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OUTCOMES AMONG 562 RECIPIENTS OF PLACENTAL-BLOOD TRANSPLANTS FROM UNRELATED DONORS

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ABSTRACT

Background A program for banking, characterizing, and distributing placental blood, also called umbilical-cord blood, for transplantation provided grafts for 562 patients between August 24, 1992, and January 30, 1998. We evaluated this experience.

Methods Placental blood was stored under liquid nitrogen and selected for specific patients on the basis of HLA type and leukocyte content. Patients were prepared for the transplantation of allogeneic hematopoietic cells in the placental blood and received prophylaxis against graft-versus-host disease (GVHD) according to routine procedures at each center.

Results Outcomes at 100 days after transplantation were known for all 562 patients, and outcomes at 1 year for 94 percent of eligible recipients. The cumulative rates of engraftment among the recipients, according to actuarial analysis, were 81 percent by day 42 for neutrophils (median time to engraftment, 28 days) and 85 percent by day 180 for platelets (median, day 90). The speed of myeloid engraftment was associated primarily with the leukocyte content of the graft, whereas transplantation-related events were associated with the patient's underlying disease and age, the number of leukocytes in the graft, the degree of HLA disparity, and the transplantation center. After engraftment, age, HLA disparity, and center were the primary predictors of outcome. Severe acute GVHD (grade III or IV) occurred in 23 percent of patients, and chronic GVHD occurred in 25 percent. The rate of relapse among recipients with leukemia was 9 percent within the first 100 days, 17 percent within 6 months, and 26 percent by 1 year. These rates were associated with the severity of GVHD, type of leukemia, and stage of the disease.

Conclusions Placental blood is a useful source of allogeneic hematopoietic stem cells for bone marrow reconstitution. (N Engl J Med 1998;339:1565-77.)

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TRANSPLANTATION of hematopoietic stem and progenitor cells from placental blood, also called umbilical-cord blood, from unrelated donors can restore the function of bone marrow and sustain hematopoietic recovery in both related and unrelated recipients.¹⁻⁵ For patients for whom no suitable related donor is available, this source of hematopoietic stem cells offers substantial advantages, notably the relative ease of procurement; the absence of risk to the donor; the small likelihood of transmitting clinically important infections, especially cytomegalovirus (CMV) and Epstein-Barr virus (EBV); the low risk of severe graft-versus-host disease (GVHD)³⁻⁶; and the rapid availability of placental blood to transplantation centers.^{6,7} The reduced severity of GVHD after the infusion of allogeneic placental blood, as compared with transplantation of bone marrow from unrelated donors, permits the use of transplants from HLA-mismatched donors and improves the odds of finding donors for patients with uncommon tissue types. Our efforts to make placental blood available for transplantation began in 1992, with the creation of the Placental Blood Program at the New York Blood Center.⁶⁻⁸ As of June 1998, the program had provided 676 placental-blood grafts for recipients unrelated to the donors. We assessed the outcomes of all 562 transplantations performed from August 24, 1992, through January 30, 1998, and examined factors related to the effectiveness of placental-blood transplantation.

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METHODS

Harvesting of Placental Blood and Collection of Data on Donors

Placental-blood units were collected from freshly delivered placentas at Mount Sinai Medical Center and Brooklyn Hospital Medical Center in New York. (A unit is the blood collected from a single donor, after processing and testing.) Trained staff members harvested blood from the placentas and obtained specimens of the mothers' blood and infants' saliva, obtained informed consent, and abstracted data from the mothers' interviews and the mothers' and infants' medical records.⁹ For purposes of consent and analysis, the mother was considered to be the donor. Variables identifying the donor are confidential and are available only under special circumstances (such as the finding of a new transmissible disease in a placental-blood recipient) to project staff members and public health authorities. Our procedures were approved by the institutional review boards of the New York Blood Center and both hospitals.

Processing and Cryoprotection

Processing of placental-blood units began within 28 hours of collection. The first 3687 units were cryopreserved by the addition of an equal volume of 20 percent dimethyl sulfoxide (Fisher Scientific, Fair Lawn, N.J.).⁹ In subsequently collected units, the volume was reduced to 20 ml by removal of excess plasma and red cells before cryopreservation with 5 ml of 50 percent dimethyl sulfoxide (Cryoserv, Research Industries, Salt Lake City) in 5 percent dextran 40 (Baxter Healthcare, Deerfield, Ill.), in the cold.¹⁰ Units were immersed in liquid nitrogen for storage, then forwarded to the transplantation centers in special containers called dry-shippers (at temperatures below -145°C) by overnight-delivery services or by transplantation-center personnel. The recommended thawing procedure has been described previously.¹⁰

Testing and Typing of Placental Blood

In addition to routine serologic screening for infectious agents,⁹ placental-blood units and samples of the mothers' blood were tested for CMV-specific IgM antibodies (CMV-M EIA diagnostic kit, Abbott Laboratories, North Chicago, Ill.). After the first 3890 units had been harvested, we began to collect saliva samples from all newborns and cultured them for CMV by a shell-vial method (carried out by Dr. Robert Pass, University of Alabama, Birmingham).¹¹ HLA-A and B antigens were determined serologically.¹² HLA-DRB1 alleles were determined at low-to-intermediate or high resolution in genomic DNA by hybridization with allele-specific oligonucleotide probes after a polymerase-chain-reaction (PCR) assay with a locus-specific or group-specific primer. "Resolution" refers to the capacity of the HLA-typing process to identify discrete alleles within a group that encodes a common antigenic determinant. Low-resolution DNA typing is similar in accuracy to serologic typing of HLA antigens. All units were selected on the basis of the results of high-resolution DRB1 DNA typing, except for the first 14. Tests for hemoglobinopathies and other genetic diseases were performed before transplantation, as determined to be appropriate, on the basis of family history and ethnic background.

Selection of HLA-Matched Units

Units from donors who were matched with a potential recipient for at least five of the recipient's six HLA-A, B, and DR antigens at low resolution (5/6 of the possible matches) or, if requested, for four of six antigens (4/6 of the possible matches) were reported as candidate units, with blanks at the same locus considered matches. The finding of such matching units is currently reported within 48 hours after the Placental Blood Program receives a completed search-request form from a transplantation center. The HLA types, including DRB1 alleles identified at high resolution, of all patients and donors were confirmed by

our laboratory and, usually, also by the laboratory at the transplantation center. Donors were selected by the physicians at the transplantation center after review of the available options with medical personnel at the Placental Blood Program.

Transplantation and Follow-up

Transplantation centers provided information on the diagnosis and stage of disease for each recipient and used their own protocols for cytoreduction and prophylaxis against GVHD. Centers reported on the outcome of transplantation and any complications at periodic intervals during follow-up. Ambiguities in these reports were resolved and missing data were obtained, whenever possible, by contact between one or more of the investigators and the staff at the transplantation center.

End Points

Data on outcomes for at least the first 100 days after transplantation were received for the first 562 consecutive patients who received placental-blood grafts. We evaluated the status of these patients at the last follow-up report (July 1997 through July 1998). Hematopoiesis by donor cells was ascertained by testing for cells with the donor's HLA antigen, sex, or microsatellite markers, or a combination, in the recipient's blood. Myeloid engraftment was defined as an absolute neutrophil count of 500 per cubic millimeter or higher on three consecutive days, and platelet engraftment as a platelet count of 50,000 per cubic millimeter or higher without transfusion support for seven consecutive days. Time to myeloid or platelet engraftment was defined as the time required to reach the first day of engraftment of the relevant cell.

