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Viral infections

High-dose acyclovir and pre-emptive ganciclovir to prevent cytomegalovirus disease in myeloablative and non-myeloablative allogeneic stem cell transplantation

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Summary:

We evaluated high-dose acyclovir and pre-emptive ganciclovir to prevent cytomegalovirus disease in myeloablative and non-myeloablative allogeneic stem cell transplantation. One hundred and seventy-four consecutive patients who were at risk for CMV infection (either recipient or donor seropositive) and received either intensive chemoradiotherapy and a T cell-depleted stem cell transplant followed by delayed add-back of donor T cells (TCDT: $n = 98$), or a non-myeloablative preparative regimen followed by an unmanipulated peripheral blood stem cell transplant (NMT: $n = 76$) from an HLA-identical sibling donor were studied. All received high-dose acyclovir (HDACV) from day -7 for 3 months post-transplant in conjunction with weekly CMV pp65 antigenemia monitoring and pre-emptive treatment with intravenous immunoglobulin (not CMV-specific) and ganciclovir. The actuarial probabilities of developing pp65 antigenemia were $83 \pm 4\%$ after TCDT and $41 \pm 6\%$ after NMT ($P < 0.00001$) with reactivation occurring earlier in the TCDT group (the median 36 days vs 55 days). We observed no reactivation of CMV in seronegative recipients with a seropositive donor ($n = 23$). A total of 11 patients (5 in TCDT, 6 in NMT) developed CMV disease within 400 days after transplantation, and one death was clearly attributable to CMV interstitial pneumonitis (IP). This strategy was associated with effective control of CMV antigenemia in the majority of patients and near-complete eradication of fatal CMV IP.

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Following allogeneic stem cell transplantation (SCT), patients previously exposed to cytomegalovirus (CMV) are at risk from potentially fatal reactivation of the virus which can cause interstitial pneumonia (IP), gastroenteritis, retinitis, myelitis, myelosuppression and occasionally graft failure.[1] Donor CMV seronegativity, T cell depletion, and SCT from donors other than HLA-identical siblings increase the risk of CMV disease.[2–4] Until the development of effective antiviral drugs, mortality from CMV reactivation could reach 20%. Ganciclovir (GCV) and foscarnet are effective agents in the prevention and treatment of CMV disease.[5,6] However, these antiviral agents are not without disadvantages: ganciclovir causes neutropenia and an increased risk of bacterial and fungal infection;[7–12] resistance to antiviral drugs can develop and the treatment can delay recovery of CMV-specific immunity.[8,12–15] In order to minimize the use of GCV or foscarnet, many investigators use a pre-emptive treatment strategy performing regular blood monitoring for CMV antigen or DNA (using techniques which have varying sensitivity) and treating at the first indication of viremia.[16–19] An alternative way to reduce the need for ganciclovir or foscarnet is to use high-dose acyclovir (HDACV) (1.5 g/m²/day) as prophylaxis for CMV disease. Although CMV is less sensitive to inhibition by ACV,[20] the agent has been used in high doses with partial success in a few studies to prevent or delay CMV reactivation.[21–23] When administered for up to 6 months post-transplant HDACV also reduced late CMV infection and improved survival when compared to standard treatment doses,[22,23] while one study showed no clear benefit using HDACV followed by prophylactic GCV.[24] Using a total body irradiation (TBI)-based, T cell-depleted transplant protocol and pre-emptive treatment of CMV antigenemia with GCV, we previously reported a high incidence (25%) of fatal CMV IP in patients at risk from CMV reactivation.[25] Subsequently, in all allogeneic transplant patients at risk for CMV infection, we have used HDACV from day -7 for 3 months post-transplant in conjunction with weekly CMV antigenemia monitoring and pre-emptive treatment with intravenous immunoglobulin and GCV. Here we report the outcome of this strategy in two large cohorts of patients receiving either intensive chemoradiotherapy and

a T cell-depleted SCT followed by delayed add-back of donor T cells (TCDT), or a non-myeloablative preparative regimen followed by an unmanipulated peripheral blood SCT (NMT). This strategy was associated with effective control of CMV antigenemia in the majority of patients and near-complete eradication of fatal CMV IP.

