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Adult Human Mesenchymal Stem Cells Added to Corticosteroid Therapy for the Treatment of Acute Graft-versus-Host Disease

Partow Kebriaei,[1] Luis Isola,[2] Erkut Bahceci,[3] Kent Holland,[4] Scott Rowley,[5] Joseph McGuirk,[6] Marcel Devetten,[7] Jan Jansen,[8] Roger Herzig,[9] Michael Schuster,[10] Rod Monroy,[11] Joseph Uberti[12]

The unique immunomodulatory properties of mesenchymal stem cells (MSCs) make them a rationale agent to investigate for graft-versus-host disease (GVHD). Human MSCs were used to treat de novo acute GVHD (aGVHD). Patients with grades II-IV GVHD were randomized to receive 2 treatments of human MSCs (Prochymal®) at a dose of either 2 or 8 million MSCs/kg in combination with corticosteroids. Patients received GVHD prophylaxis with tacrolimus, cyclosporine, (CsA) or mycophenolate mofetil (MMF). Study endpoints included safety of Prochymal administration, induction of response to Prochymal, and overall response of aGVHD by day 28, and long-term safety. Thirty-two patients were enrolled, with 31 evaluable: 21 males, 10 females; median age 52 years (range: 34-67). Twenty-one patients had grade II, 8 had grade III, and 3 had grade IV aGVHD. Ninety-four percent of patients had an initial response to Prochymal (77% complete response [CR] and 16% partial response [PR]). No infusional toxicities or ectopic tissue formations were reported. There was no difference with respect to safety or efficacy between the low and high Prochymal dose. In conclusion, Prochymal can be infused safely into patients with aGVHD and induces response in a high proportion of GVHD patients.

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From the [1]Department of Stem Cell Transplantation and Cellular Therapy, M.D. Anderson Cancer Center, Houston, Texas; [2]Mount Sinai Hospital, New York, New York; [3]Bristol-Myers Squibb, New York, New York; [4]Blood and Marrow Transplant Group, Northside Hospital, Atlanta, Georgia; [5]Bone Marrow Transplant Program, Hackensack University, Hackensack, New Jersey; [6]Blood and Marrow Transplantation, Kansas City Cancer Center, Kansas City, Missouri; [7]Hematology/Oncology, Lied Transplantation Center, University of Nebraska Medical Center, Omaha, Nebraska; [8]Indiana Bone Marrow Transplantation, St. Francis Hospital and Health Center, Indianapolis, Indiana; [9]Blood and Marrow Transplantation Program, James Graham Brown Cancer Center, Louisville, Kentucky; [10]New York Hospital, Cornell Medical Center, New York, New York; [11]Osiris Therapeutics, Inc., Columbia, Maryland; and [12]Blood and Marrow Stem Cell Transplantation Program, Karmanos Cancer Institute, Detroit, Michian.

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Correspondence and reprint requests: Partow Kebriaei, MD, Department of Stem Cell Transplantation and Cellular Therapy, Univesity of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 423, Houston, Texas 77030 (email: pkebriae@mdanderson.org).

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INTRODUCTION

Acute graft-versus-host disease (aGVHD) is a significant cause of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (HSCT). Depending on the intensity of the conditioning regimen, the extent of human leukocyte antigen (HLA) match, age of the recipient, and stage of the primary disease, the incidence of aGVHD varies from 20% to 70% [1-5]. Initial treatment with corticosteroids remains the standard for aGVHD [6]. However, even with prompt initiation, steroid therapy is suboptimal. Two large retrospective studies on the use of steroids as the primary treatment of aGVHD reported sustained complete response (CR) rates of only 18% and 35% [7,8]. Furthermore, in a more recent comparative study, the CR rate for patients treated with steroids alone was 33% [9]. A recent report suggests that the addition of mycophenolate mofetil (MMF) to steroid therapy may improve outcomes in the initial treatment of GVHD, with a 60% CR rate noted at day 28 [10]. However, other attempts to intensify the immunosuppressive therapy as part of the initial treatment of GVHD, either with higher doses of steroids

[11] or by adding antithymocyte globulin (ATG) [12] or daclizumab [13], have not improved response rates, and raise additional safety concerns. Specifically, the addition of daclizumab to corticosteroid therapy yielded significantly worse survival because of increased disease relapse and GVHD-related mortality compared to corticosteroid use alone [13]. At present, GVHD response rates to steroids are inadequate, and patients who do not respond to steroid therapy have a poor prognosis, and 1-year survival rates range from 10% to 30% [6]. Therefore, it is clear that new therapeutic agents that are both safe and effective are needed for the management of aGVHD.

