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Cellular Therapy

Long-Term Follow-Up of a Pilot Study Using Placenta-Derived Decidua Stromal Cells for Severe Acute Graft-versus-Host Disease

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A B S T R A C T

There is a need for effective therapy with few side effects for severe acute graft-versus-host disease (GVHD). The placenta protects the fetus from the mother's haploidentical immune system during pregnancy. We found that maternal stromal cells from the fetal membrane, so-called decidua stromal cells (DSCs), are more immunosuppressive than other sources of stromal cells. We prospectively treated 21 patients (median age, 49 years; range, 1.6 to 72 years) for grade II-IV acute GVHD. All 21 patients had biopsy-proven gastrointestinal GVHD. The majority of patients were either steroid-refractory or had progressive GVHD, 11 patients after >7 days or with progression after 3 days, and 10 were refractory to steroids after >3 days. We used an improved protocol in which DSCs were thawed and infused in a buffer with 5% human albumin. DSCs were given at a median dose of 1.2 (range, 0.9 to 2.9) $\times$ 10^6 cells/kg body weight with a median of 2 (range, 1 to 6) doses, given 1 week apart. The median viability of thawed DSCs was 93% (range, 69% to 100%), and the median cell passage number was 4 (range, 2 to 4). Complete resolution of GVHD was seen in 11 patients, with a partial response in the other 10. The cumulative incidence of chronic GVHD was 52%. GVHD was mild in 6 patients, moderate in 4 patients, and severe in 1 patient based on National Institutes of Health chronic GVHD severity scoring. Nine patients died, including 3 from relapse and 1 each from acute GVHD and septicemia, Zygomycetes infection, liver insufficiency, cerebral hemorrhage, multiple organ failure, and chronic GVHD with obstructive bronchiolitis. Four-year transplantation-related mortality was 28.6%, and overall survival was 57%. Survival was similar ($P = .33$) to that for all 293 patients who underwent allogeneic hematopoietic cell transplantation during the same period (2012 to 2015), with 66% overall survival. DSC infusion is a novel therapy for acute GVHD grade II-IV, and a randomized trial is currently underway (ClinicalTrials.gov NCT 02172937).

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INTRODUCTION

Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT) [1–4]. Steroids are the first-line therapy for acute GVHD, but the outcome is poor in patients with steroid-refractory acute GVHD [3,4].

There is a need for effective therapy for severe acute GVHD. Several novel immunosuppressive therapies have emerged in recent years [5]. Anti-CD52, anti-TNFα, mycophenolate mofetil, sirolimus, pentostatin, and extracorporeal photopheresis have been tried in several pilot studies, with varying response rates and no dramatic improvement in survival. The most recently introduced innovative drug to treat steroid-refractory acute GVHD is vedolizumab, an anti-$\alpha4\beta7$ integrin [6]. However, the results from a phase II trial were not encouraging, owing to limited efficacy (personal communication, Yngvar Floisand and Jonas Mattsson). In another study, the selective adhesion molecule inhibitor natalizumab (Tysabri) was administered to 18 patients with acute gastrointestinal GVHD grade II-IV [7]. The

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28-day response rate was 75%, and 6-month survival was 52%. Acceptable responses to severe acute and also chronic GVHD have been reported with ruxolitinib, a JAK-1/2 inhibitor [8]. Regulatory T cells have been tried in only a few patients with acute and chronic GVHD [9].

We introduced mesenchymal stromal cells (MSCs) as a treatment for severe acute GVHD [10,11]. A prospective, double-blind placebo-controlled study sponsored by Osiris Therapeutics did not reach the primary endpoint of a durable, complete response at 28 days; however, patients with liver and gastrointestinal GVHD who were treated with MSCs had better response rates than the placebo group [12]. In a meta-analysis, Hashmi et al [13] found a 6-month survival of 63% in responders to MSC therapy for steroid-refractory acute GVHD. One-year survival in bone marrow (BM) MSC-treated acute GVHD patients at our center was 20% [14].