Patients and methods

Patients

Between May 1995 and June 2001, 227 consecutive patients underwent allogeneic stem cell transplantation (SCT) from an HLA-identical sibling donor. Of these, 174 patients were considered at risk for CMV infection (recipient and/or donor seropositive) and were analyzed in this study. The remaining 53 patients (both recipient and donor seronegative) did not receive HDACV and were excluded from this study. All patients gave written informed consent for National Institute of Health protocols (Protocols: 93-H-0212, 94-H-0092, 94-H-0182, 95-H-0099, 97-H-0099, 99-H-0046, 97-H-196, 97-H-0202, 98-H-0006, 99-H-0050, 99-H-0064, 00-H-0001, 01-H-0010) approved by the National Heart, Lung, and Blood Institute (NHLBI) institutional review board. Details of patient characteristics and outcomes are shown in Table 1. Ninety-eight patients with hematologic malignancies received a TCDT, and 76 patients received a NMT for hematologic disorders and solid tumors. In the TCDT cohort, patients with a diagnosis of chronic myelogenous leukemia (CML) in chronic phase (CP), acute leukemia in first complete remission, or low-grade untreated myelodysplastic syndrome (refractory anemia and refractory anemia with excess blasts subtypes) were considered standard risk. All other diagnoses were considered high risk.

Standard transplantation approaches used in both TCDT and NMT

Quantitation of $CD34^+$ and $CD3^+$ cells: $CD34^+$ and $CD3^+$ cells were quantitated by automated leukocyte counting (CellDyn 3500, Abbott, Palo Alto, CA, USA) and flow cytometry using the FACScan with CellQuest software (Becton Dickinson, Mountain View, CA, USA). For flow cytometry, samples of PBSC were stained with fluorochrome-labeled antibodies to CD34, CD3, and CD45 (Becton Dickinson) and with 7-AAD for concurrent evaluation of viability. The sum of the cell doses of all infused products was expressed as a dose per recipient body weight in kg. Lymphocytes collected for post-transplant DLI were quantitated in a similar fashion.[26]

Post-transplant management: All patients received fluconazole from day −7 until day +30 for antifungal prophylaxis. Weekly trimethoprim/sulfamethoxazole was started from day +30 to day +180 as prophylaxis against *Pneumocystis* infection. The diagnosis and grading of acute and chronic GVHD were established according to the Seattle criteria.[27] Acute GVHD ≥grade II was treated with corticosteroids tapered according to response.

Prophylaxis and treatment for CMV: All patients received high-dose acyclovir (500 mg/m^2 i.v. every 8 h, or 800 mg p.o. four times a day) at least to day +100 post transplant as prophylaxis against CMV and herpesvirus reactivation.[22] Blood was tested for CMV pp65 antigen (BIOSOFT CIN-kit, Argene, N Massapequa, NY, USA) weekly until day +100 post PBSCT and longer if clinically indicated. Patients who underwent TCDT received intravenous immunoglobulin (not CMV-specific) at 500 mg/kg weekly until day +30 post-transplant. Pre-emptive GCV therapy was instituted whenever a patient had a positive pp65 antigenemia test defined as at least one positive cell per 400 000 white blood cells examined.[25] Patients received GCV at an induction dose of 5 mg/kg i.v. twice daily for 10 days, followed by maintenance therapy at 5 mg/kg three times weekly, continued until antigenemia tests remained negative for 3 weeks. Re-induction with twice-daily dose was started at recurrence of a positive pp65 antigenemia test. A dose of intravenous immunoglobulin at 1000 mg/kg was also given during the first week of antigenemia and weekly at 500 mg/kg during the period of antigenemia. Foscarnet (FSC) was used for neutropenic patients or GCV-refractory cases at the discretion of the treating physician.

