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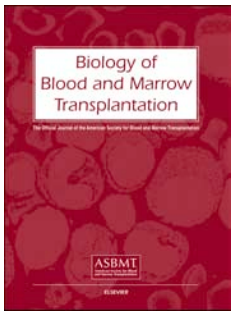

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Allogeneic Human Mesenchymal Stem Cell Therapy (remestemcel-L, Prochymal®) as a Rescue Agent for Severe Refractory Acute GvHD in Pediatric Patients

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ABSTRACT

Severe steroid-refractory acute graft versus host disease (aGvHD) is related to significant mortality and morbidity following allogeneic stem cell transplantation. Early clinical trials testing 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 human MSCs, as a rescue agent for treatment-resistant aGvHD in pediatric patients. Children with grades B-D aGvHD failing steroids and in most cases, other immunosuppressive agents were eligible for enrollment. Patients were given 8 biweekly intravenous infusions of 2×10^6 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 patients enrolled comprised a very challenging population suffering from severe disease that was non-responsive to standard of care, with 88% of the patients experiencing severe aGvHD (grade C or D).

Seventy-five patients (median age of 8 years, 58.7% male, and 61.3% Caucasian) were treated in this study. Sixty-four patients (85.3%) had received an unrelated HSCT graft and 37.3% (28 patients) of grafts were from cord blood transplants. At baseline, distribution of aGvHD grades B, C, and D was 12.0%, 28.0%, and 60.0%, respectively. Median duration of aGvHD prior to enrollment was 30 days (range 2, 1639), and patients failed a median of 3 immunosuppressive agents. Organ involvement at baseline was GI=86.7%, skin=54.7%, and liver=36.0%. Thirty-six (48.0%) patients had 2 organs involved and 11 patients (14.7%) had all 3 organs involved.

When stratified by aGvHD grade at baseline, overall response (complete and partial responders) at day 28 was 66.7%, 76.2%, and 53.3% for aGvHD grades B, C, and D, respectively. Overall

response (OR) for individual organs at day 28 was 58.5% for gastrointestinal (GI) system,75.6% for skin, and 44.4% for liver. Collectively, OR at day 28 for patients treated for severe refractory aGvHD was 61.3% and this response correlated with statistically significant improved survival 100 days post infusion of hMSCs. Patients who responded to therapy by day 28 had a higher Kaplan-Meier estimated probability of 100-day survival than patients who did not respond (78.1% vs. 31.0%, $p<0.001$). Prochymal® infusions were generally well tolerated without any evidence of ectopic tissue formation.

INTRODUCTION

The success of allogeneic hematopoietic stem cell transplantation (HSCT) and its ultimate therapeutic effect is dependent upon the control of acute graft versus host disease (aGvHD). Depending on various risk factors and the administration of prophylactic agents, 30-80% of recipients will develop acute GvHD (1, 2). Corticosteroids (steroids) are the initial intervention to control acute GvHD, however, in 30-50% of the patients, aGvHD is not controlled with first line therapy and requires additional therapeutic intervention (3). In a recent retrospective analysis of 864 patients with acute GvHD (4), patients who failed to respond to therapy at day 28 were 2.78 times more likely to experience treatment related, non-relapse, mortality (TRM) than those who were responders. Thus, the outcome for non-responders is poor. A variety of agents have been added to steroid therapy in an attempt to treat steroid-resistant aGvHD. These agents include polyclonal or monoclonal antibodies, immunotoxins, immunosuppressive agents, chemotherapeutic agents, and phototherapy. Overall, responses and outcomes of these agents in salvage therapy for steroid refractory acute GvHD have been disappointing (5-7).

Clinically, patients who fail to respond to steroids and additional immunosuppressive agents have an increased risk of morbidities associated with infections and uncontrolled aGvHD as well as an increased risk of death. The poor prognosis of severe acute GvHD is well-documented with probabilities of long-term survival for grades III and IV of 30% and less than 5%, respectively (8). These poor outcomes demonstrate that steroid refractory aGvHD represents a significant clinical challenge.

