

HEMATOPOIETIC ENGRAFTMENT AND SURVIVAL IN ADULT RECIPIENTS OF UMBILICAL-CORD BLOOD FROM UNRELATED DONORS

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ABSTRACT

Background Umbilical-cord blood from unrelated donors who are not HLA-identical with the recipients can restore hematopoiesis after myeloablative therapy in children. We studied the use of transplantation of umbilical-cord blood to restore hematopoiesis in adults.

Methods Sixty-eight adults with life-threatening hematologic disorders received intensive chemotherapy or total-body irradiation and then transplants of HLA-mismatched umbilical-cord blood. We evaluated the outcomes in terms of hematologic reconstitution, the occurrence of acute and chronic graft-versus-host disease (GVHD), relapses, and event-free survival.

Results Of the 68 patients, 48 (71 percent) received grafts of umbilical-cord blood that were mismatched for two or more HLA antigens. Of the 60 patients who survived 28 days or more after transplantation, 55 had neutrophil engraftment at a median of 27 days (range, 13 to 59). The estimated probability of neutrophil recovery in the 68 patients was 0.90 (95 percent confidence interval, 0.85 to 1.0). The presence of a relatively high number of nucleated cells in the umbilical-cord blood before it was frozen was associated with faster recovery of neutrophils. Severe acute GVHD (of grade III or IV) occurred in 11 of 55 patients who could be evaluated within the first 100 days after transplantation. Chronic GVHD developed in 12 of 33 patients who survived for more than 100 days after transplantation. The median follow-up for survivors was 22 months (range, 11 to 51). Of the 68 patients, 19 were alive and 18 of these (26 percent) were disease-free 40 months after transplantation. The presence of a high number of CD34+ cells in the graft was associated with improved event-free survival ($P=0.05$).

Conclusions Umbilical-cord blood from unrelated donors can restore hematopoiesis in adults who receive myeloablative therapy and is associated with acceptable rates of severe acute and chronic GVHD. (N Engl J Med 2001;344:1815-22.)

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TRANSPLANTATION of allogeneic hematopoietic stem cells, an effective treatment for hematologic cancers,¹ is limited by the availability of HLA-matched donors. The risk of severe graft-versus-host disease (GVHD) after the transplantation of bone marrow from unrelated donors or partially HLA-mismatched related donors

is another drawback of the procedure.²⁻⁶ Umbilical-cord blood is a source of hematopoietic stem cells that has been successfully used for transplantation, primarily in children.⁷⁻¹⁷ Grafts of umbilical-cord blood reconstitute hematopoiesis more slowly than does HLA-matched marrow from a sibling or an unrelated adult donor,²⁻⁶ but the incidence and severity of GVHD are lower with transplantation of umbilical-cord blood,¹⁴ even with HLA-mismatched umbilical-cord blood.^{8-10,12} In this study, we evaluated the safety of transplantation of umbilical-cord blood from unrelated donors in adults.

METHODS**Eligibility**

The clinical protocols for the transplantation of umbilical-cord blood were approved by the institutional review boards at participating institutions. Patients were eligible for enrollment if their disease was stable and they lacked an HLA-identical related or unrelated donor or if their disease was unstable, they lacked a related donor, and an HLA-matched unrelated donor of bone marrow could not be identified within six to eight weeks. Written informed consent was obtained from all patients. For the patients with leukemia, disease status was categorized according to the criteria of the International Bone Marrow Transplant Registry.¹⁸

Selection of Grafts

Preliminary searches of umbilical-cord-blood banks were performed with the use of the patient's HLA phenotype, as determined by serologic typing for class I HLA-A and HLA-B antigens and low-resolution DNA typing for class II HLA alleles. High-resolution molecular typing for HLA-DRB1 alleles was performed as confirmatory typing. Preferred cord-blood units were those matched at three or more of six HLA loci and containing a minimal cell count of 1×10^7 nucleated cells per kilogram of the recipient's body weight before freezing. In some cases, a less closely matched graft with a higher number of nucleated cells was selected over a more closely matched graft with fewer nucleated cells. Units of umbilical-cord blood were not depleted of T lymphocytes. Some units were reduced in volume and depleted of red cells with heparin before freezing.¹⁹ Fifty-seven grafts of umbilical-cord blood

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were obtained from the Placental Blood Program of the New York Blood Center. The remaining 11 grafts of umbilical-cord blood were obtained from other umbilical-cord-blood banks.

