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## Donor Derived Second Hematologic Malignancies after Cord Blood Transplantation

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### Abstract

Double umbilical cord blood transplantation with a reduced intensity regimen is an effective strategy for adult patients without matched donors. However, the risk of second cancers is not yet established. Ninety-eight adults with hematologic malignancies received a double umbilical cord blood transplant. Seventy patients received the reduced intensity regimen of fludarabine 30 mg/m<sup>2</sup>/day × 6 days, melphalan 100 mg/m<sup>2</sup>/day × 1 day, and rabbit antithymocyte globulin 1.5 mg/kg/day × 4 days, and 28 patients received an ablative total body radiation containing conditioning regimen. Sixty-three patients received sirolimus-based graft versus host disease prophylaxis and 35 patients received non-sirolimus based graft versus host disease prophylaxis. Median age was 48 (range 19-67) years. Eighteen patients developed a second malignancy at a median of 134 days after transplant. Sixteen patients had lymphoma and two patients had myelodysplasia/myeloproliferative disorder. Sixteen of these second cancers (both MDS/MPD and fourteen of the lymphomas) were donor derived; the origin of the others was not determined. GVHD prophylaxis, HLA matching, primary disease, age, total nucleated cell dose, and CD34+ cell dose were not associated with a higher rate of second malignancy. Second myeloid malignancies of donor origin occur after double umbilical cord blood transplantation, suggesting that a search for donor origin should be performed in all patients with suspected relapse.

### Introduction

Umbilical cord blood is an alternative stem cell source for patients without matched related or unrelated donors. A projected disease-free survival of approximately 30-60% has been observed in adults receiving umbilical cord blood-derived hematopoietic stem cell transplantation (HSCT) using either a single cord blood product (1-4) or two cord blood products. (5-7) However, most of these transplantations utilize donors that are mismatched at multiple HLA antigens. Moreover, the product includes only naïve T cells, and those are present in small numbers. Thus, there is an inherent risk of post transplantation lymphoproliferative disorders (PTLD). Stimulation of limiting numbers of stem and

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progenitor cells may increase the risk of myelodysplasia and AML. (8) Finally, mutations have been identified in post-partum umbilical cord blood that are associated with the later development of leukemia. (9)

Second malignancies may be of recipient or more rarely donor origin. (10-13) Recent analyses of the risk of second malignancy after allogeneic stem cell transplantation suggest an incidence of 6-12%. (14-15) However, the risk of solid tumors does not appear to plateau. A retrospective study of 18,000 patients reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) indicates that the incidence of PTLD was 1%, with 82% of cases occurring in the first year after transplant. (14) Risk factors for developing PTLD included Epstein Barr Virus (EBV) infection, HLA mismatch, use of antithymocyte globulin (ATG), T cell depletion, and chronic Graft versus Host Disease (GVHD). The incidence of PTLD after reduced-intensity double cord blood transplantation ranges from 3% to as high as 21%, especially if ATG is used in the conditioning regimen. (16)

Second myeloid malignancies are rare after allogeneic transplantation using living donor marrow or PBSC. There have been individual case reports of secondary myeloid malignancies of donor origin after allogeneic stem cell transplant; the incidence is estimated at less than <0.25%. (17-23)

Since relapse of the primary disease may be difficult to distinguish from donor derived secondary leukemia unless cytogenetic or molecular techniques are used, the actual incidence of donor derived second malignancies may be underreported. In this study, we describe the incidence and outcomes of secondary hematologic malignancies in 98 patients who received a double umbilical cord blood HSCT.

## Materials and Methods

### Patients

Consecutive patients with hematologic malignancies undergoing double cord blood transplantation were retrospectively analyzed. Patients were eligible for reduced intensity double cord blood HSCT if they had no 5/6 or 6/6 HLA-A, B, DR allele level matched related donor or no 6/6 HLA A, B, DR allele level matched unrelated donor. Patients with acute leukemia were eligible if they were in first remission with high-risk cytogenetics or in second or subsequent remission. Patients with refractory leukemia were not eligible. Patients with chemotherapy sensitive relapsed lymphoma were eligible. In addition, patients with myelodysplasia, chronic myelogenous leukemia refractory to tyrosine kinase inhibitors, or chronic lymphocytic leukemia progressive after at least two regimens were also eligible.

