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Clinical and laboratory features of mesenchymal stromal cells in pediatric stem cell transplantation

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Citation

Ball, L. M. (2010, March 4). *Clinical and laboratory features of mesenchymal stromal cells in pediatric stem cell transplantation*. Retrieved from <https://hdl.handle.net/1887/15035>

Version: Corrected Publisher's Version

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Note: To cite this publication please use the final published version (if applicable).

Chapter 3

Co-transplantation of *ex vivo* expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haplo-identical hematopoietic stem cell transplantation.

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BLOOD. 2007; 110(7): 2764-2767

Summary

Haploidentical hematopoietic stem cell transplantation (HSCT) is associated with an increased risk of graft failure. Adult bone marrow-derived mesenchymal stromal cells (MSCs) have been shown to support *in vivo* normal hematopoiesis and to display potent immune suppressive effects. We co-transplanted donor MSCs in 13 children undergoing transplantation of HLA-disparate CD34+ cells from a relative. While we observed a graft failure rate of 20% in historical controls, all patients given MSCs showed sustained hematopoietic engraftment, without any adverse reaction. In particular, children given MSCs did not experience more infections as compared to controls. These data suggest that MSCs, possibly thanks to their potent immunosuppressive effect on alloreactive host T lymphocytes escaping the preparative regimen, reduce the risk of graft failure in haploidentical HSCT recipients.

Introduction

T-cell–depleted hematopoietic stem-cell transplantation (HSCT) from an HLA-haploidentical relative is a feasible option for children needing an allograft and lacking an HLA-compatible donor.¹ However, both primary (defined as lack of hematologic recovery or absence of donor chimerism) and secondary (defined as loss of donor chimerism after initial engraftment)² graft failure, mainly mediated by host alloreactive T cells escaping the preparative regimen, have been reported in up to 15% to 18% of children given mismatched HSC transplants,³ despite the infusion of large numbers of hematopoietic stem cells.⁴ Recipients of T-cell–depleted HSC transplants from an HLA-disparate relative are also exposed to an increased risk of life-threatening infections, especially of viral origin, due to the delay in reconstitution of adaptive immunity.^{1,3}

Bone marrow (BM) contains pluripotent mesenchymal stromal cells (MSCs), which form cartilage, fat, bone, and muscle.⁵ MSCs have been shown to modulate the function of T lymphocytes,⁶ including that of alloreactive T cells involved in graft-versus-host disease (GvHD) pathophysiology.⁷ In adult patients undergoing transplantation from an HLA-identical sibling, MSC infusion was shown to be safe and possibly to accelerate hematopoietic recovery, as well as to reduce the incidence of both acute and chronic GvHD.⁸ However, it is still unknown whether co-transplantation of MSCs in haploidentical HSC transplant recipients can reduce graft failure.

We carried out a phase 1/2 pilot study of co-transplantation of BM-derived, ex vivo–expanded MSCs of donor origin in children undergoing transplantation of granulocyte colony stimulating factor (G-CSF)–mobilized, CD34–selected progenitor cells from an HLA-disparate relative. The procedure was intended to reduce graft failure rate compared with historic controls.

Patients, materials, and methods

Patients

Children with hematologic malignancies or nonmalignant disorders, including

primary immune deficiencies, lacking an HLA-matched donor were enrolled in the study by the 2 participating centers (Leiden University Medical Center and Fondazione IRCCS Policlinico San Matteo). Institutional Review Board approval was provided by the 2 participating centers.

Parents or legal guardians of patients provided written informed consent for inclusion in the study. Written informed consent in accordance with the Declaration of Helsinki was also obtained from donors by an independent physician trained to explain risks associated with mesenchymal and hematopoietic stem cell donation.