Studies have demonstrated the potential of human mesenchymal stem cells (MSCs) as an effective treatment for acute GvHD. Recent reviews (9, 10) indicate that human MSCs down-regulate immune and inflammatory responses, providing therapeutic potential for treating

diseases characterized by the presence of an inflammatory component. The production of anti-inflammatory cytokines and growth factors by MSCs can promote a favorable environment and facilitate tissue repair. Clinical improvement in aGvHD following intravenous treatment with human MSCs 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 one to two infusions of human MSCs. Clinical experience and pilot investigations (14) indicated that for the most severe cases of refractory aGvHD, a greater number of treatments may be required in order to reverse the course of one of the most severe complications of HSCT. Herein, we present the findings of a study of severe, multi-line refractory acute GvHD in pediatric patients treated with multiple infusions of remestemcel-L, allogeneic culture-expanded adult human MSCs (Prochymal®, Osiris Therapeutics Inc., Columbia, MD).

METHODS

Study Design

This was an open label, single-arm, prospective multi-center study of male and female pediatric patients between the ages of 2 months and 17 years (inclusive) with grades B-D acute GvHD (18), non-responsive to steroids and, in most cases, other immunosuppressive therapies. The objective was to evaluate whether the treatment plan (eight infusions of 2×10^6 hMSCs/kg, remestemcel-L, Prochymal, Osiris Therapeutics, Inc.) could induce an objective response in patients with severely 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 therapy were administered at the Investigator's discretion in accordance with site-specific institutional practices and policies. Safety assessments included 12-lead electrocardiogram, and monitoring for infusional toxicity, ectopic tissue formation, relapse of underlying malignancy, and survival. Serious adverse events (SAEs) were collected throughout the study. Patients were evaluated for efficacy and safety of remestemcel-L until death, withdrawal, or 100 days post first infusion (day 0), whichever occurred first.

Study Population

Pediatric patients (2 months to 17 years, median 7.8 years) with acute GvHD secondary to allogeneic HSCT or donor lymphocyte infusion (DLI) who had failed to respond to systemic steroid therapy for grades B-D acute GvHD (Center for International Blood and Marrow Transplant Registry grading scheme (18), were eligible. Failure to respond to steroid treatment

for acute GvHD was defined as any grades B-D acute GvHD that was not improving after at least 3 days of methylprednisolone (≥1 mg/kg/day) or equivalent. Exclusion criteria included known allergy to bovine or porcine products and if the patients' most recent HSCT was for treatment of a solid tumor. Additionally, patients must not have had evidence of a pulmonary infiltrate or diffuse alveolar hemorrhage and must have been unlikely to require more than 2 L of oxygen via face mask or an estimated FiO_2 of 28% via other delivery methods to sustain O_2 saturation of 92% for the 3 days following screening.

The protocol was submitted for ethics review and approval or acknowledgement of treatment was obtained in writing from the Institutional Review Board (IRB) or Ethics Committee (EC) of each institution. Parental signature of 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 prior to the start of remestemcel-L was collected. Prior aGvHD therapies included systemic steroids (methylprednisolone or equivalent), infliximab, etanercept, pentostatin, daclizumab, rituximab, denileukin diftitox (ontak), alemtuzumab, mycophenolate mofetil (MMF), tacrolimus, and antithymocyte globulin (ATG). Non-systemic steroids such as budesonide and beclamethasone were not counted towards second-line therapies for aGvHD treatment. Prophylactic treatments such as cyclosporine, sirolimus, and methotrexate were also not counted toward second-line therapies. If an agent was used for aGvHD prophylaxis, discontinued, and then restarted for treatment, the start date of the drug provided for aGvHD treatment must have been after the onset of aGvHD to be counted as a second-line agent for aGvHD.