The in vivo and in vitro properties of mesenchymal stem cells (MSCs), or multipotent mesenchymal stromal cells as defined in the International Society for Cellular Therapy position statement [14,15] gives rise to their potential use in a broad range of inflammatory and immune-mediated conditions such as GVHD and Crohn's disease. Bone marrow-derived MSCs are a population of undifferentiated pluripotent stem cells that modulate immune and inflammatory response, and facilitate repair of connective tissues [16,17]. MSCs have been shown to inhibit the proliferation of T cells induced by a variety of stimuli [18-20] and downregulate levels of inflammatory cytokines such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ [21]. LeBlanc et al. [22] reported the first case of successful treatment of severe refractory aGVHD using ex vivo expanded haploidentical human MSCs. In a subsequent report, these investigators demonstrated a positive therapeutic effect with allogeneic MSCs in patients experiencing steroid refractory aGVHD with no significant adverse events attributed to the cells [23]. Thus, the clinical and experimental data support the concept of MSCs as therapeutically tolerated cells without the need for donor-recipient matching.

Based upon early results of MSCs for the treatment of steroid refractory GVHD and an encouraging safety profile, an open-labeled phase II study was conducted to determine whether the addition of a preparation of unrelated, culture-expanded human MSCs formulated for intravenous delivery (Prochymal®, Osiris Therapeutics, Inc., Columbia, MD), to initial corticosteroid therapy for aGVHD would improve patient outcomes.

PATIENTS AND METHODS

Study Design

The study was a randomized, multicenter, phase II trial evaluating 2 different dose levels of Prochymal used in combination with standard corticosteroid therapy for the treatment of aGVHD. The study was approved by the institutional review board (IRB) of each of the 16 participating institutions. All patients were required to give written informed consent before enrollment in accordance with the Declaration of Helsinki. Two infusions of Prochymal were administered with the first given 24 to 48 hours following diagnosis of grade II-IV aGVHD. The second infusion was given 3 days following the first treatment. Patients were randomized with equal probability of receiving either high-dose (8×10^6 MSCs/kg) or low dose (2×10^6 MSCs/kg) Prochymal. Patients were stratified for the 2 dose levels between grades II and grades III/IV aGVHD. Standard steroid therapy consisted of methylprednisolone 2 mg/kg i.v. or prednisone 2.5 mg/kg orally daily starting on study day 1 (or up to 72 hours prior to Prochymal dosing), and when indicated, tapered as per institutional standard. Prophylactic therapy with either cyclosporine (CsA) or tacrolimus, and/or MMF was continued at therapeutic dose levels. Supportive care, including use of prophylactic antibiotics, was as per institution standards.

The primary endpoint of the study was the proportion of patients who achieved a CR of aGVHD by study day 28. Secondary endpoints included a partial response (PR), time to best response, the addition of escalated immunosuppressive therapy, and survival at study day 90. Safety endpoints included infusional toxicity, adverse events, formation of ectopic tissue, and infection. This report presents safety and efficacy data through study day 90.

Patients between 18 to 70 years of age inclusive with newly diagnosed grade II-IV aGVHD requiring systemic steroid therapy were eligible for the study. Biopsy for confirmation of GVHD was recommended, but not required. Patients developing aGVHD after receiving a HSCT from bone marrow, peripheral blood (PBSC), or cord blood stem cells, regardless of conditioning regimen, were eligible, as were patients developing GVHD following donor lymphocyte infusion (DLI). Patients were required to have adequate renal function.

Exclusion criteria included treatment for aGVHD with $\geq$2 mg/kg of methylprednisolone for $>$72 hours, and the use of any investigational agent (not approved by the FDA for marketed use in any indication) within 30 days of randomization. A central randomization was utilized for randomization between the high- or low-dose infusions according to the randomization schedule generated by the study statistician using SAS® PROC PLAN. Subjects were randomized with equal probability to the treatment arms (2 million cells/kg of Prochymal™ or 8 million cells/kg of Prochymal™) using a stratified block design. The stratification factor was aGVHD grade. For the purpose of stratification, the aGVHD grades were II and III-IV.