Secondary graft failure was defined as the loss of an engrafted transplant. Acute and chronic GVHD were diagnosed and graded for each target organ and overall at each transplantation center. Event-free survival denotes the post-transplantation period during which the patient had not received a second graft (placental blood or a bone marrow allotransplant or a frozen "backup" marrow autograft) and had no signs of autologous reconstitution or relapse. Transplantation-related complications were death, autologous reconstitution, or infusion of a second graft. In the analysis of transplantation-related events, data on patients who had relapses were censored at the time of relapse to make this end point comparable for leukemia or lymphoma and for non-neoplastic diseases.

Statistical Analysis

The proportion of patients who had engraftment at various times, the incidence of transplantation-related events, and event-free survival were estimated by the Kaplan-Meier method.¹³ In assessing the association of variables with the rates of event-free survival, transplantation-related events, and relapse, we used the generalized Wilcoxon (Breslow) statistic in univariate analyses (in which comparisons are weighted according to the number of patients at risk at each time point, a process that emphasizes the early post-transplantation period). In univariate comparisons of variables affecting the speed of engraftment, we used the non-weighted log-rank statistic. Categorical data in cross-tabulation tables were compared with use of Fisher's exact test, Pearson's chi-square, or Mantel-Haenszel's (linear-by-linear) chi-square, all two-tailed, and logistic regression (for multivariate analysis). Multivariate analyses of time to engraftment and survival distributions were performed with use of Cox logistic regression¹⁴ under the assumption of proportional hazards with all analyzed variables in the model. All statistical analyses were carried out with software from the Statistical Package for the Social Sciences (SPSS, Chicago).

RESULTS

Collection of Placental Blood

Collection of placental-blood units began on February 1, 1993; by June 30, 1998, 7705 units

were in the inventory. Roughly 45 percent of donors were white, 20 percent Hispanic, 20 percent black, 4 percent Asian, and 10 percent of mixed ancestry.

Search Requests

Between May 1993 and June 1998, we performed searches for suitable transplants for 6497 potential recipients from 290 transplantation centers. The distribution of ethnic groups among these patients resembled that of the donors, except that 72 percent of the recipients were white. Given the size of our current inventory, a 6/6 HLA match would be found for 6.8 percent of patients for whom a search request was submitted, and a 5/6 match at a conventional level of resolution would be found for another 53 percent. Sixty-six percent of white patients, 57 percent of Hispanic patients, and 38 percent of black patients would find a 5/6 or 6/6 antigen match at seroequivalent resolution.

Transplantation

By January 30, 1998, each of the 98 transplantation centers (see the Appendix) had performed at least one placental-blood transplantation; 2 were done in 1993,⁸ 15 in 1994, 89 in 1995, 209 in 1996, 228 in 1997, and 19 in January 1998, for a total of 562 patients. These patients had either no suitable bone marrow donor or urgent medical indications for transplantation. Table 1 shows the salient characteristics of the 562 recipients. According to the criteria of the International Bone Marrow Transplant Registry¹⁵ for describing the stage of acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), or chronic myelogenous leukemia (CML), 17 percent of all the patients with these types of leukemia were in the early stage and one third in the advanced stage of disease. Forty-five patients had received prior marrow transplants, 25 of which were autologous.

Myeloid and Platelet Engraftment

The oldest patient with successful myeloid engraftment was 58 years old. The heaviest patient (116 kg) in whom myeloid cells engrafted was also the patient who received the lowest number of placental-blood leukocytes (7 million leukocytes per kilogram of body weight, before blood processing). Myeloid engraftment did not occur in 160 patients; 102 died before the absolute neutrophil count reached 500 per cubic millimeter. Among the remaining 58 recipients, 13 had autologous reconstitution, 29 received backup grafts of autologous or allogeneic marrow or another unit of placental blood (between day 14 and day 89; median, day 42), and 16 relapsed before myeloid engraftment. The time to reach an absolute neutrophil count of ≥ 500 per cubic millimeter ranged from 10 days to 4 months, with medi-

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF 562 PATIENTS WHO RECEIVED TRANSPLANTS OF PLACENTAL BLOOD FROM UNRELATED DONORS.*

| CHARACTERISTIC | No. (%) |
|--------------------------------|----------|
| Sex | |
| Male | 324 (58) |
| Female | 238 (42) |
| Age at transplantation | |
| <2 yr | 114 (20) |
| 2-5 yr | 127 (23) |
| 6-11 yr | 137 (24) |
| 12-17 yr | 82 (15) |
| ≥ 18 yr | 102 (18) |
| Body weight at transplantation | |
| <10 kg | 77 (14) |
| 10-19 kg | 148 (26) |
| 20-39 kg | 152 (27) |
| 40-59 kg | 91 (16) |
| ≥ 60 kg | 94 (17) |
| Diagnosis | |
| Leukemia or lymphoma | 378 (67) |
| ALL | 177 (47) |
| AML | 124 (33) |
| CML | 48 (13) |
| JCML | 14 (4) |
| CLL | 2 (0.5) |
| Lymphoma | 13 (3) |
| Genetic disease | 137 (24) |
| Fanconi's anemia | 35 (26) |
| SCID | 24 (18) |
| Osteopetrosis | 12 (9) |
| Hurler's syndrome | 8 (6) |
| Wiskott-Aldrich syndrome | 7 (5) |
| Adrenoleukodystrophy | 6 (4) |
| Blackfan-Diamond syndrome | 5 (4) |
| Other | 40 (29) |
| Acquired disease | 47 (8) |
| Myelodysplastic disease | 21 (45) |
| Severe aplastic anemia | 21 (45) |
| Other cancer | 5 (11) |
| Stage of ALL, AML, or CML† | |
| Early | 56 (17) |
| Intermediate | 166 (49) |
| Advanced | 116 (34) |
| No. of HLA mismatches‡ | |
| 0 | 40 (7) |
| 1 | 218 (39) |
| 2 | 261 (47) |
| 3 | 37 (7) |
| 4 | 3 (0.5) |

*ALL denotes acute lymphoblastic leukemia, AML acute myelogenous leukemia, CML chronic myelogenous leukemia, JCML juvenile chronic myelogenous leukemia, CLL chronic lymphocytic leukemia, and SCID severe combined immunodeficiency. Because of rounding, percentages do not always total 100.

†For the 349 patients with ALL, AML, or CML, the stage was determined according to the criteria of the International Bone Marrow Transplant Registry. The stage could not be determined for 11 patients. Early denotes the first complete remission in acute leukemia or the first chronic phase in CML; intermediate denotes the second or subsequent complete remission in ALL or AML and accelerated or chronic disease in CML; advanced denotes relapse, refractory disease, blast phase, or failure of induction therapy.

‡HLA typing is described in the Methods section. Three patients did not undergo high-resolution typing for HLA-DRB1.