We found that maternal stromal cells from the fetal membrane, so-called decidua stromal cells (DSCs), are more immunosuppressive than other sources of stromal cells, including those from bone marrow [15]. This is not surprising, as the placenta plays an important role in fetomaternal tolerance. Our preliminary experience in using DSCs to treat acute GVHD has been reported previously [16,17]. Reporting 6-month survival in patients treated for severe acute GVHD is mandatory [18]; however, long-term follow-up in these patients is seldom reported. Here we report our experience in treating 21 patients with severe acute GVHD with DSCs using an improved protocol [17]. The median duration of follow-up in these patients was 4 years.

METHODS

Patients

This prospective study involved 21 patients who were treated for acute GVHD of grades II–IV between 2012 and 2015.

Myeloablative conditioning regimens included cyclophosphamide (120 mg/kg) or etoposide (60 mg/kg) combined with fractionated total body radiation (12 Gy) [12]. Reduced-intensity conditioning regimens included fludarabine combined with treosulfan with or without thiotepa or cyclophosphamide with or without 6 Gy whole-body irradiation [19,20].

All 21 patients had gastrointestinal GVHD confirmed by analysis of biopsy specimens obtained during colonoscopy or gastroscopy. Steroid-refractory acute GVHD was defined as disease progression after 3 days despite prednisolone treatment (1 or 2 mg/kg/day), or lack of response after 7 days. Nine patients were included just 3 days after receiving steroids, owing to older age and/or comorbidities and lack of improvement after steroid therapy. One patient with several serious medical problems who developed severe acute gastrointestinal GVHD was started on steroids and DSCs on the same day. These patients received DSCs because they were considered unable to tolerate long-term immunosuppressive therapy with high-dose steroids.

The Ethical Committee of Karolinska Institute, Stockholm approved the donation and isolation of DSCs (entry nos. 2009/418-31/4 and 2010/2061-32). The donors of placentas provided written consent. The use DSCs for GVHD was approved by the Ethical Committee (entry nos. 2010/452-31/4 and 2014/2132-32). All patients gave written consent. For children, both parents agreed and gave written consent for their child to participate in the trial. The initial patients were included because no other therapy was available for severe acute GVHD [16]. Subsequently, this developed into a more formal prospective study with safety and 1-year survival as important endpoints [17] (ClinicalTrials.gov NCT 02172937).

DSCs

Term placentas were donated from healthy mothers following elective cesarian section delivery. The procedures for isolation, expansion, and freeze-thawing of DSCs according to good manufacturing practice have been published previously [16]. The fetal membrane was dissected from the placenta and incubated with trypsin-EDTA (Thermo Fisher Scientific, Waltham, MA). The fetal membrane was cut into pieces and incubated in culture flasks. When the cells were approximately 90% confluent, they were harvested with trypsin-EDTA, washed, and seeded in new flasks. The cells were cultured to passage no. 2–4 and frozen slowly in complete Dulbecco's modified Eagle medium (Thermo Fisher Scientific) containing 10% dimethyl sulfoxide (WAK-Chemie Medical, Steinbach, Germany). The DSCs were positive for CD29, CD73, CD90, CD105, HLA class I, programmed cell death ligand 1 (PD-L1), PD-L2, and ICAM-1. DSCs were negative for HLA class II, hematopoietic markers, CD14, CD31, CD34, CD45, CD86, and CD326 [16]. DSCs showed slight differentiation to adipocytes, but they differentiated poorly into osteocytes and chondrocytes, in contrast to BM MSCs [21]. The cells were of maternal origin, as determined by microsatellite polymorphism analysis [16]. They had a normal karyotype and were able to suppress proliferation in mixed lymphocyte cultures.

