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ORIGINAL ARTICLE

Graft failure in the modern era of allogeneic hematopoietic SCT

This article has been corrected since Advance Online Publication and a corrigendum is also printed in this issue.

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Graft failure may contribute to increased morbidity and mortality after allogeneic hematopoietic SCT (allo-HSCT). Here, we present risk factors for graft failure in all first allo-HSCTs performed at our center from 1995 to mid-2010 ($n = 967$). Graft failure was defined as $> 95\%$ recipient cells any time after engraftment with no signs of relapse, or re-transplantation because of primary or secondary neutropenia ($< 0.5 \times 10^9$/L) and/or thrombocytopenia ($< 30 \times 10^9$/L). Fifty-four patients (5.6%) experienced graft failure. The majority were because of autologous reconstitution ($n = 43$), and only a few patients underwent re-transplantation because of primary ($n = 6$) or secondary ($n = 5$) graft failures. In non-malignant disorders, graft failure had no effect on survival, whereas in malignant disease graft failure was associated with reduced 5-year survival (22 vs 53%, $P < 0.01$). In multivariate analysis, *ex vivo* T-cell depletion (relative risk (RR) 8.82, $P < 0.001$), HLA-mismatched grafts (RR 7.64, $P < 0.001$), non-malignant disorders (RR 3.32, $P < 0.01$) and reduced-intensity conditioning (RR 2.58, $P < 0.01$) increased the risk for graft failure, whereas graft failures were prevented by total nucleated cell doses of $\geqslant 2.5 \times 10^8$/kg (RR 0.36, $P < 0.01$). In conclusion, graft failure was only associated with inferior survival in malignant disease. Non-malignant disorders, HLA match, conditioning intensity, immunosuppression regimen and cell dose all influenced graft failure risk.

INTRODUCTION

Graft failure still remains an important contributor to morbidity and mortality, following allogeneic hematopoietic stem cell transplantation (allo-HSCT).[1,2] The most obvious manifestation of graft failure is primary graft failure, where the patient never recovers from neutropenia (ANC $< 0.5 \times 10^9$/L), resulting in pancytopenia and an urgent need for re-transplantation. In contrast, secondary graft failure occurs because of loss of donor cells after initial engraftment. In the latter case, autologous recovery is common; however, marrow aplasia and pancytopenia may also develop. There are several biological mechanisms that may contribute to graft failure. Immunological rejection of the hematopoietic stem cell graft is a major cause of graft failure, which is a result of recipient immune responses to donor hematopoietic cells. Graft failure may also be caused by other mechanisms such as drug toxicity, septicemia and virus infections (CMV, human herpes virus type 6 and parvovirus).[3]

Different immunological mechanisms may result in graft failure. Increased risk of graft failure has been reported in HLA-mismatched[4] and major ABO-mismatched transplants.[5] Recipient T-cells are regarded as the main contributors to immunological rejection of the donor hematopoietic stem cells, although NK-mediated rejection has also been demonstrated in animal models.[6–10] NK-mediated allograft rejection can be overcome to some extent by CY or TBI administered before transplantation and anti-metabolites, such as MTX, given after transplantation.[11] Antibody-mediated rejection in allo-HSCT is controversial,[12–16] although some data suggest that pre-transplant donor-specific anti-endothelial precursor cell antibodies augment the risk of graft

failure in clinical allo-HSCT.[17] Altogether, these studies indicate that cellular mechanisms are the major contributors to graft failure in sensitized recipients, but humoral mechanisms may also be important. The increasing use of reduced intensity conditioning (RIC), and wider application of HLA-mismatched donors in recent years may have turned graft failure into an increasing problem. For this reason, we wanted to define the patients who are most at risk of graft failure in the modern era of allo-HSCT (1995–2010).

MATERIALS AND METHODS

Patients

This retrospective study included all patients ($n = 967$) who underwent first allo-HSCT at our center between 1 January 1995 and 30 June 2010. Patient and donor characteristics are given in Table 2. The diseases were: hematological malignancies (ALL, AML, CLL, CML; $n = 584$), lymphoma ($n = 60$), myelodysplastic syndrome ($n = 85$), myeloproliferative disorders (MPD)/myelofibrosis ($n = 21$), multiple myeloma ($n = 29$), aplastic anemia ($n = 45$), other non-malignant disorders such as metabolic diseases and immunodeficiency syndromes ($n = 79$), and non-hematological malignancies including solid tumors and sarcoma ($n = 64$).

Donors

Most donors were HLA-identical siblings ($n = 370$) or an HLA-A-, HLA-B- and HLA-DRB1-matched unrelated donor (MUD 6/6; $n = 455$). In MUD transplants, high-resolution HLA typing for class I and class II was performed (Olerup SSP, Olerup SSP AB, Stockholm, Sweden). In MUD transplants, 285 patients were matched for HLA-C (8/8). A few donors were HLA-identical parents (HLA-A, HLA-B and HLA-DRβ1; $n = 12$) or identical twins ($n = 6$). HLA-mismatched transplants (with at least one allele or Ag

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mismatch for HLA-A, HLA-B and HLA-DRB1) were given to 124 patients. Graft sources were BM ($n = 382$), granulocyte-CSF-mobilized peripheral blood stem cells (PBSCs, $n = 529$) or cord blood ($n = 50$). BM grafts combined with PBSCs or cord blood were given to six patients. In these cases, very young sibling donors donated both BM and cord blood, whereas one donor that did not mobilize well on granulocyte-CSF also donated BM.

