response within the first month of transplantation and 10 by day 90, (Figure 1). The pattern of recovery was not apparently influenced by the CMV serology of the donor (Table 2) The average percentage of tetramer positive CD8 cells detected at day 30 was 5.9% (95% CI, 0–13%) and at day 90 was 7% (95% CI, 0–17%). Strikingly, in five of six patients who developed CMV infection, evidence of viral activity was accompanied by a rise in CMV CTLs (Figure 2b). The mean percentage of CMV CTLs detected prior to CMV infection was 1.4% compared to 7.3% following infection, representing a mean 5.2-fold rise. Only one of the 11 patients failed to recover CMV-tetramer positive T cells by day 90. This individual

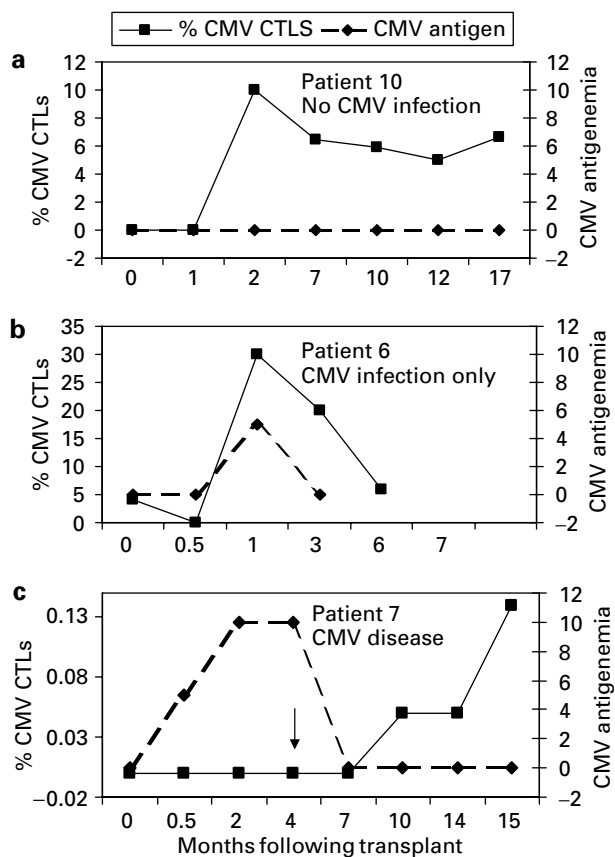


**Figure 1** Cumulative incidence of CMV infection and percentage of patients with CMV CTLs reconstitution following transplantation.

**Table 2** CMV-specific cytotoxic T lymphocytes in post transplant period

Group/patient no.	Recipient/donor CMV status	HLA allele	CMV-specific CD8 <sup>+</sup> lymphocyte		
			Day first detected	Peak % of CD8 <sup>+</sup> count	CMV infection (days post-SCT)
<i>CMV infection</i>					
1	+/+	A2	90	0.01	33, 123
2	+/-	A2	15	0.18	7
3	+/+	A2	15	5.3	23
4	+/+	A2	30	1.05	9
5 <sup>a</sup>	+/+	A2	ND	ND	39
6	+/-	A2	14	30	35
<i>CMV disease</i>					
7	+/-	A2, B7	420, 300	0.05, 0.14	24, 97
<i>No CMV infection</i>					
8	+/-	A2	14	0.92	None
9	+/-	A2	90	0.13	None
10	+/+	A2, B7	None, 60	10	None
11	+/-	A2	30	13.8	None
12	-/+	A2, B7	ND	ND	None
13	-/+	A2	30	0.05	None

<sup>a</sup>Patient died early, CTL could not be monitored beyond day 50.  
ND = not detected.



**Figure 2** Reconstitution of CMV-specific CTLs in exemplary patients following SCT. (a) patient numbered 10 did not develop CMV infection or disease; (b) patient numbered 6 developed CMV infection only; (c) patient numbered 7 developed CMV disease at 4 months. Percentage of CMV CTLs and CMV antigenemia are shown on the y-axis and months following transplant on the x-axis.

was the only patient who developed CMV disease (colitis) (Figure 2c).

#### Overall outcome

One patient developed Grade III gut GVHD. Neither univariate nor multivariate analysis revealed any relationship between the occurrence of GVHD of any grade and CMV reactivation. Five patients died of (non-CMV) infections (one each of *Staphylococcus aureus* sepsis, *Pseudomonas* sepsis, disseminated adenovirus, disseminated toxoplasmosis and *Candida* spp with vancomycin-resistant enterococcal sepsis). One patient died of idiopathic pneumonitis and one of graft rejection. Kaplan–Meier estimate of survival at 3 years was 49%. CMV infection had no impact on overall or disease-free survival.

