ASBMT
American Society for Blood and Marrow Transplantation

Efficacy and Safety of Ex Vivo Cultured Adult Human Mesenchymal Stem Cells (Prochymal™) in Pediatric Patients with Severe Refractory Acute Graft-Versus-Host Disease in a Compassionate Use Study

Vinod K. Prasad,[1] Kenneth G. Lucas,[2] Gary I. Kleiner,[3] Julie An M. Talano,[4] David Jacobsohn,[5] Gloria Broadwater,[6] Rod Monroy,[7] Joanne Kurtzberg[1]

Preliminary studies using directed-donor ex vivo expanded human mesenchymal stem cells (hMSCs) have shown promise in the treatment of acute graft-versus-host disease (aGVHD). However, their production is cumbersome and standardization is difficult. We describe the first experience of using a premanufactured, universal donor, formulation of hMSCs (Prochymal) in children (n = 12; 10 boys; 9 Caucasian; age range: 0.4-15 years) with treatment-resistant grade III and IV aGVHD who received therapy on compassionate use basis between July 2005 and June 2007 at 5 transplant centers. All patients had stage III or IV gut (GI) symptoms and half had additional liver and/or skin involvement. Disease was refractory to steroids in all cases and additionally to a median of 3 other immunosuppressive therapies. The hMSCs (8×10^6cells/kg/dose in 2 patients and 2×10^6cells/kg/dose in the rest) were infused intravenously over 1 hour twice a week for 4 weeks. Partial and mixed responders received subsequent weekly therapy for 4 weeks. HLA or other matching was not needed. The hMSCs were started at a median of 98 days (range: 45-237) posttransplant. A total of 124 doses were administered, with a median of 8 doses (range: 2-21) per patient. Overall, 7 (58%) patients had complete response, 2 (17%) partial response, and 3 (25%) mixed response. Complete resolution of GI symptoms occurred in 9 (75%) patients. Two patients relapsed after initial response and showed partial response to retreatment. The cumulative incidence of survival at 100 days from the initiation of Prochymal therapy was 58%. Five of 12 patients (42%) were still alive after a median follow-up of 611 days (range: 427-1111) in surviving patients. No infusional or other identifiable acute toxicity was seen in any patient. Multiple infusions of hMSCs were well tolerated and appeared to be safe in children. Clinical responses, particularly in the GI system, were seen in the majority of children with severe refractory aGVHD. Given the favorable results observed in a patient population with an otherwise grave prognosis, we conclude that hMSCs hold potential for the treatment of aGVHD, and should be further studied in phase III trials in pediatric and adult patients.

Biol Blood Marrow Transplant ■: *1-8 (2010)* © *2010 American Society for Blood and Marrow Transplantation*

KEY WORDS: Acute graft-versus-host disease, Human Mesenchymal stem cells, Pediatric, Hematopoietic stem cell transplantation, Compassionate use, Steroid refractory

INTRODUCTION

The treatment of severe (grades III-IV) acute graft-versus-host disease (aGVHD) continues to be very challenging. The current therapies do not offer significant benefits, and no therapy has been FDA approved for the treatment of aGVHD. In general, approximately 50% of all patients with aGVHD

From the [1]The Pediatric Blood and Marrow Transplant Program, Department of Pediatrics, Duke University Medical Center, Durham, North Carolina; [2]Pediatric Stem Cell Transplantation, Penn State Hershey Children's Hospital, Hershey, Pennsylvania; [3]Department of Pediatric Immunology, University of Miami, Miami, Florida; [4]Department of Pediatric Hematology, Oncology, Bone Marrow Transplant, Medical College of Wisconsin, Milwaukee, Wisconsin; [5]Stem Cell Transplant Program, Children's Memorial Hospital, Chicago, Illinois; [6]Cancer Center Biostatistics, Duke University Medical Center, Durham, North Carolina; and [7]Osiris Therapeutics, Inc., Columbia, Maryland.

Financial disclosure: See Acknowledgments, page 8.

Correspondence and reprint requests: Vinod K. Prasad, MD, MCRP (London), PBMT Program, Department of Pediatrics, Duke University Medical Center, DUMC 3350, Durham, NC 27710 (e-mail: vinod.prasad@duke.edu).

Received January 7, 2009; accepted April 20, 2010

1083-8791/$36.00
doi:10.1016/j.bbmt.2010.04.014

initially respond to the first-line steroid therapy. However, the response rates are lower and GVHD recurrence is higher in patients with grades III-IV disease [1,2]. At present, many steroid-refractory patients die from aGVHD and/or from complications arising from the disease or its treatment [3]. The poor prognosis of severe aGVHD was well documented in a large worldwide registry-based study of pediatric and adult patients with probabilities of overall survival (OS) for grade III and IV aGVHD to be close to 30% and 5%, respectively [4]. The 2-year survival was 10% or lower in patients failing steroid therapy in another study [5]. These poor outcomes underline the need for novel therapeutic options for patients with aGVHD in general and those with severe and refractory disease in particular.