TABLE 2. CUMULATIVE INCIDENCE OF MYELOID ENGRAFTMENT BY DAY 42 AMONG RECIPIENTS OF PLACENTAL-BLOOD TRANSPLANTS FROM UNRELATED DONORS.*

| VARIABLE | ALL RECIPIENTS | RECIPIENTS WITH ENGRAFTMENT | | P VALUE |
|---|----------------|-----------------------------|------------|---------|
| | | NO. | % (95% CI) | |
| | | no. | | |
| Sex | | | | |
| Male | 315 | 214 | 82 (77–87) | |
| Female | 231 | 151 | 79 (73–85) | 0.5 |
| Diagnosis | | | | |
| CML | 46 | 21 | 61 (44–78) | |
| Fanconi's anemia | 35 | 18 | 64 (44–84) | |
| Severe aplastic anemia | 19 | 8 | 63 (33–93) | |
| Other | 446 | 318 | 84 (81–88) | <0.001 |
| Age | | | | |
| <2 yr | 109 | 88 | 90 (84–97) | |
| 2–5 yr | 123 | 86 | 81 (73–89) | |
| 6–11 yr | 135 | 96 | 84 (77–92) | |
| 12–17 yr | 82 | 43 | 65 (53–77) | |
| ≥18 yr | 97 | 52 | 77 (66–88) | <0.001 |
| CMV antibody status before transplantation† | | | | |
| Negative | 266 | 179 | 80 (75–86) | |
| Positive | 224 | 148 | 81 (75–87) | 0.8 |
| Placental-blood status before freezing | | | | |
| Whole blood | 337 | 224 | 80 (75–85) | |
| Volume reduction | 209 | 141 | 82 (76–88) | 0.5 |
| No. of leukocytes before cryopreservation | | | | |
| ≥100 million/kg | 65 | 57 | 91 (84–98) | |
| 50 million–99 million/kg | 121 | 94 | 86 (79–93) | |
| 25 million–49 million/kg | 198 | 124 | 79 (72–86) | |
| 7 million–24 million/kg | 162 | 90 | 74 (66–82) | <0.001 |
| No. of HLA-A, B, and DRB1 mismatches‡ | | | | |
| 0 | 36 | 31 | 100 | |
| 1 | 211 | 137 | 78 (72–85) | |
| 2 | 257 | 173 | 82 (76–88) | |
| ≥3 | 39 | 22 | 69 (52–86) | 0.01 |
| Location of transplantation centers | | | | |
| U.S. | 438 | 307 | 85 (81–89) | |
| Outside U.S. | 108 | 58 | 64 (54–74) | 0.001 |
| Total | 546 | 365 | 81 (77–85) | |

*Incidence was estimated with Kaplan–Meier methods. CI denotes confidence interval, CML chronic myelogenous leukemia, and CMV cytomegalovirus. The time to an absolute neutrophil count of 500 per cubic millimeter or more was unknown for 16 patients who had engraftment. Of 56 patients who survived for more than 42 days but had not yet had engraftment, 21 later had engraftment and 8 had other evidence of donor-type myeloid engraftment but did not reach an absolute neutrophil count of ≥500 per cubic millimeter. Five other patients subsequently had autologous reconstitution, 1 relapsed without evidence of engraftment, 11 received a backup marrow transplant or second placental-blood graft, and the remaining 10 died without any of these intervening events.

†CMV antibody status was unknown for 56 recipients, 21 of whom had severe combined immunodeficiency.

‡The number of HLA-DRB1 mismatches was unknown for three recipients for whom high-resolution typing was not performed.

ans, estimated by Kaplan–Meier analysis, of 28 days for all patients who underwent transplantation and 25 days for those in whom engraftment occurred. According to Kaplan–Meier estimates, 81 percent of patients reached an absolute neutrophil count of ≥500 per cubic millimeter by day 42 (Table 2), and 91 percent by day 60. The likelihood of successful engraftment was significantly reduced among patients with Fanconi's anemia, severe aplastic anemia, or CML and for patients treated at transplantation centers outside the United States. Successful mye-

loid engraftment was associated with younger age, a higher number of nucleated cells in the placental-blood unit per kilogram of body weight, and the absence of HLA mismatching (Table 2). Except for age, each of these associations remained significant in the multivariate analysis.

The time to myeloid engraftment correlated significantly with the recipient's age, the number of leukocytes per kilogram in the graft (Fig. 1A), the type of disease, the extent of HLA disparity, and the transplantation center, although age was not inde-

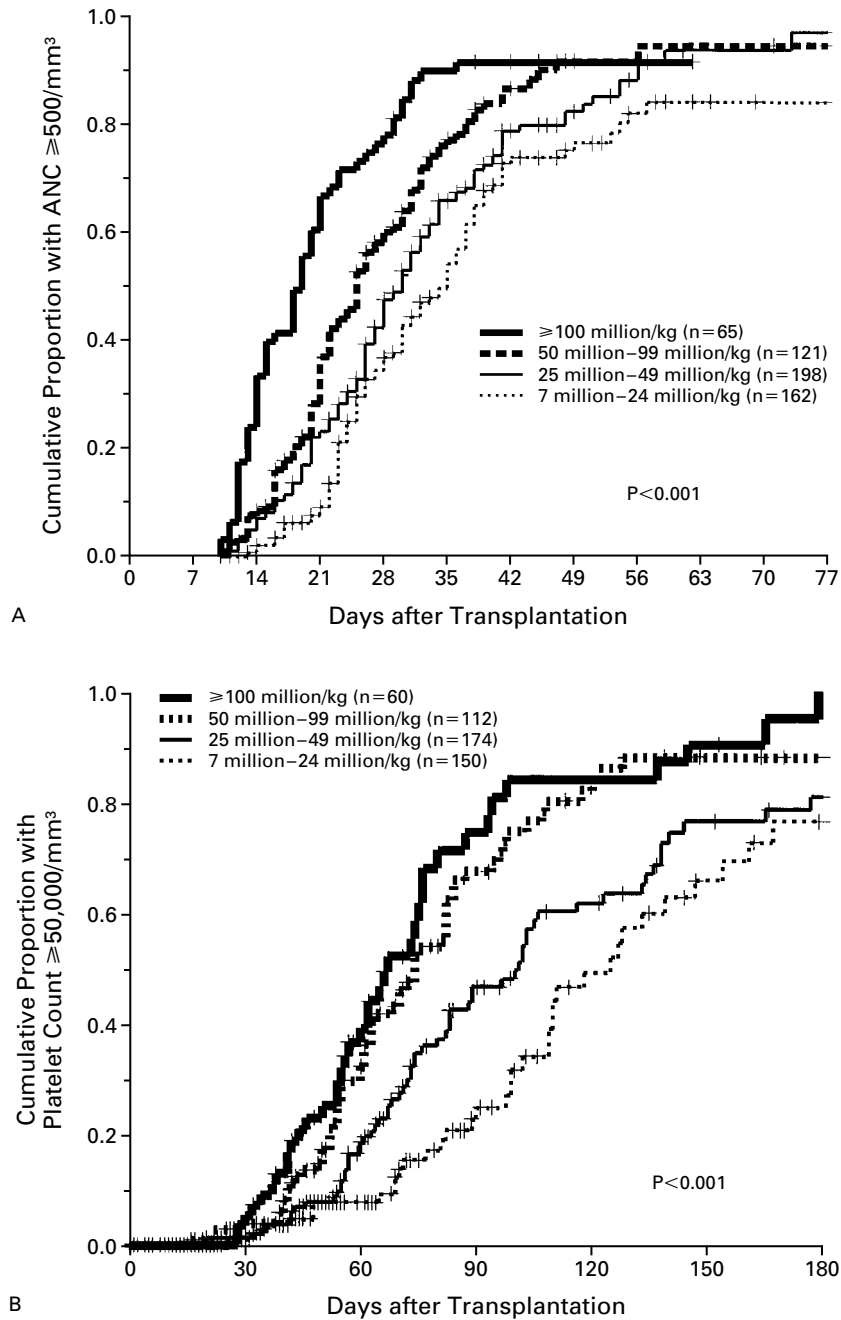


Figure 1. Kaplan-Meier Estimates of the Time to Myeloid and Platelet Engraftment after Placental-Blood Transplantation, According to the Dose of Leukocytes Transfused.