Preparative Regimens and Prophylaxis against GVHD

A regimen based on total-body irradiation was administered to 51 patients with cancer, and a regimen based on busulfan was administered to 14 patients, 6 of whom had nonmalignant disorders and 8 of whom had cancer. All patients received a total dose of 60 to 90 mg of antithymocyte globulin (ATGAM, Pharmacia-Upjohn, Kalamazoo, Mich.) per kilogram for two to three days before the infusion of umbilical-cord blood. Two patients with Fanconi's anemia received conditioning therapy as previously described.²⁰ One patient with acute myeloid leukemia received 25 mg of fludarabine per square meter of body-surface area per day for five days and 90 mg of melphalan per square meter per day for two days. Prophylaxis against GVHD consisted of cyclosporine alone or cyclosporine plus methylprednisolone.⁸ Corticosteroid therapy was generally tapered by 10 weeks after transplantation, and tapering of the dose of cyclosporine was initiated 180 to 270 days after transplantation in patients who did not have signs or symptoms of chronic GVHD.

Transplantation Procedure and Supportive Care

Cryopreserved units of cord blood were transported to the transplantation center in liquid nitrogen and were maintained in the vapor phase until the time of transplantation. The units were thawed, and some were washed with 10 percent dextran 40 (Baxter, Glendale, Calif.) and 5 percent human albumin before infusion.¹⁹ After thawing, the following tests were performed on the blood: nucleated-cell count, CD34+ and CD3+ cell count, assays for colony-forming units, tests of cell viability (exclusion of trypan blue dye), and bacterial and fungal cultures. CD34+ quantification was performed for 61 of the 68 patients enrolled. Supportive care included the administration of 5 to 10 μ g of filgrastim (Amgen, Thousand Oaks, Calif.) per kilogram per day subcutaneously or intravenously from the day of transfusion of umbilical-cord blood (day 0) until durable recovery of neutrophils was achieved. The patients received standard blood products, antibiotics, antifungal agents, and nutritional support according to the protocols of the local institution.

Hematopoietic Recovery

The time of recovery of myeloid cells was defined as the first of three consecutive days after transplantation during which the absolute neutrophil count was at least 500 per cubic millimeter. The time of recovery of platelets and red cells was defined as the first of seven days on which the platelet count was at least 20,000 per cubic millimeter and the hemoglobin level was at least 8 g per deciliter without transfusion support. Chimerism was evaluated by fluorescence in situ hybridization for the Y chromosome, DRB1 allele-specific hybridization, or quantitative polymerase-chain-reaction analysis for microsatellite DNA markers.

GVHD

During the first 100 days after transplantation, all patients were evaluated for GVHD, which was graded according to standard practice.²¹ Acute GVHD (of grade II or higher) was treated with at least 2 mg of methylprednisolone per kilogram per day for seven days or more, after which time the dose was tapered if there was an adequate response. Corticosteroid-resistant GVHD was treated with second-line agents according to institutional protocols. Patients were evaluated for chronic GVHD with the use of standard criteria.²²

Statistical Analysis

All 68 patients who received cord blood from an unrelated donor at the five participating institutions from February 1995 to September 1999 were included in the analysis. The study end