The human mesenchymal stem cells (hMSCs) arise from the mesoderm and have the ability to differentiate into various tissues including bone, cartilage, and muscle [6]. They have been shown to inhibit T cell activation, suppress inflammatory responses, and decrease the secretion of immunosuppressive factors in laboratory studies [7-10]. In addition, these cells express growth factors including vascular endothelial growth factors (VEGF), which are known to aid angiogenesis and tissue repair [11]. They also secrete various cytokines including IL-6, IL-11, leukemia inhibitory factor (LIF), stem cell factor, and macrophage colony-stimulating factor [11-13]. Coculture experiments have shown that hMSCs can induce a more anti-inflammatory and tolerant state in dendritic cells, naïve and effector T cells, and natural killer (NK) cells by altering cytokine secretion profiles [14]. These and other studies have clearly demonstrated immunosuppressive and tissue repair capabilities of hMSCs. For example, suppression of allogeneic reaction and prolongation of skin graft survival was seen in baboons following administration of major histocompatibility complex (MHC) mismatched MSCs [7]. These observations point to another attractive aspect of hMSCs; that is, the lack of HLA class II or costimulatory molecule expression of their cell surface rendering them impervious to rejection by the recipient.

Given these biological properties, hMSCs have the potential of being useful in the treatment of GVHD. In a 2004 report, ex vivo expanded haploidentical hMSCs was first used to successfully treat a patient with grade IV refractory aGVHD [15]. Subsequent human experience, albeit limited, with directed donor or universal donor hMSCs in the treatment of aGVHD has been encouraging [16,17]. Recently, a multicenter study by Le Blanc et al. [18] demonstrated efficacy of directed donor hMSCs in the treatment of adult and pediatric patients with steroid refractory aGVHD. In view of the poor prognosis of severe treatment-refractory aGVHD and lack of a good and effective treatment, we initially evaluated Prochymal, a commercial preparation of hMSCs derived from the bone marrow (BM) of universal donors [19]. Here we describe the first experience of this compassionate use study for the treatment of pediatric patients with refractory grades III and IV aGVHD.

PATIENTS AND METHODS

Study Design and Patient Eligibility

The compassionate use multicenter protocol was designed to treat children (<18 years of age) with severe steroid-refractory aGVHD and to evaluate the safety and efficacy of hMSC infusion in these patients. The patients were eligible if they had developed grade III-IV aGVHD as defined by standard criteria after HSCT transplant and were refractory to standard first-line treatment with corticosteroids and at least 1 second-line therapy. They were considered steroid refractory as defined by lack of response after at least 3 days of treatment with methylprednisone (≥2 mg/kg/day) or equivalent. Patients with uncontrolled infection, irreversible organ failure, allergy to bovine or porcine products, recipients of transplant for solid tumors, and those with conditions that might interfere with informed consent or evaluation were excluded. Safety endpoints included infusional toxicity, adverse reactions, development of ectopic tissue, infection, and death. Efficacy endpoints included improvement on the overall grade of aGVHD, response by organ and OS. Patients were evaluated for safety until death, withdrawal, or 180 days after the first infusion of hMSC, whichever occurred first.

Between July 2005 and June 2007, a total of 12 children were enrolled at Duke University Medical Center (n = 5), Milton Hershey Medical Center (n = 3), University of Miami (n = 2), Medical College of Wisconsin (n = 1), and Children's Memorial Hospital, Chicago (n = 1). Detailed demographic information is presented in Table 1. In summary, the median age was 6 years (range: 5 months to 15 years); 10 were boys, and 9 were Caucasian. The patients had undergone HSCT for acute myelogenous leukemia (n = 4), acute lymphoblastic leukemia (ALL) (n = 2), familial hemophagocytic lymphohistiocytosis (n = 2), malignant osteopetrosis (n = 1), Hurler syndrome (n = 1), adrenoleukodystrophy (n = 1), and myeloproliferative disorder with eosinophilia (n=1). Eleven patients had undergone myeloablative conditioning (MAC), whereas 1 received reduced-intensity conditioning (RIC). The graft sources were unrelated umbilical cord blood (n = 7), unrelated BM (n = 2), unrelated peripheral blood stem cell (n = 1), cord blood (CB) plus $CD34^+$ cells from a haploidentical-related parent (n = 1), and CB plus BM from a matched sibling (n = 1). Three patients were on hemodialysis for acute renal

