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# Allogeneic Human Mesenchymal Stem Cell Therapy (Remestemcel-L, Prochymal) as a Rescue Agent for Severe Refractory Acute Graft-versus-Host Disease in Pediatric Patients

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A B S T R A C T

Severe steroid-refractory acute graft-versus-host disease (aGVHD) is related to significant mortality and morbidity after allogeneic stem cell transplantation. Early clinical trials of therapy with human mesenchymal stem cells (hMSCs) in pediatric patients with severe aGVHD resistant to multiple immunosuppressive agents showed promising results. In this study, we evaluated the risk/benefit profile of remestemcel-L (Prochymal), a third-party, off-the-shelf source of hMSCs, as a rescue agent for treatment-resistant aGVHD in pediatric patients. Children with grade B-D aGVHD failing steroids and, in most cases, other immunosuppressive agents were eligible for enrollment. Patients received 8 biweekly i.v. infusions of $2 \times 10^6$ hMSCs/kg for 4 weeks, with an additional 4 weekly infusions after day +28 for patients who achieved either a partial or mixed response. The enrolled patients compose a very challenging population with severe disease that was nonresponsive to the standard of care, with 88% of the patients experiencing severe aGVHD (grade C or D). Seventy-five patients (median age, 8 yr; 58.7% male; and 61.3% Caucasian) were treated in this study. Sixty-four patients (85.3%) had received an unrelated hematopoietic stem cell graft, and 28 patients (37.3%) had received a cord blood graft. At baseline, the distribution of aGVHD grades B, C, and D was 12.0%, 28.0%, and 60.0%, respectively. The median duration of aGVHD before enrollment was 30 d (range, 2 to 1639 d), and patients failed a median of 3 immunosuppressive agents. Organ involvement at baseline was 86.7% gastrointestinal, 54.7% skin, and 36.0% liver. Thirty-six patients (48.0%) had 2 organs involved, and 11 patients (14.7%) had all 3 organs involved. When stratified by aGVHD grade at baseline, the rate of overall response (complete and partial response) at day +28 was 66.7% for aGVHD grade B, 76.2% for grade C, and 53.3% for grade D. Overall response for individual organs at day +28 was 58.5% for the gastrointestinal system, 75.6% for skin, and 44.4% for liver. Collectively, overall response at day +28 for patients treated for severe refractory aGVHD was 61.3%, and this response was correlated with statistically significant improved survival at day +100 after hMSC infusion. Patients who responded to therapy by day +28 had a higher Kaplan-Meier estimated probability of 100-d survival compared with patients who did not respond (78.1% versus 31.0%; $P < .001$). Prochymal infusions were generally well tolerated, with no evidence of ectopic tissue formation.



## INTRODUCTION

The success of allogeneic hematopoietic stem cell transplantation (HSCT) and its ultimate therapeutic effect depends on the control of acute graft-versus-host disease (aGVHD). Depending on various risk factors and the administration of prophylactic agents, 30% to 80% of recipients will develop aGVHD [1,2]. Corticosteroids are the initial intervention for controlling aGVHD; however, in 30% to 50% of patients, aGVHD is not controlled with first-line therapy and requires additional therapeutic intervention [3]. In a recent retrospective analysis of 864 patients with aGVHD [4], patients who failed to respond to therapy at day +28 after initiation were 2.78 times more likely to experience treatment-related mortality (TRM) compared with those who demonstrated response. Thus, the outcomes for nonresponders are poor. Various agents have been added to

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steroid therapy in an attempt to treat steroid-resistant aGVHD, including polyclonal and monoclonal antibodies, immunotoxins, immunosuppressive agents, chemotherapeutic agents, and phototherapy. Overall, responses to these agents and outcomes in salvage therapy for steroid-refractory aGVHD have been disappointing [5-7].

Clinically, patients who fail to respond to steroids and additional immunosuppressive agents are at increased risk for morbidity associated with infections and uncontrolled aGVHD, as well as an increased risk of mortality. The poor prognosis of severe aGVHD is well documented, with long-term survival probabilities of 20% for grade III and <5% for grade IV [8]. Thus, steroid-refractory aGVHD represents a significant clinical challenge.