#### Discussion

In our study, the cumulative incidence of CMV infection was 60% at 1 year (95% CI, 45–78%) with a median reactivation time of just 24 days (range 5–95 days). All patients with CMV reactivation were treated with ganciclovir and/or foscarnet, and only one patient developed CMV-disease. More strikingly, only 8/21 patients had relapse of CMV antigenemia once antiviral medication was withdrawn. Tetramer analysis in 13 patients with informative HLA types showed that 11 reconstituted CMV CTLs (7/11 by day 30 and 10/11 by day 90). Moreover, the development of CMV infection/reactivation in these patients was accompanied by a >5-fold rise of CMV-specific CTLs. Recurrence or persistence of CMV infection occurred only in the patients who failed to generate a CTL response to the virus.

RIC regimens have helped broaden the applicability of SCT, but the incidence of graft rejection has been reported to be between 0 and 21%, while severe/fatal GVHD may occur from 10 to 20%.<sup>5-8</sup> One way to reduce these risks may be to use lympholytic monoclonal antibodies, depleting both the host immune system (to enhance engraftment), and the engrafted donor T cells (to reduce GVHD). Alemtuzumab fulfills both these requirements. Given pre-transplant, it depletes recipient lymphocytes, thus enhancing engraftment, and because of its long half-life, it remains in circulation for several weeks following stem cell infusion, thereby depleting donor T cells and reducing GVHD.<sup>12-14</sup> Monoclonal antibodies targeting the common leucocyte antigen CD45<sup>15</sup> (an antigen present on hematopoietic precursors and all leucocytes), may complement Alemtuzumab, since they enhanced engraftment in mismatched murine stem cell transplant model<sup>16</sup> and were well tolerated and lymphodepleting in a phase I study in patients with advanced hematological malignancy.<sup>10</sup> Moreover, CD45 MAb has a short half-life therefore affect the recipient cells without damaging engraftment.<sup>10</sup> Consistent with these activities, in our study, both graft rejection and GVHD were very low; only one patient developed graft rejection and one grade III-IV GVHD.

Pharmacokinetic studies have shown that Alemtuzumab levels were detectable even up to 28 days after the last infusion, and the estimated time for clearance from the blood was 60 days.<sup>17</sup> As expected, profound and prolonged lymphopenia was observed in previous studies of Alemtuzumab in patients with advanced B cell malignancies or rheumatoid arthritis.<sup>18-20</sup> There has been a variable incidence of opportunistic infections.<sup>18-22</sup> The incidence of CMV infection following nonmyeloablative SCT containing fludarabine-based conditioning regimens but without anti-lymphocyte antibody has been reported to be from 21 to 53%.<sup>23,24</sup> The much higher incidence of CMV infections after RIC with Alemtuzumab has been reported previously.<sup>25</sup> The total dose of Alemtuzumab used in the RIC regimen in this earlier study was 100 mg compared to 40 mg in our study. Although immune reconstitution measured as recovery of CD4<sup>+</sup> T cell count was reportedly delayed, CMV-specific CD8<sup>+</sup> CTL were not measured in this study. Therefore, the effect of an increased Alemtuzumab dose on CMV CTL recovery is not yet known.

However, our data also show that the high incidence of CMV infection observed predominantly in the early post transplant period can readily be controlled with antiviral medications. Withdrawal of antiviral agents in general was not accompanied by reactivation, and tetramer studies showed the speedy reconstitution of CMV-tetramer-specific CTL. CMV-specific CTLs increased after episodes of CMV reactivation following stem cell transplant, as previously reported.<sup>26</sup> There was no evidence that addition of CD45 MAb worsens immune recovery as there was no difference between CMV infection rate/tetramer recovery between groups with or without CD45 MAb ( $P=1.0$ ). A similar, rapid recovery of T cells was shown at the end of Alemtuzumab therapy in chemo-refractory CLL patients.<sup>22</sup> Patients receiving SCT of product depleted *ex vivo* with Alemtuzumab showed rapid recovery of CD8<sup>+</sup> cells.<sup>27</sup>

Our data suggest that the preventive strategies are required to decrease the high incidence of early CMV infection following RIC with Alemtuzumab. Antiviral agents can be used in prophylactic or pre-emptive protocols but drug-related toxicities limit their use in the early post transplant period. As an alternative, adoptive transfer of CD8<sup>+</sup> CTLs can restore the antiviral immunity post-allogeneic SCT.<sup>28</sup> Monitoring CMV-specific T cell recovery after RIC may help to identify the patients at risk of CMV infections and define the appropriate candidates for immune intervention.

In conclusion, recipients of SCT using Alemtuzumab-RIC are initially profoundly immunosuppressed and have a high incidence of early CMV reactivation. However, in the majority of patients infection is transient, and antiviral T cell reconstitution is rapid. Thus, despite the 'reduced intensity' of the conditioning, these patients require early, and comprehensive pre-emptive or prophylactic therapy for opportunistic infections. Alternatively, immune intervention may be instituted in early post transplant period, and also in patients in whom recovery of viral-specific immunity is delayed. Monitoring CMV tetramer positive cells may help identify this vulnerable group.

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