### Conditioning Regimen and Graft versus Host Disease Prophylaxis

All reduced intensity patients received a conditioning regimen of fludarabine 30mg/m<sup>2</sup>/day on Days -8 through -3 (total dose 180 mg/m<sup>2</sup>), melphalan 100 mg/m<sup>2</sup>/day on Day -2, and rabbit antithymocyte globulin (Genzyme, Cambridge MA) 1.5 mg/kg/day on Days -7,-5,-3,-1 (total dose 6.0 mg/kg). Patients under age 45 with no comorbid conditions were eligible to receive a myeloablative conditioning regimen which consisted of fludarabine 25 mg/m<sup>2</sup>/day on Days -6,-5,-4 (total dose 75 mg/m<sup>2</sup>), cyclophosphamide 1800 mg/m<sup>2</sup>/day Days -6 and -5 (total dose 3600mg/m<sup>2</sup>), and total body radiation 14 Gy in 7 fractions Days -3,-2,-1,0. Four patients also received ATG in addition to the above regimen. Sixty-three patients received a sirolimus based GVHD prophylaxis with sirolimus (target trough serum concentration 3-12 ng/ml) and tacrolimus (target trough serum concentration 5-10 ng/ml) for GVHD prophylaxis. (24) GVHD prophylaxis was tapered after Day +100, if there was no evidence of acute GVHD. Thirty-five patients received GVHD prophylaxis with either tacrolimus as above or cyclosporine (CYA) continuous intravenous infusion beginning on

Day -3 and mycophenolate mofetil (MMF) 15 mg/kg twice daily starting on Day 0, with tapering of MMF after Day +60 and CYA after Day+100, if there was not evidence of acute GVHD. (6) Eleven patients received parathyroid hormone at 100 mcg a day for 28 days after transplantation on a concomitant trial. The protocols were reviewed and approved by the Institutional Review Board of the Dana Farber/Harvard Cancer Center. Written informed consent was obtained from all patients.

### Cord Unit Selection

Cord blood units were obtained from national and international registries. Cord blood units had to meet a minimum cell dose requirement of  $>1.5 \times 10^7$  NC/kg pre freeze for each individual unit and  $3.7 \times 10^7$  NC/kg pre freeze for both units combined. Cord blood units had to be at least a 4/6 A, B, DR allele level match with the patient and with each other. HLA C typing was performed on the cord units but was not used in the match strategy. Cord units were washed with dextran and albumin and were infused sequentially 1 to 6 hours apart. (25)

### Second Malignancies

All patients are followed for survival, relapse, and second cancers. When possible, donor origin of the second cancer was assessed by routine cytogenetics or chimerism studies. Chimerism assays of peripheral blood buffy coat isolates were performed by short tandem repeat (STR) analysis using a multiplex kit with primers for 10 different loci (Profiler Plus, Applied Biosystems, Foster City, CA), as previously described. (26) Patients were monitored for EBV every other week by DNA analysis, and rituximab was instituted for sustained levels greater than 1000.