Study Assessments

The severity of aGVHD was assessed using the consensus grading scale [24]. Each study site had an

investigator who determined the presence or absence of aGVHD grade II-IV of the skin, liver, and gastrointestinal (GI) tract documenting both the stage of each organ and overall grade. aGVHD assessments through day 28 of the study were performed on study days 1, 4, 7, 14, 21, and 28. Assessment of treatment response was made based on established clinical criteria. A CR was defined as the absence of symptoms referable to aGVHD in all organs. A PR was defined as a decrease by at least 1 GVHD stage in any 1 organ system without deterioration in others. The best response during the 28-day period of assessments was recorded for final response to treatment.

Therapy

Human MSCs are isolated from an unrelated, unmatched donor-derived bone marrow aspirate after donor screening and testing according to FDA requirements for Blood and Tissue-Based Products. The product lots of Prochymal used in this study were derived from 6 different donors within 18-30 years of age.

The ex vivo cultured MSC manufacturing process is a scaled adaptation of the technique described by Pittenger et al. [16]. The process consists of 2 steps: step 1, the production of an in-process intermediate and step 2, production of a unique Prochymal lot. The complete process consists of a total of 5 cell passages. All reagents, equipment, and procedures utilized in the ex vivo cultured MSC manufacturing process are according to FDA GMP.

For processing, the bone marrow aspirate undergoes isolation steps to remove hematopoietic elements, and then from the nucleated bone marrow cells, human MSC expansion occurs in culture medium supplemented with 10% fetal bovine serum (FBS) that has undergone extensive screening for safety and processing effectiveness. The cells grow as symmetric fibroblastic colonies, resulting in a cell population positive for surface antigens, CD105 (SH-2), CD 73 (SH-3, SH-4) CD29, CD44, CD71, CD90, CD106, CD120a, CD124, CD166, and negative for markers of hematopoietic lineages, CD14, CD34, and CD45. Ex vivo cultured MSCs have the ability to proliferate and retain the ability to undergo in vitro differentiation to osteogenic, adipogenic, and chondrogenic lineages when clonally expanded.

The cells are formulated in Plasma-Lyte®A containing 5% human serum albumin (HSA) and 10% dimethyl sulfoxide (DMSO) and cryopreserved at a concentration of 6.6×10^6 MSC/mL. The in-process intermediate and final lots are tested for potential viral pathogens, mycoplasma, sterility, endotoxin, cell identity, purity, potency, and viability before lots are released for clinical distribution. Specifically, 2 assays to assess the functional properties of MSCs are used to establish potency for each product lot; a quantitative assay measures the expression level of TNFR1 on MSCs and a qualitative assay measures the inhibition of IL2Rα expression on activated T cells by MSCs. Purity assays were used to detect the residual level of hematopoietic cells, bovine protein, and porcine trypsin. Prochymal lot release testing and strict acceptance criteria were incorporated to ensure lot-to-lot consistency of the manufactured product.

For administration, the cells were thawed and diluted with Plasma-Lyte®A to achieve the cell concentration needed for infusion. The final infusion product had a final viable cell concentration of 2.5×10^6 MSCs/mL. The final viability was at least 70% viable MSCs, as determined by Trypan blue testing. After dilution, the DMSO concentration of the infused product was 3.75%. The total volume administered for each patient was dependent upon dose cohort and body weight. Each patient received cells from only 1 donor. Before infusion, patients were premedicated with hydrocortisone and diphenhydramine. The infusion was administered i.v. at a rate of 4 to 6 mL/min. Vital signs and oxygen saturation were measured within 15 minutes prior to the infusion (time 0) and then at 15 minutes, 30 minutes, and then hourly until 6 hours after the infusion.

All patients received standard-of-care treatment with corticosteroids, which was initiated at the time of GVHD diagnosis. When indicated, steroids were tapered in accordance with institutional standards. However, it was recommended that a steroid taper consisting of a minimal taper rate of at least 10% of the dose per week be used. If at study evaluation day 7 the patient's aGVHD worsened, the patient was to be treated with secondary GVHD therapy determined by the investigator. Patients on secondary GVHD therapy were monitored for the 28-day period and evaluated for GVHD response.