Previous studies have demonstrated the potential of human mesenchymal stem cells (hMSCs) as an effective treatment for aGVHD. Recent reviews indicate that hMSCs down-regulate immune and inflammatory responses, providing therapeutic potential for treating diseases characterized by the presence of an inflammatory component [9,10]. The production of anti-inflammatory cytokines and growth factors by hMSCs can promote a favorable environment and facilitate tissue repair. Clinical improvement in aGVHD after i.v. infusion of hMSCs has been reported in single case reports [11], pilot studies [12-15], and phase II studies [16,17]. In these studies, the vast majority of patients received 1 or 2 infusions of hMSCs. Clinical experience and pilot investigations have indicated that for the most severe cases of refractory aGVHD, a greater number of treatments may be required to reverse the course of one of the most severe complications of HSCT [14]. Here we present the findings of a study of severe, multiline refractory aGVHD in pediatric patients treated with multiple infusions of allogeneic culture-expanded adult hMSCs (remestemcel-L [Prochymal]; Osiris Therapeutics, Columbia, MD).

## METHODS

### *Study Design*

This was an open-label, single-arm, prospective multicenter study of male and female pediatric patients between age 2 mo and 17 yr (inclusive) with grade B-D aGVHD [18] who were nonresponsive to steroids and, in most cases, other immunosuppressive therapies. The objectives were to evaluate whether the treatment plan (8 infusions of $2 \times 10^6$ hMSCs/kg) could induce an objective response in patients with severe refractory aGVHD, and also to assess the safety and tolerability of remestemcel-L infusion for the given dosing scheme.

As part of this trial, aGVHD prophylactic agents, concomitant therapies, and other supportive therapies were administered at the investigator's discretion in accordance with site-specific institutional practices and policies. Safety assessments included 12-lead electrocardiography, and monitoring for infusional toxicity, ectopic tissue formation, relapse of underlying malignancy, and survival. Serious adverse events (SAEs) were recorded throughout the study. Patients were evaluated for the efficacy and safety of remestemcel-L until death, withdrawal, or 100 d after the first infusion (day 0), whichever occurred first.

### *Study Population*

Pediatric patients (age 2 mo to 17 yr; median age, 7.8 yr) with aGVHD secondary to allogeneic HSCT or donor lymphocyte infusion who had failed to respond to systemic steroid therapy for grade B-D aGVHD (using the Center for International Blood and Marrow Transplant Registry grading scheme [18]) were eligible. Failure to respond to steroid treatment for aGVHD was defined as any grade II-IV aGVHD that did not improve after at least 3 d of treatment with methylprednisolone (≥1 mg/kg/d) or equivalent. Exclusion criteria included known allergy to bovine or porcine products and most recent HSCT performed to treat a solid tumor. In addition, patients must not have had evidence of a pulmonary infiltrate or diffuse alveolar hemorrhage, and must have been deemed unlikely to require more than 2 L of oxygen via face mask or an estimated fraction of inspired oxygen of 28% via other delivery methods to maintain oxygen saturation of 92% for the 3 d after screening.

The protocol was submitted for ethics review, and approval or acknowledgment of treatment was obtained in writing from the Institutional Review Board or Ethics Committee of each institution. Parental signed informed consent and patient assent, when applicable, were required before any study-specific procedures were undertaken. The study was registered with www.ClinicalTrials.gov (NCT00759018).

### *Failed aGVHD Therapy*

The number of therapies beyond systemic steroid therapy that each patient received before the start of remestemcel-L was recorded. Previous aGVHD therapies included systemic steroids (methylprednisolone or equivalent), infliximab, etanercept, pentostatin, daclizumab, rituximab, denileukin diftitox, alemtuzumab, mycophenolate mofetil, tacrolimus, and antithymocyte globulin. Nonsystemic steroids, such as budesonide and beclamethasone, were not counted as second-line therapy for aGVHD treatment, nor were prophylactic treatments, such as cyclosporine, sirolimus, and methotrexate. If an agent was used for aGVHD prophylaxis, discontinued, and then restarted for treatment, therapy must have been initiated after the onset of aGVHD for the agent to be counted as a second-line agent for aGVHD.

The effect of aGVHD therapies before the introduction of remestemcel-L was characterized as improving, unchanged, or worsening. Improving aGVHD was defined as at least a 1-grade reduction in aGVHD between disease onset and study baseline, worsening GVHD was defined as an increase in aGVHD grade, and maximal aGVHD was defined as grade D at both onset and study baseline.

### *Treatment Regimen*

Remestemcel-L was given i.v. at a dose of $2 \times 10^6$ hMSCs/kg of body weight twice weekly for 4 consecutive wk. Patients received all 8 infusions in the initial treatment plan by day +28. Infusions were administered at least 3 d apart. During the course of remestemcel-L treatment, all other aGVHD therapies were administered at the discretion of the investigator according to institutional practice.