Transplantation approaches used in TCDT

Of 98 patients who underwent TCDT, 96 received a conditioning regimen of fractionated TBI, 13.6 Gy in eight fractions over 4 days, followed by cyclophosphamide, 60 mg/kg for 2 days. In two patients who had received extensive previous radiation, busulphan 16 mg/kg and cyclophosphamide, at 200 mg/kg were given over 4 days. The first 27 patients received a bone marrow transplant (BMT) depleted of T cells by elutriation or the Ceprate TCD selection system (CellPro, Bothell, WA, USA) as described previously.[28,29] The remaining 71 patients received a granulocyte colony-stimulating factor (GCSF, filgrastim; Amgen, Thousand Oaks, CA, USA) mobilized peripheral blood stem cell transplantation (PBSCT) depleted of T cells either by the Ceprate TCD selection system or the Isolex 300i immunomagnetic cell selection system (Nexell Therapeutics, Irvine, CA, USA) as described previously.[26] G-CSF, 5 μg/kg, was given daily from day +7 until the neutrophil count exceeded 1×10^9/l for 3 consecutive days.

Cyclosporin A (CsA) 3 mg/kg i.v. was started on day −4 until an oral dose was tolerated and continued until at least day +130 post-PBSCT, and longer if chronic GVHD occurred. In the first 61 patients, the CsA dose was adjusted to maintain a cyclosporine plasma level between 200–400 μl/ml. Because the incidence of early acute GVHD was low and our T cell depletion using the Isolex 300i immunomagnetic cell selection system enabled delivery of a fixed CD3 cell dose at 5×10^4/kg, the target plasma level was decreased to 100–200 μl/ml in the subsequent 19 patients. The last 18 patients received no cyclosporine post transplant.

To prevent relapse and confer donor immune function, patients received cryopreserved peripheral blood mononuclear cells from the donor, obtained by leukapheresis. Three add-back schedules were evaluated: (1) 2×10^6

Table 1 Patient characteristics

	TCD (n = 98)	NMT (n = 76)	P value
Mean patient age (range)	36.8 (10–58)	48.3 (14–71)	<0.0001
Mean donor age (range)	36 (6–62)	46.6 (6–72)	<0.0001
Patient sex (F/M)	41/57	18/58	0.01 ($\chi^2 = 6.3$)
HLA-identical and related donor	100%	100%	
CMV serology			
R+/D+	77 (78.6%)	40 (52.6%)	
R+/D−	14 (14.3%)	20 (26.3%)	
R−/D+	7 (7.1%)	16 (21.1%)	0.007[a] ($\chi^2 = 7.2$)
Sex match			
Match	53	37	
M to F	23	11	
F to M	22	28	0.04[b] ($\chi^2 = 4.3$)
Transplant cell doses			
Mean CD34 $\times 10^6$/kg (range)	4.62 (0.55–14.5)	8.08 (2.2–21.1)	<0.001
Mean CD3 $\times 10^5$/kg (range)	1.22 (0.25–6.03)	3500 (1100–8800)	<0.0001
Stem cell source			
Bone marrow	27	0	<0.0001 ($\chi^2 = 24.8$)
Peripheral blood	71	76	
GVH prophylaxis			
CsA/MMF	0	31	
CsA	61	45	
1/2 CsA	19	0	
No CsA	18	0	
Diagnosis			
CML	42	10	
AML	25	1	
MDS	12	7	
ALL	7	0	
MM	6	5	
CLL	3	5	
CMML	2	2	
Lymphoma	1	6	
Solid tumors[c]	0	36	
AA/PNH	0	3	
Systemic mastocytosis	0	1	
Disease status (high/low)	54/44	NA	

R+ (−) = recipient CMV seropositive (negative); D+ (−) = donor CMV seropositive (negative); CsA = cyclosporin A; MMF = mycophenolate mofetil; RCC = renal cell carcinoma.
[a]Seronegative recipients *vs* seropositive recipients; [b]Female donor to male recipient *vs* other combinations; [c]Solid tumors include renal cell carcinoma (*n* = 26), melanoma (*n* = 6), and others (*n* = 4).

CD3$^+$ cells/kg on day +30 followed by 5×10^7 CD3$^+$ cells/kg on day +45; (2) 1×10^7 CD3$^+$ cells/kg on day +30; (3) 1×10^7 CD3$^+$ cells/kg on day +45, followed by 5×10^7 CD3$^+$ cells/kg on day +100. Patients developing grade II or greater acute GVHD after transplant were excluded from DLI if GVHD was still active and requiring corticosteroid treatment. Patients with CML-CP in cytogenetic remission on day +100, and therefore at low risk for relapse, were excluded from day +100 DLI.[26,28,30] Thirteen patients received no DLI, and the remaining 85 patients received total DLI doses ranging between $0.2–22.8 \times 10^7$/kg (median 5.2×10^7/kg).