The effect of aGvHD therapies prior to the introduction of Prochymal was presented as improving, unchanged, or worsening. Improving aGvHD meant at least one grade reduction in aGvHD between disease onset and study baseline, worsening GvHD represented an increase in aGvHD grade, and maximal aGvHD was grade D at both onset and study baseline.

Treatment Regimen

Remestemcel-L was given intravenously at a dose of 2×10^6 hMSCs/kg of body weight twice per week for 4 consecutive weeks. Patients received all 8 infusions in the initial treatment plan by day 28. Infusions were administered at least 3 days apart. During the course of treatment with remestemcel-L, all other aGvHD therapies were administered at the discretion of the Investigator according to institutional practice.

Patients who demonstrated a partial or mixed response to remestemcel-L at study day 28 and had no safety issues to the therapy after the first eight doses were eligible for continued therapy of an additional 4 infusions of 2×10^6 hMSCs/kg administered once per week for 4 weeks.

Prior to administration, patients received pre-medication with hydrocortisone (0.5-1.0 mg/kg, up to 50mg/dose) and diphenhydramine (0.5-1mg/kg, up to 50mg/dose), within 30 minutes of the infusion. The product was thawed and reconstituted with PlasmaLyte®A (Baxter) to a final cell concentration of 2.5×10^6 hMSC/mL. The DMSO concentration of the final infused product was 3.75%. The infusion was administered intravenously at a controlled rate of 4 to 6 mL/min for patients weighing ≥ 35 kg, and over 60-minutes for those < 35 kg. The total volume administered for each patient was dependent on body weight. Vital signs and oxygen saturation were monitored for each infusion. Oxygen saturation was monitored by pulse oximetry for at least 30 minutes prior to and until 2 hours after the start of the treatment.

All patients received standard of care treatment with corticosteroids and could receive other second line agents at the discretion of the investigator.

Source of hMSC, remestemcel-L

The product lots of remestemcel-L used in this study were derived from the bone marrow of 7 different donors aged 18-30 years who had been screened and tested according to FDA requirements for Blood and Tissue Based Products. The product lots were manufactured by a scaled adaptation of the technique described by Pittenger (19) according to Good Manufacturing Practices (GMP), as described previously (17). All lots passed established quality release criteria for viral pathogens, mycoplasma, sterility, endotoxin, cell identity, purity, viability, and potency prior to use.

GvHD Assessment

The severity of acute GvHD was assessed using Center for International Blood and Marrow Transplant Registry (CIBMTR) grading (18). Patients were evaluated by the treating physician for the presence or absence of acute GvHD of the skin, liver, and gut. Organ stage and overall grade were recorded. Acute GvHD assessments were performed at baseline prior to initiation of remestemcel-L, day 28, and day 100/end-of-treatment.

Response to treatment was determined using established clinical criteria (4). Overall Response (OR) was a Complete Response (CR) or a Partial Response (PR). No Response (NR) was defined as Mixed Response (MR), Stable disease, or Worsening disease. Definitions of each response are summarized in Table 1.

Responders were defined as achieving at least an OR, while those patients experiencing an MR, NR or who died prior to or on day 28 were counted as non-responders at day 28.

Statistical Analysis

Objective assessment of 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 summarized by each organ as improving, stable, progressing, or death.

To assess the effect of continuing therapy (greater than 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, two Kaplan-Meier (KM) survival analyses were conducted through day 100. A KM curve was generated for patients who had achieved response (OR) at day 28 and another KM curve for non-responders at day 28. The null hypothesis of no difference in overall survival between the groups was tested with the log-rank test using PROC LIFETEST in SAS®. The testing was performed at a significance level of $P < 0.05$.