Clinical Use of DSCs

The cells were thawed to 37°C in Clini-MACS PBS/EDTA buffer (Am Cell; Miltenyi Biotech, Gladbach, Germany) supplemented with 5% human serum albumin (CSI Behring, King of Prussia, PA). The DSCs were washed 3 times, counted, filtered through a 70-mM cell strainer (BD, Franklin Lakes, NJ), supplemented and suspended in an infusion solution (NaCl) (B. Braun Melsingen, Melsingen, Germany), and transferred to a heparinized syringe (2×10^6 DSCs/mL). Sterility testing was performed on both the infusion solution and the supernatant of the culture medium. The cells were introduced within 5 minutes via the central venous line. Before and after addition of the DSCs, the infusion line was washed with 5% saline containing 50 IE heparin/mL in adults. Children weighing >15 kg received 25 IE heparin/mL, and children weighing <15 kg received 12.5 IE heparin/mL. According to the protocol, patients should receive 1×10^6 per kg and at least 2 doses given 1 week apart. The patients received a median of 2 treatments (range, 1 to 6) with DSCs. The median DSC dose per kg was 1.2 (range, 0.9 to 2.9) $\times 10^6$. The median cell passage number was 4 (range, 2 to 4). The median cell viability was 95% (range, 69% to 100%). The number of DSC doses given depended on the GVHD response. Clinical response was evaluated independently by 2 clinicians (O.R. and J.M.). Complete response was defined as the disappearance of all GVHD symptoms, and partial response was defined as a significant improvement by at least 1 grade of acute GVHD, but with some signs of GVHD remaining [1]. Chronic GVHD was evaluated according to National Institutes of Health criteria [22].

Statistical Analysis

Time to survival and time to leukemia-free survival were determined with the life table method using the Mantel-Haenszel log-rank test and taking censored data into account. Chronic GVHD, GVHD-related mortality, transplantation-related mortality (TRM), and hematologic relapse were estimated using a nonparametric estimator of cumulative incidence, taking competing events into consideration. GVHD-free, relapse-free survival (GRFS) was defined as the absence of severe acute GVHD, severe chronic GVHD, and relapse as defined previously [23]. Competing events included death without GVHD for GVHD, death from other causes for GVHD/mortality, relapse for TRM, and TRM for relapse. Patients were censored at the time of death, relapse, or last follow-up. Analyses were performed using the freely available EZR software and Statistica (StatSoft, Tulsa, OK).

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. The study cohort included 3 children, and the median patients age was 49 years (range, 1 to 72 years). Cyclosporine A and methotrexate [24] were administered as prophylaxis against GVHD. Six patients received tacrolimus and sirolimus as part of a randomized trial [25]. Three patients, 1 with a haploidentical donor and 2 with matched unrelated donors (MUDs), received cyclophosphamide after transplantation [26]. Fourteen patients underwent HSCT was performed with an MUD in 14 patients, a matched-related donor (MRD) in 6 patients, and a haploidentical graft in 1 patient. Two patients developed severe acute GVHD following donor lymphocyte infusion. Immunosuppressive therapy given for acute GVHD included calcineurin inhibitors, steroids, and DSCs. All patients received prophylaxis for fungal infection with posaconazole.

Organ involvement in GVHD was as follows: skin, 10 patients with grade 0, 3 patients with grade 1, 7 patients with grade 2, and 1 patients with grade 3; gastrointestinal tract, 8 patients with grade 1, 6 patients with grade 2, 5 patients with grade 3, and 2 patients with grade 4; and liver, 2 patients with grade 2.

Response to DSC Therapy

At 28 days after initiation of DSC therapy, a complete response regarding acute GVHD was seen in 11 patients and a

Table 1
Patient Characteristics

Characteristic	Value
Sex, female/male, n	5/16
Age, yr, median (range)	49 (1-72)
Malignant/nonmalignant disease, n	17/4
Disease status, low risk/high risk, n	7/14
Conditioning, MAC/RIC, n	4/17
GVHD prophylaxis, n	
Cyclosporine + methotrexate	13
Tacrolimus + sirolimus	6
Post-transplantation cyclophosphamide	2
ATG, pretransplantation	14
Donor type, n	
Matched sibling donor	6
Matched unrelated donor	14
Haploidentical donor	1
Stem cell source, peripheral blood/bone marrow, n	16/5
Cytomegalovirus serology: donor and recipient both negative, n	7
Acute GVHD grade at intervention, II-IV/III-IV, n	6/15
GVHD organs involved, skin/gut/liver, n	11/21/2
GVHD after DLI, n	2
Time from HSCT/DLI to start of steroids, d, median (range)	64 (5-265)
Time from start of steroids to DSC treatment, d, median (range)	7 (0-35)

