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ORIGINAL ARTICLE

Transplantation of *ex-vivo* culture-expanded parental haploidentical mesenchymal stem cells to promote engraftment in pediatric recipients of unrelated donor umbilical cord blood: results of a phase I–II clinical trial

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Suboptimal neutrophil and platelet recovery after unrelated donor umbilical cord blood transplantation (UCBT) may be due in part to an impaired microenvironment after intensive chemoradiotherapy. In an attempt to speed hematopoietic recovery, 15 pediatric patients with high-risk acute leukemia were enrolled on a single-institution phase I–II clinical trial in which *ex-vivo* culture-expanded MSCs from haploidentical parental donors were infused at the time of UCBT. Eight patients received MSCs on day 0, with three patients having a second dose infused on day 21. No serious adverse events were observed with any MSC infusion. All eight evaluable patients achieved neutrophil engraftment at a median of 19 days. Probability of platelet engraftment was 75%, at a median of 53 days. With a median follow-up of 6.8 years, five patients remain alive and disease free. The results of this pilot study show that infusion of *ex-vivo* culture-expanded haploidentical MSCs into unrelated pediatric UCBT recipients can be performed safely. This encouraging safety profile with haploidentical MSCs supports the investigation of unrelated 'off the shelf' allogeneic HLA-mismatched MSC products.
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Introduction

The importance of the BM microenvironment in the maintenance and regulation of hematopoietic stem cells has been reviewed widely.[1,2] MSCs reside in the BM and give rise to multiple mesodermal tissue types, including bone, cartilage, tendon, muscle, fat and marrow stroma.[3–5] Marrow stroma supports hematopoiesis by creating an optimal microenvironment by providing cytokines and other regulatory factors to stimulate and enhance proliferation of the hematopoietic elements.[6–10]

In the last decade, hematopoietic stem cell transplantation using umbilical cord blood (UCB) grafts has increasingly been used.[11,12] Transplantation of partially HLA-matched UCB units has been shown to be safe. However, cell dose limits the use of UCB, particularly in adults and adolescents.[13] Even for those for whom cell dose is adequate, rate of neutrophil and platelet recovery after unrelated donor umbilical cord blood transplantation (UCBT) is suboptimal.[13] The slow recovery may be because of residual graft resistance and impaired microenvironment after intensive doses of chemoradiotherapy,[14,15] in addition to limited numbers of hematopoietic and progenitor cells. Concomitant infusion of stromal cells or MSCs at the time of transplantation may enhance marrow regrowth. Therefore, we investigated the safety and potential impact of haploidentical MSCs as a strategy to promote hematopoietic recovery after UCBT either by secretion of hematopoietic growth factors and chemokines or by immune modulative effect leading to the recipient's tolerance to the graft.

This report summarizes the results of the first phase I–II trial to establish the safety of *ex-vivo* culture-expanded allogeneic human MSCs from haploidentical related donors co-transplanted during unrelated donor UBC transplantation.

Materials and methods

Study design

This was a phase I–II study to evaluate the feasibility, safety and potential efficacy of MSCs obtained from a haploidentical parental donor in recipients of unrelated donor UCBT. By study design, each patient was to receive two doses of MSCs; patients in group 1 were to receive 5.0×10^6 MSCs/kg recipient on days 0 and 21 after unrelated donor UCBT; if less than two of the first 10 patients in group 1 had grade III–IV infusional toxicity

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within 2 weeks following the second dose of MSCs, additional group 2 patients were eligible to receive 10.0×10^6 MSCs/kg recipient on day 0.

The study protocol and consent forms were approved by the Institutional Review Board of the University of Minnesota. All parents gave informed signed consent. The trial was sponsored by Osiris Therapeutics (Baltimore, MD, USA).

Patients

To be eligible for the study, patients had to be <18 years of age, have high-risk ALL, high-risk AML or juvenile myelomonocytic leukemia. Further eligibility criteria included having a 3–4/6 HLA-matched parental MSC donor, a 4–6/6 HLA-matched unrelated UCB donor with a dosage of $\geqslant 1.0 \times 10^7$ nucleated cells/kg recipient, adequate organ function and an ECOG performance status of 0–1 or Karnofsky score of >70. Patients were excluded if they had a HLA-identical related or unrelated marrow donor, had a history of invasive fungal infection within 6 months, had an active infection at the time of transplant, were enrolled on other investigational agent concurrently or had any medical condition in the opinion of the clinical investigator that would interfere with the evaluation of the patient.