Myeloid engraftment (Panel A) was defined as the achievement of an absolute neutrophil count (ANC) of 500 per cubic millimeter or higher on three consecutive days, and platelet engraftment (Panel B) as the achievement of a platelet count of 50,000 per cubic millimeter or higher without transfusion for seven consecutive days. The dose of leukocytes was expressed as the number of nucleated leukocytes in the transplant per kilogram of the recipient's body weight. Each cross denotes a patient whose data were censored (because of death, autologous reconstitution, relapse, or receipt of a second marrow or placental-blood transplant) before engraftment. Data on myeloid engraftment were unavailable for 16 patients, and data on platelet engraftment were unavailable for 66. P values were derived with use of the log-rank statistic.

TABLE 3. GRAFT-VERSUS-HOST DISEASE (GVHD) AMONG RECIPIENTS OF PLACENTAL-BLOOD TRANSPLANTS FROM UNRELATED DONORS.*

| VARIABLE | NO. OF PATIENTS | GRADE OF GVHD | | | P VALUE |
|---------------------------------------|-----------------|--|---------|-----------|---------|
| | | 0 OR I | II | III OR IV | |
| | | no. (%) | | | |
| Sex | | | | | 0.07 |
| Male | 229 | 112 (49) | 58 (25) | 59 (26) | |
| Female | 170 | 102 (60) | 37 (22) | 31 (18) | |
| Age | | | | | 0.01 |
| <2 yr | 93 | 55 (59) | 26 (28) | 12 (13) | |
| 2–11 yr | 200 | 108 (54) | 50 (25) | 42 (21) | |
| ≥12 yr | 106 | 51 (48) | 19 (18) | 36 (34) | |
| | | Linear-by-linear association, P=0.02 | | | |
| No. of HLA-A, B, and DRB1 mismatches† | | | | | 0.15 |
| 0 | 34 | 25 (74) | 6 (18) | 3 (9) | |
| 1 | 156 | 81 (52) | 40 (26) | 35 (22) | |
| ≥2 | 207 | 107 (52) | 49 (24) | 51 (25) | |
| | | Linear-by-linear association, P=0.056 | | | |
| | | Linear-by-linear association, 0 vs. ≥1, P=0.01 | | | |
| Infection after transplantation‡ | | | | | 0.03 |
| No | 93 | 53 (57) | 28 (30) | 12 (13) | |
| Yes | 291 | 153 (53) | 65 (22) | 73 (25) | |
| Location of transplantation center | | | | | 0.008 |
| U.S. | 327 | 184 (56) | 79 (24) | 64 (20) | |
| Outside U.S. | 72 | 30 (42) | 16 (22) | 26 (36) | |
| Total | 399 | 214 (54) | 95 (24) | 90 (23) | |

*Data are included for 381 patients who had an absolute neutrophil count of ≥ 500 per cubic millimeter, 12 who had some evidence of engraftment but did not reach an absolute neutrophil count of ≥ 500 per cubic millimeter, and 6 who had no evidence of engraftment. Not included in this table were 21 patients who had engraftment but for whom data on GVHD status were unavailable and 142 with no evidence of engraftment or GVHD. Because of rounding, percentages do not always total 100.

†The number of HLA-DRB1 mismatches was unknown for two recipients for whom high-resolution typing was not performed.

‡Data were not available for 15 patients.

pendently predictive in multivariate tests. With the sample limited to patients who reached an absolute neutrophil count of ≥ 500 per cubic millimeter, only the number of leukocytes per kilogram in the placental-blood graft correlated with time to myeloid engraftment.

The status of platelet engraftment was known for 496 patients. The time to reach a platelet count of $\geq 50,000$ per cubic millimeter ranged from 16 to 250 days (median, 90 days for all patients and 71 days for patients who reached this end point). According to Kaplan–Meier analysis, 58 percent of patients (95 percent confidence interval, 52 to 66 percent) had platelet engraftment by day 100 and 85 percent by day 180 (95 percent confidence interval, 79 to 91 percent). In the univariate analysis, the timing of platelet engraftment was associated with

the recipient's disease, age, number of leukocytes per kilogram (Fig. 1B), whether infection occurred after transplantation, and presence or absence of GVHD, but not with the extent of HLA disparity or the transplantation center. In the multivariate analysis, only age and infection after transplantation were significant in a model that included all these variables except GVHD (GVHD was also significant when included in the model).

Secondary graft failure occurred in only six patients (whose grafts failed one to four months after transplantation), all of whom had active, ganciclovir-treated, post-transplantation CMV infection ($P < 0.001$ for the comparison with recipients without secondary graft failure). All six patients had anti-CMV antibodies before transplantation, whereas no CMV-specific IgM antibodies were detectable in any of the six infants or their mothers. Three of the six patients had severe acute GVHD ($P = 0.09$), and four were 12 years of age or older ($P = 0.07$). There was no association between secondary graft failure and the number of leukocytes in the graft.

GVHD

Information concerning acute GVHD was available for 399 patients. Of these, engraftment occurred in 381, 6 had no detectable engraftment, and 12 had donor cells but did not attain an absolute neutrophil count of ≥ 500 per cubic millimeter. GVHD status has not yet been reported for 21 patients with engraftment and was not considered relevant by the center for 142 others without engraftment. Transplantation centers graded the overall severity of GVHD in 381 of the 399 patients for whom data on GVHD were available; grade 0 (absence of signs of GVHD) in 118 patients (31 percent), grade I in 94 (25 percent), grade II in 84 (22 percent), grade III in 43 (11 percent), and grade IV in 42 patients (11 percent). We used published guidelines¹⁶ to assign an overall grade to another 18 patients whose reports gave organ-specific grades only; 2 were classified as having grade I GVHD, 11 as having grade II disease, and 5 as having grade III or IV disease. The severity of acute GVHD correlated with the patient's age, the extent of HLA incompatibility, the presence or absence of post-transplantation infection (though not with infection with any particular organism), and the transplantation center (Table 3). The frequency of severe GVHD (grades III and IV) was lower in patients with six of six HLA antigen matches than in other patients ($P = 0.008$) but did not otherwise correlate with the number of mismatches. In the multivariate analysis of GVHD, age (≥ 12 vs. < 12 years) and location of center (United States vs. foreign) were variables significantly and independently associated with GVHD (grade 0 to II vs. grade III or IV; $P = 0.005$ and $P = 0.006$, respectively), and HLA mismatch-

ing (0 vs. ≥ 1 mismatches) approached significance ($P=0.06$).

Chronic GVHD, generally limited, was diagnosed in 48 patients but was a cause of death or contributed to death in only 3 cases. Among 158 patients who survived for six months or more, 39 (25 percent) had chronic GVHD (data on GVHD in an additional 52 surviving patients were unavailable). Chronic GVHD occurred in 80 percent of patients who had previously had severe acute GVHD, as compared to 18 percent of those who had not ($P < 0.001$), but the incidence of chronic GVHD did not correlate with the extent of HLA disparity or other study variables.