Patients who demonstrated a partial response (PR) or mixed response (MR) to remestemcel-L at study day +28 and had no safety issues related to therapy after the first 8 doses were eligible for continued therapy with an additional 4 infusions of $2 \times 10^6$ hMSCs/kg administered once weekly for 4 wk.

Within 30 min before remestemcel-L infusion, patients were premedicated with hydrocortisone (0.5-1.0 mg/kg, up to 50 mg/dose) and diphenhydramine (0.5-1 mg/kg, up to 50 mg/dose). The product was thawed and reconstituted with PlasmaLyteA (Baxter, Deerfield, Illinois) to a final cell concentration of $2.5 \times 10^6$ hMSCs/mL. The dimethyl sulfoxide (DMSO) concentration of the final infused product was 3.75%. The infusion was given i.v. at a controlled rate of 4-6 mL/min in patients weighing ≥35 kg and over 60 min in those weighing <35 kg. The total volume administered to each patient was dependent on body weight. Vital signs and oxygen saturation were monitored during each infusion. Oxygen saturation was monitored by pulse oximetry for at least 30 min before and up to 2 hr after the start of infusion.

All patients received standard of care treatment with corticosteroids, as well as other second-line agents at the discretion of the investigator.

### *Source of hMSCs, Remestemcel-L*

The product lots of remestemcel-L used in this study were derived from the bone marrow of 7 different donors, age 18-30 yr, who had been screened and tested in accordance with Food and Drug Administration requirements for blood and tissue-based products. The product lots were manufactured using a scaled adaptation of the technique described by Pittenger et al. [19] in accordance with good manufacturing practices, as described previously [17]. All lots met established quality release criteria for viral pathogens, mycoplasma, sterility, endotoxin, cell identity, purity, viability, and potency before use.

### *GVHD Assessment*

The severity of aGVHD was assessed using the Center for International Blood and Marrow Transplant Research grading system [18]. Patients were evaluated by the treating physician for the presence or absence of aGVHD of the skin, liver, and gastrointestinal (GI) system. Organ stage and overall grade were recorded. aGVHD assessments were performed at baseline before initiation of remestemcel-L, at day +28 after initiation, and at day +100/end of treatment.

Response to treatment was evaluated based on established clinical criteria [4]. Overall response (OR) was either a complete response (CR) or a PR. No response (NR) was defined as a MR, stable disease, or worsening disease. Definitions of responses are summarized in Table 1.

**Table 1**
Response Definitions

| Term | Definition |
|---|---|
| Complete response (CR) | Resolution of aGVHD in all involved organs |
| Partial response (PR) | Organ improvement of at least 1 stage without worsening in any other organ system |
| Overall response (OR) | CR or PR |
| Mixed response (MR) | Improvement by at least 1 organ stage in at least 1 evaluable organ with worsening by at least 1 organ stage in at least 1 other organ |
| Stable disease | The absence of any clinically significant differences (improvement or worsening) sufficient to meet minimal criteria for improvement or deterioration in any evaluable organ |
| Worsening disease | Deterioration in at least 1 evaluable organ by 1 stage or more |
| No response | MR or stable disease or worsening disease |

Responders were defined as achieving at least an OR at day +28. Patients experiencing an MR or NR or who died on or before day +28 were counted as nonresponders at day +28.

***Statistical Analysis***

Objective assessment of the response of aGVHD to treatment with remestemcel-L was determined as the OR rate at day +28. To present changes in aGVHD organ stage, response data from baseline to day +28 was classified for each organ as improving, stable, progressing, or death.

To assess the effect of continuing therapy (>8 infusions), response from day +28 to day +100 was summarized, stratified by aGVHD grade at baseline and overall. To evaluate the effect of response on overall survival, 2 Kaplan-Meier survival analyses were conducted through day +100. A Kaplan-Meier curve was generated for patients who had achieved OR at day +28, and another Kaplan-Meier curve was generated for nonresponders at day +28. The null hypothesis of no difference in overall survival between the 2 groups was tested with the log-rank test using PROC LIFETEST in SAS (SAS Institute, Cary, NC). The testing was performed at a significance level of $P < .05$.

Categorical variables were summarized as frequencies and percentages. Continuous variables were summarized using descriptive statistics (number, mean, standard deviation [SD], median, and range). All confidence intervals had a 95% confidence level.