Preparation of MSCs

Approximately 5 weeks before HSCT, mononuclear cells were isolated from 50 to 70 mL donor BM by density gradient centrifugation on Ficoll. These were plated in noncoated 75- to 175-cm² polystyrene culture flasks at a density of 160 000/cm² in complete culture medium (LG-DMEM [Invitrogen, Paisley, United Kingdom] supplemented with penicillin and streptomycin [Lonza, Logan, UT] and 10% fetal bovine serum [FBS; HyClone, Verviers, Belgium]). We used characterized and defined FBS batches preselected for their potential to support MSC expansion. All procedures were carried out under strict Good Manufacturing Practice (GMP) conditions. Flasks were incubated at 37°C in a CO₂ incubator and culture medium was replaced twice weekly. After reaching at least 70% confluence, MSCs were replated at 4000 cells/cm² using trypsin/EDTA (Lonza). MSCs were infused, fresh or after cryopreservation, at passage 3 or less to reduce the risk of genetic instability. MSC release criteria for clinical use were as follows: spindle-shape morphology, absence of contamination by pathogens, viability, and an immune phenotype proving the expression of CD73, CD90, and CD105 surface molecules and the absence of CD34, CD45, and CD31. The target dose for infusion was 1 x 10⁶/kg to 5 x 10⁶/kg body weight. MSCs were infused at a final concentration of 1 x 10⁶ to 2 x 10⁶ cells/mL.

Cotransplantation of MSCs and haploidentical peripheral blood stem cells at day 0, under monitoring of vital signs, patients were given MSCs intravenously via a central venous catheter and 4 hours later received T-cell-depleted, G-CSF+ mobilized CD34+ cells, positively selected using the CliniMac^s 1-step procedure (Miltenyi Biotech,

Bergisch Gladbach, Germany). The target number of CD34+ cells to be infused was 20×10^6 CD34+ cells/kg recipient weight.

Statistics

A Student *t* test, Fisher exact test, and chi-square test with Yates correction were used to assess differences between study and historic control groups. A *P* value of less than .05 was considered to be significant.

Results and discussion

Table 1 shows the characteristics of the 14 study patients compared with 47 historic controls that received transplants in either one of the 2 centers and were selected for an equivalent number of CD34+ cells infused and matched for transplant indication. There was no significant difference between patients and controls in terms of age, sex, malignant versus nonmalignant disease, method of CD34+ cell selection, and number of CD3+ cells infused. In all donors, both expansion of MSCs and mobilization of CD34+ cells were successful. Patients received a mean of 1.6×10^6 MSCs/kg (range, 1×10^6 MSCs/kg to 3.3×10^6 MSCs/kg). No MSC infusion–related toxicity was observed. Either primary or secondary graft failure occurred in 7 of the 47 children of the control group, whereas no rejection occurred in children who received cotransplants of haploidentical MSCs (*P* = .14). The number of CD34+ cells infused was superimposable in the study patients (mean, 21.5×10^6 /kg; range, 11.6×10^6 /kg to 38.6×10^6 /kg), in controls with sustained engraftment (mean, 21.2×10^6 /kg; range, 12.1×10^6 /kg to 47.5×10^6 /kg), and in those who experienced either primary (mean, 21.7×10^6 /kg; range, 14.7×10^6 /kg to 39.4×10^6 /kg) or secondary (mean, 21.1×10^6 /kg; range, 12.4×10^6 /kg to 26.6×10^6 /kg) graft failure.

Neutrophil and platelet recovery was comparable in study patients and controls (see Table 1 for definitions and details). However, patients given MSCs had faster recovery of a total leukocyte count above 1.0×10^9 /L in comparison to historic controls (mean, 11.5 days [95% confidence interval [CI] 9.0–14.8] versus 14.9 days [95% CI 10.1–26.0], respectively, *P* = .009).