### Statistical analysis

The time and each outcome event were measured from Day 0 of cord blood infusion. Overall survival was measured until the date of death or was censored at the last follow-up for patients who were still alive. Disease-free survival was measured until the earlier date of relapse or death, while it was censored at the last follow-up for patients who were alive and free of relapse. Overall and disease-free survivals were estimated by the Kaplan-Meier method. Treatment-related mortality was based on the cumulative incidence estimated in the presence of relapse as a competing risk. (27) The absolute risk of second cancer was based on the cumulative incidence estimated by considering death as a competing risk using Gray's method. Patients who had not developed a second malignancy or died were censored at their last follow-up. To adjust for confounding, the associations between patient or cord unit characteristics and second cancer incidence were analyzed in a multivariate model. In particular, competing risks regression using the Fine and Gray method (28) was used to assess the joint effects of age, primary disease, conditioning regimen, GVHD prophylaxis, HLA matching, nucleated cell dose and CD34+ dose. The relative risk of developing a second cancer was based on the hazard ratio estimate, with the 95% confidence interval (95% CI) calculated by taking exponents of the 95% CI for the regression coefficient. SAS 9.1 was used to perform the general computations, while the *cmprsk* package in R version 2.5.1 was used in the analysis of competing risks. All p-values are based on two-sided hypothesis tests. Due to the modest sample size available for a multivariate model with several covariates, p-values  $<0.200$  were considered to be suggestive of a trend within a hypothesis-generating framework.

## Results

### Patient Characteristics

Patient characteristics are outlined in Table 1. The median age of the 98 patients was 48 years (range 19-67 years). The majority of patients had acute leukemia (40 patients) or relapsed non-Hodgkin lymphoma (19 patients). Eighty percent of patients were Caucasian. Twenty percent of leukemia patients were considered standard risk and 80% high risk. The median follow-up was 29 months (range 6-69 months) with 43 patients still alive. Twenty-nine percent of patients received an ablative regimen. Patients with myelodysplasia, chronic myelogenous leukemia and aplastic anemia were more common in the myeloablative group (56% vs 21%) whereas patients with lymphoma were more often treated with the reduced intensity regimen (28% vs 3%).

### Cord Unit Characteristics

Cord unit characteristics are outlined in Table 2. The median nucleated cell (NC) dose infused for the combined two cord blood units was  $4.4 \times 10^7$  NC/kg (range 1.3-8.5). The median CD34+ dose infused for the combined two cord blood units was  $2.4 \times 10^5$  CD34+ cells/kg (range 0.3-11.5). Seventy-six percent of patients received two 4/6 HLA A, B, DR allele level matched cords. Fourteen percent received one 4/6 and one 5/6 matched cord unit. The remaining 8% received two 5/6 matched cord units. No patients received a 6/6 matched cord unit.

### Second Malignancies

Eighteen patients (18%) developed second malignancies, corresponding to an actuarial rate of 19% at 26 months after transplantation. The median time to second cancers was 134 days (range 39 days to 26 months). Thirteen of these patients died, all from second malignancy. Table 3 outlines the patients with second malignancies. Eleven patients in this study received parathyroid hormone after transplantation; one of these patients developed a second cancer. No solid tumors were observed in this population with a median follow-up after transplantation of 29 months.

### Epstein Barr Virus Lymphoproliferative Disorders

Sixteen patients had EBV-PTLD; 14 patients had donor derived disease as measured by chimerism assay, and the origin of the disease in the other two patients was not tested. In one patient, the bone marrow showed chimerism from one cord blood unit and the lung tissue with the lymphoproliferative disorder showed chimerism from the other cord blood unit. Post transplantation lymphoma developed at a median of 117 days after transplantation (range 39 to 368 days). The primary diseases were Hodgkin lymphoma (3), non-Hodgkin lymphoma (3), chronic lymphocytic leukemia (1), chronic myelogenous leukemia (1), aplastic anemia (2), myelodysplasia (3), and acute myelogenous leukemia (3). The median maximum EBV viral load was 9500 copies/ml (range 240-5.4 million). Thirteen patients had widespread organ involvement at the time of diagnosis, and two patients were treated for a rising EBV viral load. Twelve patients were treated with rituximab; three patients received rituximab and CHOP (cyclophosphamide, adriamycin, vincristine, and prednisone) therapy. Eight patients died with fulminant disease within 2 weeks of the diagnosis. Three patients also received EBV-specific cytotoxic T lymphocytes after failure of rituximab, and one of these patients is alive in a sustained remission. EBV PTLT resulted in the deaths of 11 patients.