points were the probability of neutrophil, red-cell, and platelet recovery; the occurrence of acute or chronic GVHD; and event-free and overall survival. Primary graft failure was defined as an absence of donor-derived myeloid cells in patients who survived for more than 28 days after transplantation. The time required to attain an absolute neutrophil count of at least 500 per cubic millimeter was censored at the date of death, recovery of the patient's myeloid cells, or relapse or at 42 days after transplantation, whichever occurred first. Overall survival was measured from the date of transplantation to the date of death and was censored as of the date of the last follow-up visit for survivors. Event-free survival was measured from the date of transplantation to the date of relapse or death, whichever occurred first, and was censored at the date of the patient's hematopoietic recovery or the date of the last follow-up, whichever occurred first. Analyses of data obtained at the last follow-up visit were performed for all patients on August 2, 2000. Variables related to the patient, the disease, and the transplantation procedure were compared with the use of the chi-square test for categorical variables. Those included in these analyses were the age of the recipient, the sex of the recipient, the degree of HLA and ABO matching between the donor and recipient, the weight of the recipient, the disease (acute myeloid leukemia, chronic myeloid leukemia, acute lymphocytic leukemia, or other), the results of serologic tests for cytomegalovirus (CMV) in the recipient before transplantation, whether the recipient received total-body irradiation or chemotherapy-only conditioning therapy, and characteristics of the graft (total nucleated-cell content and results of HLA typing before freezing and nucleated-cell counts, CD34 content, and colony-forming-unit content after thawing). The probability of neutrophil engraftment and of event-free survival was estimated by the Kaplan-Meier method and evaluated with the use of the log-rank test or Wilcoxon test for univariate analyses. All reported P values are two-sided.

RESULTS

Characteristics of the Patients

Of 54 patients with hematologic cancers, 50 were classified as having intermediate or advanced disease according to the criteria of the International Bone Marrow Transplant Registry.¹⁸ Fourteen patients underwent transplantation of cord blood for nonmalignant disease. The median age of the recipients was 31.4 years, and the median weight was 69.2 kg (Table 1).

Characteristics of the Grafts of Umbilical-Cord Blood

The six possible HLA matches between the recipient and the graft were scored serologically for HLA-A and B and genetically for DRB1 alleles. The results were six of six possible matches in 2 patients, five of six in 18 patients, four of six in 37 patients, and three of six in 11 patients. The median number of nucleated cells in the grafts, measured before freezing, was 2.1×10^7 per kilogram of the recipient's body weight (range, 1.0×10^7 to 6.3×10^7 per kilogram), and the median number measured after thawing was 1.6×10^7 per kilogram (range, 0.6×10^7 to 4×10^7 per kilogram). After thawing, the median number of CD34+ progenitor cells was 1.2×10^5 per kilogram (range, 0.2×10^5 to 16.7×10^5 per kilogram), the median number of colony-forming units was 1.2×10^4 per kilogram (range, 0 to 25.4×10^4 per kilogram), and the median number of CD3+ cells was 4.6×10^6 per kilogram (range, 0.9×10^6 to 9.1×10^6 per kilogram). The number of nucleated

TABLE 1. CHARACTERISTICS OF THE 68 PATIENTS.

CHARACTERISTIC	VALUE
Age (yr)	
Median	31.4
Range	17.6–58.1
Weight (kg)	
Median	69.2
Range	40.9–115.5
No. of patients in whom previous transplantsations failed	7
Diagnosis (no. of patients)	
Cancer	
Acute lymphoblastic leukemia	15
First or second complete remission	8
Advanced disease	7
Acute myeloid leukemia	19
First or second complete remission	10
Advanced disease	9
Chronic myelomonocytic leukemia	1
Chronic myeloid leukemia	15
First chronic phase	10
Advanced disease	5
Chronic lymphocytic leukemia	1
Non-Hodgkin's lymphoma	1
Refractory Hodgkin's disease	2
Bone marrow failure syndromes	
Severe aplastic anemia	4
Blackfan–Diamond anemia*	1
Fanconi's anemia	4
Myelofibrosis	2
Myelodysplastic syndrome	2
Inborn error of metabolism	
Adrenoleukodystrophy	1

*This patient received regular transfusions for 20 years.

cells in the graft before freezing correlated with the number of CD34+ cells present after thawing ($P < 0.001$) (data not shown).