MSC donors

To be eligible as a MSC donor, the parent had to be 18–55 years of age, 2–3 HLA antigen disparate with the recipient, have been tested negative for HIV-I, HIV-II, HTLV-I/II, hepatitis B and C, CMV, syphilis, Creutzfeldt-Jakob disease and tuberculosis, have been free of active infection at the time of BM harvest, in good health and have signed informed consent.

BM (35–112 ml^3) was harvested from the haploidentical parent donor at the University of Minnesota at the time of referral. Cells were express-shipped overnight to Osiris Therapeutics, where MSCs were isolated and culture expanded. The aim was to manufacture sufficient number of cells to administer a total of 10.0×10^6 MSC/kg recipient in two divided doses, on day 0 and 21 (group I), or as a single dose on day 0 (group 2) after UCBT, in addition to performing quality control testing. Quality control testing included gram stain, bacterial culture, mycoplasma rapid testing (PCR ELISA) and culture, endotoxin, and MSC phenotype.

The harvested MSCs were formulated in PlasmaLyte A (Baxter, Deerfield, IL, USA) containing 5% human serum albumin and 10% dimethyl sulfoxide (Tera Pharmaceuticals, Los Angeles, CA, USA) and aseptically transferred into a sterile cryocyte freezing container (Baxter). After formulation, the product was immediately placed inside a controlled rate freezer and frozen to $-90\,^{\circ}C$ at $1\,^{\circ}C$/min. The final product was cryopreserved and stored in the vapor phase (between -130 and $-160\,^{\circ}C$) of liquid nitrogen.

A single UCB unit had to have at least 1.0×10^7 total nucleated cells per kg actual body weight (based on the cryopreserved cell count of the UCB product). Umbilical cord blood units were stored within the UCB bank processing facilities in liquid nitrogen freezers (between -130 and $-160\,^{\circ}C$). Units were shipped to the University of Minnesota immediately before initiation of the transplant preparative therapy.

Transplant procedure

Patients received a conditioning regimen of cyclophosphamide 60 mg/kg i.v. once daily for 2 days fractionated TBI 1350 cGy total dose. Patients less than 1 year of age received a non-TBI-based preparative therapy consisting of busulfan 30 mg/m^2/dose orally every 6 h for 4 days and melphalan 60 mg/m^2 i.v. once daily for 3 days. All patients also received equine anti-thymocyte globulin 30 mg/kg i.v. once daily for 3 days and methylprednisolone 1 mg/kg i.v. twice daily for 3 days to promote engraftment. On day 0, patients received the specified MSC dose i.v. 4 h before infusion of the UCB unit. Group 1 patients were eligible to receive a second dose of MSCs on day 21. On the day of the transplant, the UCB unit was rapidly thawed at $37\,^{\circ}C$ and infused through the central indwelling line at the rate of 5 ml/min. GVHD immunoprophylaxis for all patients consisted of CSA and short-course methylprednisolone (2 mg/kg/day, day $+5$ to day $+19$, with a 25% taper every other day; off by day $+24$ if no evidence of GVHD). If there was no evidence of GVHD, CSA was tapered by 10% per week beginning 6 months after UCBT.

Toxicity assessment

Vital signs were monitored 1 h before MSC infusion and 15, 30 min, 1, 2 and 4 h after infusion, at which time the UCB was given. Vital signs were monitored for 2 h after UCB infusion. In addition, patients were monitored continuously for MSC infusional toxicity, defined as alterations in vital organ functions within 14 days of MSC infusion that cannot be explained by other intercurrent complications such as infection or chemoradiotherapy. To assess the presence of ectopic tissue formation, patients were evaluated by skeletal survey at 1 year and 2 years after transplant.