Categorical variables were summarized using frequencies and percentages. Continuous variables were summarized using descriptive statistics (n, mean, standard deviation [SD], median, minimum, and maximum). 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, 20) was administered to the enrolled patients with all patients receiving at least 1 infusion. Patient characteristics are summarized in Table 2. The patients comprised 44 males (58.7%) and 31 females (41.3%), with a median age of 7.8 years (0.2, 17.5). Forty-five patients (60.0%) were transplanted for hematological malignancies and the remaining patients were transplanted for non-malignant diseases, primarily of genetic origin. The most common underlying malignancies or leukemic diseases at transplant were acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) with 18 patients (24.0%) and 16 patients (21.3%) respectively. Most of the HSCT donors were unrelated (85.3%), nearly evenly divided between HLA matched (52%) and mismatched (48%).

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

Prior Failed aGvHD Therapy

The median time from acute GvHD onset to start of treatment with remestemcel-L was 30 days. Between aGvHD onset and start of treatment with remestemcel-L, patients were maintained on aGvHD prophylaxis, systemic steroids and often one or more second line agents for the treatment of aGvHD (Table 3). All patients were refractory to steroid therapy. The majority (60.0%) of patients 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 MMF (24.0%). Virtually all of the patients (96.0%) were not improving despite treatment with steroids and other aGvHD immunosuppressive therapies prior to study entry.

GvHD Treatment Response

Response to study treatment at day 28 is summarized in Table 4 and Table 5. At day 28, forty-six patients (61.3%) were responders. At day 28, sixty-three (63%) of responding grade D patients improved with at least a 2-grade reduction. Overall, in 87% of evaluable patients, no new aGvHD medications were introduced after initiation of remestemcel-L, and response rate for these patients was 65.5%.

Individual organ response to remestemcel-L was assessed from organ staging (Table 5). Of the 65 patients who had aGvHD of the lower gastrointestinal tract (GI) at baseline, 70.7% were experiencing clinical symptoms consistent with severe (stages 3 and 4) GI aGvHD. At day 28, 58.5% of these patients showed an improvement in their clinical symptoms and, for patients experiencing severe GI aGvHD, 46.2% had a 2-grade or greater improvement in their GI aGvHD. Seventeen patients (26.2%) had complete resolution of GI aGvHD. Three patients (4.6%) with GI aGvHD at baseline experienced GI aGvHD progression.

Twenty-seven patients (36.0%) had liver aGvHD at baseline and 44.4% (12/27) showed an improvement in their liver disease at day 28, with 9 cases (33.3%) completely resolving. Two patients (7.4%) with liver aGvHD at baseline experienced liver aGvHD progression.

Forty-one patients (54.7%) had skin aGvHD at baseline. Fourteen of these patients had skin rash covering 50-100% of their body and 6 patients had severe rash with bullae. At day 28, 75.6% (31/41) showed an improvement in their skin disease with 43.9% completely resolving. No patients with skin aGvHD at baseline experienced progression of their skin disease.

Effect of Continuing Therapy

Patients were eligible for continued therapy if they had either a PR or MR at day 28. The benefit of continuing remestemcel-L treatment beyond the initial four weeks (8 infusions) was assessed by aGvHD grade and is summarized in Table 6. Only patients receiving continuing therapy were included in the analysis. For a patient to be considered a responder to continuing therapy, they must have experienced additional improvement in at least 1 organ, of at least 1 stage, without worsening in any other organ from day 28 to day 100. Patients who maintained a CR after day 28 were also considered responders. Patients who had a PR at day 28, but had no change in organ staging between day 28 and day 100, were considered non-responders.

Overall, 40 of 75 (53.3%) patients received more than 8 remestemcel-L infusions and were included in the continuing therapy analysis. Over half of these patients (57.5%) showed an additional improvement in their aGvHD with 16 patients achieving a complete resolution of their aGvHD.

Survival

The probability of survival from study entry based on whether a patient was a responder or not at day 28 was estimated using a Kaplan-Meier analysis (Figure 1). Of those patients with an OR at day 28, 76.1% survived at least 100 days past the first infusion. In contrast, patients without an OR at day 28 had a 27.6% survival rate. The log-rank test for the comparison of survival probability for responders versus non-responders demonstrated a significant 100-day survival advantage for those patients with an OR at day 28 ($p<0.001$).