Conditioning

Myeloablative conditioning consisted of CY given at a dose of 60 mg/kg/day on two consecutive days in combination with either TBI (10 Gy single dose or 12-Gy fractionated), or BU at 16 mg/kg.[18] Therapeutic drug monitoring from the first dose was used for patients treated with BU, followed by adjustment for patients with a higher area under the curve than 5 000 ng/h/mL.[19] Non-myeloablative conditioning (NMA) was mainly given to patients with solid tumors or aplastic anemia. The conditioning regimen in solid tumors consisted of fludarabine combined with 2 Gy TBI[20] and patients with aplastic anemia received CY (200 mg/kg). RIC was given to 300 patients and consisted of fludarabine (30 mg/m^2/day) for 5–6 days combined with BU (8 mg/kg) or CY (120 mg/kg); and in some cases, patients also received fractionated TBI up to 6 Gy.[21,22]

GVHD prophylaxis

As prophylaxis against GVHD, CSA was combined with four planned doses of MTX in 744 patients.[23,24] CSA combined with prednisolone was given to recipients of cord blood transplants.[25] Some recipients of mismatched grafts received ex vivo T-cell-depleted grafts ($n = 17$).[26] Other immunosuppressive protocols consisted of tacrolimus combined with sirolimus or MTX.[27,28] Anti-thymocyte globulin (Thymoglobulin; Genzyme, Cambridge, MA, USA) at doses ranging from 4–10 mg/kg was given to recipients of unrelated or HLA-mismatched grafts and to all patients with non-malignant diseases.[29]

Supportive care

Most patients were treated in reversed isolation;[23] some were treated at home during the pancytopenic phase.[30] Acyclovir prophylaxis was given to patients with a high HSV titer.[31] From 2002 onward, all patients were treated with ursodiol at 12 mg/kg/day for 3 months, to prevent liver toxicity.[32] Pre-planned granulocyte-CSF was started at or before day +10 post transplant to fasten engraftment in 324 patients. This was, however, discontinued when some studies reported that granulocyte-CSF given before day +15 may increase the risk of GVHD.[33,34]

Definition of graft failure

Graft failure was defined as >95% recipient CD3+ or CD34+ cells at any single time after engraftment, re-infusion of donor cells because of permanent loss of neutrophils ($<0.5 \times 10^9$/L) and/or platelets $<30 \times 10^9$/L or >50% recipient CD3+ cells and treatment with donor lymphocyte infusion (DLI). Reinfusion of donor cells before engraftment (ANC $\geqslant 0.5 \times 10^9$/L) was considered to be primary graft failure and all hematopoietic cell infusions after engraftment were considered to be secondary graft failures.[35] Cases of relapse within 1 month of graft failure, were coded as no graft failure.

Chimerism analysis

Chimerism analysis was first performed on an experimental basis, and thereafter implemented as a clinical routine in 2001. PCR amplification of variable numbers of tandem repeats was used to evaluate the degree of donor and recipient chimerism in various cell types in peripheral blood or BM (CD3+, CD19+, CD33+, CD34+), using immunomagnetic beads (Dynal, Oslo, Norway), as previously described.[36] Between 2003 and 2005, some tandem repeat markers were replaced by microsatellites; and in 2005, a real-time PCR method based on single-nucleotide polymorphisms was also adopted for chimerism analysis.[37]

Statistical analysis

The results were analyzed as of 30 September 2010, allowing for a median follow-up time of 78 months (range 3–185 months) in all subjects who were still alive. All statistical analyses were performed using Statistica 9.1.206.0 (Statsoft Inc., Tulsa, OK, USA). Times to graft failure were analyzed by the life-table method with the log-rank test, taking censored data into

account. When evaluating risk factors for graft failure, the Cox regression model was used in univariate and multivariate analyses. Variables with a P-value of <0.10 in the univariate analysis were included in the following multivariate analysis, which was performed using backward elimination. In all analyses, P-values <0.05 were considered significant.

RESULTS

The incidence of graft failure is depicted in Table 1. In the univariate analysis, there were several characteristics that differed between patients with graft failure ($n = 54$) or without graft failure ($n = 913$), following allo-HSCT (Table 2). There was a tendency of an increased incidence of graft failure from 3% before the year 2000 to 6–7% in more recent years ($P = 0.05$). Recipient age was similar for graft failures and when there was no graft failure, and there was a tendency of lower donor age in donor–recipient pairs with graft failure ($P = 0.08$). Moreover, we noted a tendency of a higher probability of graft failure in males than in females ($P = 0.07$).

Diagnosis

There was a correlation between type of disease and graft failure. Patients with non-malignant disorders had a three times higher incidence of graft failure than those with malignant disease (Figure 1). In malignant disease, the incidences of graft failure were 20% for non-hematological malignancies, 10% in both CLL and myeloproliferative disorders (MPD)/myelofibrosis and 5% in myelodysplastic syndrome (5%), whereas the incidences of graft failure were much less (2–3%) in acute and chronic leukemia (Table 2). For some diseases such as CLL and MPD/myelofibrosis, the number of cases is fairly low, which means that these findings must be handled with caution. In multivariate analysis, patients with other non-malignant disorders had a higher probability of graft failure (relative risk (RR) 3.32, $P < 0.01$) than patients with acute leukemia (Table 3).

Conditioning

Pre-transplant conditioning was important for incidence of graft failure (Figure 2). In patients who received RIC and NMA, the incidence of graft failure was 8% and 19%, respectively. In contrast, only 3% graft failures were observed in patients who received myeloablative conditioning (MAC) (Table 2). In multivariate analysis, patients conditioned with NMA (RR 4.50, $P < 0.01$) or RIC (RR 2.58, $P < 0.01$) had a much higher risk of graft failure than those treated with MAC (Table 3).

Table 1. Incidence of graft failure

	N	Percent
Primary graft failure		
ANC never recovered[a]	6	0.6%
Secondary graft failure		
>95% recipient chimerism[b]	43	4.5%
ANC<0.5 or TPK<30[c]	5	0.5%
Overall graft failure		
Primary and secondary graft failure	54	5.6%

[a]All patients were re-transplanted (median 27, range 22–32 days post transplant). [b]All patients had >95% CD34+ or CD3+ cells of recipient origin or were clinically regarded as graft failures because of having >50% recipient cells ($n = 8$), which resulted in treatments such as donor lymphocyte infusions (median 68, range 13–377 days post transplant). [c]All patients received booster infusion of donor cells (median 80, range 43–223 days post transplant).