Transplantation-Related Events and Event-free Survival

By 100 days after transplantation, 261 patients (46 percent) had had transplantation-related events: 13 had had autologous reconstitution, 30 had received second transplants (9 with placental blood and 21 with autologous or allogeneic bone marrow, 1 of them for secondary graft failure), and 218 died. In addition, 28 patients with leukemia or lymphoma had relapses. Event-free survival for the first 100 days and the overall incidence of transplantation-related events other than relapse (Table 4 and Fig. 2) correlated with recipient's diagnosis, age, the number of leukocytes in the transplant, the extent of HLA disparity, and the location of the transplantation center in univariate and multivariate analyses. The presence or absence of anti-CMV antibodies before transplantation was not associated with the incidence of transplantation-related events or with event-free survival. When we limited the multivariate analysis to patients with engraftment, the incidence of transplantation-related events correlated with age, the extent of HLA mismatching, and the location of the center, but not with the dose of leukocytes or the diagnosis (Table 4). The incidence of transplantation-related events among patients who received grafts with only one HLA mismatch (152 with a mismatch at HLA-A or HLA-B and 66 with a mismatch at HLA-DRB1) was not influenced by the class of HLA mismatch or the level of resolution in the typing of DR antigens (11 with high-resolution and 55 with low-resolution typing). Among recipients of grafts with two HLA-antigen mismatches, there was also no association between the incidence of transplantation-related events and the class of mismatch (9 with mismatches at HLA-DRB1 only; 124 with mismatches at HLA-A, B, or both; and 128 with mismatches at both HLA-A or B and DRB1) or the resolution level of the detection of HLA-DR mismatches.

Viral Infection

The risk of CMV infection after transplantation correlated strongly with the recipient's initial CMV-

antibody status. Of the 500 patients for whom the pretransplantation CMV antibody status was known, CMV infection after transplantation occurred in 23 percent of 211 seropositive recipients and 3 percent of 256 seronegative recipients ($P < 0.001$). There was no evidence of CMV infection or CMV-specific IgM antibodies in the placental-blood units given to the seven initially seronegative patients in whom CMV infection developed or in the donors. EBV-associated lymphoproliferative disease has been reported thus far in two patients. The source of these infections is unknown. One patient had EBV encephalitis.

Relapse

Fifty-one patients with leukemia (14 percent) have had relapses thus far (20 of 177 with ALL, 23 of 124 with AML, 4 of 48 with CML, and 4 of 14 with juvenile chronic myelogenous leukemia), as have 2 of the 13 patients with lymphoma. The actuarial probability of leukemic relapse in patients with ALL, AML, or CML correlated with the stage of disease; by one year, 19 percent of the patients with early disease, 24 percent of those with intermediate-stage disease, and 35 percent of those with advanced disease¹⁵ had relapsed ($P=0.02$). The incidence of relapse at one year was higher for patients with AML than for those with CML or ALL (30 percent, 18 percent, and 24 percent, respectively; $P=0.003$), partly because 50 percent of cases of AML were advanced at the time of transplantation, as compared with 27 percent for ALL and CML ($P < 0.001$). Thus far, only one relapse followed grade III or IV GVHD ($P=0.05$), although only 12 patients with leukemia who had severe GVHD had survived six months or more as of the last reported follow-up evaluation.

Causes of Death

Infection was reported to have contributed to death in 47 percent of the deaths, pulmonary disease in 26 percent, multiorgan failure in 12 percent, GVHD in 11 percent, and veno-occlusive disease of the liver in 7 percent. The extent of HLA matching was not associated with any specific cause of death.

DISCUSSION

Our study includes data on most of the placental-blood transplantations from unrelated donors performed in the world thus far. The results indicate that placental-blood transplants regularly engraft, cause GVHD at a relatively low rate, and produce survival rates similar to those with transplantation of bone marrow from unrelated donors. The data from multiple transplantation centers on the outcomes of the 562 consecutive recipients of placental blood permit accurate estimates of the major end points. These data also provide the study with the

TABLE 4. RELATIVE RISK OF TRANSPLANTATION-RELATED EVENTS.*

| VARIABLE | NO. OF PATIENTS | RELATIVE RISK (95% CI) | |
|---|-----------------|------------------------|-----------------------|
| | | UNIVARIATE ANALYSIS | MULTIVARIATE ANALYSIS |
| All patients† | | | |
| Diagnosis of CML, FA, or severe aplastic anemia | | | |
| No | 458 | 1.0 | 1.0 |
| Yes | 102 | 2.0 (1.5–2.6) | 1.5 (1.1–2.0) |
| Age | | | |
| <2 yr | 114 | 1.0 | 1.0 |
| 2–11 yr | 263 | 1.5 (1.1–2.1) | 1.3 (0.9–1.9) |
| ≥12 yr | 183 | 2.6 (1.9–3.7) | 1.5 (0.96–2.5) |
| CMV-antibody status before transplantation‡ | | | |
| Negative | 271 | 1.0 | |
| Positive | 228 | 1.1 (0.9–1.5) | |
| No. of leukocytes before cryopreservation | | | |
| ≥50 million/kg | 191 | 1.0 | 1.0 |
| 25 million–49 million/kg | 203 | 1.4 (1.02–1.8) | 1.1 (0.8–1.6) |
| <25 million/kg | 166 | 2.2 (1.6–2.9) | 1.6 (1.1–2.3) |
| No. of HLA-A, B, and DRB1 mismatches§ | | | |
| 0 | 40 | 1.0 | 1.0 |
| 1 | 217 | 1.8 (1.02–3.2) | 2.0 (1.1–3.6) |
| ≥2 | 300 | 2.5 (1.4–4.4) | 2.5 (1.4–4.5) |
| Location of transplantation center | | | |
| U.S. | 449 | 1.0 | 1.0 |
| Outside U.S. | 111 | 1.7 (1.3–2.2) | 1.5 (1.2–2.0) |
| Patients with myeloid engraftment¶ | | | |
| Diagnosis of CML, FA, or severe aplastic anemia | | | |
| No | 351 | 1.0 | 1.0 |
| Yes | 51 | 1.6 (1.05–2.4) | 1.1 (0.7–1.7) |
| Age | | | |
| <2 yr | 94 | 1.0 | 1.0 |
| 2–11 yr | 198 | 1.6 (0.99–2.5) | 1.6 (0.97–2.6) |
| ≥12 yr | 110 | 2.5 (1.6–4.0) | 2.0 (1.1–3.7) |
| CMV-antibody status before transplantation‡ | | | |
| Negative | 195 | 1.0 | |
| Positive | 162 | 1.3 (0.96–1.8) | |
| No. of leukocytes before cryopreservation | | | |
| ≥50 million/kg | 160 | 1.0 | 1.0 |
| 25 million–49 million/kg | 142 | 1.1 (0.8–1.6) | 0.8 (0.5–1.3) |
| <25 million/kg | 100 | 1.8 (1.2–2.6) | 1.2 (0.7–2.0) |
| No. of HLA-A, B, and DRB1 mismatches | | | |
| 0 | 35 | 1.0 | 1.0 |
| 1 | 153 | 1.3 (0.6–2.5) | 1.4 (0.7–2.8) |
| ≥2 | 212 | 2.1 (1.1–4.0) | 2.1 (1.1–4.1) |
| Location of transplantation center | | | |
| U.S. | 336 | 1.0 | 1.0 |
| Outside U.S. | 66 | 1.8 (1.2–2.6) | 1.7 (1.1–2.4) |

*CI denotes confidence interval, CML chronic myelogenous leukemia, FA Fanconi's anemia, and CMV cytomegalovirus.