Overall, survival at day 100 was 57.3%. By aGvHD Grade at baseline, 66.7% of grade B, 66.7% of grade C and 51.1% of grade D patients survived to day 100.

Safety

Infusion of remestemcel-L was well tolerated in these patients. The mean total number of infusions received was 9.7 ± 3.97, with a median of 10.0 (1, 20) infusions per patient. Thirty-five patients (46.7%) received ≤8 infusions and 40 patients (53.3%) received >8 infusions. Duration of exposure ranged from 0 days (one patient received only one infusion) to 116 days, with a mean of 40.5 ± 24.59 days. Infusional toxicity was evaluated by monitoring vital signs (HR, respiration rate, temperature, and BP) and oxygen saturation from 30 minutes prior to infusion through 2 hours post infusion. Only one patient (1.3%) experienced infusion-related reactions (rise in body temperature, increased breathing and decreased oxygen saturation) following the 3rd and 4th infusions that resolved without sequelae.

Forty-six out of 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%), multi-organ failure in 6 patients (8.0%), and hypertension and graft versus host disease in 3 patients (4.0%) each. A single patient had acute respiratory distress that led to withdrawal. No patients experienced an SAE considered to be probably or definitely related to remestemcel-

L by the investigator. Seven SAEs in 6 patients were considered possibly related: neutropenia (1 patient, 1.3%), tachycardia (1 patient, 1.3%), infusion-related reaction (2 events in 1 patient, 1.3%), respiratory distress (1 patient, 1.3%), pulmonary hemorrhage (1 patient, 1.3%), and hypertension (1 patient, 1.7%).

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

Patients were monitored for ectopic tissue formation using CT scans prior to the first infusion and at the time of the day 100 visit. No findings indicating ectopic tissue formation were reported. In addition, there were no SAEs reported as possibly representing ectopic tissue foci. There were also no remarkable findings on ECG post-treatment with remestemcel-L.

DISCUSSION

We report results of a single-arm, multi-institutional study of remestemcel-L in pediatric patients with severe end-stage acute GvHD who had exhausted conventional treatment options. This is the largest prospective study of its kind in pediatric patients with severe, multi-line refractory aGvHD. The patients enrolled comprised a very challenging population suffering from severe disease that was non-responsive to steroids and in most cases, other immunosuppressive agents. The 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 were experiencing severe aGvHD, 91% had visceral organ involvement, and 63% had multi-organ involvement. Despite aggressive treatment for a median of 30 days prior to remestemcel-L, 96% of patients were worsening or not improving at study entry.

Due to the aggressive refractory nature of the disease in these patients, achieving a substantial level of response is a meaningful and positive observation. In this study, 61% of patients responded to treatment with remestemcel-L 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 grade B, 76% of grade C and 53% of grade D patients responding 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.

Since 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 determined to be 77%.

Continued therapy beyond the initial regimen of 8 biweekly infusions was found to be beneficial. The treatment of 40 patients beyond the initial 8 infusions produced 23 additional overall responses (57.5%), including 16 patients with complete resolution of their aGvHD.

The data available for pediatric patients with refractory aGvHD are limited, primarily consisting 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 acute GvHD reporting data at day 28, response was observed in 6 of 17 patients (35%) overall and a relationship between response and survival was observed, with 5 of 6 responding patients surviving (22). Overall median survival was 60 days post initiation of daclizumab, demonstrating the life-threatening nature of aGvHD. In a study of infliximab in 18 pediatric patients with acute GvHD less severe than the current study (39% were Grade I/II aGvHD and 61% were Grade III/IV aGvHD), survival ranged from 40% to 62% at day 100 post start of infliximab (25).