## RESULTS

### *Patient Characteristics*

Seventy-five pediatric patients were enrolled in 7 countries (the United States, Canada, the United Kingdom, Italy, Finland, New Zealand, and Australia). A median of 10.0 doses (range, 1 to 20) was administered, with all patients receiving at least 1 infusion. Patient characteristics are summarized in Table 2. The study cohort comprised 44 males (58.7%) and 31 females (41.3%), with a median age of 7.8 yr (range, 0.2 to 17.5 yr). Forty-five patients (60.0%) underwent HSCT for a hematologic malignancy, and the remaining patients underwent HSCT for nonmalignant disease, primarily of genetic origin. The most common underlying malignancies or leukemic diseases at the time of transplantation were acute lymphoblastic leukemia (ALL; n = 18, 24.0%) and acute myelogenous leukemia (AML; n = 16, 21.3%). The majority of the HSCT graft donors were unrelated (85.3%), nearly evenly divided between HLA-matched (52%) and HLA-mismatched (48%).

Baseline aGVHD disease characteristics are detailed in Table 2. The median time from HSCT to aGVHD onset was 28.0 d (range, 7 to 270 d). At the time of onset, 33.3% of patients had grade C aGVHD and 32.0% had grade D aGVHD. At the start of remestemcel-L treatment, the vast majority of patients (88%) had grade C (28.0%) or grade D (60.0%) aGVHD, indicating the aggressive nature of their disease. Sixty-five patients (86.7%) experienced GI aGVHD, 39 (52.0%) with maximal GI involvement (stage 4). Forty-one patients (54.7%) had skin involvement, and 27 (36.0%) had liver involvement. Approximately one-half of the patients had 2 organs involved, and 14.7% of had all 3 organs (skin, liver, and GI system) involved.

**Table 2**
Patient Characteristics

| Characteristic | Value |
|---|---|
| No. of patients | 75 |
| Age, yr | |
| Mean (SD) | 8.6 (5.78) |
| Median (range) | 7.8 (0.2-17.5) |
| Sex, n (%) | |
| Male | 44 (58.7) |
| Female | 31 (41.3) |
| Race, n (%) | |
| American Indian or Alaska Native | 1 (1.3) |
| Asian | 5 (6.7) |
| Black or African American | 15 (20.0) |
| Hawaiian Native or Pacific Islander | 0 (0.0) |
| White | 46 (61.3) |
| Other | 8 (10.7) |
| Weight, kg | |
| Mean (SD) | 32.1 (20.54) |
| Median (range) | 26.9 (5.4-103.7) |
| Underlying disease, n (%) | |
| Malignant | 45 (60.0) |
| Nonmalignant | 30 (40.0) |
| Underlying disease, n (%) | |
| ALL | 18 (24.0) |
| AML | 16 (21.3) |
| CML | 1 (1.3) |
| MDS | 7 (9.3) |
| NHL | 1 (1.3) |
| Genetic disease | 16 (21.3) |
| Other | 16 (21.3) |
| Donor type, n (%) | |
| Unrelated | 64 (85.3) |
| Related | 11 (14.7) |
| Donor compatibility, n (%) | |
| HLA-matched | 39 (52.0) |
| HLA-mismatched | 36 (48.0) |
| HSCT graft source, n (%)* | |
| Bone marrow | 25 (33.3) |
| PBSCs | 16 (21.3) |
| Cord blood | 28 (37.3) |
| Donor lymphocyte infusion | 5 (6.7) |

* Source was not available for 1 patient.

### *Previous Failed aGVHD Therapy*

The median time from aGVHD onset to the start of remestemcel-L treatment was 30 d. In the interval between aGVHD onset and initiation of remestemcel-L, patients were maintained on aGVHD prophylaxis, systemic steroids, and, in many cases, 1 or more second-line agents for the treatment of aGVHD (Table 3). All patients were refractory to steroid therapy. The majority (60.0%) received 2 or more additional aGVHD agents after failing steroids. The most common agents were infliximab (54.7%), tacrolimus (42.7%), daclizumab (25.3%), and mycophenolate mofetil (24.0%). Virtually all of the patients (96.0%) did not improve despite treatment with steroids and other aGVHD immunosuppressive therapies before study entry.