Low risk: first remission or nonmalignant diseases; high risk: all other stages. MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; ATG, antithymocyte globulin; DLI, donor lymphocyte infusion.

partial response was seen in 10 patients. The overall response rate was 100% at day 28 and 95% at day 56. A 72-year-old man who underwent HSCT for myelodysplastic syndrome died of septicemia with signs of acute GVHD on day 59 after initiation of DSC therapy.

Infections

Five of the 21 patients who received DSCs to treat acute GVHD contracted a systemic or serious infection. Two patients had Epstein-Barr virus (EBV) reactivation, 1 patient had varicella zoster virus reactivation, and 2 patients developed Zygomycetes infection. Both patients with Zygomycetes infection had low concentrations of posaconazole in their plasma (<.7 ng/mL). No other systemic or serious infections were diagnosed in any patients.

Chronic GVHD

Eleven patients developed chronic GVHD. The global National Institutes of Health score was mild in 6 patients, moderate in 4 patients, and severe in 1 patient. The patient with severe chronic GVHD developed obstructive bronchiolitis and died 885 days after HSCT (754 days after treatment with DSCs). The cumulative incidence of chronic GVHD was 52% (Fig. 1).

Survival

Of the 21 patients treated with DSCs, nine patients died. Three patients died from leukemic relapse. One patient died of liver insufficiency at 158 days after transplantation and 140 days after DSC infusion. This patient had highly elevated bilirubin and liver enzyme levels, and liver biopsy showed no signs of GVHD. Posaconazole and other hepatotoxic drugs were discontinued, but hepatotoxicity progressed. The patient had no signs of acute GVHD. One patient died of acute GVHD and septicemia at 2 months after DSC infusion. One patient died from multiple organ failure on day 613 after HSCT, 1 patient died from cerebral hemorrhage on day 624 after HSCT, 1 patient died from Zygomycetes infection at 278 days after HSCT (132 days after DSC infusion), and 1 patient died from obstructive bronchiolitis. Altogether, 6 patients died from transplantation-related causes, for a cumulative incidence of TRM of 28.6% (Fig. 2). (Unexplained abbreviation) Twelve patients were alive at 3 to 5 years after transplantation, with an overall 4-year survival of 57% (Fig. 3). Statistically, this was not significantly worse than the 4-year survival rate of 66% for all 293 patients who underwent HSCT at our center between 2012 and 2015, the same years in which the study patients were treated with DSCs for severe acute GVHD (Fig. 4). Eleven patients were steroid-refractory for at least 7 days before DSC therapy. These patients had a 3-year TRM of 27.3% and a 4-year survival of 55%.

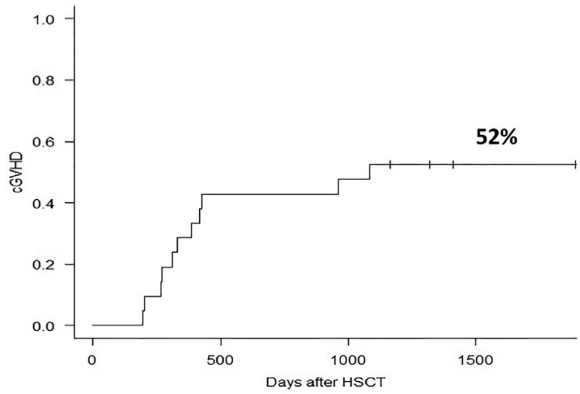

Figure 1. Time to and cumulative incidence of chronic GVHD in patients treated with DSCs for severe acute GVHD.

GRFS

GRFS for all patients was 54% at 4 years (Fig. 5). When only patients with malignancies (n = 17) were analyzed, the 4-year GRFS was 57%.