†For the analyses of all recipients of placental-blood transplants, the events included are death, autologous reconstitution, and receipt of a second transplant. Data were censored for patients who had a relapse. Two patients who died on the day of transplantation have been excluded.

‡CMV-antibody status before transplantation was unknown for 61 recipients, 24 of whom had severe combined immunodeficiency, and was therefore not included in the multivariate models.

§The number of HLA-DRB1 mismatches was unknown for three recipients for whom high-resolution typing was not performed.

¶Myeloid engraftment was defined as the achievement of an absolute neutrophil count of 500 per cubic millimeter or higher on three consecutive days. For this analysis, only deaths were included as events.

||The number of HLA-DR mismatches was unknown for two recipients for whom high-resolution typing was not performed.

statistical power for a more rigorous examination of the relation between end points and characteristics of the recipients and donors than was possible previously — for example, with the Eurocord Transplant Group's analysis.⁵ This analysis of 65 recipients of placental-blood transplants from unrelated donors (47 of whom received grafts supplied by the New York Blood Center) suggested that the CMV-antibody status before transplantation predicts the occurrence of GVHD and survival.⁵ By contrast, we found that the presence of antibodies to CMV was unrelated to either end point in our analysis of 562 patients, including the 47 cited above. Instead, the presence of anti-CMV antibodies in patients before transplantation was significantly associated with active post-transplantation CMV disease, the foremost correlate of secondary graft failure in our study.

Among the variables associated with engraftment and transplantation-related events, the number of leukocytes per kilogram in the graft and the age of the recipient were correlated strongly with each other, confounding their individual associations with outcome. Multivariate analyses allowed us to separate these relations; although both the number of leukocytes per kilogram and the recipient's age were associated with the incidence of transplantation-related events, the number of transfused leukocytes per kilogram, but not age, correlated with the time to myeloid engraftment. Conversely, after engraftment, age correlated significantly with event-free survival, but the number of transplanted leukocytes per kilogram did not. The leukocyte content of the graft may relate principally to the speed and overall success of engraftment and only secondarily to transplantation-related events and event-free survival. Consequently, larger doses of leukocytes from larger placental-blood collections, or perhaps from hematopoietic precursors expanded *ex vivo*,¹⁷ may accelerate engraftment, but improvement of event-free survival is less certain, particularly for older patients. In contrast to inferences about platelet reconstitution in other studies,⁴ both the probability and the timing of platelet engraftment in this study were similar to those observed after transplantation of bone marrow from unrelated donors.¹⁸⁻²⁰

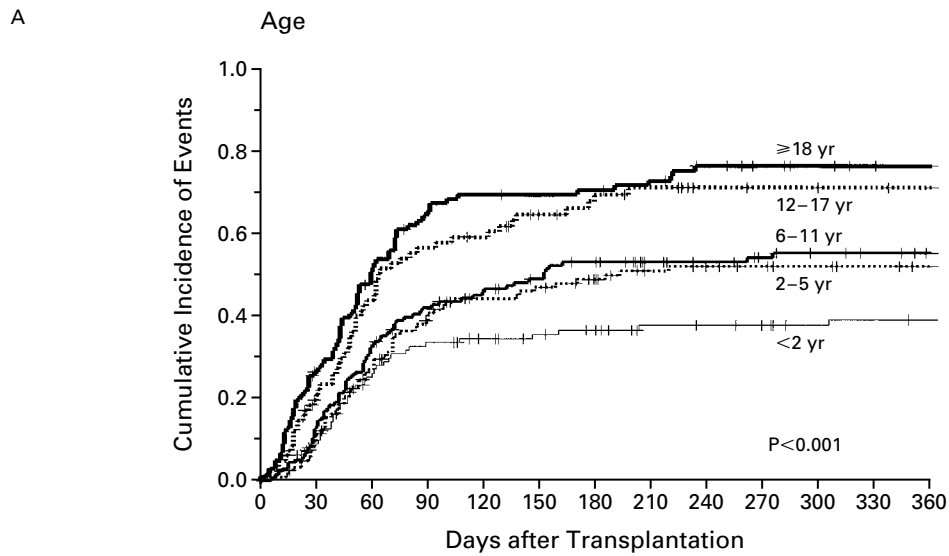
The rate and speed of myeloid engraftment were also associated with the degree of HLA compatibility in univariate and multivariate tests, as in some studies of transplantation of bone marrow^{19,20} but not all.¹⁸ In the subgroup of patients with engraftment, however, there was no association between the degree of HLA compatibility and time to engraftment. HLA incompatibility was more frequent in recipients in whom engraftment failed than in recipients with engrafted transplants. This suggests a role for HLA alloimmunization in at least some placental-blood graft failures. Host factors may also

underlie the poor engraftment seen in patients with Fanconi's anemia, severe aplastic anemia, and CML. As we anticipated,⁶⁻⁸ severe acute or chronic GVHD was less common in this study, despite the multiple HLA mismatches, than after transplantation of bone marrow from unrelated donors.¹⁸⁻²¹ Thus, it appears that placental-blood grafts that are mismatched for up to two HLA antigens can be used effectively in patients without HLA-identical related donors.

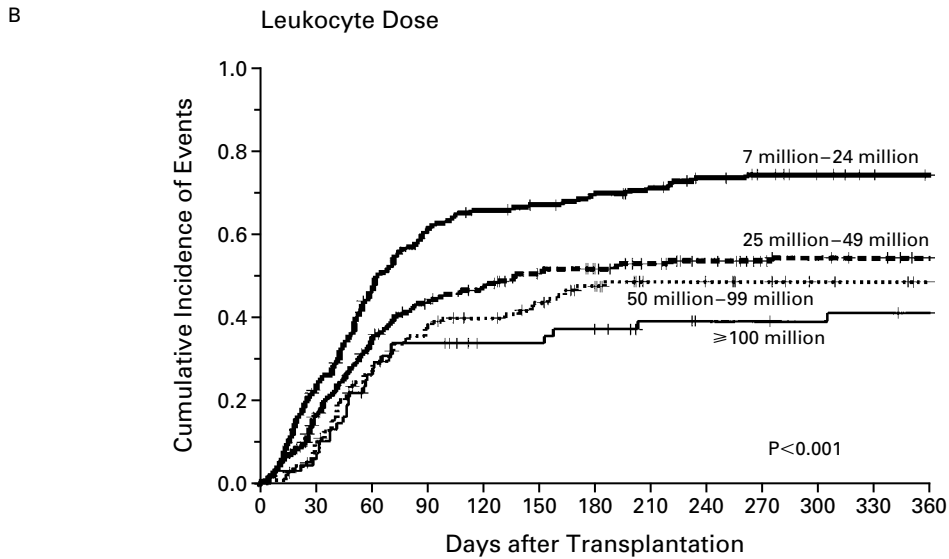
Among the arguments in favor of storing placental blood for later use in autologous transplantation in the event that leukemia or other diseases develop is the fear of post-transplantation morbidity, including GVHD, with grafts from unrelated donors.²² The transplantation of HLA-identical marrow is associated with a higher frequency of leukemic relapse, however, since such transplants induce weaker GVHD and graft-versus-leukemia effects.^{23,24} Our data, though insufficient to prove graft-versus-leukemia effects, are in agreement with the results of bone marrow transplantation²¹ and suggest that the use of grafts of autologous placental blood may not be desirable in treating leukemia. An even more compelling reason to avoid the use of autologous placental blood grafts is the recent finding that leukemic cells are already present in the fetal and neonatal blood of patients diagnosed with leukemia at ages up to 9 and 10 years.²⁵⁻²⁸