Hematopoietic Recovery

The estimated probability of neutrophil recovery during the first 42 days after transplantation was

0.90 (95 percent confidence interval, 0.85 to 1.0), and the median time required to attain an absolute neutrophil count of at least 500 per cubic millimeter was 27 days (range, 13 to 59). Eight patients died before day 28 without myeloid recovery. Of the 60 patients who survived for 28 days or more after transplantation, primary graft failure occurred in 5 (Table 2). In three patients, engraftment of the cord-blood cells occurred after day 42. There was no statistically significant association between graft failure and the extent of HLA mismatching ($P = 0.50$ for the comparison of three groups of patients: those receiving grafts with five of six or six of six HLA matches, with four of six HLA matches, and with three of six HLA matches), mismatching at HLA class II alleles ($P = 0.21$), seropositivity for CMV in the recipients ($P = 0.35$), whether the patient's condition was malignant or non-malignant ($P = 0.97$), or diagnosis (acute lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, or others; $P = 0.90$) (data not shown).

Figure 1A shows the relation between neutrophil recovery and nucleated-cell counts in the umbilical-cord blood before it was frozen. Figure 1B shows neutrophil recovery according to the degree of HLA matching. The closeness of HLA matching was unrelated to the speed of recovery. The speed of recovery was also unrelated to the number of CD34+ cells ($P = 0.26$) and CD3+ cells ($P = 0.15$) in the graft and to the use or nonuse of hetastarch for the depletion of red cells before freezing ($P = 0.77$). In 30 patients who could be evaluated, platelet recovery took a median of 58 days (range, 35 to 142), and in 28 patients who could be evaluated, red-cell recovery took a median of 60 days after transplantation (range, 21 to 273). Recovery of platelet counts of more than 50,000 per cubic millimeter and more than 100,000 per cubic millimeter occurred at a median of 99 days (range, 42 to 228) and 124 days (range, 76 to 280),

TABLE 2. PRIMARY GRAFT FAILURES.

PATIENT No.	No. OF CELLS BEFORE FREEZING no. $\times 10^{-7}/\text{kg}$	RECIPIENT/DONOR HLA MISMATCHES	No. OF HLA MATCHES	DIAGNOSIS	OUTCOME
1	2.20	DRB1 1303/1503; DRB1 blank/1602	4 of 6	Severe aplastic anemia	Death due to multiorgan failure, day 35
2	1.41	HLA-A 24/1; HLA-A 32/2; HLA-B blank/38	3 of 6	Secondary acute myeloid leukemia	Death due to multiorgan failure, day 35
3	1.55	HLA-A 1/29	5 of 6	Acute lymphocytic leukemia in relapse	Death due to sepsis, day 33
4	1.75	HLA-A 24/1; HLA-A 28/23; HLA-B 35/70	3 of 6	Acute myeloid leukemia in second complete remission	Death due to sepsis, day 42
5	1.38	HLA-B 51/7; HLA-B 60/35	4 of 6	Accelerated phase of chronic myeloid leukemia	Recovery of patient's cells, day 28