DISCUSSION

Patients with severe acute GVHD have a high morbidity and mortality due to infection, hemorrhage, and other

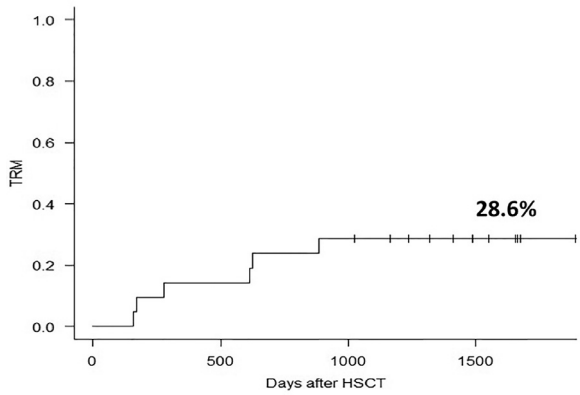

Figure 2. Time to and cumulative incidence of TRM in patients treated with DSCs for severe acute GVHD.

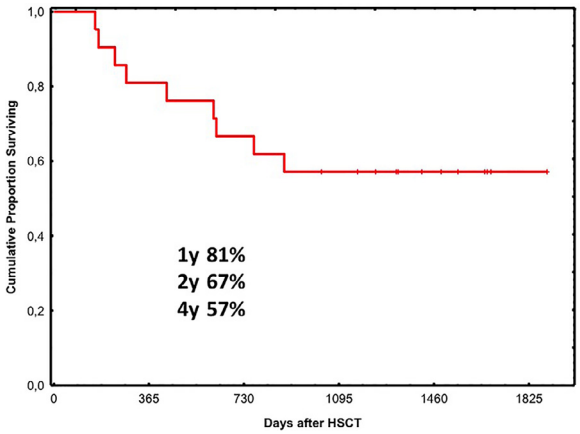

Figure 3. Overall survival in patients treated with DSCs for severe acute GVHD.

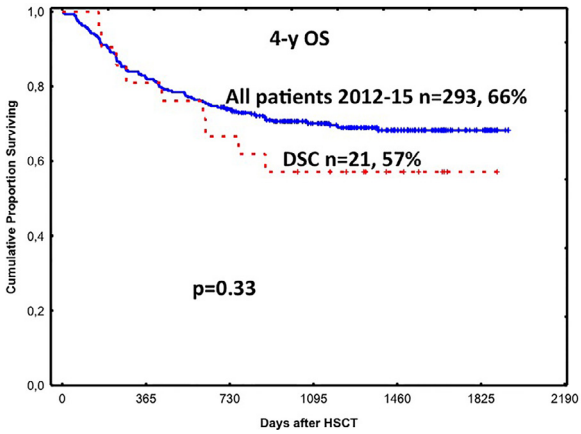

Figure 4. Overall survival in all patients who underwent HSCT at the Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital during 2012-2015 and in 21 patients treated with DSCs for severe acute GVHD. Four-year survival was 66% and 57%, respectively, in the 2 groups.

complications. Mortality is generally very high, 50% to 90% [2]. Several studies using novel immunosuppressive therapies have shown acceptable 6-month survival, but long-term follow-up data are limited [18]. Owing to the high mortality in patients with severe acute GVHD, long-term survival analysis

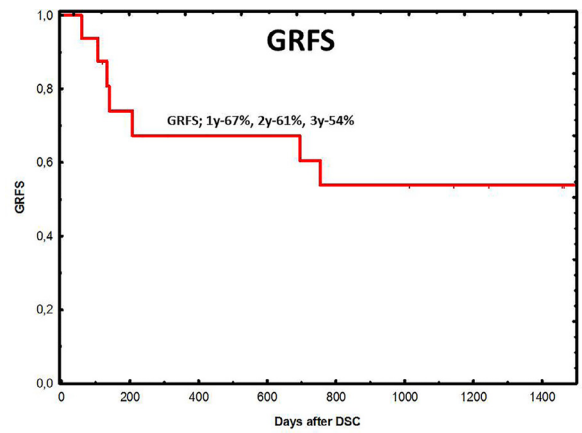

Figure 5. Probability of GRFS in all 21 patients from the time of DSC treatment.

is important. In our patients who received DSCs to treat acute GVHD, the 4-year survival rate was statistically similar to that of all patients who underwent HSCT at our institution during the same time period (57% versus 66%).