Table 1. Patient Demographic Information

Patient No.	Patient Sex	Patient Age (Years)	Diagnosis	Conditioning Regimen	Graft	Donor HLA Match	GvHD Prophylaxis
1	Male	0.4	ALD	MAC	BM + UCB (sibling)	6/6	CsA
2	Male	0.7	M. OP	RIC	UCB	4/6	FK/MP
3	Male	6	HLH	MAC	BM	10/10	CsA/MTX
4	Male	2	Hurler	MAC	UCB	4/6	CsA/MMF
5	Male	13	AML	MAC	UCB	4/6	CsA/MMF
6	Male	2	AML	MAC	UCB	5/6	CsA/MMF
7	Male	15	HLH	MAC	UCB + haplo PBSC	5/6	CsA/MP
8	Male	4	ALL	MAC	PBSC	9/10	FK/MTX/ATG
9	Male	15	AML	MAC	UCB	5/6	CsA/MP
10	Female	2	MPD	MAC	UCB	6/6	CsA/MP
11	Female	6	AML	MAC	UCB	4/6	CsA/MMF
12	Male	13	ALL	MAC	BM	10/10	CsA/MTX

ALD indicates adrenoleukodystrophy; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; HLH, hemophagocytic lymphohistiocytosis; Hurler, Hurler's syndrome; M OP, malignant osteopetrosis; MPD, myeloproliferative disorder with eosinophilia; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; BM, bone marrow; UCB, umbilical cord blood; PBSC, peripheral blood stem cells; haplo PBSC, CD34-selected PBSC from haploidentical parent; CsA, cyclosporine A; FK, tacrolimus; MTX, methotrexate; MMF, mycophenolate mofetil, MP, methylprednisone.

insufficiency at the time of hMSC therapy. The last follow-up on the patients was in August to September 2008. Written informed consent according to the declaration of Helsinki was obtained in all cases from parent or legal guardian. All patients were approved by institutional review boards of participating centers and by the FDA with parental signature by FDA regulations (21 CFR Part 50), written authorization for use and disclosure of personal health information (45 CFR 164), and International Conference on Harmonization guidelines, as applicable.

hMSC Production

The hMSC were manufactured and provided by Osiris Therapeutics, Inc. (Columbia, MD, USA). The product lots of hMSC used in this study were derived from the BM of 4 different donors aged 18 to 30 years, who had been screened and tested according to FDA requirements for Blood and Tissue Based Products, and were manufactured under GMP guidelines by a scaled adaptation of the technique described by Pittenger et al. [6]. The cells were grown as symmetric fibroblastic colonies, resulting in a homogeneous cell population positive for surface antigens, CD105 (SH-2), CD 73 (SH-3, SH-4) CD29, CD44, CD71, CD90, CD106, CD120a, CD124, and CD166 and negative for markers of hematopoietic lineages CD14, CD34, and CD45. The cells were formulated in PlasmaLyte®A containing 5% human serum albumin (HSA) and 10% dimethyl sulfoxide (DMSO) in a final volume of 15 mL and cryopreserved. The in-process intermediate and final lots were tested for potential viral pathogens, mycoplasma, sterility, endotoxin, cell identity, purity, and viability before being released for clinical distribution. The cells were shipped frozen in a dry shipper to each site and stored in the vapor phase of liquid nitrogen at each investigating transplant center.

hMSC Infusion

On the day of administration, appropriate numbers of bags were thawed and 25 mL of PlasmaLyte®A added to each bag. The reconstituted cellular product was expected to have a viability of ≥70% and the DMSO concentration of the infused product was 3.75%. The hMSCs were administered intravenously (i.v.) within 5 hours of thawing at a rate of 4 to 6 mL/min by infusion pump for patients weighing ≥35 kg and over 60 minutes for those <35 kg. Patients were premedicated with hydrocortisone and diphenhydramine both at 0.5 to 1 mg/kg up to 50 mg i.v., given within 30 minutes prior to infusion. Vital signs and oxygen saturation were monitored for each infusion. Oxygen saturation (SaO_2/SAT) was monitored by pulse oximeter for at least 30 minutes prior to and until 2 hours after the start of hMSC infusion. The infusion could be halted if there was respiratory distress, a decrease in SaO2/SAT to <85% for over 3 minutes, or if there was another adverse event that the physician believed was related to hMSC infusion.

Therapy Schedule

Patients were scheduled to receive hMSC infusions twice a week (administered at least 3 days apart) for 4 consecutive weeks. The first 2 patients received 8×10^6 hMSCs/kg per infusion. The cell dose for the subsequent 10 patients was decreased to 2×10^6 hMSCs/kg per dose after protocol amendment. A lower dose was being used in a definitive adult study at that time [16]. At the conclusion of "induction therapy," aGVHD assessment was performed on day +32 (±2 days) to evaluate for response. No additional infusions were administered to patients showing a complete response (CR) or no response. However, patients continued to receive weekly hMSC infusions as "maintenance therapy" at the same cellular dose for an additional 4

weeks if day 32 assessment showed a partial or mixed response.