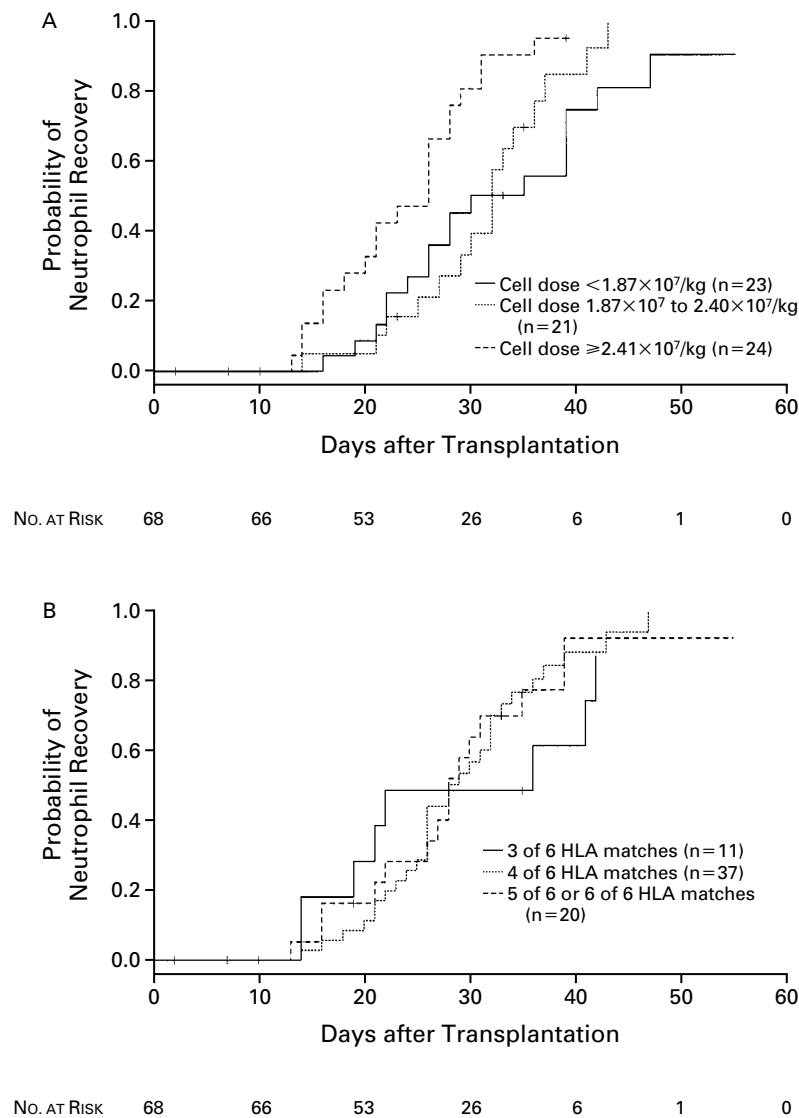


Figure 1. Recovery of Neutrophils after Transplantation of Umbilical-Cord Blood.

The kinetics of neutrophil engraftment after transplantation are plotted against the number of cells in the umbilical-cord blood before freezing (Panel A); $P=0.003$ for the comparison of a cell dose of less than 1.87×10^7 per kilogram with a cell dose of 1.87×10^7 to 2.40×10^7 per kilogram and with a cell dose of 2.41×10^7 or more per kilogram. The kinetics of neutrophil engraftment after transplantation are plotted against the degree of HLA matching between the graft of umbilical-cord blood and the recipient (Panel B); $P=0.82$ for the comparison of three groups of patients: those receiving grafts with five of six or six of six HLA matches, with four of six HLA matches, and with three of six HLA matches.

respectively. In all patients in whom neutrophil recovery occurred, the blood contained over 98 percent donor cells and there were no late graft failures.

GVHD

Among the 55 patients in whom engraftment of the transplanted cells occurred and who survived for 28 days or more, 22 had grade 0 to I acute GVHD,

22 had grade II, 7 had grade III, and 4 had grade IV. The probability of grade II to IV and grade III or IV acute GVHD by 100 days after transplantation was 0.60 (95 percent confidence interval, 0.49 to 0.71) and 0.20 (95 percent confidence interval, 0.11 to 0.29), respectively. There was no statistically significant association between the grade of acute GVHD and the degree of HLA mismatching ($P=0.70$), mis-

matching in HLA class II alleles ($P=0.68$), seropositivity for CMV in the recipient before transplantation ($P=0.34$), or use of total-body irradiation or busulfan as conditioning therapy ($P=0.68$). Among the 33 patients who survived for more than 100 days after transplantation, chronic GVHD developed in 12; all but 1 of the 12 patients had limited-stage disease, as defined by the involvement of a single organ.²² The probability of chronic GVHD was 0.38 (95 percent confidence interval, 0.23 to 0.52) from day 100 after transplantation until the date of the last follow-up.

Relapse and Survival

As of August 2, 2000, 19 of the 68 patients who underwent transplantation are alive and 18 are disease-free, with a median follow-up of 22 months (range, 11 to 51). Table 3 shows the causes of death, and Figure 2A displays the probability of event-free survival after transplantation (a univariate analysis of prognostic factors in event-free survival is available with the full text of this article at <http://www.nejm.org>). Within the first three months after transplantation, 32 patients died of either toxicity related to the preparative regimen (multiorgan failure, hepatic veno-occlusive disease, or interstitial pneumonitis) or infection. Four patients with cancer relapsed within the first year after transplantation (three had acute lymphocytic leukemia and one had Hodgkin's disease). The degree of HLA mismatching between the donor of the graft and the recipient was not related to event-free survival ($P=0.07$), nor was older age (more than 40 years vs. 25 to 40 years vs. less than 25 years; $P=0.42$). There were no statistically significant differences in event-free survival according to the type of malignant disorder ($P=0.61$). Event-free survival in patients who received grafts containing more than 1.2×10^5 CD34+ cells per kilogram was longer than in patients who received fewer CD34+ cells ($P=0.05$) (Fig. 2B).