An effect of the location of the transplantation center emerged when we analyzed the rates of engraftment and survival after engraftment. The reasons for the disparity between U.S. and foreign centers were not conclusively identified, although some centers outside the United States reported that they did not reduce the concentration of dimethyl sulfoxide in placental blood after thawing, as recommended. The high osmolarity gradient facing cells on infusion causes cell death, thus reducing the dose of cells.¹⁰ The location of the center also correlated with the rate of severe acute GVHD in multivariate analyses that included the dose of cells before freezing, age, and the degree of HLA compatibility, suggesting the involvement of additional unidentified factors, possibly differences in diagnostic criteria or in prophylaxis against and treatment of GVHD.

We conclude that stored placental blood is a useful source of hematopoietic stem cells for patients who do not have a related histocompatible donor. Its effectiveness as a source could be enhanced by wider accessibility and by improvements that would speed engraftment and lessen early morbidity. These enhancements would have to involve adequate international standards to permit worldwide cooperation among placental-blood banks. Other potential improvements will emerge from studies that focus on variables that influence pla-

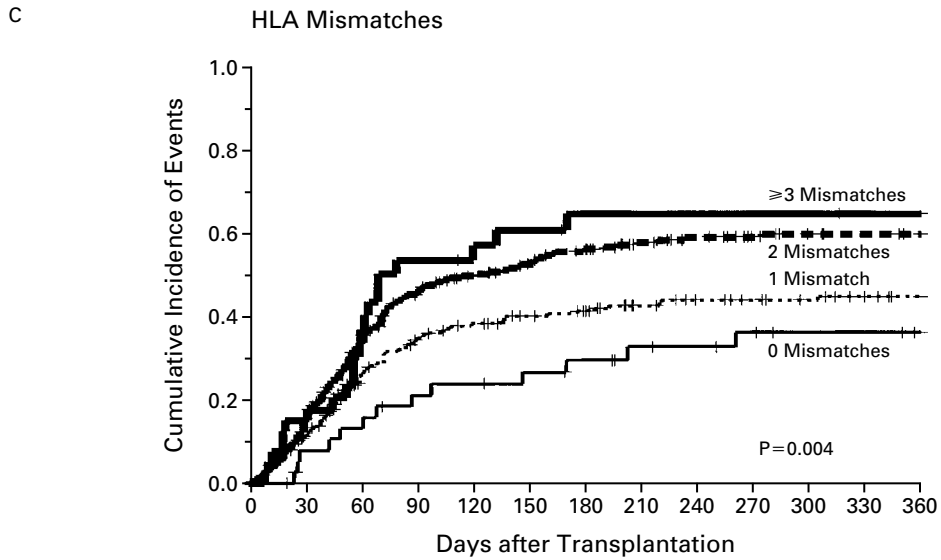


| AGE | | | | | |
|---------------------|-----|----|----|----|----|
| 0-1 yr | 114 | 71 | 58 | 50 | 44 |
| 2-5 yr | 127 | 70 | 51 | 39 | 33 |
| 6-11 yr | 137 | 77 | 56 | 40 | 32 |
| 12-17 yr | 82 | 34 | 19 | 12 | 10 |
| $\geq 18\text{ yr}$ | 102 | 33 | 27 | 16 | 11 |

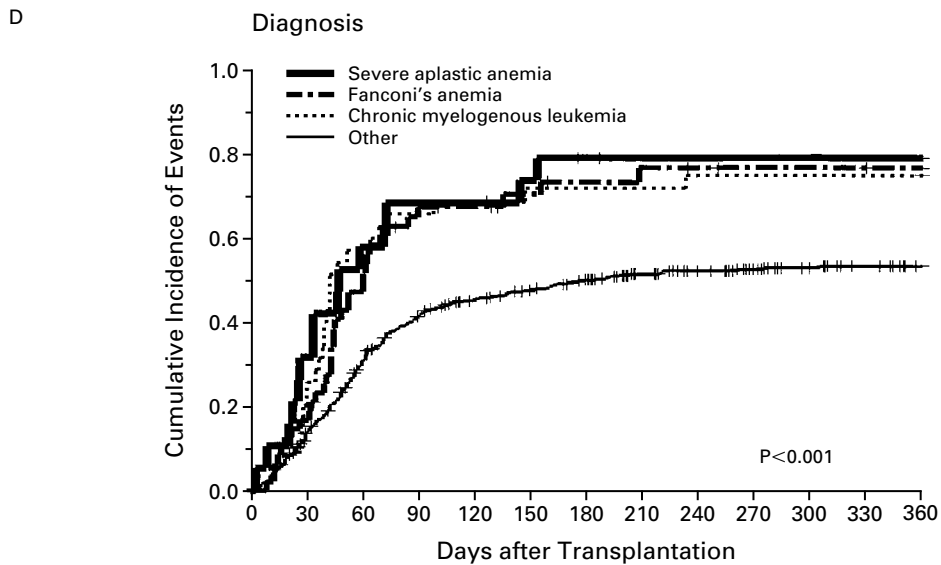


| LEUKOCYTE DOSE | | | | | |
|------------------------------|-----|-----|----|----|----|
| 7 million-24 million/kg | 166 | 63 | 44 | 31 | 22 |
| 25 million-49 million/kg | 205 | 106 | 79 | 58 | 50 |
| 50 million-99 million/kg | 122 | 72 | 52 | 39 | 33 |
| $\geq 100\text{ million/kg}$ | 69 | 44 | 37 | 30 | 27 |

Figure 2. Kaplan-Meier Estimates of the Cumulative Incidence of Transplantation-Related Events Other Than Relapse. Events included were death, autologous reconstitution, and receipt of a second transplant. Data were censored at the time of relapse or at the time of the last follow-up evaluation for patients without events (indicated by crosses). P values were derived with use of the generalized Wilcoxon statistic. Panel A shows events according to age, Panel B according to the leukocyte dose (the number of nucleated leukocytes in the transplant per kilogram of the recipient's body weight), Panel C according to the number of mismatches at the HLA-A, B, and DR loci, and Panel D according to diagnoses. Numbers below the graphs are the numbers of patients at risk.



| No. OF MISMATCHES | 0 | 1 | 2 | ≥ 3 |
|-------------------|-----|-----|----|----------|
| 0 | 40 | 29 | 24 | 17 |
| 1 | 218 | 117 | 94 | 72 |
| 2 | 261 | 124 | 85 | 58 |
| ≥ 3 | 40 | 14 | 9 | 9 |



| DIAGNOSIS | 0 | 1 | 2 | ≥ 3 |
|------------------------------|-----|-----|-----|----------|
| Chronic myelogenous leukemia | 48 | 14 | 8 | 5 |
| Fanconi's anemia | 35 | 12 | 9 | 9 |
| Severe aplastic anemia | 19 | 6 | 3 | 2 |
| Other | 460 | 253 | 192 | 142 |

cental-blood engraftment and on the prevention of GVHD.