Assessment of aGVHD and Statistical Analyses

Baseline aGVHD prior to start of hMSC therapy was graded according to standard criteria [20]. Using the same criteria, aGVHD was graded on day +32 from the start of hMSC therapy and again after completion of therapy if the patient received maintenance dosing. Responses were defined as follows: CR, resolution of aGVHD in all evaluable involved organs; partial response (PR), a decrease of at least 1 GVHD stage in any 1 organ system without a worsening in any other organ system; mixed response (MR), a decrease of at least 1 GVHD stage in any 1 organ system with worsening in other organ system; no response (NR), no change in any organ system or worsening in 1 or more organ system without improvement in any other organ system. Details of steroid and other concurrent immunosuppressive therapy were recorded. hMSC therapy was administered in combination with preexisting GVHD therapies in accordance with the individual institutional guidelines. The primary response endpoint was assessed at day +32 from the start of hMSC therapy and the responses were scored as CR, PR, MR, and NR as defined earlier. Clinical status including any aGVHD or chronic GVHD (cGVHD) was assessed at the most recent follow-up in August to September 2008. OS in the whole cohort and various response groups were calculated using
Kaplan-Meier method. Safety and other efficacy measures are expressed using descriptive statistics.

RESULTS

Patient and aGVHD Characteristics

All enrolled patient (n = 12) received hMSC therapy. At the initiation of hMSC therapy, 7 patients had grade IV and 5 had grade III aGVHD. Severe gastrointestinal involvement was seen in all patients with 7 patients showing stage 4 and 5 patients showing stage 3 symptoms. In addition to the gut, 2 patients had liver and skin, 1 patient had liver, and 3 patients had skin involvement. All 12 patients were steroid refractory and in addition had failed 2 to 5 (median of 3) other immunosuppressive therapies (Table 2). The patients were a median of 98 days posttransplantation (range: 45-237) and the median duration between the diagnosis of aGVHD and initiation of hMSC therapy was 46 days (range: 18-157).

Prochymal Infusion

A total of 124 doses of hMSC were administered, with a median of 8 infusions (range: 2-21) per patient and detailed in Table 3. The median total cumulative

Table 2. Detailed Information about Acute GVHD and Prochymal Treatment Plan

Pt. No.	Day of Onset of aGVHD	Days of aGVHD prior to hMSC	aGVHD Grade at start of hMSC	GI/Skin/Liver Stages at Start of hMSC	Prior and Concurrent Therapies	No. of Infusion	Cells/kg per Infusion ($\times 10^6$)	Cumulative Dose cells/kg ($\times 10^6$)	Treatment Plan
1	70	20	IV	4/1/3	MMF, **MP, FK, IFL, DAC**	21*	8; 2	108	2/week for 4 weeks; 1/week for 13 weeks
2	81	45	III	3/3/0	MMF, **MP, FK, DAC**	2	8	16+16	Days 1 and 4
3	22	46	IV	4/2/0	IFL, **MP, CsA, EPT**	12	2	24+12	2/week for 4 weeks; 1/week for 4 weeks
4	98	119	III	3/0/0	BUD, **MP, FK, IFL, DAC, MMF**	12	2	24	2/week for 4 weeks; 1/week for 4 weeks
5	56	181	IV	4/0/2	**MP, DAC, MMF**	9	2	18	2/week for 4 weeks; 1/week for 4 weeks
6	72	30	IV	4/0/0	IFL, OKT3, **CsA, MP, MMF**	8	2	16	2/week for 4 weeks
7	27	18	IV	4/1/3	MMF, IFL, RIT, **MP, FK, DAC**	12	2	24	2/week for 4 weeks; 1/week for 4 weeks
8	22	76	IV	4/1/0	IFL, ECP, **MP, FK**	7	2	14	2/week for 4 weeks
9	84	19	III	3/0/0	**CsA, MP, BUD**	8	2	16	2/week for 4 weeks
10	33	38	III	3/0/0	**CsA, MP, MMF, IFL**	3†	2	6	2/week for 4 weeks
11	93	125	III	3/0/0	**MP, DAC, MMF**	8	2	16	2/week for 4 weeks
12	80	157	IV	4/0/1	MMF, **MP, DAC, FK**	12+8‡	2	40	2/week for 4 weeks; 1/week for 4 weeks; 2/week for 4 weeks

CsA, cyclosporine A; FK, tacrolimus; MTX, methotrexate; MMF, mycophenolate mofetil; MP, methylprednisone; IFL, infliximab; DAC, daclizumab; RIT, rituximab; EPT, etanercept; ECP, extra-corporeal photopheresis; BUD, budesonide.
Abbreviations in bold lettering indicate therapies that patients received concurrently with human mesenchymal stem cells (hMSC).
*Eleven infusions were administered at 8×10^6 hMSC/kg, and 10 at 2×10^6 hMSC/kg.
†Patient was discontinued at parents' request.
‡Patient was continued on therapy to allow for discontinuation of steroids.

hMSC cell dose per patient was 21 × 10^6 cells/kg (range: 6-108 × 10^6 cells/kg). Patient 2 received only 2 doses during the initial induction therapy. After initial response, 2 patients (#2 and #3; Table 2) had a flare-up of GVHD 2 and 4 weeks after the last infusion and were treated again with hMSC after an amendment to the original protocol was granted.