In the largest double-blind, placebo-controlled, randomized study to date of MSCs for the treatment of steroid-refractory aGvHD, a subset of 28 pediatric patients exhibited a day 28 Overall Response rate of 64% for the Prochymal group, versus 36% for standard of care treatment group, which is very similar to the results of the presented study. The patient populations between the two studies were comparably severe, as 79% of the 28-patient pediatric subset entered the study as a Grade III or Grade IV (26, 27).

The best-documented 28-day response rate for the treatment of aGvHD is for high dose systemic corticosteroids used as a first line agent (4). That response rate is 65%; essentially identical to the response rate obtained in this study, even though the steroid data was collected from a population with significantly milder disease. The 65% OR for steroids was in a patient population (n=864)

where 85% had Grade I or II, 14% had Grade III, and only 1% had Grade IV. In the current study, 60% of patients had Grade D aGvHD at the time of enrollment.

The treatment regimen in the current study lead to response and objective clinical benefit in severe refractory acute GvHD. Multiple infusions were selected for the treatment of severe refractory aGvHD for several reasons. The extensive inflammation occurring in severe aGvHD may limit the persistence of MSCs (28), and thus additional infusions are needed to quell the active ongoing inflammatory response. In addition, while not assessed in these patients, multiple dosing may promote tolerance of the HSC graft through mechanisms such 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 the MSCs (31).

Response to treatment at day 28 is an important endpoint because of its link to the probability of survival. According to a recent consensus of experts (32), response to treatment at day 28 is the most relevant endpoint for evaluation of a aGvHD therapy, as it is an important predictor of survival at later times. Recent data (4) confirmed that overall response was correlated with improved survival in aGvHD patients. The data from this study supports that relationship. Survival to day 100 after the first infusion of remestemcel-L was improved in patients who responded at day 28. Of the 46 patients who experienced an OR at day 28, 35 patients (76%) survived to day 100 while only 8 of the 29 patients (28%) who were non-responders survived. The effect of achieving a response at day 28 on survival was highly significant ($p<0.001$).

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 over 500 infusions administered during the course of the study. There were no cases of ectopic tissue formation. The number and type of events reported is consistent with a severely immunocompromised acute GvHD 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 prophylaxis against or treatment of acute GvHD.

The prognosis of severe refractory aGvHD continues to be poor, and development of better therapies for these patients is 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 due to the high observed response rates and positive safety profile.

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FINANCIAL DISCLOSURE STATEMENT

Dr. Charles R. Mills is employed at Osiris Therapeutics, Inc.

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TABLES

Table 1. Response Definitions

Complete Response (CR)	Resolution of acute GvHD 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 one organ stage in at least one evaluable organ with worsening by at least one organ stage in at least one 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 one evaluable organ by one stage or more.
No Response (NR)	MR or Stable Disease or Worsening Disease

Table 2. Patient Characteristics

	n=75
Age	
Mean (SD)	8.6 (5.78)
Median (minimum, maximum)	7.8 (0.2, 17.5)
Gender	
Male	44 (58.7%)
Female	31 (41.3%)
Race	
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 (minimum, maximum)	26.9 (5.4, 103.7)
Underlying Disease	
Malignant	45 (60.0%)
Non-malignant	30 (40.0%)
Underlying Disease	
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	
Unrelated	64 (85.3%)
Related	11 (14.7%)
Donor Compatibility	
HLA-Matched	39 (52.0%)
HLA-Mismatched	36 (48.0%)
HSCT Source[1]	
Bone Marrow	25 (33.3%)
PBSC	16 (21.3%)
Cord Blood	28 (37.3%)
DLI	5 (6.7%)

[1] HSCT source was not available for one patient.