Table 2. Univariate analysis for graft failure				
Characteristics	N	No graft failure	Graft failure	P-value
Number of patients	967	913	54	
Year of transplantation				0.05
1995–1999	268	260	8 (3%)	
2000–2004	330	310	20 (6%)	
2005–2010	369	343	26 (7%)	
Recipient age[a]	967	37 (0.5–77)	33 (1.0–66)	0.87
Donor age[a]	967	35 (0–72)	32 (0–71)	0.08
Recipient sex				0.07
Male	560	522	38 (7%)	
Female	407	391	16 (4%)	
Donor sex				0.82
Male	556	525	31 (6%)	
Female	396	375	21 (5%)	
CMV mismatch				0.12
D−/R−	137	124	13 (9%)	
D+/R−	78	76	2 (3%)	
D+/R+	438	417	21 (5%)	
D−/R+	247	234	13 (5%)	
Sex mismatch				0.14
Female to male	175	163	12 (7%)	
Male to male	374	350	24 (6%)	
Male to female	182	175	7 (4%)	
Female to female	221	212	9 (4%)	
Disease				<0.001
AML	256	249	7 (3%)	
ALL	169	166	3 (2%)	
CML	129	126	3 (2%)	
CLL	30	27	3 (10%)	
Lymphoma	60	59	1 (2%)	
MDS	85	81	4 (5%)	
MPD/myelofibrosis	21	19	2 (10%)	
Multiple myeloma	29	29	0 (0%)	
Aplastic anemia	45	42	3 (7%)	
Other non-malignant disorders	79	64	15 (19%)	
Non-hematological malignancies	64	51	13 (20%)	
Conditioning				<0.001
NMA	57	46	11 (19%)	
RIC	300	275	25 (8%)	
MAC	610	592	18 (3%)	
Cell source				0.08
BM	382	361	21 (6%)	
PBSCs	529	505	24 (5%)	
Cord blood	50	41	9 (18%)	
BM and PBSCs or cord blood	6	6	0 (0%)	
ABO mismatch				0.02
No	467	447	20 (4%)	
Minor	218	206	12 (6%)	
Major	270	248	22 (8%)	
HLA match				0.02
HLA-identical sibling	370	360	10 (3%)	
Unrelated donor (6/6)	170	159	11 (6%)	

Table 2. (Continued)				
Characteristics	N	No graft failure	Graft failure	P-value
Unrelated donor (8/8)	285	268	17 (6%)	
Other matched[b]	18	18	0 (0%)	
Other mismatched[c]	124	108	16 (13%)	
Total nucleated cell dose (× 10^8 per kg)				0.03
0–2.4	200	181	19 (10%)	
2.5–7	277	264	13 (5%)	
7.1–12.5	244	232	12 (5%)	
>12.5	236	226	10 (4%)	
CD34+ cell dose (× 10^6/kg)				0.04
0–3.0	206	182	24 (12%)	
3.1–6.3	212	204	8 (4%)	
6.4–9.8	210	207	3 (1%)	
>9.8	214	198	16 (7%)	
GVHD prophylaxis				<0.001
CSA+MTX	744	722	22 (3%)	
CSA+Prednisolone	65	57	8 (12%)	
CSA+MMF	47	40	7 (15%)	
Ex vivo T-cell depleted	17	12	5 (29%)	
Fk+rapamune	70	64	6 (9%)	
Fk+MTX	5	2	3 (60%)	
Other	19	16	3 (16%)	
G-CSF preplanned[d]				0.41
Yes	324	309	15 (5%)	
No	643	604	39 (6%)	

Abbreviations: MDS, myelodysplastic syndrome; NMA, non-myeloablative conditioning; RIC, reduced intensity conditioning. [a]Median and range. [b]Identical twin/HLA-A, -B, -DR identical parent. [c]Haploidentical, mismatched unrelated donor. [d]Preplanned G-CSF starting within 10 days of transplant.
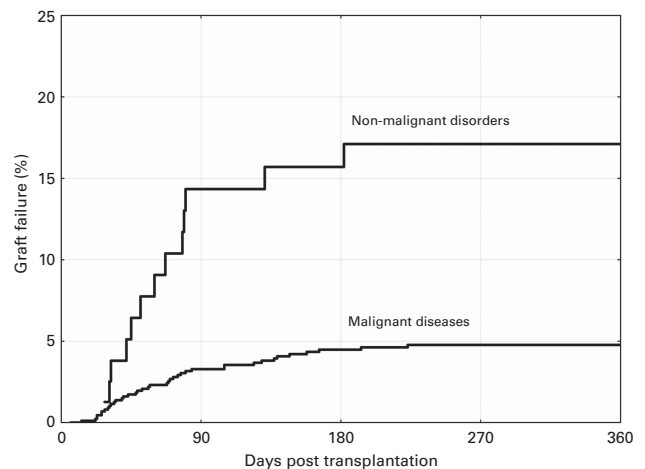

Figure 1. Time to and cumulative incidence of graft failure in malignant and non-malignant diseases ($P<0.001$).

Cell source

Cell source appears to be important for graft failure, and recipients of cord blood transplants had a higher incidence of graft failure (18%) than recipients of PBSCs (5%) or BM (6%), although this did not reach statistical significance in univariate analysis ($P = 0.08$).
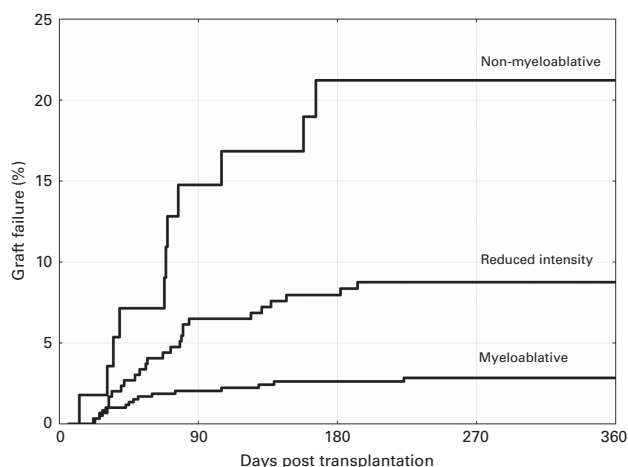
Figure 2. Time to and cumulative incidence of graft failure with intensity of conditioning regimen ($P < 0.001$).
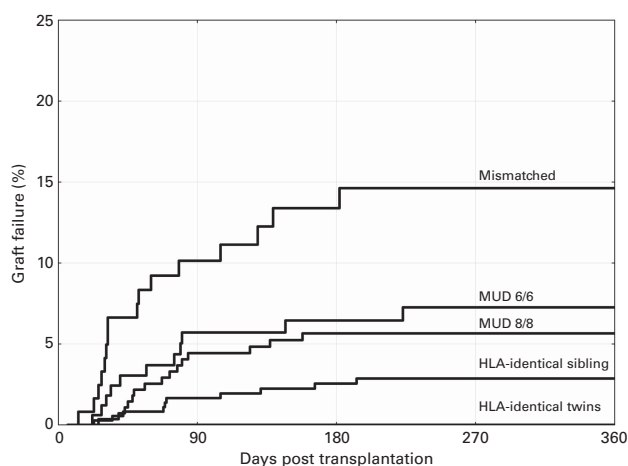

Figure 3. HLA match and cumulative incidence of graft failure ($P < 0.001$).