Supportive care

Patients were hospitalized in single rooms with high-efficiency particulate air filtration with positive pressure until neutrophil engraftment. Patients received irradiated leukocyte-depleted red blood cell and platelet transfusions if hemoglobin level was $\leqslant 8$ g/l and platelet count was $\leqslant 10.0 \times 10^9$/l. Patients received antimicrobial prophylaxis with ciprofloxacin, fluconazole and acyclovir prophylaxis if they or their donors were seropositive for herpes simplex virus and/or CMV. Oral trimethoprim-sulfamethoxazole was given for pneumocystis carinii pneumonia prophylaxis after engraftment for 1 year. CMV-seronegative recipients received CMV-seronegative or filtered blood products. Patients received i.v. granulocyte colony-stimulating factor 5 mcg/kg/day after UCBT until ANC was $\geqslant 2.5 \times 10^9$/l for 2 consecutive days.

Statistical analysis

The primary objective of this study was to show lack of infusional toxicity after the infusion of varying doses of

MSCs derived from haploidentical related donors. Infusional toxicity was defined as alterations in vital organ functions within 14 days of MSC infusion that could not be explained by other intercurrent complications such as infection or chemotherapy. Rates of neutrophil engraftment, severe GVHD and other adverse events were continuously monitored throughout the study. Transplant outcomes were compared with those of a cohort of similar patients treated at our institution during the same period, in exactly the same manner except that they did not receive MSCs. Analyses, including toxicity monitoring, were carried out by the Biostatistical Group at the University of Minnesota. Stopping rules were determined such that if two of five patients failed to achieve neutrophil engraftment by day 100 enrollment would be put on hold, pending evaluation by the investigators and medical monitor. Donor (UCB and MSC) chimerism was determined serially on BM at days 21, 100, 180, 360 and 720 after transplantation, with additional time points as needed. The method of analysis was quantitative PCR of informative polymorphic variable number tandem repeat or short tandem repeat regions in the recipient and donor.[16,17]

Results

Patients' characteristics

Between May 2000 and July 2002, 15 patients were enrolled into the study and parental BM was obtained for MSC production. MSC expansion data for all parental BM samples are shown in Table 1, including volume and cell count of parental BM, MSC culture volume, cell count, quality control and final MSC cell dose infused. Seven patients did not receive MSC infusions because UCBT did not occur ($n=3$), UCBT occurred at a different center ($n=1$) or because of insufficient MSC availability at the time the patient was ready for transplantation ($n=3$).

For the eight patients who received haploidentical MSCs and UCB transplantation, patient and graft characteristics are shown in Table 2. All patients had high-risk acute leukemia, including Philadelphia chromosome positive ALL in first CR ($n=1$), ALL in CR2 ($n=5$), AML in CR2 ($n=1$) and infant AML in CR1 ($n=1$). The median age at the time of transplant was 7.5 (range, 0.2–16.0) years. Umbilical cord blood units were HLA-A, -B, -DRB1 matched ($n=1$) or mismatched at 1 Ag ($n=2$) or 2 Ag ($n=5$). Median UCB cell doses were 3.1×10^7 (range, $2.0–12.4\times10^7$) nucleated cells/kg and 5.9×10^5 (range, $3.1–34.8\times10^5$) CD34 dose/kg. Median MSC doses were 2.1×10^6 (range, 0.9–5.0)/kg on day 0. Only three patients (UPN 202-10-001, 202-10-003 and 202-10-012) received MSC on day 21 (1.0×10^6, 0.06×10^6 and 5.0×10^6/kg). For the remaining five patients insufficient growth of MSCs from the parent precluded a second infusion of cells.

Adverse events

Transplant outcomes are shown in Table 3. No serious adverse events occurred within 24 h of MSC infusions. Three patients developed transient hives and one patient experienced nasal congestion after MSCs. No long term no evidence of ectopic tissue formation was present in any patient, with six patients evaluated at 1 year and 2 years after transplant.

Hematopoietic recovery

All eight patients achieved neutrophil engraftment (first of three consecutive days of ANC $\geqslant 0.5\times10^9$/l) at a median of 19 (range, 9–28) days. Six patients achieved platelet engraftment (first of seven consecutive days of platelet count $\geqslant 50\,000/mm^3$) at a median of 53 (range, 36–98) days. BM biopsies were performed in all patients on days 21, 60, 100, 180 and 365 after transplantation to assess engraftment. All patients achieved 100% UCB donor chimerism at day 21 after UCBT, which was sustained except for one patient (UPN 202-10-006) who relapsed with disease 2 years after transplantation. No evidence of MSC donor chimerism was detectable in any patient at any time point after transplant.