The number of nucleated cells in the graft of umbilical-cord blood before freezing or after thawing, serologic status with respect to CMV, the preparative regimen used, International Bone Marrow Transplant Registry classification (early vs. intermediate or advanced disease), diagnosis, use or nonuse of heparin for depletion of red cells, and the grade of acute GVHD (grade I or II vs. grade III or IV) were not predictors of event-free survival at six months (data not shown).

DISCUSSION

We found that umbilical-cord blood from unrelated donors engrafted and reconstituted myeloid hematopoiesis in 90 percent of adult recipients, most of whom weighed more than 60 kg. However, the time to hematopoietic engraftment was longer than that reported for allogeneic grafting with marrow from

TABLE 3. CAUSES OF DEATH.

TIME AFTER TRANSPLANTATION	REGIMEN-RELATED TOXICITY	INFECTION	RELAPSE	SECOND CANCER	GRAFT-VERSUS-HOST DISEASE	no. of deaths					
Before day 28	5	3	0	0	0						
Day 28 to 100	10	14	1	0	2						
After day 100	2	5	3	3	1						

HLA-matched siblings or unrelated adult donors.^{2,4-6} In a recent report summarizing the outcomes of transplantation in a cohort of 1423 patients with chronic myeloid leukemia who received marrow transplants from unrelated adult donors, the actuarial incidence of engraftment of donor cells by day 42 after transplantation was 92 percent (95 percent confidence interval, 90 to 94 percent), and the median time to neutrophil recovery was 20 days (range, 8 to 42).⁴

In our 68 patients, there were 17 deaths related to the preparative regimen and 22 deaths secondary to infection after transplantation (Table 3). These high death rates may be attributable in part to the selection of patients at high risk. The slow myeloid engraftment may also have contributed to death within the first three months after the transplantation of umbilical-cord blood. As noted in children, the number of nucleated cells in the umbilical-cord blood before freezing and the number of CD34+ cells after thawing were associated with a shorter time to myeloid engraftment and with improved event-free survival in our adult recipients.^{7,9,10,12,13} Since the CD34+ content of cord blood is currently not always measured before freezing, clinicians must rely on nucleated-cell counts and HLA analyses to guide the selection of a graft. We observed that the number of nucleated cells in the umbilical-cord blood before freezing correlated with the number of CD34+ cells present after thawing.

Recipients of umbilical-cord blood receive approximately $\frac{1}{10}$ as many CD34+ cells as recipients of allogeneic marrow. This explains the delayed hematopoietic recovery in recipients of umbilical-cord blood.^{23,24} Rapid engraftment of marrow from siblings and unrelated adult donors is related to the dose of cells, the number of CD34+ cells, and the degree of HLA matching.²³⁻²⁷ In our patients who received transplants of umbilical-cord blood, HLA class I mismatching did not correlate with graft failure. Additional causes of delayed hematopoietic recovery in patients who received umbilical-cord blood may relate to characteristics of grafts of umbilical-cord-blood progenitor cells that affect homing or maturation.²⁸⁻³¹ CD34+