Table 3. Multivariate analysis of risk factors for graft failure

Characteristics	N	RR	95% CI	P-value
Disease				
Acute leukemia	425	1		
Chronic leukemia	159	1.02	0.37–2.79	0.96
Lymphoma	60	0.44	0.06–3.46	0.43
MDS	85	1.30	0.36–4.71	0.69
MPD/myelofibrosis	21	2.67	0.57–12.5	0.21
Multiple myeloma	29	0	0–12.51	1.00
Aplastic anemia	45	0.54	0.13–2.30	0.41
Other non-malignant disorders	79	3.32	1.43–7.74	0.005
Non-hematological malignances	64	2.60	0.85–7.94	0.09
Conditioning				
MAC	610	1		
RIC	300	2.58	1.33–5.01	0.005
NMA	57	4.50	1.77–11.46	0.002
Total nucleated cell dose[a]				
0–2.4	200	1		
2.5–7	277	0.36	0.17–0.75	0.006
7.1–12.5	244	0.27	0.12–0.59	0.001
>12.5	236	0.23	0.10–0.52	<0.001
HLA match				
HLA-identical sibling	370	1		
Matched unrelated donor (8/8)	285	3.41	1.53–7.58	0.003
Matched unrelated donor (6/6)	170	5.09	2.07–12.51	<0.001
Other matched[b]	18	0	0–12.51	1.00
Other mismatched[c]	124	7.64	3.16–18.46	<0.001
GVHD prophylaxis				
CSA + MTX	744	1		
CSA + prednisolone	65	2.54	0.99–6.54	0.05
CSA + MMF	47	1.22	0.43–3.48	0.70
Ex vivo T-cell depleted	17	8.82	2.98–26.08	<0.001
Fk + rapamune	70	1.86	0.74–4.69	0.18
Other	24	3.88	1.43–10.50	0.008

Abbreviations: NMA, non-myeloablative conditioning; MDS, myelodysplastic syndrome; RIC, reduced intensity conditioning. [a]Total nucleated cell dose ($\times 10^8$/kg). [b]Identical twin/HLA-A, -B, -DR identical parent. [c]Haploidentical, mismatched unrelated donor.

HLA and ABO

In univariate analysis, both HLA and ABO mismatch were associated with increased incidence of graft failure (Table 2). This association was most pronounced for HLA-mismatched grafts (Figure 3), and in the multivariate analysis both MUD transplants and HLA-mismatched grafts had markedly increased risk of graft failure (Table 3). Moreover, graft failure rates were similar in 8/8 or 6/6 HLA-matched donor pairs ($P = 0.23$), and ABO incompatibility was almost a significant risk factor for graft failure (RR 1.36, $P = 0.06$) in the multivariate analysis.

Cell dose

Patients who received a total nucleated cell dose below 2.5×10^8/kg had a risk of graft failure of 10%, as opposed to 5% in patients who received a higher cell dose (Figure 4a). Patients with a CD34+ cell dose below 3×10^6/kg had an incidence of graft failure of 12%, which was significantly higher than the 1–7% seen in patients who received higher cell doses (Figure 4b). Total nucleated cell dose $\geqslant 2.5 \times 10^8$ cells/kg was associated with a reduced risk of graft failure in multivariate analysis (Table 3), whereas CD34+ cell dose did not reach statistical significance.

Immunosuppression

With regard to GVHD prophylaxis, patients who received CSA + MTX had a graft failure rate of 3%. This was lower than for all other

regimens ($P < 0.001$, Table 2). In multivariate analysis (Table 3), there was a tendency of increased risk of graft failure using CSA + prednisolone (RR 2.54, $P = 0.05$), and the risk was markedly increased using *ex vivo* T-depleted grafts (RR 8.82, $P < 0.001$). Moreover other GVHD prophylaxis was associated with increased risk of graft failure (RR 3.88, $P = 0.008$).

OS, relapse, TRM and causes of death

At 5 years post transplantation, the OS for the whole cohort was 55%. In patients with non-malignant disorders, the 5-year OS was 85% independent of the preceding graft failure. In contrast, in patients with malignant disease, graft failure was associated with a markedly inferior 5-year OS (22 vs 53%, $P < 0.01$) (Figure 5). Moreover, 5-year relapse rates were similar in patients with malignant disease irrespective of graft failure (22 vs 32%, $P = 0.37$). TRM was also similar (34 vs 22%, $P = 0.16$).