### *GVHD Treatment Response*

Patients' responses to study treatment at day +28 are summarized in Tables 4 and 5. At day +28, 46 patients (61.3%) were responders, and 63 (63%) of the responding

**Table 3**
Baseline GVHD Characteristics

| Characteristic | Value | | | | |
|---|---|---|---|---|---|
| Number of patients | 75 | | | | |
| Interval from HSCT to GVHD onset, d | | | | | |
| Mean (SD) | 49.6 (54.02) | | | | |
| Median (range) | 28.0 (7-270) | | | | |
| GVHD grade at baseline, n (%) | | | | | |
| Grade B | 9 (12.0) | | | | |
| Grade C | 21 (28.0) | | | | |
| Grade D | 45 (60.0) | | | | |
| Organ staging at baseline, n (%) | Stage 0 | Stage 1 | Stage 2 | Stage 3 | Stage 4 |
| Skin | 34 (45.3) | 6 (8.0) | 15 (20.0) | 14 (18.7) | 6 (8.0) |
| GI | 10 (13.3) | 3 (4.0) | 9 (12.0) | 14 (18.7) | 39 (52.0) |
| Liver | 48 (64.0) | 4 (5.3) | 8 (10.7) | 9 (12.0) | 6 (8.0) |
| One organ involvement, n (%) | 28 (37.3) | | | | |
| Skin | 7 (9.3) | | | | |
| GI | 18 (24.0) | | | | |
| Liver | 3 (4.0) | | | | |
| Two organ involvement, n (%) | 36 (48.0) | | | | |
| GI, skin | 23 (30.7) | | | | |
| GI, liver | 13 (17.3) | | | | |
| Three organ involvement, n (%) | 11 (14.7) | | | | |
| Interval from GVHD onset to first infusion, d* | | | | | |
| Mean (SD) | 70.3 (190.51) | | | | |
| Median (range) | 30.0 (2-1639) | | | | |
| Number of failed GVHD agents, n (%) | | | | | |
| Systemic steroids only | 12 (16.0) | | | | |
| One agent | 18 (24.0) | | | | |
| Two agents | 25 (33.3) | | | | |
| Three agents | 12 (16.0) | | | | |
| Four or more agents | 8 (10.7) | | | | |
| Previous GVHD agents, n (%) | | | | | |
| Etanercept | 11 (14.7) | | | | |
| Pentostatin | 4 (5.3) | | | | |
| Infliximab | 41 (54.7) | | | | |
| Daclizumab | 19 (25.3) | | | | |
| Denileukin difitox | 1 (1.3) | | | | |
| Alemtuzumab | 2 (2.7) | | | | |
| Antithymocyte globulin | 4 (5.3) | | | | |
| Mycophenolate mofetil | 18 (24.0) | | | | |
| Tacrolimus | 32 (42.7) | | | | |
| Rituximab | 7 (9.3) | | | | |
| GVHD status before first infusion, n (%) | | | | | |
| Improving | 2 (2.7) | | | | |
| Unchanged | 22 (29.3) | | | | |
| Worsening or maximal GVHD | 50 (66.7) | | | | |

The date of GVHD onset was not available for 1 patient.
* The high SD and range are attributed largely to 1 patient who started treatment 1639 d after being diagnosed with GVHD. Omitting this patient, the time was mean 48.8 ± 46.39 d.

grade D patients had improved, with at least a 2-grade reduction. Overall, in 87% of the evaluable patients, no new aGVHD medications were introduced after initiation of remestemcel-L, and the response rate in these patients was 65.5%.

Individual organ response to remestemcel-L was assessed from organ staging (Table 5). Among the 65 patients with aGVHD of the lower GI tract at baseline, 70.7% were experiencing clinical symptoms consistent with severe (stage 3 to 4) GI aGVHD. At day +28, 58.5% of these patients showed improvement in their clinical symptoms, and 56.2% of the patients experiencing severe GI aGVHD had a ≥2-grade improvement in GI aGVHD. Seventeen patients (26.2%) experienced complete resolution of GI aGVHD. Three patients (4.6%) with GI aGVHD at baseline experienced GI aGVHD progression.

Of the 27 patients (36.0%) with liver aGVHD at baseline, 12 (44.4%) demonstrated improvement in liver disease at day +28, with 9 cases (33.3%) resolving completely. Two patients (7.4%) with liver aGVHD at baseline experienced progression of liver disease.

Forty-one patients (54.7%) had skin aGVHD at baseline. Fourteen of these patients had skin rash covering 50%-100% of the body, and 6 patients had severe rash with bullae. At day +28, 31 patients (75.6%) showed improvement in skin disease, with 43.9% of cases resolving completely. No patients with skin aGVHD at baseline experienced progression of skin disease.