Safety

hMSC therapy was well tolerated, and no infusional or other treatment-related toxicities were observed. One patient (patient #4) with preexistent respiratory distress related to the underlying diagnosis and recent history of endotracheal intubation and surgery was transferred to the intensive care unit within 24 hours of the second hMSC infusion. The event was deemed not likely related to the hMSC infusion. He received subsequent infusion per induction protocol and tolerated them well. During this period his respiratory status improved while hMSC therapy was continued. He eventually made complete respiratory and aGVHD recovery and was discharged home. Almost 2 years later, he remains well, is off all immunosuppressive therapy, and has normal total and fractionated lymphocyte count and normal immunoglobulin levels. In patient #2 (malignant osteopetrosis) calcified ectopic lesions developed in the scalp and foot after hMSC therapy. However, excision biopsies of these lesions revealed no evidence of DNA from the infused hMSC, and thus the lesions were not considered to be a result of hMSC therapy. These DNA analyses were carried out at the Immunogenetics Testing Laboratories (IMGL) of the University of Maryland Medical Center (Baltimore, MD) and performed using a multiplex short tandem repeat (STR) method. In addition, these lesions did not pose a serious health issue and the patient remains alive to date. Three patients were on dialysis prior to hMSC therapy. No patient developed organ dysfunction related to MSCs while on the study.

Response

At day 32 from the initiation of therapy, 2 patient achieved CR, while 6 had PR and 3 Mr. By completion of therapy, 9 patients exhibited positive response to hMSC therapy (Table 3). Seven patients (58%) achieved CR, 2 (17%) PR, and 3 (25%) MR. Two patients (# 2 and #3) achieved CR after initial hMSC therapy but later had a flare-up of GVHD, 1 with grade IV skin and the other with grade IV gut. They were retreated with hMSC, and both achieved partial response and improved to grade I. All 12 patients had severe gut involvement and responded to hMSC to achieve a 75% (9/12) CR rate (Figure 1). The remaining 3 patients responded with a 1 to 2 stage improvement in their gut symptoms. Two of these

Table 3. Response to Therapy: Overall Grade, Organ System, and Survival

	Prior to Prochymal Therapy		Following 32 Days of Prochymal Therapy			At completion of Prochymal Therapy			Last Follow-up		
Patient No.	GI/Skin/Liver (stages)	GVHD Grade	GI/Skin/Liver (stages)	GVHD Grade	Response	GI/Skin/Liver (stages)	GVHD Grade	Response	Day from hMSC therapy initiation	Cause of Death
1	4/1/3	IV	1/0/4	IV	MR	0/0/4	IV	MR	Dead; day 101	Fungal sepsis
2	3/3/0	III	0/2/0	I	PR	0/0/0	0	CR	cGVHD skin/gut; on SL and PD; days 1, 111	
3	4/2/0	IV	1/1/0	II	PR	0/0/0	0	CR	Dead; day 185	Bacterial sepsis
4	3/0/0	III	2/0/0	III	PR	0/0/0	0	CR	No GVHD or therapy; day 707	
5	4/0/2	IV	3/1/2	III	MR	3/1/2	III	MR	Dead; day 36	Encephalitis
6	4/0/0	IV	0/0/0	0	CR	0/0/0	0	CR	Limited skin cGVHD; no systemic therapy; day 611	
7	4/1/3	IV	1/0/0	I	PR	0/0/0	0	CR	Dead; day 85	MSOF
8	4/1/0	IV	0/0/0	0	CR	0/0/0	0	CR	cGVHD skin and mouth; on FK, PS, PD; day 519	
9	3/0/0	III	1/0/0	I	PR	1/0/0	I	PR	Dead: day 54	MSOF
10	3/0/0	III	–			2/0/0	II	PR	Dead; day 58	EBV-LPD
11	3/0/0	III	0/1/1	II	MR	0/1/1	II	MR	Dead; day 49	MSOF; fungus
12	4/0/1	IV	1/0/0	I	PR	0/0/0	0	CR	No GVHD; on FK, SL, PD; day 427	

GI indicates gastrointestinal; CR, complete response; PR, partial response; MR, mixed response; cGVHD, chronic graft-versus-host disease; SL, sirolimus; PD, prednisone; PS, pentostatin; FK, tacrolimus; EBV-LPD, Epstein Barr virus lymphoid proliferative disorder; MSOF, multisystem organ failure.