During the study period, 433 out of 913 patients died. In 403 patients with no graft failure, the causes of death were as follows: 182 (45%) relapse, 114 (28%) infections, 49 (12%) GVHD and 58 (14%) organ failure. In patients with graft failure the causes of death were as follows: 15 (50%) relapse, 11 (37%) infections, 0 GVHD and 4 (13%) organ failure.
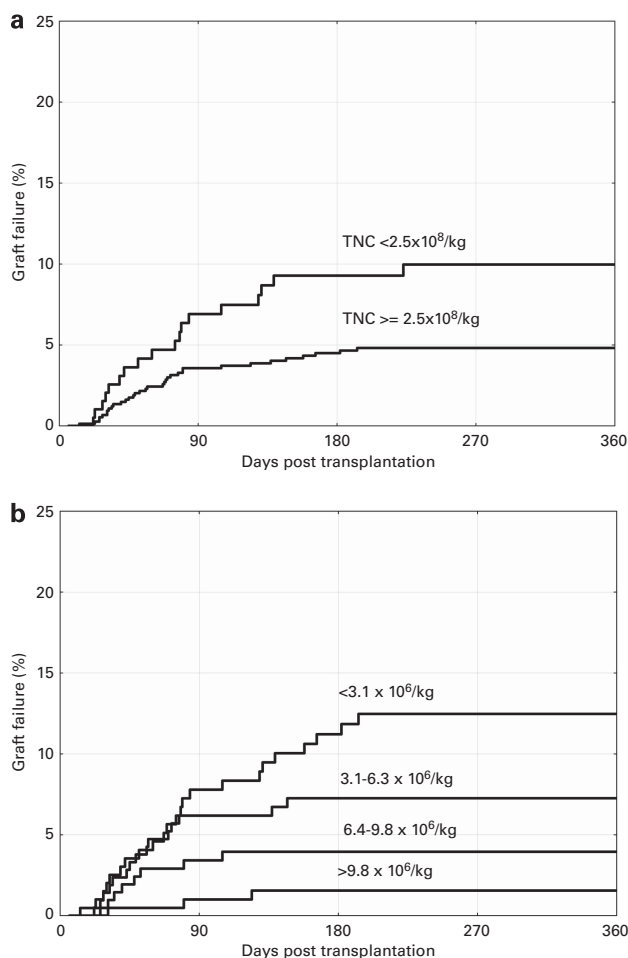
Figure 4. Total nucleated cell dose (TNC) ($P<0.01$) (**a**) and CD34+ cell dose ($P<0.001$) (**b**), and cumulative incidence of graft failure.
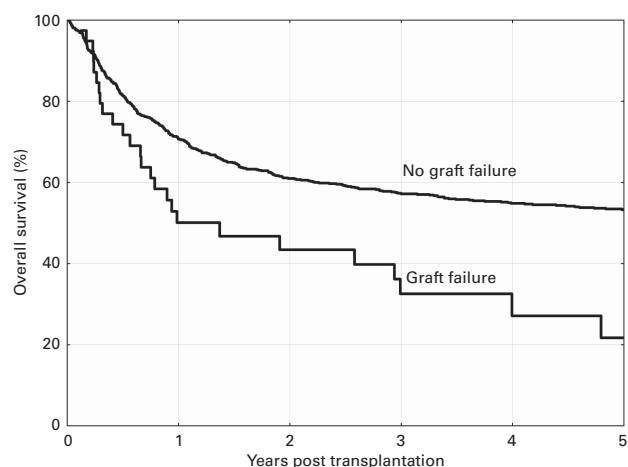

Figure 5. OS in patients with malignant disease, who experiences graft failure compared with those with no graft failure, following their first allogeneic HSCT ($P<0.01$).

DISCUSSION

In the present study, we determined risk factors for graft failure at our center during the modern era of allogeneic HSCT. From 1995 to mid-2010, we performed 967 first allogeneic HSCTs and there were 54 graft failures (5.6%). The majority of graft failures were secondary graft failures, and we only found six primary graft failures (0.6%). It is likely that the same risk factors contribute to both primary and secondary graft failures, and as we had such a low incidence of primary failures, we chose to analyze risk factors for all graft failures as defined by chimerism analysis or clinical reinfusion of donor cells. In univariate analyses, variables such as disease, intensity of conditioning, ABO match, HLA match, total nucleated cell dose, CD34+ cell dose and type of GVHD prophylaxis influenced the risk of graft failure. These analyses were followed by a multivariate analysis that revealed a 3-times higher risk of graft failure in patients with non-malignant disorders. Furthermore, RIC or NMA resulted in a 3–4 times increased risk of graft failure compared with MAC, and a total nucleated cell dose of $\geqslant 2.5 \times 10^8$/kg markedly reduced the risk of graft failure. Unrelated donor transplants were also associated with an increased risk of graft failure; however, graft failure rates were similar in 6/6 and 8/8 matched unrelated donor-recipient pairs. We also noted a marked increase in graft failures following *ex vivo* T-cell depletion compared with our standard GVHD prophylaxis with CSA and MTX.

The majority of patients were transplanted in the treatment for acute leukemia, and the incidence of graft failure among them was only a few percent. The incidence of graft failure was slightly higher in other disorders such as myelofibrosis, but this was NS in the multivariate analysis, where only non-malignant disorders showed an increased risk of graft failure. This is in accordance with previously published data, where non-malignant disorders such as metabolic diseases and non-malignant hematological disorders were found to have an increased risk of graft failure.[38,39]

Apart from killing of remnant malignant cells, the purpose of the conditioning regimen is mainly to suppress the recipient's immune system and prevent an immunological rejection of the infused donor hematopoietic cells. RIC regimens are; however, used in the elderly as well as in patients with comorbidities, who would probably not be able to tolerate the toxicity associated with myeloablative conditioning regimens.[40,41] Thus, it is reasonable that conditioning regimens with less myelotoxicity would result in an increased risk of graft failure, as seen in the present study.

The source of hematopoietic stem cells may also be of importance for successful engraftment. In some previous studies, it has been found that cord blood transplants have an increased risk of graft failure, which may be explained by the lower cell dose and the increased acceptance for HLA disparities associated with cord blood compared with other graft sources.[42–44] However, in this study, there was only a tendency of increased incidence of graft failure in recipients of cord blood grafts compared with other grafts. We also found similar graft failure incidences for PBSC and BM grafts. Furthermore, both CD34+ and total nucleated cell dose appears to be of importance in a dose-dependent manner to reduce the risk of graft failure, where a total nucleated cell dose of $\geqslant 2.5 \times 10^8$/kg was found to markedly reduce the risk of graft failure in the present multivariate analysis.

That HLA compatibility between donor and recipient is of major importance for graft failure is well known.[45] The fact that we, in this study, found that HLA-mismatched grafts were more likely to fail was therefore to be expected. Moreover, we found that the recipients of HLA-identical sibling grafts are less prone to reject their grafts, whereas the risk of graft failure is increased in MUD transplants. Previous studies have shown that HLA-C mismatch is associated with an increased risk of graft failure.[46] Even so, in a previous study, we did not find that HLA-C mismatch was associated with graft failure or GVHD,[47] and now we can again report that we observed similar graft failure risk in recipients of 6/6 and 8/8 MUD grafts.