GVHD

The cumulative incidence of grade II–IV acute GVHD by day 100 was 38% (95% CI, 10–66%). All three cases were grade II GVHD and were treated successfully with methylprednisolone 48 mg/m^2. No patient developed chronic GVHD.

Survival

The Kaplan–Meier estimate of survival 1 year after transplantation was 75% (95% CI, 10–66%). With a median follow-up of 6.8 years, five patients remain alive, well and disease free. Two patients died from invasive Aspergillus infections 53 and 63 days, respectively, after transplantation. One patient relapsed 2 years after UCBT and died 6 weeks later.

Historical control comparison

The historical control group consisted of 23 pediatric patients with high-risk ALL or AML who received a single UCBT at our institution from May 2000 to June 2002, after myeloablative therapy, with CSA and methylprednisolone as GHVD prophylaxis. The median cell dose for these control patients was 3.2×10^7 (range, $1.3–10.3\times10^7$) nucleated cells/kg, similar to the dose given to patients on the trial. All patients in both the MSC trial and the historical control groups achieved neutrophil engraftment, at a median of 19 days (range 8–28 days) for the MSC group, and at a median of 15 days (range 11–30) for the control group ($P=0.55$), as shown in Figure 1. Similarly, the probability of achieving a platelet count $\geqslant 50\,000/mm^3$ by 6 months after transplant was comparable, being 75% (95% CI, 46–100%) at a median of 53 (range, 36–98) days for the MSC group, and 74% (95% CI, 51–97%) at a median of 69 (range, 31–129) days for the historical control group ($P=0.55$), as shown in Figure 2. The cumulative incidence of grade II–IV acute GVHD by day 100 as similar between the MSC (38% (95% CI, 10–66%)) and historical cohorts (22%, (95% CI, 6–38%), $P=0.44$). Similarly, the incidence of chronic GVHD was not statistically different between the MSC (0%) and historical cohorts (17%, 95%

Table 1 MSC expansion data

Donor ID #	*Volume BM (ml)*	*BM cell count*	*MNCs recovered*	*Number of days cells were cultured*	*Passages cultured*	*Number of MSCs cultured*	*QC/sterility*	*Number of days MSCs were infused after cryopreservation*	*MSC dose infused*
202-10-901	73	3.40×10^9	4.00×10^8	40	P0 → P3	2.60×10^8	Passed	35	5.0×10^6/kg
								56	10.0×10^6/kg
202-10-902	112	1.60×10^9	1.20×10^8	29	P0 → P2	3.50×10^7	Passed	7	1.0×10^6/kg
202-10-903	78	2.70×10^9	3.00×10^8	31	P0 → P3	1.30×10^8	Passed	25	2.5×10^6/kg
								38	0.06×10^6/kg
202-10-904	110	2.20×10^9	2.50×10^8	40	P0 → P3	4.90×10^7	Passed	2	0.9×10^6/kg
202-10-905	87	8.20×10^9	2.80×10^8	46	P0 → P4	4.70×10^7	Passed	23	1.5×10^6/kg
202-10-906	57	1.60×10^9	4.80×10^8	35	P0 → P3	3.00×10^8	Passed	35	4.9×10^6/kg
202-10-907	65	3.80×10^8	3.60×10^8	52	P0 → P4	3.39×10^8	Passed Pt. in remission	Not infused	NA
202-10-908	38	3.00×10^8	2.90×10^7	NA	P0 → P1	NA	N/A Growth failure	Not infused	NA
202-10-909	56	1.09×10^9	1.52×10^8	49	P0 → P4[a]	1.33×10^8	Passed	34	1.7×10^6/kg
202-10-910	41	1.15×10^9	3.03×10^8	NA	P0	NA	N/A Discontinued Contaminated	Not infused	NA
202-10-911	54.5	1.50×10^9	7.80×10^8	NA	P0	NA	N/A Discontinued Growth Failure	Not infused	NA
202-10-912	55	2.86×10^9	5.70×10^8	21	P0 → P1	4.06×10^8	Passed	50	5.0×10^6/kg
								71	5.0×10^6/kg
202-10-913	36	9.30×10^8	2.10×10^8	42	P0 → P3	9.50×10^8	N/A	Not infused	NA
202-10-914	60	1.04×10^9	7.09×10^8	81	P0 → P4	1.20×10^8	Passed	Not infused	NA
202-10-915	63	1.42×10^9	7.86×10^8	69	P0 → P3	5.56×10^8	Passed	Not infused	NA

Abbreviations: CF = cell factory; MNC = mononuclear cells; NA = not applicable; Pt. = patient; QC = quality control.
[a]P1 seeded into Nunc CF.
901-906 QC summary recorded on G-SOP-D010 rev.02, 907-915 recorded on G-SOP-D010 rev.03.