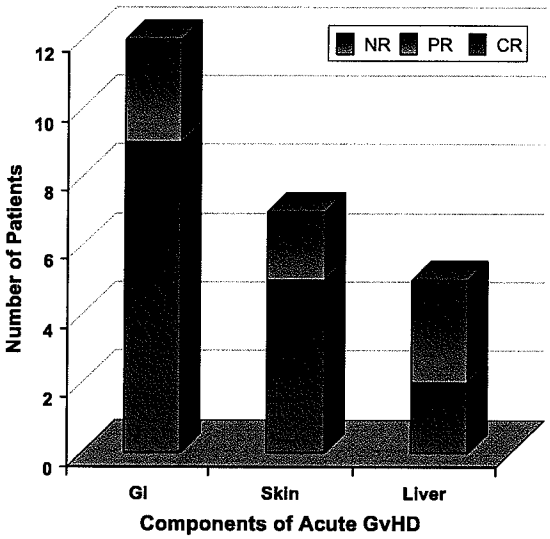

Figure 1. Responses in various organs at the end of Prochymal therapy.

patients showed a small increase in their skin disease, from stage 0 to stage 1. All patients with skin GVHD refractory to steroid therapy achieved resolution of their skin disease after hMSC therapy. However, 2 patients (#5 and #11) who had no skin involvement at the initiation of therapy subsequently developed stage 1 skin involvement. Of the 4 patients with liver involvement 1 achieved CR, 1 had PR,whereas 2 patients increased their stages (1 patient from stage 0 to I and 1 patient from stage III to IV).

Survival

The cumulative incidence of survival at day 32 and day 100 from the initiation of hMSC therapy was 100% and 58%, respectively. Five of 12 patients (42%) were still alive after a median follow-up for surviving patients of 611 days (range: 427-1111). All surviving patients had achieved CR following hMSC therapy. The KM estimates of OS for patients achieving CR was 68% (95% CI 40%-100%) at 2 years. For the whole group, the probability of OS at 2 years was 40% (95% confidence interval [CI] 20%-*0%) (Figure 2). For the deceased (n = 7), the median time to death was 58 days (range: 36-185) from the initial hMSC infusion. The causes of death are listed in Table 3. One patient who died 58 days after starting hMSC had been withdrawn from the protocol at the parents' request after only 3 infusions. She had developed posterior reversible encephalopathy syndrome, a complication that was most likely a result of her preexistent calcineurin-inhibitor therapy. Two of the CR patients died of infectious complications (*Xanthomonas* and *Pseudomonas aeruginosa*), which led to sepsis and multiorgan failure. The causes of death in 5 patients with PR or MR included respiratory failure from multimicrobial pneumonia, renal failure, lymphoproliferative disorder, and cytomegalovirus (CMV) encephalitis. One CR patient required a liver transplantation on day 19 of hMSC induction therapy for pretransplant hepatic insufficiency secondary to the patient's underlying disease (hemophagocytic

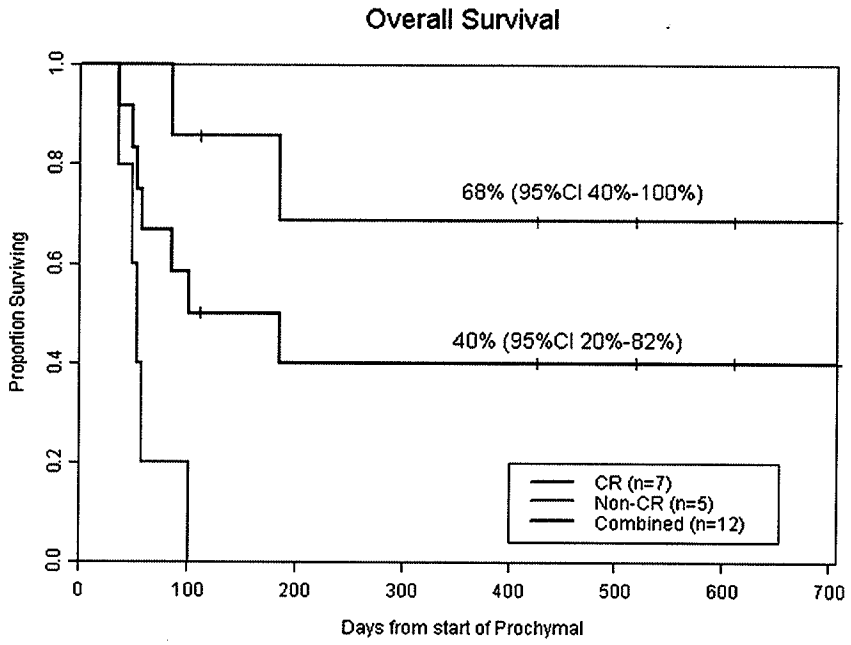

Figure 2. Kaplan-Meier estimates of the probability of 2-year OS according to GVHD response following Prochymal therapy. Seven patients had a complete response to Prochymal, and whereas the remaining 5 patients (Non-CR) had partial or mixed response. Surviving patients have been followed for a median of 611 days (range, 427-1111).