Statistical Methods

To detect statistical differences between treatment groups, the categoric variables of patient demographics were compared with Fisher's exact test; age was compared with a 2-sided *t*-test, and time of onset of GVHD since bone marrow transplant was compared with the Kruskal-Wallis test. CR and PR rates between treatment groups were compared by Fisher's exact test. Time to response after MSC treatment was analyzed by exact logistic regression. CR was examined for contributing effects of certain baseline characteristics (donor relation, organ involvement at screening, and initial GVHD grade) and *P*-values were calculated from the Breslow-Day test. Survival between complete responders and noncomplete responders was analyzed using log rank test. All statistical testing was performed at a significance level of $P < .05$.

RESULTS

Patient Characteristics

Thirty-two patients were enrolled between April 2005 and June 2006. One patient with a history of GI bleeding and increased diarrhea at randomization withdrew informed consent on study day 10. Therefore, data for 31 patients are reported. The patient characteristics are listed in Table 1. The median age of the patients was 52 years (range: 34-67 years) with 21 males and 10 females. Fifteen patients were randomized to receive high-dose Prochymal at 8×10^6 MSC/kg, and 16 received low dose at 2×10^6 MSCs/kg. The groups were balanced with respect to age, sex, donor type, preparative regimen, GVHD prophylaxis, and GVHD severity. Eighteen patients received HSCT from matched related donors (MRD), and 13 patients received HSCT from matched unrelated donors (MUD). Stem cell source for HSCT was peripheral blood for all patients except for 1 patient who received bone marrow. Fifteen patients received myeloablative preparative regimens and 12 received a reduced or nonmyeloablative regimen. Four patients developed GVHD following DLI.

GVHD events are described in Table 1. The median day to the development of GVHD was 37 days (range: 14-121). All patients had biopsy proven GHVD: 21 had grade II (12 MRD, 9 MUD) 7 grade III (3 MRD, 4 MUD), and 3 had grade IV aGVHD (3 MRD). Of the 21 patients with grade II aGVHD, 12 had skin involvement, 6 had GI involvement, and 3 had GI and skin. Of the 10 patients with grade III-IV aGVHD, 5 had GI, 2 had liver and GI, 2 had skin and GI, and 1 had grade IV of the skin.

Table 1. Patient Characteristics

	High Dose (8×10^6 MSCs/kg) No. Patients	Low Dose (2×10^6 MSCs/kg) No. Patients
Sex		
Male	10	11
Female	5	5
Median Age, years (range)	49 (34-67)	53 (42-67)
Disease		
AML/MDS	8	6
NHL	2	3
CLL	1	3
ALL	3	1
MMF	0	2
MM	1	0
Hodgkin	0	1
Donor		
Related	9	10
Unrelated	6	6
GVHD Grade		
II	10	11
III	4	3
IV	1	2
Stem cell source		
Bone marrow	0	1
PBSC	15	15
Conditioning		
Myeloablative	8	7
Reduced intensity	3	5
Nonmyeloablative	2	2
DLI	2	2
Onset of GVHD		
Median days (range)	37 (14-121)	31 (18-115)
GVHD prophylaxis		
Cyclosporine	3	3
Tacrolimus	4	6
Tacrolimus + MMF or MTX	6	5

MSC indicates mesenchymal stem cells; GVHD, graft-versus-host disease; MMF, mycophenolate mofetil; MTX, methotrexate; DLI, donor leukocyte infusions; NHL, non-Hodgkin lymphoma; PBSC, peripheral blood stem cells; AML/MDS, acute myelogenous leukemia/myelodysplastic syndromes; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; MF, myelofibrosis.