Table 1. Characteristics of patients and controls

	Patients (n = 13)	Controls (n = 52)	p value
Transplant years (range)	Oct. 2004 – Feb. 2007	March, 1998- Oct. 2004	
Mean age (range) years	8 (1-16)	8 (1-17)	NS
Patient gender			
Male	8 (61%)	31 (60%)	NS
Female	5 (39%)	21 (40%)	NS
Original diseases			
Haematological malignancies	10 (77%)	40 (77%)	(Distribution) 0.2)
ALL	4 (40%)	21 (52.5%)	
CR1	0	2	
CR 2	3	11	
>CR2	1	8	
	6 (60%)	12 (30%)	
AML	0	2	
CR1	3	5	
CR 2	0	2	
>CR2	1	1	
Secondary	2	2	
Refractory	0 (0%)	5 (12.5%)	
MDS		1	
RC		1	
RAEB		2	
RAEBt		1	
Aplastic MDS	0 (0%)	2 (5%)	
CML Chronic phase			
Immune deficiencies	2 (15%)	2 (4%)	
Other non-malignant disorders	1 (8%)	10 (19%)	
Fanconi anemia	1	4	
Hemoglobinopathies	-	1	
HLH	-	4	
Other	-	1	
Donor gender			
Male: Female	7 : 6	29 : 23	0.9
Conditioning regimen			
TBI-based vs. Chemotherapy-based	8:5 (62 vs. 38%)	30:22 (58 vs. 42%)	0.8
Graft characteristics			
Number of CD34+ cells infused x 10 ⁶ /kg (median, range)	25.2 (11.6 – 38.6)	23.0 (12.1 – 47.5)	NS
Number of CD3+ cells infused x 10 ⁷ /kg (mean, SD)	0.3 (0.3)	0.5 (0.7)	NS
Haematopoietic recovery			
Number of days to PMN recovery (median, range)	12 (10-17)	14 (9-28)	0.1
Number of days to PLT recovery (median, range)	11 (10-18)	13 (9-100)	0.15
Number of days to reticulocyte recovery (median, range)	11 (10-31)	23 (9-41)	0.02
Number of days to leucocyte recovery (median, range)	11.6 (9-15)	16.5 (10-26)	0.005
Post-HSCT complications			
Graft failure	0 (0%)	11 (20%)	0.06
Primary	-	7	
Secondary	-	4	
Acute GvHD			0.08
Grade I-II	2 (15%)	12 (23%)	
Grade III-IV	0 (0%)	2 (3%)	
Chronic GvHD	1 (8%)	6 (12%)	0.2
Limited	1	4	
Extensive	0	2	

Table 1 Legend

ALL = acute lymphoblastic leukaemia; AML = acute myeloid leukaemia; MDS = myelodysplastic syndrome; RC = refractory cytopenia; RAEB = refractory anemia with excess of blasts; RAEBt = refractory anemia with excess of blasts in transformation; CML = chronic myeloid leukaemia; HLH = hemophagocytic lymphochistiocytosis; CR = complete remission; TBI = total body irradiation; PMN = polymorphonuclear neutrophils; PLT = platelets; HSCT = hematopoietic stem cell transplantation; GvHD = graft-versus-host disease; SD = standard deviation; NS = non-significant.

Lymphocyte recovery accounted for this finding: the absolute numbers of natural killer (NK) cells 1 month after HSCT being 497/ μ L (95% CI 347-646) in the study group and 252/ μ L (95% CI 173-330) in controls (P= .02). However, at 3 months, NK and T-cell recovery was quantitatively no different between study patients and controls. Chimerism analysis of ex vivo-expanded MSCs derived from recipient BM at 3-month intervals up to 1 year after HSCT using polymerase chain reaction (PCR) for