The European Group for Blood and Marrow Transplantation (EBMT) performed a retrospective landmark analysis of 1,738 patients who survived for >6 months after HSCT between 2002 and 2014 [27]. Two-year survival starting 6 months after HSCT was 49% in patients with acute GVHD of grade III-IV. It was 59% in those with grade II and 61% in those with no GVHD or only mild acute GVHD. A similar evaluation for DFC treated acute GVHD patients at our center at 6 months indicated a 2-year survival of 66%, which compares favorably with that for the patients with severe acute GVHD in the European Group for Blood and Marrow Transplantation report. Zeiser et al [28] reported a 1-year survival rate of 62% in 54 patients treated with ruxolitinib for severe acute GVHD, compared with 81% in our 21 patients (Fig. 3). Moreover, in another study, treatment with inolimomab or etanercept in 127 adult patients with steroid-refractory acute GVHD resulted in a 2-year survival rate of 19% [29].

When novel therapies are introduced, side effects and severe adverse events are important issues. DSCs appear to be well tolerated, with few side effects [30]. BM MSC treatment has been associated with EBV lymphoma, pneumonia-related death, and invasive fungal infection [31–33]. Severe adverse events in patients with severe acute GVHD include infections, graft failure, and multiple organ failure [17]. Using BM MSCs, we saw a trend toward more invasive fungal infections [14]. Two patients treated with DSCs in this study had Zygomycetes infections, and both had low serum concentrations of posaconazole. These infections might have been due to the low serum drug concentrations, highlighting the importance of monitoring posaconazole concentration to allow for appropriate dose adjustments [34]. Larger studies are needed to investigate the risk of invasive fungal infection in patients treated for GVHD with various types of stromal cells. BM MSC treatment has been associated with a significantly increased risk of developing EBV lymphoma post-transplantation lymphoproliferative disease [32]. To date, 2 patients have had EBV reactivation following treatment with DSCs, but neither developed post-transplantation lymphoproliferative disease.

Strongly immunosuppressive therapies may abrogate the graft-versus-leukemia effect [35–37]. Leukemic relapse occurred in 3 of our 21 patients, not a remarkably high rate. However, patients who survive severe acute GVHD are at reduced risk of leukemic relapse [27].

DSCs appear to differ from BM MSCs in many ways. DSCs seem to have better immunosuppressive effects both in vitro and in vivo [11,13,15–17], as we found in this pilot study. INF-γ, prostaglandin E2, indoleamine dioxygenase, and PD-L1 appear to be involved in the immunosuppressive mechanism of DSCs, as indicated by blocking experiments.

Eleven patients treated for steroid-refractory acute GVHD with DSCs for >7 days had a 4-year survival of 55%, which is similar to the 57% 4-year survival when patients treated earlier due to comorbidities and older age were included. However, 7 days of being refractory to steroids is not a very good predictor of outcome of acute GVHD. Biomarkers, such as ST2 and REG3α, may be better predictors of long-term survival [38]. One limitation of the present study was that biomarkers were not analyzed.

Severe chronic GVHD is associated with a poor quality of life [39,40]. Holtan et al [41] suggested a composite endpoint that better reflects patient quality of life and not simply survival or leukemia-free survival. We used an adjusted version of

this endpoint that included severe acute GVHD and severe chronic GVHD [23]. GRFS was 55% following DSC treatment, comparable to the rate seen in thousands of patients treated with HSCT for acute myelogenous leukemia in Europe [23].

Although our results appear to support the use of DSCs as treatment for acute GVHD grades II-IV, this is only a small pilot study. Randomized studies comparing DSCs with the best available therapy, such as ruxolitinib or vedolizumab, are currently underway.

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