lymphohistiocytosis) that had progressed through the HSCT. This patient continued to receive hMSC infusions shortly after receiving his liver transplant. Unfortunately, he died 85 days after the first hMSC infusion because of respiratory failure resulting from a bacterial infection. One patient (patient #4) in the CR group and 1 (patient #11) in the MR group developed Fusarium infection. The first patient was successfully treated and is alive without evidence of GVHD for 25 months. The second patient died of multisystem organ failure while on therapy for the Fusarium infection. No hMSC therapy-related mortality was observed.

DISCUSSION

We report the first experience using Prochymal to treat refractory aGVHD in children undergoing allogeneic HSCT. Availability of hMSC as a premanufactured, quality-controlled product ready for use without either HLA matching or lengthy on-site manipulation makes it an attractive therapeutic option for patients with severe aGVHD. hMSC was administered at varying dosing schedules and for a length of 1 to 7 months. Responses were seen in all patients in 1 or more organ systems. All 3 patients requiring dialysis were able to receive hMSC therapy. Ectopic tissue was seen in 1 patient but molecular studies showed it to be composed of recipient and hematopoietic donor DNA and not of the infused hMSC cells. In general, Prochymal was well tolerated by the whole cohort and no infusional or acute toxicity was observed during 124 infusions.

Despite small numbers, it is encouraging to see CR in 58% of children with severe aGVHD that were resistant to steroid and other immunosuppressive agents in this compassionate study. In addition, CR increased the probability of OS at 2 years to 68%. Importantly, only 2 of 7 patients achieving CR died, whereas none of the non-CR patients survived. The deaths were either related to infection or multi organ system failure both likely to be consequences of GVHD, immunosuppression, and from cumulative toxicity of all therapies. It is noteworthy that serious infection was lower in CR patients, and only 1 CR patient died of overwhelming infection. It is difficult to assess causality in heavily treated immunosupressed patients, but it is likely that cumulative toxicity of preceding or on-going therapies or active GVHD contributed to the observed infections in nonresponders. Lower infection in responders may reflect improved gut integrity following therapeutic response. All 12 patients in this report were heavily pretreated. Significant responses in this cohort may point to a better response to hMSC therapy in pediatric patients, a finding supported by Le Blanc et al.'s report of a better response rate in children compared to adults (84% versus 60%) receiving directed donor hMSCs [18].

A high response rate (75%) in severe gut GVHD is particularly noteworthy because they have historically been more difficult to treat [2]. One may hypothesize that the high CR rate in the GI disease may reflect the combination of homing, broad immunomodulatory, and tissue repair properties of MSCs. Multiple animal models of injury including cerebral ischemia, radiation, and myocardial infarction have demonstrated the homing property of MSC [21-24]. Other studies have also demonstrated that MSCs can preferentially improve clinical symptoms of lower GI tract [18]. Tissue repair properties of hMSCs may provide additional help in healing the mucosal breakdown, which is responsible for the severity of gut symptoms. The patients achieving CR had a much better OS and longer term outcome than those who achieved a PR or MR. A long gap of 6 to 7 weeks between the diagnosis of aGVHD and therapy with hMSC and inclusion of only severe and resistant patients in this study may be responsible for some of the therapeutic failures and poor outcome in those not achieving CR. All patients have been on other immunosuppressive agents, and it is possible that some of the responses seen after hMSC therapy may reflect late responses to prior and concurrent therapies. Only large controlled studies could resolve this question. At present, many other questions about hMSC's remain unanswered. These include clinical questions like the appropriate dosing and schedule of administration and biological questions related to their homing, longevity, half-life, and differences related to the various donors.

Other studies have also demonstrated the potential usefulness of hMSCs as well as their tolerability and safety. A recent study utilizing hMSCs derived from family and other donors to treat severe therapy-resistant aGVHD was published [18]. In that study, of the 55 patients treated, 30 achieved CR (55%) and 9 (16%) achieved PR. Similar to our findings, CR patients had improved OS compared to patients with less than CR (2-year posttransplant survival, 53% versus 16%, $P = .018$). In an earlier study, GVHD symptoms improved in 6 of 8 patients, with complete resolution in the gut (n = 6), liver (n = 1), and skin (n = 1) [17]. In a clinical trial of 32 adults with grades II-IV aGVHD treated with a combination of corticosteroids (2 mg/kg/day for at least 1 week) and 2 doses of either 2×10^6 hMSCs/kg (n = 17) or 8×10^6 hMSCs/kg (n = 15), 94% patients achieved overall response and 77% achieved CR by 28 days [16]. There was no response difference between the 2 dosing arms in that study.