Table 3. Baseline GvHD Characteristics

			n = 75		
HSCT to GvHD Onset (Days)					
Mean (SD)			49.6 (54.02)		
Median (minimum, maximum)			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 (%)	0	1	2	3	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			36 (48.0%)		
GI-Skin			23 (30.7%)		
GI-Liver			13 (17.3%)		
Three Organ Involvement			11 (14.7%)		
GvHD Onset to 1st Infusion (Days)					
Mean (SD)[1]			70.3 (190.51)		
Median (minimum, maximum)[1]			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%)		
Prior 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 prior to 1st Infusion, n (%)					
Improving			2 (2.7%)		
Unchanged			22 (29.3%)		
Worsening or maximal GvHD			50 (66.7%)		

Note: The date of GvHD onset was not available for one patient.

[1]The high SD and range is largely due to a single patient who started treatment 1639 days after being diagnosed with GvHD. Omitting this patient, the time was 48.8 ± 46.39 days.

Table 4. Summary of Overall Response to remestemcel-L at Day 28

	Baseline GvHD Grade			
	Grade B (n=9)	Grade C (n=21)	Grade D (n=45)	Overall (n=75)
Responder , n (%)	6 (66.7%)	16 (76.2%)	24 (53.3%)	46 (61.3%)
Non-Responder , 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	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%)

Notes: Only subjects having organ involvement at Baseline (stage >0) are included in the percentages.
Complete resolution = reduction to organ stage 0.
Improving = reduction by at least one stage.
Stable = no change in the stage.
Progressing = an increase by at least one stage.

Table 6. Effect of Continuing Therapy with remestemcel-L, stratified by Baseline Grade

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

Table 7. Summary of Serious Adverse Events

	Number of Subjects (n=75) n (%)	Number of Events
Subjects with at least one 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

FIGURES

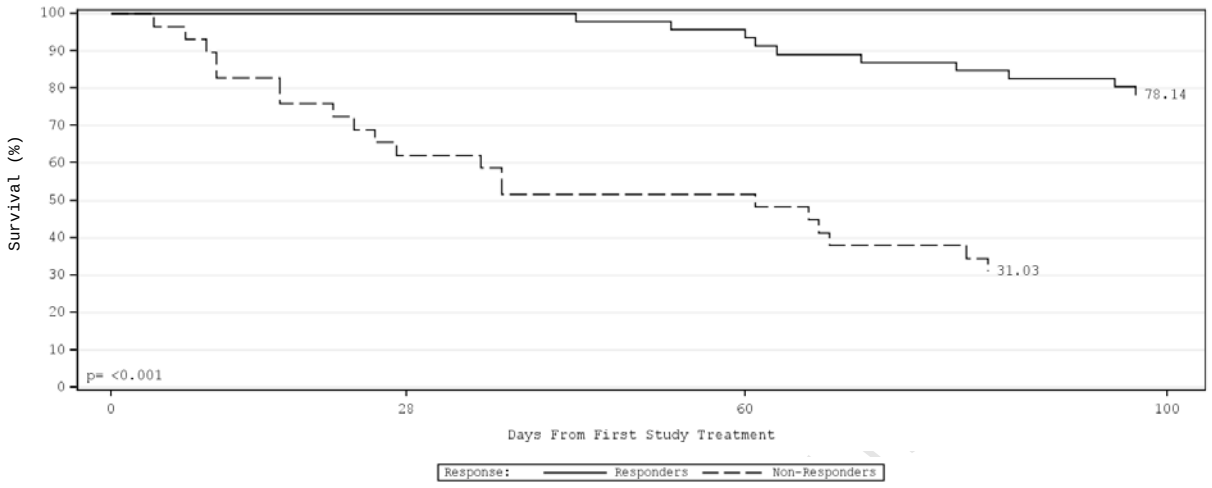

Figure 1. The Kaplan-Meier plot of survival from study entry based on whether a patient was a responder or not at day 28. The log-rank test for the comparison of survival probability for responders versus non-responders demonstrated a significant 100-day survival advantage for responders ($p<0.001$). Two patients (one a responder and the other a non-responder) who completed the study prior to day 100 (day 92 and day 99), were censored.