Overall, five patients are alive and in remission, a median of 12 months from the diagnosis of PTLT. These surviving patients developed PTLT at a median of 5 months post transplant (range 4-12 months), similar to the general cohort of PTLT patients. The median EBV viral

load in these 5 patients was 7000 (range 603-340,000). All five patients received rituximab, one patient received R-CHOP and one patient, as above, also received EBV-specific cytotoxic T lymphocytes.

Patients were monitored every other week until Day +180 for the development of EBV viremia. After Day +180, patients were monitored as clinically indicated. An additional 12 patients developed an EBV viremia, but did not progress to a lymphoproliferative disorder. The median EBV viral load for these patients was 500 copies/ml with a range of 200 to 3300 copies/ml. The EBV viremia occurred at a median of 89 days after transplantation (range 22-1460 days). Two of these patients received rituximab, as part of treatment for relapsed disease.

### **Second Cancer with Myelodysplasia/Myeloproliferative Disorder**

Two patients developed myelodysplasia or myeloproliferative disorders, and both patients died of their second cancers. The actuarial rate of donor derived myeloid disease was 3%. Both patients received reduced intensity transplantations with the conditioning regimen of fludarabine, melphalan, and rabbit ATG. One occurred in a male patient transplanted for non-Hodgkin lymphoma at 21 months after double cord blood transplantation. He had been slow to engraft and was receiving filgrastim. He developed myelodysplasia with bone marrow showing 11% myeloblasts consistent with refractory anemia with excess blasts, and 100% XX cytogenetics. He was treated with 5 azacytidine without improvement. A second patient (female) was transplanted for AML, and developed a confirmed donor derived secondary MDS/MPD at 26 months after HSCT. Bone marrow revealed a hypercellular marrow with left shifted myeloid cells, erythroid dysplasia, and 100% XY cytogenetics. She was treated with steroids and splenectomy without improvement.

Both cases of donor derived second myeloid malignancies were reported to the cord blood banks, which provided the cord blood units. In one case, follow up revealed that both cord blood donors were healthy. No feedback was given from the cord blood banks in the second instance.

### **Predictors for Second Malignancies**

Age, primary disease, conditioning regimen, GVHD prophylaxis, HLA matching, nucleated cell dose and CD34+ dose were studied in a multivariate analysis to determine independent predictors of second cancers (Table 4). Reduced intensity conditioning was associated with a trend towards increased incidence of second cancers compared to myeloablative regimens, 24% versus 7%, but the effect is confounded with the effect of ATG. ATG was used in all patients receiving a reduced intensity regimen, compared to only 4 (14%) patients receiving a myeloablative regimen. A reduced intensity regimen with ATG was associated with a trend towards an increased risk of developing a second cancer, but the effect was not significant. ( $p=0.23$ ). Patients with primary Hodgkin lymphoma had a 38% incidence of second cancers that was higher than the 18% rate associated with other diseases, but this effect was also not significant ( $p=0.06$ ). Older age, GVHD prophylaxis, HLA matching, total nucleated cell dose and CD34+ cell dose had no association with an increased incidence of second cancers. Both patients with donor derived MDS/MPD had received ATG but risk factors for MDS/MPD could not be determined due to the low patient numbers.

### **Survival**

As reported previously, the reduced intensity CYA/MMF group had overall and disease-free survivals of 71% and 62% at one year, respectively. (6) Two year overall and disease-free survival was 57% and 57%. The reduced intensity group with either CYA/MMF or



sirolimus/tacrolimus GVHD prophylaxis had overall and disease-free survivals of 63% and 51% respectively at one year and 48% and 33% at two years respectively. In the myeloablative group, overall and disease-free survivals were 35% and 35% at one and two years, respectively. For the entire cohort, 100 day transplant related mortality (TRM) was 26%, with infection the leading cause of death. The majority of deaths from second cancers occurred after 100 days and therefore these patients are not included in this 100-day TRM. With a median follow up of 29 months among the 43 patients still alive, the overall and disease-free survivals for the entire cohort were 55% and 47% at one year, and 44% and 32% at 2 years, respectively. (Figures 1 and 2) Twenty-four patients had relapse of their disease; in ten of these patients the relapse was documented by cytogenetics to be of recipient origin.