Treatment Response

GVHD response according to MSCs dose is shown in Table 2. Ninety-four percent of patients had an initial response to Prochymal (29/31), where 24 patients (77%) had a CR and 5 (16%) had a PR. Of the 24 patients who achieved a CR, 10 received high-dose MSCs, and 14 received low-dose MSCs infusions. Five patients in the high-dose group achieved a PR and 2 patients in the low-dose group did not respond. The CR by time showed that 42% of the patients had a CR at day 7; 52% at day 14 and 77% at day 28. CR was further examined for contributing effects of donor relation, GVHD organ involvement and GVHD grade, and no statistically significant effect was noted (Table 3). Of the 24 patients who achieved a CR, 19 maintained their response for at least 90 days without requiring second-line GVHD therapy. Five of the 24 patients who achieved an initial CR required additional therapy during the first 28 days. Two patients received second-line therapy with infliximab, 1 on day 6 and 1 on day 10, and both achieved a CR at day 28. Three patients, who initially achieved a CR, had flares of their GVHD between days 10 and 12 and required second-line therapy on days 12, 13, and 15. One of these 3 patients showed an improvement in their GVHD symptoms after initiating second-line agents.

Five patients experienced a PR. Three of these patients received second-line therapy for their GVHD. None responded, and all 3 died of GVHD-related complications within 90 days of their first treatment. The remaining 2 patients achieved significant

Table 2. Induction of GVHD Response to Treatment

Response Type	High-Dose (8×10^6 MSCs/kg) No. Patients (%)	Low-Dose (2×10^6 MSCs/kg) No. Patients (%)	Total (N = 31) No. Patients (%)
Complete Response	10 (66.7)	14 (87.5)	24 (77.4)
Partial Response	5 (33.3)	0	5 (16.1)
No Response	0	2 (12.5)	2 (6.5)

GVHD indicates graft-versus-host disease; MSCs, mesenchymal stem cells.

Table 3. Summary of Complete Response by Patient Characteristics

	High-Dose 8×10^6 MSCs/kg No. Pts (%)	90% CI	Low-Dose 2×10^6 MSCs/kg No. Pts (%)	90% CI	*P* value (BreslowDay test)
Donor matching					
Matched unrelated Donor					
Complete response	3 (20)	7.8%-41.4%	6 (35)	19.4%-55.2%	
No complete response	2 (13)	3.6%-34.3%	2 (12)	3.1%-30.9%	
Matched related donor					
Complete response	7 (47)	27.7%-66.7%	8 (47)	29.0%-66.0%	
No complete response	3 (20)	7.8%-41.4%	1 (6)	0.0%-24.0%	.76
Organ involvement					
Skin Only					
Complete response	3 (20)	7.8%-41.4%	8 (47)	29.0%-66.0%	
No complete response	2 (13)	3.6%-34.3%	0 (0)	0.0%-10.5%	
Other					
Complete response	7 (47)	27.7%-66.7%	6 (35)	19.4%-55.2%	
No complete response	3 (20)	7.8%-41.4%	3 (18)	6.7%-37.5%	.09
GVHD grade					
Grade II					
Complete response	9 (60)	39.2%-77.7%	10 (59)	39.3%-75.9%	
No complete response	2 (13)	3.6%-34.3%	1 (6)	0.0%-24.0%	
Grade III-IV					
Complete response	1 (7)	0.0%-26.6%	4 (24)	10.7%-43.6%	
No complete response	3 (20	7.8%-41.4%	2 (12)	3.1%-30.9%	.61

GVHD indicates graft-versus-host disease; MSCs, mesenchymal stem cells; CI, confidence interval; Pts., patients.

improvement in their GVHD without second-line therapy: 1 patient had stage iii skin aGVHD that improved to stage i by day 7, but had a traumatic fall and died of intracranial hemorrhage on day 13. The second patient had stage iv skin aGVHD that improved to stage i, but the patient's underlying leukemia relapsed and the patient died on day 24.

Only 2 patients did not show response to therapy by study day 28. One patient's GVHD therapy was not escalated because of concurrent CMV infection. The second patient was placed on infliximab but continued to have diarrhea associated with GI GVHD.

Four patients developed GVHD following DLI; 2 patients received chemotherapy preceding DLI. Three of the 4 patients achieved a CR with treatment; 1 of these patients had grade II skin, 1 had grade III GI, and 1 had grade IV GI and liver involvement. One patient who developed grade III skin GVHD achieved a PR with treatment.