Transplantation approaches used in NMT

The preparative regimen consisted of cyclophosphamide 60 mg/kg on days −7 and −6, followed by fludarabine 25 mg/m^2 intravenously (i.v.) on days −5 to −1. Antithymocyte globulin 40 mg/kg days −5 to −2 was added to the conditioning regimen in patients with a history of multiple transfusion (*n* = 3). Cyclosporin A was given from day −4, initially as an i.v. dose of 3 mg/kg daily. Oral CsA, 5 mg/kg twice daily, was substituted when tolerated. Levels were maintained at a plasma level between 200–400 μl/ml. Because of significant GVHD in the first 31 patients, MMF was added as GVHD prophylaxis in the last 45 patients: MMF was started on day 0 and discontinued in conjunction with CsA. Decisions regarding tapering of CsA and administration of DLIs following NST were based on the presence or absence of GVHD, status of the underlying malignancy, and degree of donor/host T cell chimerism.[31,32] Briefly, for patients with complete donor T cell chimerism on day 30, CsA taper was begun on day 60 and discontinued on day 100 if GVHD did not occur. For patients with mixed T cell chimerism on day 30, CsA was tapered over a 2-week period. Patients not converting to 100% donor T cell chimerism after CsA withdrawal received monthly escalating doses of DLI with weekly reassessment

of chimerism until 100% donor T cell chimerism, GVHD, disease regression, or graft rejection occurred.

Definition of CMV disease

Cytomegalovirus disease was defined as the demonstration of CMV by biopsy specimen from visceral sites (by culture or histology) or the detection of CMV by shell vial culture or direct fluorescent antibody stain on bronchoalveolar lavage fluid in the presence of new or changing pulmonary infiltrates. Cytomegalovirus retinitis was diagnosed based on ophthalmological clinical examinations.[33]

Statistical analysis

Patient characteristics in the two cohorts were compared using Pearson's chi-square test or the Student's t-test, where appropriate. Actuarial probabilities of acute GVHD, relapse, survival and time to the first positive antigenemia were calculated by the method of Kaplan and Meier.[34] Differences between outcomes were compared using the log-rank test. Cox multivariate analysis was used to determine relative risk for the independent risk factors. The following factors were entered into the model as discontinuous variables: age (above or below the median), donor sex match (female into male recipient vs other combinations), transplant CD34$^+$ and CD3$^+$ cell doses (above or below the median), and serological CMV status. Additionally, source of stem cells (BMT vs PBSCT) and disease risk status (high vs standard risk) in TCDT, and the use of MMF and underlying disease (hematologic vs solid tumor) in NMT were also entered into the model.

Results

Patients

Characteristics of the 176 consecutive patients analyzed are shown in Table 1. Follow-up data, including cases of CMV disease and causes of death, were available for all patients. No patients developed permanent nephrotoxicity directly from high-dose ACV. All surviving patients were followed for at least 100 days after transplantation.

Transplant outcomes

Transplant outcomes in TCDT and NMT are summarized in Table 2. After a median follow-up of 1005 days (range 104–2225) for surviving patients, 49 TCDT patients are alive with an actuarial survival of $45 \pm 6\%$. In NMT, 33 patients are alive with a median follow-up of 690 days (151–2005) with an actuarial survival of $38 \pm 6\%$. Of 49 deaths in TCDT and 43 deaths in NMT, 58 were due to relapse or disease progression. Of the remaining 34 patients, one died of CMV pneumonitis, 13 patients died of other infectious causes (viral other than CMV, 3; bacterial, 5; fungal, 5), eight died of GVHD, and four died of idiopathic interstitial pneumonitis/ARDS (Table 2).