## Discussion

Approximately 10,000 umbilical cord blood transplants have been performed worldwide, the majority for hematologic malignancies. (1-4) Recently, double umbilical cord blood transplantation has been employed in adult patients to shorten the engraftment times and reduce the risk of infections. (5-7,29) While second malignancies have been reported after double cord blood transplantation, the magnitude of the risk is uncertain. We report a high incidence (19%) of second cancers, including a surprising 3% incidence of donor derived myeloid cancers.

As has been reported previously by the Minnesota group, we found a high incidence of PTLD in our double cord blood patients. (16) Our incidence of PTLD is higher than that reported in some double cord blood transplant series, but comparable to the incidence reported by the Minnesota group in patients receiving reduced intensity transplants with ATG. Brunstein and colleagues reported a 3% incidence of PTLD in patients who received a myeloablative conditioning regimen containing ATG (3% with ATG vs none with no ATG). However, the incidence of PTLD rose sharply when a reduced intensity preparative regimen was employed, to 21% with ATG vs 2% with no ATG. The increased incidence of PTLD after cord blood transplantation may be related to the absence of EBV specific memory T cells in cord blood grafts. (30) The increase with reduced intensity conditioning is not completely understood but may be related to the number of residual recipient B cells remaining after conditioning. (31) Although weekly or every other weekly EBV monitoring may help identify early viral reactivation, the PTLD after cord blood HSCT appears to be more aggressive than after solid organ transplantation, with rapid progression to multi organ involvement and lack of responsiveness to rituximab. (31,32) However, the risk of death in our series in patients who developed PTLD (69%) was similar to that seen in other stem cell transplantation series. For example, the German group reported 82% mortality in their stem cell transplant patients who developed PTLD. (33,34) PTLD after hematopoietic stem cell transplant of all sources has a poorer prognosis than PTLD after solid organ transplant.

In prior analyses in conventional stem cell transplantation, risk factors for PTLD include T cell depletion, HLA disparity, GVHD, and the use of ATG. (14,33,34) After the first year, however, the major risk factor identified is the presence of chronic GVHD. (14) In this cord blood cohort, no variables were found to be statistically significant, although reduced intensity conditioning with the use of ATG was associated with a trend to an increased risk of second malignancies. Intensive monitoring, lowering the dose of ATG, prompt use of EBV specific cytotoxic T cells, or adding rituximab to the transplantation regimen are strategies that might lower the incidence of PTLD. (35,36,37) For example, the Italian group employs a single dose of rituximab on Day +5 after cord blood transplantation. (38)

We did not find second solid tumors, although the follow-up in our series is too short at a median of 30 months after transplantation. Squamous cell cancers have also been reported with increased frequency in older patients with chronic GVHD, and some of these squamous cell cancers are of donor origin. (39-41)

The incidence of donor-derived MDS/MPD found here (3%) is higher than that reported in the literature after adult-donor bone marrow or peripheral blood stem cell transplantation (<0.25%). (17-23) Our incidence is higher than that reported in other cord blood transplant series; however, donor-derived myeloid malignancy may be underreported as donor derived AML would be difficult to distinguish from relapsed leukemia, in the absence of cytogenetic or chimerism data. (4,5,7) Donor derivation by chimerism or cytogenetics may not be routinely done after transplantation so the true incidence after cord blood or standard transplantation is unknown. The biology for the development of second cancers is unclear. Both patients in this series with donor derived MDS/MPD had received reduced intensity regimens containing ATG but the small numbers preclude determining any specific risk factors.