Response by organ system is shown in Table 4. A total of 13 patients had only skin involvement and all 13 responded, with 11 achieving a CR (85%). Of the 11 patients with GVHD only in the GI tract, 9 of 11 (82%) responded, and 8 (73%) had a CR. Seven patients had multiorgan involvement, and all 7 responded, with 5 patients having CR (71%). Response by initial GVHD grade is also provided in Table 4. Additionally, the source of MSCs was evaluated with regards to impact on response. Twenty-three of the 32 patients treated received MSCs from 2 different donors, and the remaining patients received MSCs from 4 different MSC donors. A comparison of response between the 2 donors who supplied the majority of patients showed no difference ($P = .224$, Fisher's exact test). The remaining 4 MSC

Table 4. Summary of Response by Initial Organ System Involved

Organ System No. Patients	Grade	Response	High-Dose 8×10^6 MSCs/kg No. Patients	Low-Dose 2×10^6 MSCs/kg No. Patients	Total No. Patients (%)
Skin only	II	CR	3	8	11 (85)
N = 13		PR	1	0	1 (8)
	III/IV	PR	1	0	1 (8)
GI only	II	CR	3	2	5 (45)
N = 11					
	III/IV	CR	1	2	3 (27)
		PR	1	0	1 (9)
		NR	0	2	2 (18)
Multiorgan	II	CR	2	1	3 (43)
N = 7					
	III/IV	CR	1	1	2 (29)
		PR	2	0	2 (29)

MSCs, mesenchymal stem cells; CR, complete response; PR, partial response; GI, gastrointestinal.

donors supplied too few patients to make a statistical comparison.

Survival

Twenty-two patients on the study survived to day 90. Patients who achieved a CR to GVHD therapy had significantly higher survival rate ($P = .00008$) at day 90. Out of 24 patients that achieved a CR, 21 (88%) were alive at day 90. Of the 7 patients that did not achieve a CR, only 1 (14%) was alive at day 90.

Nine patients on the study died a median of 44 days (range: 13-63) from the first Prochymal infusion. Three patients who achieved a CR died; the causes of death were pneumonia, meningitis, and aspergillus enteritis. All 5 patients with a PR died. Three died of progressive GVHD, 1 of underlying disease relapse, and 1 of a central nervous system (CNS) bleed. Of the 2 patients who had no response, 1 died of progressive GVHD and 1 survived to day 90.

The effect on survival of adding second-line therapy to treat progressive GVHD was examined. Twenty of 22 patients (91%) did not need second-line therapy and were alive at day 90. In contrast, 9 patients required second-line therapy within the first 28 days, and only 3 (33%) survived to day 90 ($P = .0011$).

Safety

There were a total of 62 infusions of Prochymal administered to 31 patients. All infusions were well tolerated with no acute infusional toxicity and no adverse events associated with Prochymal infusions. Ectopic tissue was not detected by computed tomography (CT) imaging in any patients at study day 28. Furthermore, continued follow-up with CT scans at 1 and 2 years following MSC infusion on the long-term study has not shown any evidence for ectopic tissue formation. Twelve grade 3 infections, defined as per CTC versus 3, were reported: adenovirus (n = 1), bacteremia (n = 4), cytomegalovirus (CMV) viremia (n = 5), and BK virus-associated cystitis (n = 2). Three grade 4 infections were reported: pseudomonal pneumonia (n = 1), eneteroccocal meningitis (n = 1) and aspergillus enteritis (n = 1). There was no correlation between Prochymal dose and toxicity grade. During the 90-day period, 1 patient with acute lymphoblastic leukemia (ALL) who underwent a second HSCT had disease relapse. Two additional patients relapsed during the long-term follow-up study: 1 patient had refractory relapsed ALL and 1 had relapsed Hodgkin disease (HD), and had received a prior autologous SCT.

DISCUSSION

The use of MSCs is a promising new strategy in the treatment of aGVHD. MSCs give rise to mesodermal tissue types including bone, cartilage, tendon, muscle, and fat [25,26], and are capable of modulating immune response and inflammation [18-21]. MSCs also secrete factors that stimulate angiogenesis, tissue repair, and hematopoietic stem cell engraftment [25,27]. One of the key advantages of MSCs is that histocompatibility matching has not been required for therapeutic effect [19]. The MSCs do not express HLA class II histocompatibility antigens, and furthermore, do not express accessory molecules, CD40, CD80, and CD86, required for immune cell activation. An important biologic property of MSCs is their chemotactic response to inflammatory factors generally reserved for the migration of neutrophils and other immune responsive cells. This homing property has been demonstrated in a number of animal models of injury including cerebral ischemia [28], total-body irradiation (TBI) [29,30], and myocardial infarction [31,32]. Once at the site of injury or inflammation, it has been proposed that MSCs modulate immune and inflammatory reactions at the microenvironment level and stimulate tissue repair of the affected organs.