Table 2 Transplant outcomes

	TCD (n = 98)	NMT (n = 76)	P
Median follow-up (range)	1005 days (104–2225)	690 days (151–2005)	
Overall survival[a]	$45 \pm 6\%$	$38 \pm 6\%$	0.3
(survival day 100)[a]	$(85 \pm 4\%)$	$(84 \pm 4\%)$	
Acute GVHD grade II–IV[a]	$48 \pm 6\%$	$56 \pm 6\%$	0.3
CMV pp65 antigenemia[a]			
≥ 1	$83 \pm 4\%$	$41 \pm 6\%$	<0.00001
≥ 2[b]	$59 \pm 7\%$	$28 \pm 5\%$	0.00001
CMV disease			
<100 days	2 (2%)	5 (6.5%)	
100–400 days	3 (3%)	1 (1%)	
Causes of non-relapse death			
CMV pneumonitis	1	0	
Infections other than CMV (total)	(7)	(6)	
Viral	2	1	
Bacterial	2	3	
Aspergillosis	3	2	
GVHD	4	4	
Idiopathic IP/ARDS	4	0	
Others	5	3	

GVHD = graft-versus-host disease; IP = interstitial pneumonitis; ARDS = adult respiratory distress syndrome.
[a]Values were calculated by the Kaplan–Meier method, and P values were given by log-rank test.
[b]Reactivation on more than two occasions (either recurrent or persistent antigenemia).

CMV pp65 antigenemia

A total of 104 patients developed positive pp65 antigenemia. Thirty-four patients had only one positive test; the remaining 70 patients developed a second positive test, either as persistent antigenemia ($n = 44$, 63%) or as recurrent antigenemia ($n = 25$, 37%). Ninety percent of second occurrences of pp65 antigenemia were within 8 weeks from the first reactivation. Forty-nine patients had one positive cell on their first pp65 antigenemia, and the remaining 55 patients had two or more positive cells (range, 2–73 cells; median: 4 cells). Of the 55 patients with an initial antigenemia level ≥ 2 positive cells, 46 (84%) developed persistent or recurrent pp65 antigenemia, compared to 24 (45%) of 49 patients whose initial antigenemia level was only one positive cell ($P < 0.001$) (Figure 1). The actuarial probabilities of developing pp65 antigenemia were $83 \pm 4\%$ in TCDT and $40 \pm 6\%$ in NMT ($P < 0.00001$), and the median days of the first pp65 antigenemia were 36 days with TCDT and 55 days with NMT (Table 1, Figure 2). Among the NMT patients who had pp65 antigenemia, 83% (24/29) were persistent or recurrent antigenemia, compared to 61% (46/75) in TCDT ($P = 0.04$). Similarly, at the first reactivation, the proportion of patients with high level antigenemia (pp65 positive cells >2) was higher with NMT (22/29) compared with TCDT ($P = 0.04$). The results indicate that although the risk of developing pp65 antigenemia was much greater in TCDT, a significant proportion of patients were able to clear the pp65 antigenemia, whereas with NMT, while the incidence of reactivation was lower,
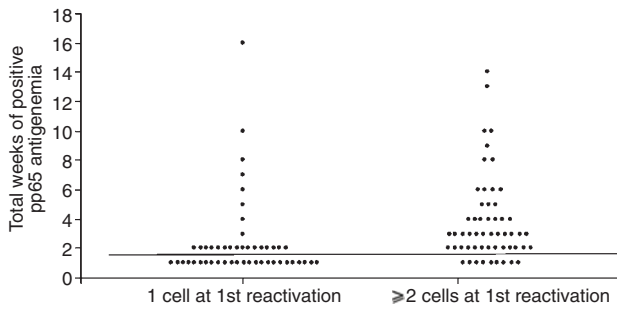
Figure 1 Two or greater positive cells at first CMV pp65 antigenemia was associated with higher rate of subsequent reactivation (persistent or recurrent reactivation). In 49 patients with one cell positive at the first reactivation, 45% ($n = 24$) developed subsequent positive antigenemia compared to 84% ($n = 46$) in patients with two or greater cells positive ($n = 55$) ($P = 0.0002$).
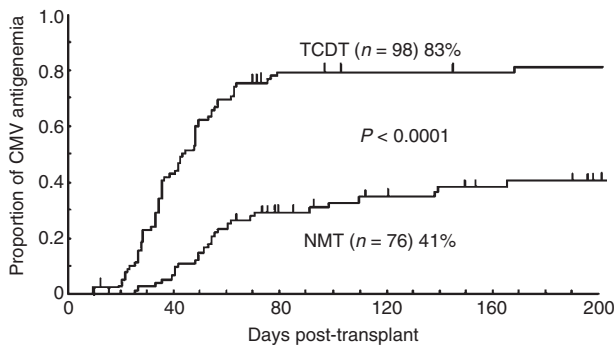