We have previously reported that major ABO blood group mismatch increases the risk of graft failure after HSCT using unrelated donors.[5] In the present study, both related and unrelated donors were included, and in univariate analysis ABO incompatibility was associated with graft failure ($P = 0.02$).

However, in the following multivariate analysis, the augmented risk of graft failure using major ABO blood group-incompatible grafts was no longer significant ($P = 0.06$). This may suggest that ABO mismatch *per se* does not induce graft failure. Notably, erythrocytes are removed from ABO-incompatible BM transplants, and this procedure reduces the graft stem cell dose by ~30%. The lower number of stem cells may therefore be a likely contributor to graft failures using ABO-incompatible transplants, which is supported by the effect of total nucleated cell dose on graft failure in the present analysis (Table 3). A low cell dose not only increases the risk of graft failure, but also affects other outcomes after HSCT such as death from invasive fungal infection, death from GVHD, overall treatment failure and infection.[48–53]

With regard to the immunosuppressive therapy, we found that CSA + MTX had the lowest incidence of graft failure compared with all other immunosuppressive protocols. CSA + MTX is the gold standard, and so far no immunosuppressive protocol has been proven to be superior.[23,54] In the multivariate analysis, *ex vivo* T-cell depletion markedly increased the risk of graft failure and there was a tendency of increased risk when using CSA and prednisolone. Although CSA + MTX was associated with a lower incidence of graft failure in the multivariate analysis, there may be other factors that influenced the high rate of graft failure using these other immunosuppressive protocols. CSA and prednisolone were used in cord blood transplants and here the graft source with a low marrow cell dose is probably more important than the immunosuppressive regimen. CSA and MMF was given to patients with solid tumors who received NMA, and here the low conditioning rather than the immunosuppressive protocol was the reason for the increased risk of graft failure. Both cord blood, as well as CSA and MMF immunosuppression have previously been reported to be associated with an increased risk of graft failure.[55,56] Moreover, patients who received *ex vivo* T-cell depletion had a 29% probability of graft failure. It is well established that T-cell depletion both increases the risk of graft failure and leukemic relapse because of reduced cellular allo-reactivity of the graft.[57] In more recent years, we have mainly used *ex vivo* T-cell depletion in recipients of transplants with HLA-mismatched unrelated donors.[25]

It is important to identify patients who are at risk of graft failure to limit the number of risk factors to prevent this severe complication occurring after allo-HSCT. Patients with non-malignant disorders have not received chemotherapy before transplantation, and their disease will not progress as fast as hematological malignancies. Accordingly, graft failure may not be as deleterious in non-malignant disorders, where the patient has a fair chance of a successful re-transplantation.[1] To reduce the risk of graft failure, the conditioning regimen may be intensified and the highest possible cell dose should be given. The best immunosuppressive protocol to prevent graft failure appears to be CSA + MTX. Other possible strategies are early therapies against graft failure including donor lymphocyte infusions, boost of hematopoietic stem cells and granulocyte CSF.[1,58,59] All of these procedures are, however, hazardous because they may induce severe GVHD. A less risky procedure may be to infuse mesenchymal stromal cells, which cause much less side effects and contribute with immunomodulatory effects, especially on T-cell alloreactivity.[60] This strategy is also supported by the work of Meuleman et al.[61] who, in a pilot study, were able to reverse the graft failure in a third of their patients using mesenchymal stromal cell infusions.

In conclusion, in the modern era of allo-HSCT, graft failure remains a clinical problem—especially in patients with non-malignant disorders, in recipients of RIC or HLA-mismatched grafts, when using grafts with low cell dose, or after T-cell depletion. In these cases, we suggest that special precautions should be taken, and results of chimerism analysis must be monitored to enable early intervention.

REFERENCES

1 Remberger M, Mattsson J, Olsson R, Ringden O. Second allogeneic hematopoietic stem cell transplantation (HSCT): a treatment for graft failure. *Clin Transplant* 2011; **25**: E68–E76.

2 Schriber J, Agovi MA, Ho V, Ballen KK, Bacigalupo A, Lazarus HM et al. Second unrelated donor hematopoietic cell transplantation for primary graft failure. *Biol Blood Bone Marrow Transplant* 2010; **16**: 1099–1106.

3 Mattsson J, Ringden O, Storb R. Graft failure after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2008; **14**(Supplement 1): 165–170.

4 Crocchiolo R, Ciceri F, Fleischhauer K, Oneto R, Bruno B, Pollichieni S et al. [HLA matching affects clinical outcome of adult patients undergoing haematopoietic SCT from unrelated donors: a study from the Gruppo Italiano Trapianto di Midollo Osseo and Italian Bone Marrow Donor Registry]. *Bone Marrow Transplant* 2009; **44**: 571–577.

5 Remberger M, Watz E, Ringden O, Mattsson J, Shanwell A, Wikman A. Major ABO blood group mismatch increases the risk for graft failure after unrelated donor hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2007; **13**: 675–682.

6 Cudkowicz G, Bennett M. Peculiar immunobiology of bone marrow allografts. I. Graft rejection by irradiated responder mice. *J Exp Med* 1971; **134**: 83–102.

7 Kiessling R, Hochman PS, Haller O, Shearer GM, Wigzell H, Cudkowicz G. Evidence for a similar or common mechanism for natural killer cell activity and resistance to hemopoietic grafts. *Eur J Immunol* 1977; **7**: 655–663.

8 Murphy WJ, Kumar V, Bennett M. Acute rejection of murine bone marrow allografts by natural killer cells and T cells. Differences in kinetics and target antigens recognized. *J Exp Med* 1987; **166**: 1499–1509.

9 Raff RF, Deeg HJ, Loughran Jr TP, Graham TC, Aprile JA, Sale GE et al. Characterization of host cells involved in resistance to marrow grafts in dogs transplanted from unrelated DLA-nonidentical donors. *Blood* 1986; **68**: 861–868.

10 Raff RF, Sandmaier BM, Graham T, Loughran Jr TP, Pettinger M, Storb R. 'Resistance' to unrelated, DLA-nonidentical canine marrow grafts is unrestricted by the major histocompatibility complex. *Exp Hematol* 1994; **22**: 893–897.