Table 2 Patient and graft characteristics

UPN	*Age (years)/gender*	*Diagnosis*	*UCB HLA match*	*UCB dose*	*MSC donor*	*MSC HLA match to patient*
202-10-001	8/M	ALL pre-B, CR2	4/6	3.8	Father	4/6
202-10-002	5/M	ALL pre-B, CNS+, CR2	5/6	10.8	Father	3/6
202-10-003	8/M	Pre-B ALL, early relapse CNS, testes, CR2	4/6	2.7	Father	3/6
202-10-004	7/M	AML M5, early relapse, CR2	4/6	3.2	Mother	3/6
202-10-005	4/M	ALL Ph+, CR1	5/6	2.4	Mother	3/6
202-10-006	14/M	ALL pre-B, CR2	4/6	2.0	Mother	3/6
202-10-009	16/F	ALL Ph+, early relapse, CR2	4/6	2.9	Mother	4/6
202-10-012	0.25/F	AML M5 CNS+, CR1	5/6	12.4	Mother	3/6

Abbreviations: CNS = central nervous system; UCB = umbilical cord blood; UPN = unique patient number

CI 2–32%, $P = 0.23$). Although there was a trend toward improved 3-year survival after transplant in the MSC group (75%, 95% CI 45–100%) compared with the historical controls (46%, 95% CI 26–66%), it was not statistically significant ($P = 0.38$), as shown in Figure 3.

Discussion

In this phase I–II clinical trial of eight pediatric patients, the results showed that infusion of *ex-vivo* culture-expanded haploidentical MSCs into UCBT recipients is safe and is associated with minimal side effects. No patient had severe infusional toxicity and all patients achieved neutrophil engraftment.

The MSC manufacturing process is based on the method developed at Osiris,[18,19] which is similar to the MSC *ex-vivo* expanding methods described by other researchers. The data presented in this and in other studies[20,21] show that variability in cell growth time, yield and cell passage between MSC donors does not affect the MSC safety profile and biological effects. The variability between MSC donors in this study (Table 1) is similar to MSC donor variability observed by other investigators. In addition, the dose of MSCs used in our study is similar to the MSC doses in other reports in the literature.[22–25] The results generated from our study can be used in future for establishment of the acceptable normal range of biological variability between MSC donors.

A potential limitation of the use of haploidentical MSCs in the setting of unrelated UCBT, however, is timing. The rapid availability of unrelated UCB often makes it an attractive hematopoietic stem cell source for patients with high-risk disease who require immediate transplant therapy. However, *ex-vivo* expansion of MSCs took approximately 4–6 weeks. To prevent delay of UCBT, related MSCs may need to be collected and expanded before it is confirmed that an unrelated UCBT is to occur. Other potential limitations are insufficient *ex-vivo* expansion of MSCs and potential bacterial contamination of the cells. For example, MSC donor age is one of the major contributors to cell yield as MSC numbers in BM significantly decrease with age.[26] The use of age-restricted pre-screened unrelated young healthy donors for MSC *ex-vivo* manufacturing will help to narrow the biological variability. The use of unrelated HLA-unmatched donors, together with the ability to cryopreserve manufactured MSCs, opens the possibility to manufacture MSCs in advance and have an 'off the shelf' drug for clinical use without limitations.