### *Effects of Continuing Therapy*

Patients were eligible for continued therapy if they had either a PR or MR at day +28. The benefit of continuing

**Table 4**
Summary of Overall Response to Remestemcel-L at Day +28

| | Baseline GVHD Grade | | | Overall (n = 75) |
|---|---|---|---|---|
| | Grade B (n = 9) | Grade C (n = 21) | Grade D (n = 45) | |
| Responders, n (%) | 6 (66.7) | 16 (76.2) | 24 (53.3) | 46 (61.3) |
| Nonresponders, n (%) | 3 (33.3) | 5 (23.8) | 21 (46.7) | 29 (38.7) |

**Table 5**
GVHD Organ Stage Response by Baseline Organ Involvement at Day +28

| | GI (n = 65) | Liver (n = 27) | Skin (n = 41) |
|---|---|---|---|
| Complete resolution, n (%) | 17 (26.2) | 9 (33.3) | 18 (43.9) |
| Improving, n (%) | 21 (32.3) | 3 (11.1) | 13 (36.4) |
| Stable, n (%) | 15 (23.1) | 7 (25.9) | 5 (12.2) |
| Progressing, n (%) | 3 (4.6) | 2 (7.4) | 0 (0) |
| Death, n (%) | 9 (13.8) | 6 (22.2) | 5 (12.2) |

Only subjects with organ involvement at baseline (stage >0) are included in the percentages.
Definitions: complete resolution, reduction to organ stage 0; improving, reduction by at least 1 stage; stable, no change in the stage; progressing, an increase by at least 1 stage.

remestemcel-L treatment beyond the initial 4 wk (8 infusions) was assessed by aGVHD grade (Table 6). Only patients receiving continuing therapy were included in the analysis. For a patient to be considered a responder to continuing therapy, he or she must have experienced additional improvement in at least 1 organ of at least 1 stage without worsening in any other organ between day +28 and day +100. Patients who maintained a CR after day +28 were considered responders as well. Patients who had a PR at day +28 but experienced no change in organ staging between day +28 and day +100 were considered nonresponders.

Overall, 40 of 75 patients (53.3%) received more than 8 remestemcel-L infusions and were included in the continuing therapy analysis. More than one-half of these patients (57.5%) demonstrated additional improvement in aGVHD, with 16 patients achieving complete resolution of aGVHD.

### *Survival*

The probability of survival from study entry based on whether a patient was a responder or not at day +28 was estimated by Kaplan-Meier analysis (Figure 1). Of those patients with an OR at day +28, 76.1% survived at least 100 d past the first infusion compared with 27,6% of patients without an OR at day +28. The log-rank test for the comparison of survival probability in responders versus nonresponders revealed a significant 100-d survival advantage for those patients with an OR at day +28 ($P < .001$).

Overall survival at day +100 was 57.3%. By aGVHD grade at baseline, 66.7% of patients with grade B, 66.7% of those with grade C, and 51.1% of those with grade D survived to day +100.

### *Safety*

The remestemcel-L infusion was well tolerated by all patients. The mean total number of infusions received was 9.7 ± 3.97, with a median of 10.0 (range, 1 to 20). Thirty-five patients (46.7%) received ≤8 infusions, and 40 patients (53.3%) received >8 infusions. Duration of exposure ranged from 0 d (1 patient received only 1 infusion) to 116 d, with a mean of 40.5 ± 24.59 d. Infusional toxicity was evaluated by monitoring vital signs (heart rate, respiration rate, temperature, and blood pressure) and oxygen saturation from 30 min before infusion to 2 hr after infusion. Only 1 patient (1.3%) experienced an infusion-related reaction (ie, rise in body temperature, increased breathing, and decreased oxygen saturation) after the third and fourth infusions, which resolved without sequelae.

Forty-six of the 75 patients (61.3%) reported at least 1 SAE, and a total of 105 SAEs were reported (Table 7). The most frequently reported SAEs were respiratory failure (in 7 patients; 9.3%), multiorgan failure (6 patients; 8.0%), and hypertension and GVHD (3 patients each; 4.0%). One patient experienced acute respiratory distress that led to study withdrawal. No patients experienced an SAE deemed likely or definitely related to remestemcel-L by the investigator. Seven SAEs in 6 patients were considered possibly related: neutropenia in 1 patient, tachycardia in 1, infusion-related reaction (2 events) in 1, respiratory distress in 1, pulmonary hemorrhage in 1, and hypertension in 1 (all 1.3%).