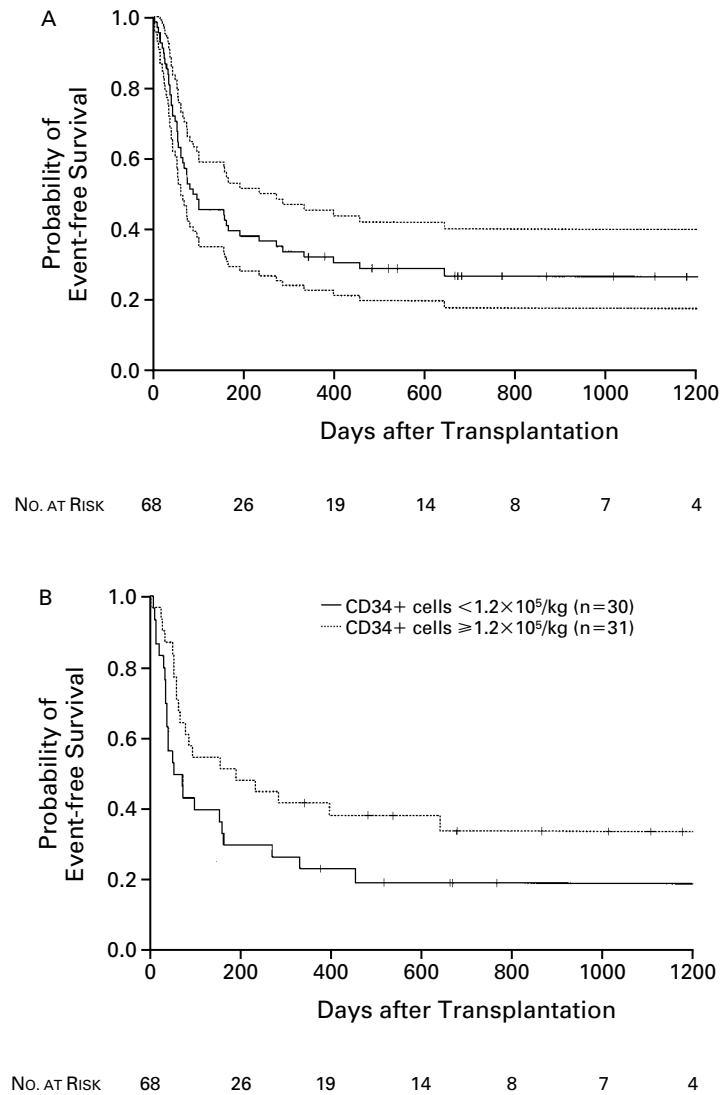


Figure 2. Event-free Survival.

Event times were analyzed as of August 2, 2000, and included data for all patients obtained at the last follow-up visit. Overall event-free survival (solid line) with 95 percent confidence interval (dotted lines) is shown in Panel A. The estimated probability of event-free survival is plotted against the number of CD34+ cells transfused in the graft of umbilical-cord blood in Panel B; $P=0.05$ by the Wilcoxon test for the comparison of less than 1.2×10^5 per kilogram with 1.2×10^5 or more per kilogram. CD34+ quantification was not performed for seven patients.

progenitor cells in umbilical-cord blood have a less mature phenotype than those in adult marrow and peripheral blood.^{32,33} Nonetheless, the durability of these grafts of umbilical-cord blood is clear; to date, there have been no late graft failures in the surviving patients (median follow-up, 22 months). Since all patients in this study received filgrastim at a dose of 5 to 10 μg per kilogram per day until neutrophil recovery occurred, the effect of filgrastim on neutrophil recovery could not be evaluated.

Although 66 of the 68 patients in this study received grafts that had HLA mismatches, the probability of severe acute GVHD (grade III or IV) was only 20 percent. This low probability compares favorably with the 35 to 55 percent reported in recipients of HLA-matched bone marrow from unrelated adult donors who received standard prophylaxis against GVHD.^{2-6,34} The probability of chronic GVHD in our series was 38 percent, and all but one patient had limited-stage disease. In comparison, chronic GVHD de-

velops in 55 to 75 percent of patients receiving HLA-matched bone marrow transplants from unrelated donors.⁴⁻⁶ These differences in the incidence and severity of acute and chronic GVHD may be related to reduced numbers of CD3+ T lymphocytes in the graft of umbilical-cord blood, the immunologic immaturity of lymphocytes in umbilical-cord blood, or both.³⁵⁻³⁷

In summary, the results of this study demonstrate that HLA-mismatched umbilical-cord blood from unrelated donors is a feasible alternative source of hematopoietic stem cells for transplantation in adults. Hematopoietic reconstitution occurred in 90 percent of our patients, and the incidence and severity of GVHD were low despite HLA mismatching. This approach should be considered in the cases of adult patients for whom an HLA-matched unrelated donor is not readily available.

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