It is possible that donor cord blood may be more likely than adult blood to contain pre-leukemic cells and contribute to a first “hit” in the “two-hit” development of a second cancer. For example, the TEL-AML 1 translocation was detectable in 6 of 567 cord blood samples. (9) The investigators estimate that up to 5% of cord blood units may contain pre-leukemic clones. (42) The use of growth factors to drive a low number of viable hematopoietic stem cells may also foster pre leukemic mutations, as has been postulated to occur in patients with aplastic anemia. (8,43,44)

Comparison of the incidence of second myeloid malignancies seen after cord blood vs. adult-donor bone marrow or stem cell transplantation is difficult. In our institution, donor origin has not been routinely tested at the time of suspected relapse. We believe these findings, which have altered our own practices, would encourage other investigators to monitor donor origin for second cancers after all allogeneic, unrelated or cord blood transplantations.

Donor derived second myeloid malignancies present unique ethical issues, especially when the donor is an infant. A consistent system of testing for donor origin in all patients with suspected relapse would help distinguish relapsed disease from a donor derived second cancer. Should the donor and their parents be notified whenever a donor derived second malignancy is diagnosed? Should these donors have any special follow-up or monitoring? These issues will need to be addressed as we perform more cord blood transplants with modern technology that permits determination of donor origin.