Figure 2 Kaplan–Meier estimates of cumulative incidence of CMV pp65 antigenemia in TCD and NMT groups (83% vs 41%, $P < 0.0001$).

there was a tendency towards a higher antigen load and more prolonged antigenemia. Nevertheless, the overall incidence of recurrent or persistent pp65 antigenemia was still higher with TCDT compared to NMT (59% vs 28%, $P = 0.00001$) (Table 2).

CMV disease and outcomes

A total of 11 patients (5% in TCD, 7% in NMT) developed CMV disease within 400 days after transplantation (Table 3). Of these, one developed CMV pneumonitis, four developed gastroenteritis, one developed CMV hepatitis, and two developed CMV retinitis. Four patients (UPN 180, 221, 253, 259) had a positive shell vial culture from bronchoalveolar lavage (BAL) samples in the presence of new or changing pulmonary infiltrates. Two patients are still alive at days 276 and 1304. Four deaths were unrelated to CMV disease; disease progression in two, diabetes and hypoglycemic coma in one, and metastatic adenocarcinoma in one. One patient died of CMV pneumonitis on day +265. The remaining four patients had a positive CMV shell vial culture from BAL and died within 6 weeks. These four patients also had fungal or bacterial isolates from the lungs (UPN 221, 253, 259), or had systemic sepsis (UPN 180) at the time of CMV isolation. These other organisms persisted during the clinical course, and were the predominant infectious problem rather than the CMV infection. Deaths were attributed to GVHD in two patients (UPN 180 and 259)

and to other infectious organisms in the remaining two patients (UPN 221, persistent adenovirus infection; UPN 253, aspergillosis and *Pseudomonas* pneumonia).

Notably, nine of these 11 patients (82%) had grade II or more acute GVHD compared to 71 (43%) of the remaining 164 patients ($P = 0.02$). Although variable, some of these patients had high numbers of pp65 positive cells at first reactivation (Table 3). With NMT, five patients with hematologic malignancies developed CMV disease whereas only one patient with a solid tumor developed CMV disease. Five NMT patients developed CMV disease without preceding pp65 antigenemia. Of these, one (UPN 155) developed CMV retinitis, one (UPN 86) was not on surveillance at the time of CMV disease and was found to be antigenemia positive a week later. The remaining three had a positive CMV culture from the BAL specimen without preceding positive antigenemia.

Risk factors for CMV reactivation

With this HD ACV prophylaxis, we observed no reactivations of CMV in seronegative recipients with a seropositive donor. With TCDT, a positive pp65 antigenemia test was seen in 75 of 91 seropositive recipients (83%) compared to none of seven seronegative recipients ($P < 0.0001$, $\chi^2 = 24.6$). With NMT, a positive pp65 antigenemia test was seen in 27 of 60 seropositive compared to none of 16 seronegative recipients ($P = 0.0001$, $\chi^2 = 11.2$). As shown earlier (Figure 2), we observed a significant difference in the probability of pp65 antigenemia between TCDT and NMT. When only seropositive recipients were analyzed, the actuarial probabilities of developing pp65 antigenemia were 88.7% with TCDT and 52.4% with NMT ($P < 0.00001$). Because patient characteristics with TCDT and NMT were widely different, further risk factor analysis for CMV events was performed separately for each group, and only for seropositive recipients. In both cohorts, age (above or below the median), donor sex match (female into male recipient *vs* other combinations), or transplant CD34^{+} and CD3^{+} cell doses (above or below the median) were not significant factors for CMV reactivation in univariate analysis. Likewise, source of stem cells (BMT *vs* PBSCT) or disease risk status (high *vs* standard risk) in TCDT, and use of MMF or disease type (hematologic *vs* solid tumor) in NMT had no significant impact on the incidence of CMV reactivation.