In this trial, parental MSC chimerism could not be assessed in the patients. Other investigators have had similar difficulty in identifying engrafted MSCs. Cilloni *et al.*[27] investigated the engraftment capacity of BM-derived mesenchymal cells in 41 patients who had received a sex-mismatched, T-cell-depleted allograft from HLA-matched or -mismatched family donors. PCR analysis of the human androgen receptor (HUMARA) or the amelogenin genes was used to detect donor-derived mesenchymal cells. Only 14 (34%) marrow samples from 41 consenting patients generated a marrow stromal layer adequate for PCR analysis. Only seven of 14 analyzed samples showed the existence of a mixed chimerism at the stromal cell level.[27] The difficulties in detecting engrafted MSCs may be explained by the limitations of sampling for testing and by the limitations of the current detection methods. However, despite the difficulties in identifying engrafted MSCs, positive effects of MSC therapy in patients are observed.[20,21,28,29]

There have been three recent reports in the literature on the use of MSC during allogeneic transplantation to enhance engraftment. An Italian group gave mesenchymal cells to 26 adult patients receiving HLA-identical sibling BM ($n = 8$) or G-CSF mobilized peripheral blood cells ($n = 18$) after reduced intensity conditioning.[30] Twenty-three patients achieved sustained engraftment. Forty-eight marrow samples harvested from 22 patients and placed in long-term culture conditions generated sufficient marrow stromal layers for PCR analysis. Eight of these 22 patients showed a partial donor origin of stromal cells. Interestingly, none of the eight patients showing chimeric stroma developed grade II–IV acute GVHD. In our study, only three of eight patients developed grade II–IV acute GVHD. Although this rate did not differ from that of our historical controls, Le Blanc's group has shown promising results of the effectiveness of MSC for treatment of severe acute GHVD.[20,28] Further prospective clinical trials need to be performed to determine the role of MSC in the prevention and treatment of acute GVHD.

Le Blanc *et al.*[21] recently reported on the use of haploidentical MSC in conjunction with allogeneic hematopoietic stem cell transplantation to enhance engraftment. Seven patients (three with previous graft failure) underwent allogeneic transplantation with peripheral blood stem cells

Table 3 Transplant outcomes

UPN	*MSC infusion toxicity*	*Toxicities*	*Days to ANC >500/mm³*	*Acute GVHD*	*Chronic GVHD*	*Infections*	*Survival*
202-10-001	Hives	CSA-associated seizures (day 81)	14	2 (skin stage 3)	0	HSV stomatitis (day 16)	7.7 years +
202-10-002	None	None	9	1 (skin stage 2)	0	None	7.8 years +
202-10-003	Hives	Pulmonary and gastric hemorrhage secondary to invasive Aspergillosis	24		NA	*Aspergillus fumigatus* lung and stomach	Died day +63 from Aspergillosis
202-10-004	None	None	24	2 (skin grade 1, rectal grade 1)	0	Staphylococcus bacteremia (day 30) CMV viremia (day 41, day 111) Coagulase negative staphylococcus bacteremia (day 117) Sinusitis (day 140) Shingles (day 271) Reactive airway disease (day 464)	7.5 years +
202-10-005	None	Autoimmune hemolytic anemia (day 267)	27	0	0	*Clostridium difficile* colitis (day 44) Coagulase negative Staphylococcus bacteremia (day 40) Enterococcus bacteremia (day 40)	7.3 years +
202-10-006	None	Acalculous cholecystitis (day 12) Hemolytic anemia (day 113) Seizures (day 113) Hypogonadism (day 349)	28	2 (UGI stage 1, rectal stage 1)	0	*Clostridium difficile* colitis (day 1)	BM relapse 2 years after HCT, died 6 weeks later
202-10-009	Hives	None	13	0	NA	Aspergillosis CNS infection (day 43)	Died day +53 from Aspergillosis
202-10-012	Nasal congestion	None	11	0	0	*Clostridium difficile* colitis (day 57)	6.3 years +

Abbreviations: CNS = central nervous system; HSV = herpes simplex viurs; UGI = upper gastrointestinal; UPN = unique patient number.

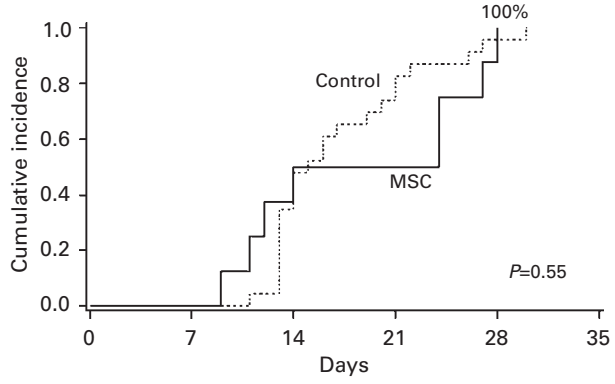

Figure 1 Probability of neutrophil engraftment: current MSC recipients versus historical control umbilical cord blood (UCB) recipients.