Current therapy for severe aGVHD refractory to steroids is limited and ineffective in a large proportion of patients. In addition, a proportion of steroid

responsive patients will develop steroid dependence and suffer from multiple toxicities associated with prolonged systemic steroid therapy. hMSC does not appear to have overlapping toxicities with the conventional immunosuppressive agents used to treat patients with refractory aGVHD. Importantly, histocompatibility matching between the patient and the MSCs is not required for either efficacy or safety because MSCs do not express either HLA class II or accessory molecules like CD40, CD80, and CD86 [9]. Given encouraging results from this limited study, hMSC should be further studied to assess its usefulness in the treatment of patients with GVHD.

In conclusion, hMSC therapy appears to be a safe and potentially effective treatment option for pediatric patients with aGVHD who have failed steroids and other immunosuppressive therapies. The lack of overlapping toxicities with conventional immunosuppressive medications allows it to be a considered as an additional agent for patients with steroid refractory aGvHD. A larger multicenter, prospective, randomized placebo-controlled Phase III trial evaluating hMSC infusion for treatment of steroid-refractory aGVHD in both pediatric and adult patients was recently completed.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

REFERENCES

1. Couriel D, Caldera H, Champlin R, Komanduri K. Acute graft-versus-host disease: pathophysiology, clinical manifestations, and management. *Cancer.* 2004;101:1936-1946.
2. Macmillan ML, Weisdorf DJ, Wagner JE, et al. Response of 443 patients to steroids as primary therapy for acute graft-versus-host disease: comparison of grading systems. *Biol Blood Marrow Transplant.* 2002;8:387-394.
3. Deeg HJ. How I treat refractory acute GVHD. *Blood.* 2007;109: 4119-4126.
4. Cahn JY, Klein JP, Lee SJ, et al. Prospective evaluation of 2 acute graft-versus-host (GVHD) grading systems: a joint Societe Francaise de Greffe de Moelle et Therapie Cellulaire (SFGM-TC), Dana Farber Cancer Institute (DFCI), and International Bone Marrow Transplant Registry (IBMTR) prospective study. *Blood.* 2005;106:1495-1500.
5. Pavletic S, Tarantolo S, Lynch J, et al. Acute graft-versus-host disease after allogeneic blood stem cell transplantation: factors determining treatment failure and survival. *Blood.* 1999;94:153a.
6. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999;284: 143-147.
7. Bartholomew A, Sturgeon C, Siatskas M, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol.* 2002;30:42-48.
8. Di NM, Carlo-Stella C, Magni M, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood.* 2002;99:3838-3843.
9. Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scand J Immunol.* 2003;57: 11-20.
10. Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation.* 2003;75:389-397.
11. Kinnaird T, Stabile E, Burnett MS, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circ Res.* 2004;94:678-685.
12. Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha. *J Cell Physiol.* 1996; 166:585-592.
13. Majumdar MK, Thiede MA, Haynesworth SE, Bruder SP, Gerson SL. Human marrow-derived mesenchymal stem cells (MSCs) express hematopoietic cytokines and support long-term hematopoiesis when differentiated toward stromal and osteogenic lineages. *J Hematother Stem Cell Res.* 2000;9:841-848.
14. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood.* 2005;105: 1815-1822.
15. Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet.* 2004;363:1439-1441.
16. Kebriaei P, Isola L; Bachechi E, et al. Phase II trial of Prochymal™ (ex-vivo cultured adult human mesenchymal stem cells) and corticosteroids as primary treatment for acute graft-vs-host disease (aGVHD). *Blood.* 2006;108:3231a.
17. Ringden O, Uzunel M, Rasmusson I, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation.* 2006;81:1390-1397.
18. Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet.* 2008;371:1579-1586.
19. Prasad VK, Lucas KG, Kleiner GI, et al. Use of mesenchymal stem cells to treat pediatric patients with severe (grade III IV) acute graft versus host disease refractory to steroid and other agents on a compassionate use basis. *Blood.* 2007;110:2971.
20. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant.* 1995; 15:825-828.
21. Pittenger MF, Martin BJ. Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res.* 2004;95:9-20.
22. Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood.* 2003; 101:2999-3001.
23. Chapel A, Bertho JM, Bensidhoum M, et al. Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J Genet Med.* 2003;5:1028-1038.
24. Price MJ, Chou CC, Frantzen M, et al. Intravenous mesenchymal stem cell therapy early after reperfused acute myocardial infarction improves left ventricular function and alters electrophysiologic properties. *Int J Cardiol.* 2006;111:231-239.