Discussion

Since we experienced a high mortality of 25% from CMV pneumonitis in our first cohort,[25] we have adopted an approach using HDAVC for prophylaxis, and early institution of GCV at a low level of CMV pp65 antigenemia (one cell/400 000 cells). With this approach, the overall incidence of CMV disease (early and late) was 5% in TCD and 8% in NMT, and in only one patient death was attributable to CMV pneumonitis in 174 patients at risk for CMV infection. These results are superior to the CMV outcomes in our historic control, in which a pre-emptive GCV approach was also used, starting at the same level of CMV

Table 3 Transplant characteristics and outcomes in patients who developed CMV disease

UPN	Age	Sex	Diagnosis	CMV serostatus	CD34+ cell dose (10^6/kg)	CD3+ cell dose (10^5/kg)	GVHD prophylaxis	aGVHD (days)	Days of 1st antigenemia	Weeks of antigenemia	Positive cells at first reactivation	CMV disease (days)	Outcome
TCD													
78	48	M	CML-AP	R+/D+	4.84	0.51	CsA	II (78)	41	1	1	lung (228)	died (265) CMV-IP
108	50	M	MDS (REAB)	R+/D+	2.43	0.95	CsA	II (29)	43	10	41	liver (92)	died (320) adeno ca
225	33	F	AML refractory	R+/D+	7.99	0.5	1/2 CsA	III (64)	28	13	26	GI (68)	died (180) relapse
254	42	M	CML-CP	R+/D+	6.75	0.5	no CsA	no	43	4	12	retinitis (136)	alive (276)
259	45	M	MDS RAEB(T)	R+/D−	7.42	0.5	no CsA	II (77)	36	2	1	GI, BAL (203)	died (230) GVHD
NMT													
86	50	M	RCC	R+/D−	9.1	2000	CsA	no	140	1	26	GI (133)	alive (1304)
155	57	M	NHL	R+/D+	5.1	4000	CsA	III (42)	no antigenemia	0	NA	retinitis (64)	died (748) encephalopathy
169	31	M	HD	R+/D−	6.7	3800	CsA	III (87)	36	14	13	GI (353)	died (369) progressive disease
180	44	F	MM	R+/D+	5.9	3900	CsA	IV (22)	no antigenemia	0	NA	BAL (31)	died (41) GVHD
221	71	M	MM/NHL	R+/D+	5.9	8800	CsA/MMF	II (53)	no antigenemia	0	NA	BAL (49)	died (75) adenovirus
253	67	M	CLL	R+/D+	8.7	3600	CsA/MMF	II (29)	40	2	9	BAL (31)	died (75) fungal/bacterial pneumonia

CML-CP or AP = CML in chronic phase or accelerated phase; RAEB (T) = refractory anemia with excess blasts (in transformation); NHL = non-Hodgkin lymphoma; HD = Hodgkin's disease; MM = multiple myeloma; CLL = chronic lymphocytic leukemia; R = recipient; D = donor; CsA = cyclosporin A (1/2: half dose); MMF = mycophenolate mofetil; CMV disease: lung, GI, and liver = tissue diagnosis, retinitis = clinical diagnosis by ophthalmology; BAL = positive shell vial culture from BAL samples (see text); PD = progressive disease.

pp65 antigenemia. Therefore, besides differences in patient clinical characteristics, the only practical change for preventing CMV disease was the introduction of HDACV.

Cytomegalovirus is less sensitive to inhibition by ACV than herpes simplex or varicella-zoster viruses *in vitro*.[20] Two studies have shown improved survival in patients given HDACV;[21–23] however, one study showed no clear benefit in using HDACV until engraftment followed by GCV prophylaxis.[24] There has been no report using HDACV in combination with pre-emptive GCV. Because this was a single institution, non-randomized study, it was not possible to directly compare our results with other approaches using various CMV detection methods and prophylactic regimens. In a randomized study comparing prophylactic and pre-emptive GCV,[9] the incidence of early CMV disease (occurring before day +100) was 2.7% in the prophylactic arm compared to 14.1% in the other arm (*P* = 0.002). However, survival was similar at all times because the early improvement was offset by increased late CMV disease (16.5% *vs* 8.3%) and many fungal infections. Subsequently, the same group reported the results of a modified protocol treating pp65 antigenemia at any level (1/300 000 cells modified from 3/300 000 cells in the earlier study). With this, they observed an incidence of early CMV disease of 3.8% and late CMV disease of 13.1%.[35]