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APPENDIX

The clinicians and transplantation centers that performed the placental-blood transplantations and provided outcome data were as follows: A. Abdel-Mageed, Shands Hospital, University of Florida, Gainesville; M. Abecasis, Instituto Portugues de Oncologia, Lisbon, Portugal; M. Amylon, Stanford University Medical Center, Palo Alto, Calif.; A. Anelli, Hospital A.C. Camargo, São Paulo, Brazil; W. Arcese, Università La Sapienza, Rome; C. August, Miami Children's Hospital, Miami; I. Badell, Hospital Universitari of Barcelona University, Barcelona, Spain; B. Bambach, Roswell Park Cancer Institute, Buffalo, N.Y.; F. Barriga, Catholic University of Chile, Santiago; Y. Beguin, Centre Hospitalier Universitaire-Sart Tilman University Hospital Liege, Liege, Belgium; J.N. Brochstein, Hackensack University Medical Center, Hackensack, N.J.; M. Brunvand, Oregon Health Sciences University, Portland; N. Bunin, Children's Hospital of Philadelphia, Philadelphia; J.Y. Cahn, Centre Hospitalier Universitaire, Besançon, France; M. Cairo, Children's Hospital of Orange County, Orange, Calif.; F. Campilho, Instituto Portugues de Oncologia, Porto, Portugal; J. Casper, Midwest Children's Cancer Center, Milwaukee; M. Champagne, Hôpital Sainte Justine, Montreal; K. Chan, M.D. Anderson Cancer Center, Houston; L.-L. Chan, University Hospital, Kuala Lumpur, Malaysia; N. Ciobanu, Schneider Children's Hospital, New Hyde Park, N.Y.; M. Cowan, University of California at San Francisco, San Francisco; J. Cruz, Bowman Gray School of Medicine, Winston-Salem, N.C.; P. de Alarcon, University of Virginia Medical Center, Charlottesville; P. Dinndorf, Children's National Medical Center, Washington, D.C.; P. Falk, Kaiser Permanente, Anaheim, Calif.; C. Favre, University of Pisa, Pisa, Italy; S. Feig, University of California at Los Angeles, Los Angeles; E. Ferreira, Hospital Israelita Albert Einstein, São Paulo, Brazil; D. Friedman, Cook Children's Medical Center Fort Worth, Fort Worth, Tex.; S. Fruchtman, Mount Sinai Medical Center, New York; A.S. Gamsi, Children's Mercy Hospital, Kansas City, Mo.; E. Gluckman, Hôpital Saint-Louis, Paris; U. Göbel, University of Dusseldorf, Dusseldorf, Germany; S. Goldman, University of Chicago-Wyler Children's Hospital, Chicago; M. Graham, University Medical Center of Arizona, Tucson; C. Grande, Hospital Universitario 12 de Octubre, Madrid; G. Grayson, Southwest Texas Methodist Hospital, San Antonio; T. Gross, University of Nebraska Medical Center, Omaha; A. Grovas, Columbus Children's Hospital, Columbus, Ohio; E. Guinan, Brigham and Women's Hospital and Children's Hospital, Boston; G. Hale, University of Kentucky Medical Center, Lexington; R. Harris, Children's Hospital Medical Center, Cincinnati; R. Hutchinson, University of Michigan Medical Center, Ann Arbor; G. Jaimovich, Instituto Medico Antartida, Buenos Aires, Argentina; M. Joyce, Wolfson Children's Hospital, Jacksonville, Fla.; H. Kaiser, Rush-Presbyterian-St. Luke's Medical Center, Chicago; D. Karakasis, Evangelismos Hospital, Athens, Greece; N. Kernan, Memorial Sloan-Kettering Cancer Center, New York; M. Klemperer, All Children's Hospital, St. Petersburg, Fla.; M. Kletzel, Children's Memorial Medical Center, Chicago; R. Kline, Kosair Children's Hospital, Louisville, Ky.; G. Kusminsky, Alexander Fleming Institute, Buenos Aires, Argentina; J. Kurtzberg, Duke University Medical Center, Durham, N.C.; J.-P. LaPorte, Hôpital Saint Antoine, Paris; J. Laver, Medical University of South Carolina, Charleston; F. Locatelli, Università di Pavia, Pavia, Italy; L. Lombardini, Azienda Ospedaliera di Careggi, Florence, Italy; L.M. Lopez, Hospital Niño Jesus, Madrid; J.A. Lucero, Hospital Clinico Universidad de Chile, Santiago; R. Orvilla, Hospital G. R. No. 1 Gabriel Mancera, Mexico City, Mexico; G. Michel, Hôpital d'Enfants la Timone, Marseilles, France; M. Mogul, Emory University School of Medicine, Atlanta; A. Nademanee, City of Hope Medical Center, Duarte, Calif.; A. Nagler, Hadassah University Hospital, Jerusalem, Israel; A. Ogdan, Texas Children's Hospital, Houston; J. Ortega, Hospital Infantil Vall d'Hebron, Barcelona, Spain; R. Parkman, Children's Hospital of Los Angeles, Los Angeles; R. Pasquini, Hospital de Clinicas Universidade do Parana, Curitiba, Brazil; A. Pession, Università di Bologna-Saint Orsola Hospitale, Bologna, Italy; S. Queiroz, Hospital Duran i Reynals and Hospital Sant Creu i San Pau, Barcelona, Spain; R. Quinones, University of Colorado Children's Hospital, Denver; R. Reiss, Columbia University-Presbyterian Medical Center, New York; M.N.F. Rodriguez, Clinica Puerta de Hierro, Madrid; A. Rubin, St. Joseph's Hospital and Medical Center, Paterson, N.J.; J. Russell, Foothills Hospital, Calgary, Alta., Canada; I. Sahdev, North Shore Hospital, Manhasset, N.Y.; E. Sandler, Children's Medical Center of Dallas, Dallas; G.F. Sanz, Hospital Universitari La Fe, Valencia, Spain; E.F. Saunders, Hospital

for Sick Children, Toronto; G. Selby, Children's Hospital of Oklahoma, Oklahoma City; P.J. Shaw, New Children's Hospital, Sydney, Australia; E.J. Shpall, University of Colorado Health Sciences Center, Denver; W.E. Spruce, Children's Hospital San Diego, San Diego, Calif.; E. Sievers, Fred Hutchinson Cancer Research Center, Seattle; M.-K. Siren, Helsinki University Central Hospital, Helsinki, Finland; F.O. Smith, Riley Hospital for Children-Indiana University Medical Center, Indianapolis; P. Stiff, Loyola University Medical Center, Maywood, Ill.; L. Teague, Starship Children's Health, Auckland, New Zealand; K. Tiedemann, Royal Children's Hospital, Melbourne, Victoria, Australia; M. Vowels, Prince of Wales Children's Hospital, Sydney, Australia; J. Wagner, University of Minnesota Hospital, Minneapolis; D. Wall, Cardinal Glennon Children's Hospital, St. Louis; A. Wayne, University of Miami School of Medicine, Miami; S. Weinreb, Bay-State Medical Center, Springfield, Mass.; J. Weinthal, Medical City Dallas Hospital, Dallas; S. Wiersma, University of Wisconsin Center for Health Sciences, Madison; L. Yu, Children's Hospital-New Orleans, New Orleans.

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