By system organ class, the most common SAEs leading to death were respiratory, thoracic, and mediastinal disorders, of which the most frequent was respiratory failure (5 patients; 6.7%). Other frequent SAEs leading to death were multiorgan failure (4 patients; 5.3%) and aGVHD, mucormycosis, and aspergillosis (2 patients each; 2.7%). Two deaths were associated with relapse of the underlying malignancy (ALL and AML).

Patients were monitored for ectopic tissue formation with computed tomography scans before the first infusion and at the day +100 visit. No findings indicating ectopic tissue formation were detected, and no SAEs possibly representing ectopic tissue foci were reported. In addition, there were no remarkable post-treatment electrocardiography findings.

**Table 6**
Effect of Continuing Therapy with Remestemcel-L, Stratified by Baseline Grade

| | Baseline GVHD Grade | | | Overall (n = 75) |
|---|---|---|---|---|
| | Grade B (n = 9) | Grade C (n = 21) | Grade D (n = 45) | |
| Subjects with >8 infusions, n | 5 | 13 | 22 | 40 |
| Responders, n (%) | 3 (60.0) | 10 (76.9) | 10 (45.5) | 23 (57.5) |
| Nonresponders, n (%) | 2 (40.0) | 3 (23.1) | 12 (54.5) | 17 (42.5) |

## DISCUSSION

We report the results of a single-arm, multi-institutional study of remestemcel-L in pediatric patients with severe end-stage aGVHD who had exhausted conventional treatment options. This is the largest prospective study of its kind reported to date in pediatric patients with severe, multiline refractory aGVHD. The enrolled patients composed a very challenging population suffering from severe disease that was nonresponsive to steroids and, in most cases, other immunosuppressive agents. Their aGVHD was aggressive in nature, with 65% of the patients experiencing severe (grade C/D) aGVHD at disease onset. At study baseline, 88% of the patients had severe aGVHD, 91% with visceral organ involvement and 63% with multiorgan involvement. Despite aggressive treatment for a median of 30 d before initiation of remestemcel-L treatment, 96% of the patients were worsening or not improving at study entry.

Owing to the aggressive refractory nature of aGVHD in these patients, the achievement of a substantial level of response is a meaningful and positive observation. In this study, 61% of the patients responded to remestemcel-L treatment at day +28. The vast majority (87%) of the patients did not receive any new aGVHD medication during the remestemcel-L treatment window. Clinical response was observed across all grades of aGVHD, with 67% of patients with grade B, 76% of those with grade C, and 53% of those with grade D demonstrating response at day +28. Objective improvement was also observed in all organ systems, with 76% of skin cases, 58% of GI cases, and 44% of liver cases improving at day +28.

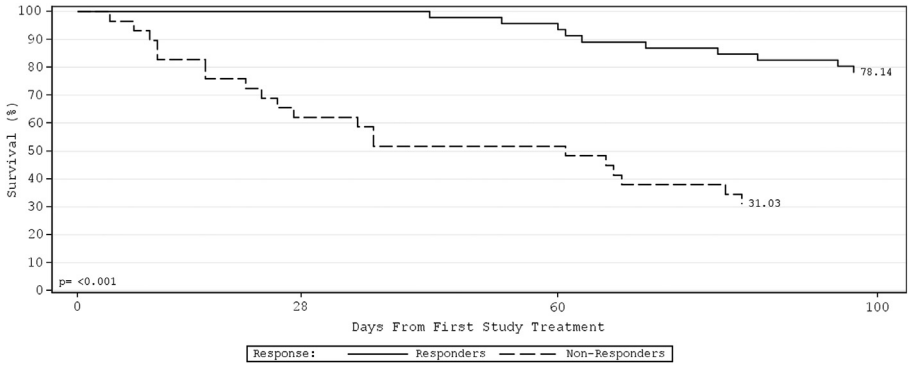


**Figure 1.** Kaplan-Meier plot of survival from study entry based on whether the patient was a responder or a nonresponder at day +28. The log-rank test for the comparison of survival probability for responders versus nonresponders demonstrated a significant 100-d survival advantage for responders ($P < .001$). Two patients (1 responder and 1 nonresponder) who completed the study before day +100 (at day +92 and day +99) were censored.

Given that incomplete responses at day +28 could be associated with either continued improvement or progression at subsequent time points, response by day +100 was also assessed, and was found to be 77%.

Continued therapy beyond the initial regimen of 8 biweekly infusions proved beneficial. The treatment of 40 patients beyond the initial 8 infusions produced 23 additional ORs (57.5%), including 16 patients with complete resolution of aGVHD.