11 Deeg HJ, Sale GE, Storb R, Graham TC, Schuening F, Appelbaum FR et al. Engraftment of DLA-nonidentical bone marrow facilitated by recipient treatment with anti-class II monoclonal antibody and methotrexate. *Transplantation* 1987; **44**: 340–345.

12 Barge AJ, Johnson G, Witherspoon R, Torok-Storb B. Antibody-mediated marrow failure after allogeneic bone marrow transplantation. *Blood* 1989; **74**: 1477–1480.

13 Moeller E. Contact-induced cytotoxicity by lymphoid cells containing foreign isoantigens. *Science* 1965; **147**: 873–879.

14 Storb R, Floersheim GL, Weiden PL, Graham TC, Kolb HJ, Lerner KG et al. Effect of prior blood transfusions on marrow grafts: abrogation of sensitization by procarbazine and antithymocyte serum. *J Immunol* 1974; **112**: 1508–1516.

15 Taylor PA, Ehrhardt MJ, Roforth MM, Swedin JM, Panoskaltsis-Mortari A, Serody JS et al. Preformed antibody, not primed T cells, is the initial and major barrier to bone marrow engraftment in allosensitized recipients. *Blood* 2007; **109**: 1307–1315.

16 Warren RP, Storb R, Weiden PL, Su PJ, Thomas ED. Lymphocyte-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity in patients with aplastic anemia: distinguishing transfusion-induced sensitization from possible immune-mediated aplastic anemia. *Transplant Proc* 1981; **13**(1 Part 1): 245–247.

17 Nordlander A, Mattsson J, Sundberg B, Sumitran-Holgersson S. Novel antibodies to the donor stem cell population CD34 + /VEGFR-2 + are associated with rejection after hematopoietic stem cell transplantation. *Transplantation* 2008; **86**: 686–696.

18 Ringden O, Ruutu T, Remberger M, Nikoskelainen J, Volin L, Vindelov L et al. A randomized trial comparing busulfan with total body irradiation as conditioning in allogeneic marrow transplant recipients with leukemia: a report from the Nordic Bone Marrow Transplantation Group. *Blood* 1994; **83**: 2723–2730.

19 Hassan M. The role of busulfan in bone marrow transplantation. *Med Oncol* 1999; **16**: 166–176.

20 Niederwieser D, Maris M, Shizuru JA, Petersdorf E, Hegenbart U, Sandmaier BM et al. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. *Blood* 2003; **101**: 1620–1629.

21 Hentschke P, Barkholt L, Uzunel M, Mattsson J, Wersall P, Pisa P et al. Low-intensity conditioning and hematopoietic stem cell transplantation in patients with renal and colon carcinoma. *Bone Marrow Transplant* 2003; **31**: 253–261.

22 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**: 756–763.

23 Ringden O, Remberger M, Persson U, Ljungman P, Aldener A, Andstrom E et al. Similar incidence of graft-versus-host disease using HLA-A, -B and -DR identical unrelated bone marrow donors as with HLA-identical siblings. *Bone Marrow Transplant* 1995; **15**: 619–625.

24 Storb R, Deeg HJ, Fisher L, Appelbaum F, Buckner CD, Bensinger W et al. Cyclosporine v methotrexate for graft-v-host disease prevention in patients given marrow grafts for leukemia: long-term follow-up of three controlled trials. *Blood* 1988; **71**: 293–298.

25 Ringden O, Okas M, Uhlin M, Uzunel M, Remberger M, Mattsson J. Unrelated cord blood and mismatched unrelated volunteer donor transplants, two alternatives in patients who lack an HLA-identical donor. *Bone Marrow Transplant* 2008; **42**: 643–648.

26 Ringden O, Pihlstedt P, Markling L, Aschan J, Baryd I, Ljungman P et al. Prevention of graft-versus-host disease with T cell depletion or cyclosporin and methotrexate. A randomized trial in adult leukemic marrow recipients. *Bone Marrow Transplant* 1991; **7**: 221–226.

27 Nash RA, Pineiro LA, Storb R, Deeg HJ, Fitzsimmons WE, Furlong T et al. FK506 in combination with methotrexate for the prevention of graft-versus-host disease after marrow transplantation from matched unrelated donors. *Blood* 1996; **88**: 3634–3641.

28 Cutler C, Kim HT, Hochberg E, Ho V, Alyea E, Lee SJ et al. Sirolimus and tacrolimus without methotrexate as graft-versus-host disease prophylaxis after matched related donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant* 2004; **10**: 328–336.

29 Remberger M, Svahn BM, Mattsson J, Ringden O. Dose study of thymoglobulin during conditioning for unrelated donor allogeneic stem-cell transplantation. *Transplantation* 2004; **78**: 122–127.

30 Svahn BM, Remberger M, Myrback KE, Holmberg K, Eriksson B, Hentschke P et al. Home care during the pancytopenic phase after allogeneic hematopoietic stem cell transplantation is advantageous compared with hospital care. *Blood* 2002; **100**: 4317–4324.

31 Lundgren G, Wilczek H, Lonnqvist B, Lindholm A, Wahren B, Ringden O. Acyclovir prophylaxis in bone marrow transplant recipients. *Scand J Infect Dis Suppl* 1985; **47**: 137–144.

32 Ruutu T, Eriksson B, Remes K, Juvonen E, Volin L, Remberger M et al. Ursodeoxycholic acid for the prevention of hepatic complications in allogeneic stem cell transplantation. *Blood* 2002; **100**: 1977–1983.

33 Remberger M, Naseh N, Aschan J, Barkholt L, LeBlanc K, Svennberg P et al. G-CSF given after haematopoietic stem cell transplantation using HLA-identical sibling donors is associated to a higher incidence of acute GVHD II-IV. *Bone Marrow Transplant* 2003; **32**: 217–223.

34 Ringden O, Hassan Z, Karlsson H, Olsson R, Omazic B, Mattsson J et al. Granulocyte colony-stimulating factor induced acute and chronic graft-versus-host disease. *Transplantation* 2010; **90**: 1022–1029.