This study represents the first prospective trial of third-party, unmatched MSCs for the treatment of de novo aGVHD. These results corroborate the reports of MSCs used in the treatment of steroid refractory GVHD [22,33]. The results of this study provide evidence that MSCs can effectively induce a response in a high percentage of GVHD cases, and when used in combination with existing therapy, may improve overall outcome. Seventy-seven percent of patients had an intial CR following the initiation of steroids and Prochymal therapy. In the majority of these patients (19 of 24), the response was maintained for 90 days.

Of particular interest is the low number of patients that do not respond to treatment. It is hypothesized that the broad immunomodulatory properties of MSCs, responsible for the secretion of multiple anti-inflammatory cytokines, are more effective than targeted small molecules or biologics for the treatment of GVHD. In line with previous observations from individual case studies using Prochymal to treat GI GVHD [22], a high response rate was observed in patients with GI GVHD. In this study, 18 patients had GI GVHD (GI alone or GI with skin or liver), 16 of 18 (89%) responded and 13 (72%) achieved a CR of their GI component. Other studies have also demonstrated that administering third-party MSCs to patients with steroid refractory aGVHD improved the clinical symptoms of lower GI tract [22,33]. In a recent report, data from a multicenter study were presented summarizing treatment with MSCs for 55 children and adults with steroid-refractory aGVHD. Overall response was 71%, with 55% CR. In corroboration with our findings, CR patients had improved overall survival (OS) at 2 years after HSCT compared to patients with less than CR, 53% versus 16%, $P = .018$ [33]. Furthermore, there was suggestion of a better response rate in

children compared to adults, (84% versus 60%) [33]. Similarly, positive clinical responses and improved survival were recently reported for pediatric patients treated with MSCs for steroid refractory aGVHD. Seven of 12 (58%) treated patients achieved CR with the best responses in patients with GI involvement (75%). Five (42%) were alive at a median follow-up time of 229 days [35].

Our study was not designed to assess the optimal dose and schedule of Prochymal administration. Five patients who achieved a CR had flares of their GVHD that required second-line therapy, suggesting that 2 doses of Prochymal may be insufficient to maintain a CR. Le Blanc et al. [33] reported that multiple infusions were needed for sustained response in more that half of the patients treated with MSCs for steroid refractory GVHD. Furthermore, the low dose of Prochymal at 2×10^6 cells/kg appeared as effective as the higher dose in inducing a response, consistent with other MSC GVHD study reports [22,33,35].

Although many studies have shown that methods to prevent GVHD often result in increased relapse rates, it is unclear if more effective treatment for established aGVHD will increase relapse. Three patients in our study relapsed during a follow-up period of 2 years. All 3 had advanced disease, including 1 with a prior transplant, and had a high likelihood of relapse. Ning et al. [28] published a small randomized trial in which human MSCs were cotransplanted with hematopoietic stem cells in efforts to improve engraftment time; there was no impact on engraftment, but they noted a higher relapse rate in the group treated with MSCs compared to the non-MSC group, (6 of 10 versus 3 of 15, respectively). Of interest, the MSC group had a lower incidence of aGVHD and chronic GVHD (cGVHD). This increase in relapse rate was not noted in an earlier study by Lazarus et al. [36], in which MSCs were also cotransplanted with HSC to improve engraftment.

No infusional toxicity was attributable to Prochymal infusions. Furthermore, adverse events and rates of infection were similar across both Prochymal dose groups. There were 3 deaths resulting from infection in the absence of ongoing GVHD. This rate (~10%) is within expectations for this heavily immunosuppressed population.

In conclusion, MSCs represent a new potential therapeutic option in the treatment of GVHD. Third-party human MSCs can be infused safely into patients with aGVHD and may improve clinical symptoms. A larger, blinded, placebo-controlled trial with Prochymal is warranted to fully investigate these encouraging results.

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