Available data for pediatric patients with refractory aGVHD are limited, consisting primarily of retrospective studies generally of fewer than 20 patients treated with daclizumab [20-23] or infliximab [24,25]. In a daclizumab study of steroid-refractory aGVHD reporting data at day +28, response was observed in 6 of 17 patients (35%) overall, and a relationship between response and survival was seen, with 5 of 6 responding patients surviving [22]. Overall median survival was 60 d after initiation of daclizumab, demonstrating the life-threatening nature of aGVHD. In a study of infliximab in 18 pediatric patients with less severe aGVHD compared with the present study (39% with grade I/II aGVHD and 61% with grade III/IV aGVHD), survival ranged from 40% to 62% at day +100 after initiation of infliximab [25].

In the largest double-blind, placebo-controlled, randomized study of hMSCs for treating steroid-refractory aGVHD reported to date [26,27], a subset of 28 pediatric patients had a day +28 OR rate of 64% for the Prochymal group versus 36% for the standard of care treatment group, very similar to our present results. The patient populations in the 2 studies had comparably severe disease, with 79% of the 28-patient pediatric subset entering the study with grade III or IV aGVHD.

**Table 7**
Summary of SAEs

| | Subjects (n = 75), n (%) | Events, n |
|---|---|---|
| Subjects with at least 1 SAE | 46 (61.3) | 105 |
| Relationship to study drug | | |
| Possibly related | 6 (8.0) | 7 |
| Not related | 40 (53.3) | 98 |
| Severity grade | | |
| Mild (grade 1) | 0 (0.0) | 2 |
| Moderate (grade 2) | 4 (5.3) | 12 |
| Severe (grade 3) | 6 (8.0) | 39 |
| Life-threatening (grade 4) | 6 (8.0) | 20 |
| Death (grade 5) | 30 (40.0) | 32 |
| Subjects with an SAE leading to withdrawal | 1 (1.3) | 1 |

The best-documented 28-d response rate for the treatment of aGVHD is for the use of high-dose systemic corticosteroids as first-line therapy [4]. The response rate in that study was 65%, essentially identical to the response rate obtained in the present study, even though the steroid data were collected from a patient population (n = 864) with significantly milder disease (85% with grade I or II aGVHD, 14% with grade III, and only 1% with grade IV). In contrast, in the present study, 60% of the patients had grade D aGVHD at the time of enrollment.

In the present study, the treatment regimen led to responses and an objective clinical benefit in severe refractory aGVHD. Multiple infusions were administered to treat severe refractory aGVHD for several reasons. The extensive inflammation occurring in severe aGVHD may limit the persistence of hMSCs [28], thus requiring additional infusions to quell the active ongoing inflammatory response. In addition, although not assessed in these patients, multiple dosing may promote tolerance of the stem cell graft through such mechanisms as increased numbers of regulatory T cells [29,30]. Other investigations have implemented multiple infusions over the course of several weeks to prolong the therapeutic effect of hMSCs [31].

Response to treatment at day +28 is an important endpoint with a proven link to the probability of survival. According to a recent consensus of experts, response to treatment at day +28 is the most relevant endpoint for evaluation of aGVHD therapy, because it is an important predictor of later survival [32]. Recently reported data have confirmed that overall response is correlated with improved survival in patients with aGVHD [4]. Our present data support that relationship. Survival to day +100 after the first infusion of remestemcel-L was improved in the patients who responded at day +28. Of the 46 patients who experienced an OR at day +28, 35 (76%) survived to day +100, compared with only 8 of the 29 nonresponders (28%). The effect on survival of achieving a response at day +28 was highly significant ($P < .001$).

Remestemcel-L appears to have a benign safety profile. Infusions were well tolerated, with only 2 reported reactions occurring in the same patient out of more than 500 infusions administered during the course of the study. There were no cases of ectopic tissue formation. The number and type of events reported are consistent with a severely immunocompromised aGVHD patient population. At study entry, these patients have a complicated history and suffer from a variety of severe medical conditions. Treatment with

remestemcel-L did not lead to apparent additional toxicities and was well tolerated in this population. Furthermore, this therapy was not associated with hematologic or renal toxicity, which is commonly seen with other approaches to aGVHD prophylaxis or treatment.

The prognosis of severe refractory aGVHD remains poor, and better therapies for these patients are urgently needed. Remestemcel-L is a promising alternative to second-line immunosuppressive agents. The risk/benefit profile for remestemcel-L for the treatment of this life-threatening disease is in favor of treatment, based on the high observed response rates and positive safety profile.

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