In our TCDT cohort, the incidence of early CMV disease was 2%, which was comparable to the incidences of the prophylactic arm or of the modified pre-emptive protocol. The incidence of late CMV disease in our TCDT cohort was lower than that reported by other groups. However, it is difficult to attribute this low incidence of late CMV disease to either HDACV or pre-emptive GCV. We continued HDACV and weekly pp65 antigenemia monitoring until day +100 (or longer only if clinically indicated), and it is unclear why there would be any protective effect beyond day +100 in our regimen. This might rather be due to differences in patient characteristics, such as a lower incidence of acute GVHD (48% compared to 75–78%), and no mismatched or unrelated transplants in our cohorts. It is possible that our delayed DLI on days +45 (or 30) and +100 might have contributed to immune reconstitution beyond day +100, thereby preventing CMV disease. However, when we analyzed the pattern of CMV pp65 antigenemia before and after day +45 post-transplant, we did not find clear differences between patients who received day +45 (or 30) DLI and those who did not (data not shown). A favorable effect of HDACV can also be discerned by the fact that there was no CMV reactivation or CMV disease in seronegative recipients who received a transplant from a seropositive donor (*n* = 7 in TCDT, *n* = 16 in NMT), although the number was small. Overall, our results compared favorably with the original randomized study evaluating HDACV (HDACV arm, day −5 to day +180) with regard to the incidence of CMV disease (eight of 105 patients) and infectious death (eight deaths).[22]

We used GCV for patients with CMV pp65 antigenemia at any level. With this approach, about a third (34/103) of CMV infections never recurred after one course of induc-

tion therapy. We identified that a first CMV pp65 antigenemia level greater than 2/400 000 cells was associated with subsequent persistence or relapse, requiring prolonged antiviral therapy. With regard to CMV disease, acute GVHD and a high number of pp65 positive cells at first reactivation appeared to be associated with subsequent CMV disease. While persistent or recurrent CMV pp65 antigenemia was also common in patients who developed CMV disease (4/5) after TCDT, CMV pp65 antigenemia appeared to be poorly correlated with CMV disease in our NMT cohort.[36] In the current study, five of the six patients had no preceding CMV pp65 antigenemia before developing CMV disease. However, one patient, beyond day +100 post transplant was not on regular surveillance at the time of CMV disease. Three other patients underwent bronchoscopy when they developed pulmonary infiltrates and had a positive shell vial culture. All three of these patients also had other microorganisms, and subsequent deaths were attributed to persistent adenovirus infection (UPN 180), grade IV GVHD (UPN 180), or bacterial/fungal pneumonia (UPN 253). Therefore, it is possible that we incidentally found CMV reactivation rather than true CMV disease when they underwent bronchoscopy during the period (days 31–49) at which a screening BAL is usually performed for pre-emptive GCV therapy.[7]

We observed a significantly lower incidence of CMV reactivation in NMT compared to TCDT. Although there were more seronegative recipients in the NMT cohort, the difference remained significant when we analyzed CMV outcomes only in seropositive recipients. Because the baseline patient characteristics and transplant methods were so different, we did not believe that we could clearly identify the factors responsible for the difference in the CMV outcome. Possible factors include large T cell dose in NMT, lack of TBI in the conditioning regimen, post-transplant immunosuppression, or different underlying diseases. Studies on CMV outcomes in NMT have been reported mostly in abstract form, and results were mixed. Several studies using antithymocyte globulin or CAMPATH-1H had high rates of CMV infection,[37–39] whereas a few other groups reported a lower rate of CMV infection and CMV disease.[40,41] Further understanding of immune reconstitution and infection risks after NMT is necessary to optimize the prophylaxis/pre-emptive strategies in this population.

In summary, HDACV followed by pre-emptive GCV was associated with effective control of CMV antigenemia in the majority of patients and near-complete eradication of fatal CMV IP. The results also showed that the rate of CMV infection after NMT is significantly less and delayed compared to that of TCDT.

Acknowledgements

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