Table 2. Patient follow up data

UPN	Sex	Age at HSCT	Donor	Diagnosis	Follow up	Chimerism analysis	Time to last BM chimerism	Time to last PB chimerism	Outcome
1	M	15y 6mo	Mother	Refractory AML	+7 mo†	100% donor	+6 mo	NE	Died Candida sepsis - CR
2*	Mm	2y	Father	X-LPD	+28 mo	100% donor	+ 24 mo	+26 mo	Alive and well
3*	M	2y 4mo	Father	X-LPD	+24 mo	95% donor granulocyte 88% donor CD3 (BM)	+20 mo	+23 mo	Alive and well
4	M	13y 1mo	Mother	Refractory AML	+4 mo	100% donor	+3 mo	+3 mo	Died relapse
5	F	8y 9mo	Father	Fanconi anemia	+16 mo	100% donor	+14 mo	+11 mo	Alive and well transfusion independent
6	F	3y 8mo	Father	ALL 2CR	+7 mo†	100% donor	+ 3 mo	+3 mo	Died relapse
7	F	13y 4mo	Father	monosomy 7 refractory AML	+4 mo†	100% donor	+2 mo	+2mo	GVHD Died adenovirus hepatitis - CR
8	F	7y 1mo	Mother	AML CR 2	+12 mo	100% donor	+7 mo	+10 mo	Alive and well CR
9	M	5y	Father	ALL-T CR4	+10 mo	100% donor	+8mo	+7mo	Alive and well CR
10	M	5y 4 mo	Mother	ALL CR2	+8 mo	100% donor	+7mo	+8mo	Alive and well CR
11	F	8y 5mo	Sister	ALL CR2	+7 mo	100% donor	+5mo	+6mo	Alive and well CR
12	F	8y 2mo	Mother	AML CR2	+6 mo	95% donor BM 80% donor PB Recipient % CD4/CD8 positive	+5 mo	+6mo	Alive and well CR
13	M	16y	Father	AML CR2	+3 mo	100% donor	+3 mo	+3mo	Alive and well CR

Table 2 Legend

UPN = unique patient number; BM = bone marrow; PB = peripheral blood; HSCT = hematopoietic stem cell transplantation; M = male; F = female; y = years; mo = months; ALL= acute lymphoblastic leukaemia; AML= acute myeloid leukaemia; X-LPD = X linked lymphoproliferative disorder; NE = not evaluated; CR = complete remission; † = dead. * Patients UPN 2 and 3 are identical twins transplanted from the same haploidentical father

informative donor recipient polymorphisms⁹ did not show any evidence of donor cells in the majority of patients. In 3 patients, minimal (1%-2%) transient engraftment of donor MSCs was found at 3 months. Hematopoietic chimerism is detailed in Table 2.

Four study patients died (Table 2), 2 due to relapse and 2 due to infection, compared with 11 controls (7 relapse, 2 infections, 2 GvHD). Episodes of viral reactivation were common in both patients and controls, occurring in 50% of patients belonging to the study group and in 35% of historic controls. However, only 1 study patient died, as a result of disseminated adenovirus infection complicated by grade 2 acute GvHD requiring steroid treatment, compared with 2 historic controls. Since the follow-up of patients in the study group is shorter (range, 3-28 months) than that of historic controls (range, 32-110 months), both relapse rate and probability of overall survival in the study cohort (18% and 72%, respectively) and in controls (26% and 63%, respectively) are not comparable.

Our results indicate that in patients given a T-cell-depleted, HLA-disparate-related allograft from a relative, expansion of donor MSCs is feasible and their clinical use is safe. Moreover, our data suggest that MSC co-transplantation may modulate host alloreactivity and/or promote better engraftment of donor hematopoiesis, reducing the risk of early graft failure.

A case-controlled study, with longer follow-up to exclude the risk of late rejections, can more precisely define the role played by co-transplantation of haploidentical donor MSCs on the outcome of patients given haploidentical, T-cell-depleted HSCT.

Acknowledgments

The authors wish to acknowledge the cooperation of patients and families who have participated to date in this study, the medical and ancillary staff associated with the transplant centers for contributing to the overall excellent care of the patients and the data managers for assisting in the preparation of data for the manuscript.

This work has been partly supported by grants from Istituto Superiore di Sanità (National Program on Stem Cells), European Union (FP6 program ALLOSTEM), Regione Lombardia (Research Project: 'Trapianto di cellule staminali adulte per scopi di terapia cellulare sostitutiva, riparativa e rigenerativa'), Fondazione CARIPLO to F.L. and the Dutch Program for Tissue Engineering.

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