35 Remberger M, Ringden O, Ljungman P, Hagglund H, Winiarski J, Lonnqvist B et al. Booster marrow or blood cells for graft failure after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1998; **22**: 73–78.

36 Barrios M, Jimenez-Velasco A, Roman-Gomez J, Madrigal ME, Castillejo JA, Torres A et al. Chimerism status is a useful predictor of relapse after allogeneic stem cell transplantation for acute leukemia. *Haematologica* 2003; **88**: 801–810.

37 Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C et al. Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. *Blood* 2002; **99**: 4618–4625.

38 Lucke T, Das AM, Hartmann H, Sykora KW, Donnerstag F, Schmid-Ott G et al. Developmental outcome in five children with Hurler syndrome after stem cell transplantation: a pilot study. *Dev Med Child Neurol* 2007; **49**: 693–696.

39 Wagner JE, Eapen M, MacMillan ML, Harris RE, Pasquini R, Boulad F et al. Unrelated donor bone marrow transplantation for the treatment of Fanconi anemia. *Blood* 2007; **109**: 2256–2262.

40 Castagna L, Furst S, Marchetti N, El Cheikh J, Faucher C, Mohty M et al. Retrospective analysis of common scoring systems and outcome in patients older than 60 years treated with reduced-intensity conditioning regimen and alloSCT. *Bone Marrow Transplant* 2011; **46**: 1000–1005.

41 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**: 4531–4536.

42 Brunstein CG, Noreen H, DeFor TE, Maurer D, Miller JS, Wagner JE. Anti-HLA antibodies in double umbilical cord blood transplantation. *Biol Blood Marrow Transplant* 2011; **17**: 1704–1708.

43 Rocha V, Gluckman E. Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. *Br J Haematol* 2009; **147**: 262–274.

44 Ruggeri A, Eapen M, Scaravadou A, Cairo MS, Bhatia M, Kurtzberg J et al. Umbilical cord blood transplantation for children with thalassemia and sickle cell disease. *Biol Blood Marrow Transplant* 2011; **17**: 1375–1382.

45 Petersdorf EW, Gooley TA, Anasetti C, Martin PJ, Smith AG, Mickelson EM et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. *Blood* 1998; **92**: 3515–3520.

46 Ringden O, Schaffer M, Le Blanc K, Persson U, Hauzenberger D, Abedi MR et al. Which donor should be chosen for hematopoietic stem cell transplantation among unrelated HLA-A, -B, and -DRB1 genomically identical volunteers? *Biol Blood Marrow Transplant* 2004; **10**: 128–134.

47 Morishima Y, Sasazuki T, Inoko H, Juji T, Akaza T, Yamamoto K et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. *Blood* 2002; **99**: 4200–4206.

48 Bortin MM, Gale RP, Kay HE, Rimm AA. Bone marrow transplantation for acute myelogenous leukemia. Factors associated with early mortality. *Jama* 1983; **249**: 1166–1175.

49 Ringden O, Barrett AJ, Zhang MJ, Loberiza FR, Bolwell BJ, Cairo MS et al. Decreased treatment failure in recipients of HLA-identical bone marrow or peripheral blood stem cell transplants with high CD34 cell doses. *Br J Haematol* 2003; **121**: 874–885.

50 Ringden O, Nilsson B. Death by graft-versus-host disease associated with HLA mismatch, high recipient age, low marrow cell dose, and splenectomy. *Transplantation* 1985; **40**: 39–44.

51 Sierra J, Storer B, Hansen JA, Bjerke JW, Martin PJ, Petersdorf EW et al. Transplantation of marrow cells from unrelated donors for treatment of high-risk acute leukemia: the effect of leukemic burden, donor HLA-matching, and marrow cell dose. *Blood* 1997; **89**: 4226–4235.

52 Storb R, Prentice RL, Thomas ED. Marrow transplantation for treatment of aplastic anemia. An analysis of factors associated with graft rejection. *N Engl J Med* 1977; **296**: 61–66.

53 Tollemar J, Ringden O, Bostrom L, Nilsson B, Sundberg B. Variables predicting deep fungal infections in bone marrow transplant recipients. *Bone Marrow Transplant* 1989; **4**: 635–641.

54 Storb R, Deeg HJ, Whitehead J, Farewell V, Appelbaum FR, Beatty P et al. Marrow transplantation for leukemia and aplastic anemia: two controlled trials of a combination of methotrexate and cyclosporine v cyclosporine alone or methotrexate alone for prophylaxis of acute graft-v-host disease. *Transplant Proc* 1987; **19**(1 Part 3): 2608–2613.

55 Le Blanc K, Remberger M, Uzunel M, Mattsson J, Barkholt L, Ringden O. A comparison of nonmyeloablative and reduced-intensity conditioning for allogeneic stem-cell transplantation. *Transplantation* 2004; **78**: 1014–1020.

56 Stevens CE, Carrier C, Carpenter C, Sung D, Scaradavou A. HLA mismatch direction in cord blood transplantation: impact on outcome and implications for cord blood unit selection. *Blood* 2011; **118**: 3969–3978.

57 Marmont AM, Horowitz MM, Gale RP, Sobocinski K, Ash RC, van Bekkum DW et al. T-cell depletion of HLA-identical transplants in leukemia. *Blood* 1991; **78**: 2120–2130.

58 Bishop MR, Tarantolo SR, Pavletic ZS, Lynch JC, Morris ME, Zacharias D et al. Filgrastim as an alternative to donor leukocyte infusion for relapse after allogeneic stem-cell transplantation. *J Clin Oncol* 2000; **18**: 2269–2272.

59 Kolb HJ, Mittermuller J, Clemm C, Holler E, Ledderose G, Brehm G et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990; **76**: 2462–2465.

60 Ringden O. Mesenchymal stromal cells as first-line treatment of graft failure after hematopoietic stem cell transplantation. *Stem Cells Dev* 2009; **18**: 1243–1246.

61 Meuleman N, Tondreau T, Ahmad I, Kwan J, Crokaert F, Delforge A et al. Infusion of mesenchymal stromal cells can aid hematopoietic recovery following allogeneic hematopoietic stem cell myeloablative transplant: a pilot study. *Stem Cells Dev* 2009; **18**: 1247–1252.