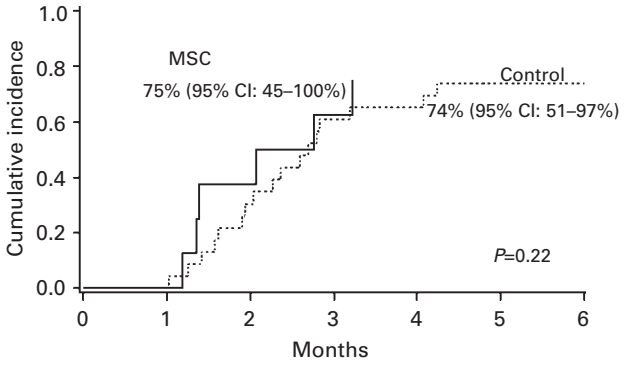

Figure 2 Probability of platelet engraftment: current MSC recipients versus historical control umbilical cord blood (UCB) recipients.

($n=4$), BM ($n=2$) or cord blood ($n=1$) after myeloablative ($n=4$) or non-myeloablative ($n=3$) conditioning. MSCs from HLA-identical sibling ($n=3$) or haploidentical ($n=4$) donors were infused within 4 h of hematopoietic stem cell infusion. Similar to our experience, there was no infusional toxicity and no signs of ectopic tissue after hematopoietic stem cell transplantation. All patients, including the three patients with previous graft failure, achieved neutrophil and platelet engraftment and 100% donor chimerism. The results of our study using a uniform preparative therapy and stem cell source lend further evidence to the safety of haploidentical MSC infusion during hematopoietic stem cell transplantation.

A group in Leiden recently reported on the results of a pilot study in which 14 children undergoing hematopoietic stem cell transplantation using G-CSF-mobilized, CD34 selected progenitor cells from a HLA-disparate relative were co-transplanted with BM-derived *ex-vivo* expanded MSCs from the same donor.[29] The MSCs were well tolerated and all patients achieved sustained hematopoietic cell engraftment, a significant improvement compared with the 47 historical controls of whom 15% developed primary or secondary graft failure.

This study was the first to evaluate the safety and feasibility of haploidentical MSCs in a cohort of children undergoing unrelated donor UCBT. The results of this pilot study showed that infusion of *ex-vivo* culture-

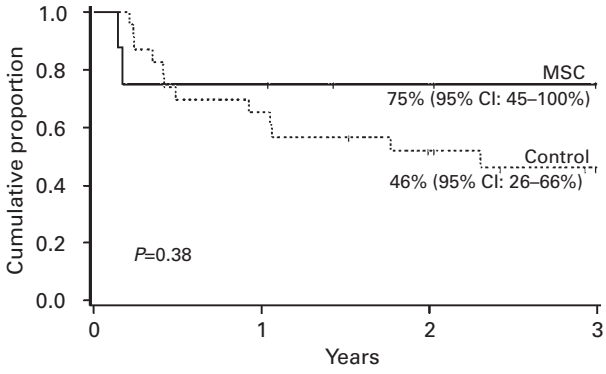

Figure 3 Probability of survival: current MSC recipients versus historical control umbilical cord blood (UCB) recipients.

expanded haploidentical MSCs into UCBT recipients can be performed safely. As haploidentical MSCs are allogeneic MSCs, these data support the use of allogeneic MSCs from unrelated donors, an approach that may help solve time and donor variability problems.

In September 2002, at our institution, a 6-month-old female with infant AML received unrelated allogeneic MSCs on the day of her 6/6-HLA-matched UCB transplantation after myeloablative conditioning therapy. As with haploidentical MSCs, she did not experience any infusional toxicity, achieved neutrophil engraftment on day 11 after transplant, platelet engraftment on day 38 after transplant, did not develop GVHD and remains alive and well 5.8 years later. Further trials are needed to study the role of unrelated allogeneic HLA-mismatched MSCs in optimizing outcomes after transplantation. The valuable MSC data from this trial, together with other data from published reports in the literature, will help to select the most appropriate patient populations that will benefit the most from MSC treatment. In addition, these data will assist in the establishment of MSC acceptable donor criteria and in the design of effective treatment regimens.

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