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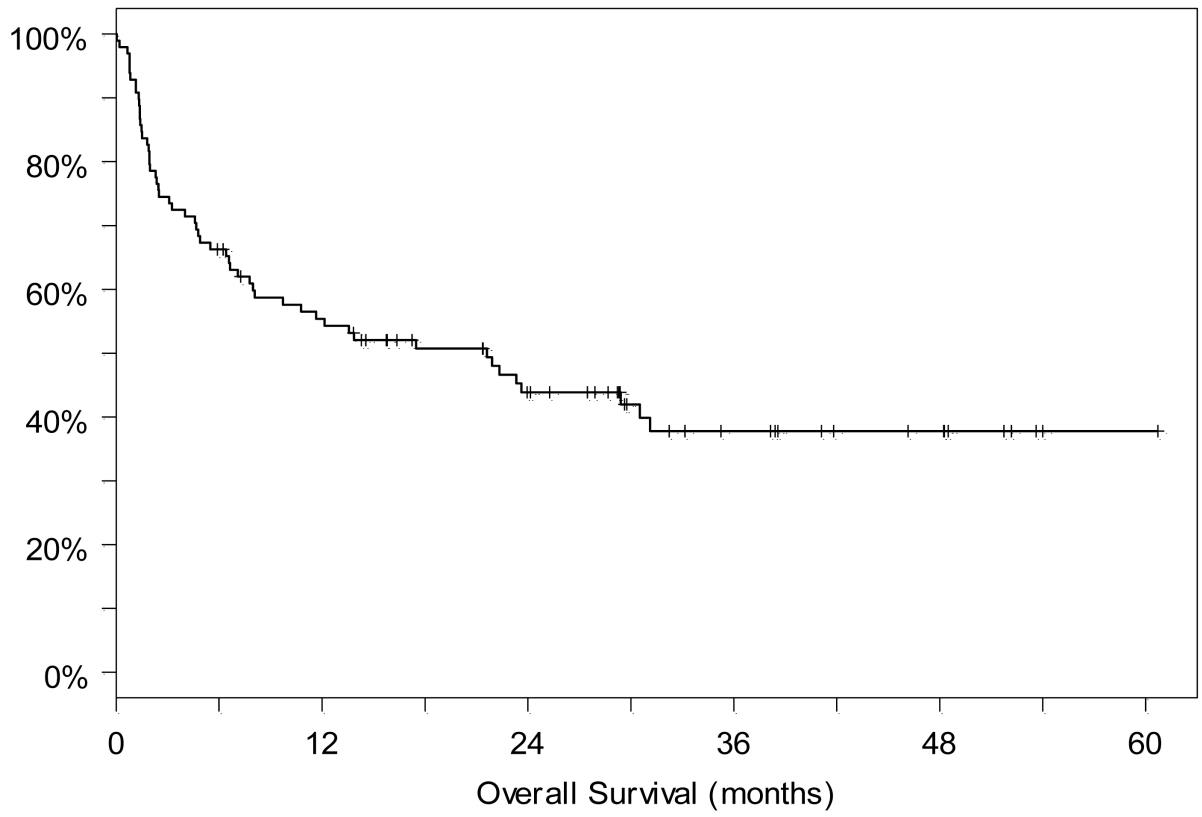
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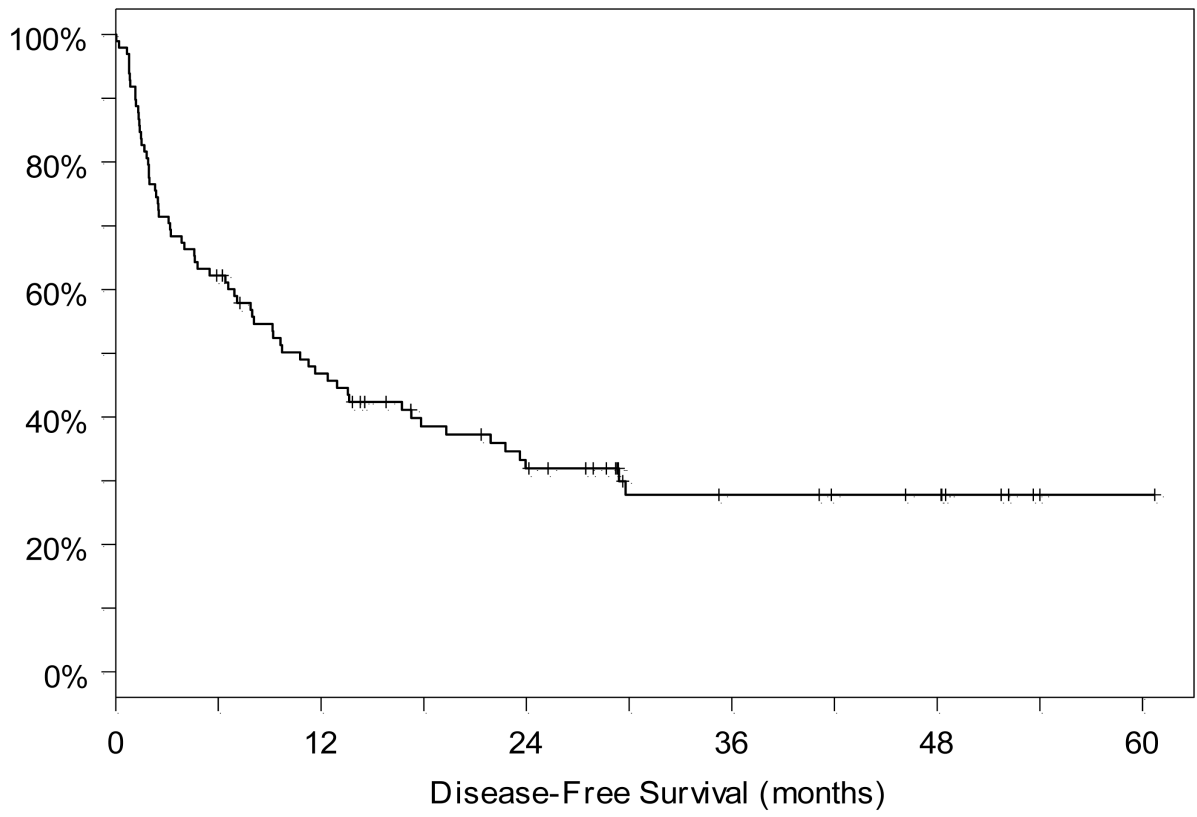
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**Figure 1.** Overall survival for the entire cohort from the time of transplantation.



**Figure 2.** Disease-free survival for the entire cohort from the time of transplantation.

**Table 1**

## Patient Characteristics

<b>Median Age (range)</b>	<b>48 years (19-67)</b>
Male Sex	57%
Malignancy	
AML	33 (34%)
Non-Hodgkin lymphoma	19 (19%)
Hodgkin lymphoma	8 (8%)
Aplastic Anemia	7 (7%)
MDS	12 (12%)
CLL/PLL	6 (6%)
ALL	6 (6%)
CML	4 (4%)
Other	3 (3%)
Transplant Risk	
Standard	18 (20%)
High Risk	70 (80%)
Conditioning Regimen	
Reduced Intensity	70 (71%)
Myeloablative	28 (29%)
GVHD Prophylaxis	
Sirolimus-based	63 (64%)
Non-sirolimus Based	35 (36%)

Standard Risk: AML/ALL CR 1, MDS RA/RARS, CML CP1

High Risk: All others, excluding aplastic anemia and lymphoma

**Table 2**

## Cord Blood Characteristics

Infused Cell Doses Combined Cord Blood Units	Median (Range)
Nucleated Cells/kg	$4.4 \times 10$ (7) (1.3-8.8)
CD 34+ cells/kg	$2.4 \times 10$ (5) (0.3-11.5)
HLA Match (A, B, DRB1)	N (percent)
4//6, 4/6	76 (78%)
4/6, 5/6	14 (14%)
5/6, 5/6	8 (8%)
5/6,6/6	0
6/6, 6/6	0

**Table 3**

## Second Malignancies

Primary Disease	Type of Second Cancer	Derivation	Outcomes
AML	MDS/MPD	Donor	Fatal
Hodgkin lymphoma	PTLD	Donor	Fatal
Non-Hodgkin lymphoma (NHL)	MDS/MPD	Donor	Fatal
Hodgkin lymphoma	PTLD	Unknown	Fatal
CLL	PTLD	Donor	Fatal
MDS	PTLD	Donor	Fatal
AML	PTLD	Donor	Fatal
MDS	PTLD	Donor	Alive, in CR
NHL	PTLD	Donor	Fatal
Hodgkin lymphoma	PTLD	Donor	Fatal
AML	PTLD	Donor	Fatal
AML	PTLD	Donor	Alive, in CR
NHL	PTLD	Donor	Fatal
NHL	PTLD	Donor	Fatal
MDS	PTLD	Donor	Fatal
Aplastic Anemia	PTLD	Donor	Alive, in CR
Aplastic Anemia	PTLD	Donor	Alive, in CR
CML	PTLD	Unknown	Alive, in CR



**Table 4**

## Predictors for Second Cancers

Patient or Cord Unit Characteristic	Risk Groups	Cumulative Incidence	Hazard Ratio (95% CI)	p-value
Age	> 50 years	26%	1.8 (0.8-4.8)	0.270
	≤ 50 years	14%		
Primary disease	Hodgkin lymphoma	38%	2.8 (0.6-4.8)	0.064
	Other malignancies	18%		
Conditioning Regimen	Reduced Intensity	24%	2.5 (0.6-10.6)	0.230
	Myeloablative	7%		
GVHD prophylaxis	Sirolimus Based	20%	1.1 (0.5-2.9)	0.800
	Other Agents	18%		
HLA matching	4/6,5/6 or 5/6, 5/6	30%	0.9 (0.3-2.6)	0.350
	4/6, 4/6	16%		
Nucleated cell dose infused	> $4.4 \times 10^7/\text{kg}$	21%	1.5 (0.6-3.9)	0.350
	≤ $4.4 \times 10^7/\text{kg}$	17%		
CD34+ cell dose infused	≤ $2.4 \times 10^5/\text{kg}$	21%	1.5 (0.6-3.6)	0.380
	> $2.4 \times 10^5/\text{kg}$	15%		