Placenta-Derived Decidua Stromal Cells for Treatment of Severe Acute Graft-Versus-Host Disease

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ABSTRACT

Severe acute graft-versus-host disease (GVHD) is a life-threatening complication after allogeneic hematopoietic stem cell transplantation (HSCT). The placenta protects the fetus from the mother’s immune system. We evaluated placenta-derived decidua stromal cells (DSCs), which differ from bone marrow mesenchymal stromal cells (BM-MSCs), as a treatment for severe acute GVHD. DSCs were obtained from term placentas. The DSCs were given to 38 patients with severe acute GVHD; 25 were steroid-refractory (SR). DSCs were thawed and infused in buffer supplemented with either 10% AB plasma (group 1, n = 17), or 5% albumin (group 2, n = 21). The viability of cells was higher when thawed in albumin rather than AB plasma (p < .001). Group 1 received a higher cell dose (p < .001), cells of lower passage number (p < .001), and fewer infusions (p = .002) than group 2. The GVHD response (no/partial/complete) was 7/5/5 in group 1 and 0/10/11 in group 2 (p = .01). One-year survival in the two groups was 47% (95% confidence interval [CI] 23–68) and 76% (95% CI 51–89), respectively (p = .016). For the SR patients, 1-year survival was 73% (95% CI 37–90) in SR group 2 (n = 11), which was better than 31% (95% CI 11–54) in SR group 1 (n = 13; p = .02), 20% (95% CI 5–42) in BM-MSC treated (n = 15; p = .0015), and 3% (95% CI 0–14) in historic controls (n = 32; p < .001). DSCs are a promising new treatment for severe acute GVHD. Prospective randomized trials are needed for evaluation of efficacy. (Clinical trial NCT-02172937.)

SIGNIFICANCE STATEMENT

There has been no effective therapy for severe acute graft-versus-host disease (GVHD), a life-threatening complication after allogeneic hematopoietic stem cell transplantation. Bone marrow-derived mesenchymal stromal cells were introduced as a novel therapy for acute GVHD, which cured some, but not all, patients with severe acute GVHD. The placenta plays an important role in fetomaternal tolerance and has been used in Africa for 100 years to successfully treat burn injuries. It was found that placenta-derived decidua stromal cells (DSCs) are immunosuppressive in vitro and in vivo and may cure severe acute GVHD. In this pilot study, an optimal protocol was found using DSCs at 1 × 106 cells/kg dissolved in saline with 5% human albumin instead of 10% AB-plasma, given at least one dose a week. All patients receiving this treatment showed partial or complete responses and the best one-year survival. This was a small pilot study, but all patients with severe acute GVHD were cured using the new protocol. There were no major side effects. In conclusion, DSCs are a novel, promising therapy for acute GVHD and other inflammatory immunological disorders.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a well-established treatment for advanced leukemias and severe hematological and metabolic diseases [1, 2]. Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after HSCT [3–5]. There is no effective treatment for severe acute GVHD, and the outcome has been poor for patients with acute GVHD that are refractory to steroids [4, 6–8]. The use of mesenchymal stromal cells (MSCs) to treat GVHD was introduced by us more than a decade ago [9–11]. Despite promising results initially, long-term overall survival was not any better than in the controls, which was not so encouraging [12, 13]. A meta-analysis found a survival rate of 63% at 6 months in patients with severe acute GVHD that responded completely to MSC therapy [14]. However, the outcome is poor in partial responders and nonresponders [10].

HUMAN CLINICAL ARTICLE

STEM CELLS TRANSLATIONAL MEDICINE 2018;7:325–332 www.StemCellsTM.com © 2018 The Authors

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The placenta protects the fetus from the mother’s immune system during pregnancy and provides a readily available source of stromal cells [15, 16]. We have isolated decidua stromal cells (DSCs), which are of maternal origin and inhibit alloreactive T cells in vitro better than other sources of stromal cells [17, 18]. DSCs induce FOXP3-positive regulatory T cells and inhibit alloreactivity in vitro in a contact-dependent manner and not by soluble factors like MSCs [19]. DSCs are half the size of MSCs and do not differentiate well to chondrocytes and osteocytes [20, 21]. In the allogeneic setting, DSCs promote an anti-inflammatory cytokine profile [5, 19]. DSCs also have stronger hemostatic properties than MSCs. DSCs have typical MSCs surface markers, but a stronger expression of programmed death-ligand 1 (PD-L1), PD-L2, and CD49d (a marker for homing to inflamed tissue) than MSCs from bone marrow [18]. Taken together, these differences may explain why DSCs have a stronger immunomodulatory effect as opposed to other sources of MSCs. Here we report our experience using DSCs for treatment of severe acute GVHD. This is a pilot study using DSCs for severe acute GVHD, in which two specified protocols have been explored.

### Materials and Methods

**Patients**

This is a retrospective analysis of the safety and efficacy of DSC treatment for acute GVHD. Between 2011 and 2015, 38 patients were treated with DSCs for acute GVHD after HSCT (Table 1), in keeping with the Declaration of Helsinki. The patients and donors of DSCs gave their written, informed consent. Patients with severe GVHD of grade 2–4 were included, based on clinical evaluation by the treating physician and with inclusion criteria stated below. They were all consecutive patients, and no patient declined to be enrolled in the DSC study. The patients were treated with prednisolone and calcineurin inhibitors but no other immunosuppressive therapy. The last follow-up was on November 12, 2015. Eight patients have been reported previously [18]. The cells were previously named fetal membrane cells.

The regional ethical committee of Stockholm approved the donation and isolation of DSCs (entry nos. 2009/418-31/4 and 2010/2061-32) and the use of DSCs for GVHD (entry nos. 2010/452-31/4 and 2014/2132-32).

**Procedures and Definitions**

Before HSCT, the patients received either myeloablative or reduced-intensity conditioning. The conditioning was myeloablative in 12 patients who were given cyclophosphamide (120 mg/kg) combined with busulfan (16 mg/kg), or fractionated whole-body irradiation (12 Gy). Twenty-six patients had reduced-intensity conditioning regimens with fludarabine phosphate combined with various cytotoxic drugs such as busulfan or treosulfan, or 2 Gy whole-body irradiation.

### Table 1. Patient characteristics for all the patients treated with DSCs

| Characteristics                  | Group 1, n = 17 | Group 2, n = 21 | p value |
|----------------------------------|-----------------|-----------------|---------|
| Sex (M/F)                        | 9/8             | 16/5            | .18     |
| Age at GVHD, years, median (range) | 54.5 (0.9–65.6) | 48.9 (1.6–72.4) | .33     |
| Children (<19 years of age)      | 2               | 3               | .33     |
| Diagnosis (malignant/nonmalignant)| 14/3            | 17/4            | 1.00    |
| Disease status (high risk/low risk) | 9/8             | 14/7            | .51     |
| Conditioning (MAC/RIC)           | 8/9             | 4/17            | .09     |
| ATG (yes/no)                     | 10/7            | 14/7            | .74     |
| GVHD prophylaxis                 |                 |                 | .63     |
| CsA/MTX                          | 13              | 13              |         |
| TAC/SIR                          | 3               | 6               |         |
| CsA/MTX/Cy                       | 1               | 2               |         |
| Donor SIB/MUD/CB/haplo           | 6/10/1/0        | 6/14/0/1        | .52     |
| Gift source (PBSCs/BM/CB)        | 14/2/1          | 16/5/0          | .36     |
| GVHD grade at time of intervention (2/3) | 2/15          | 6/15            | .26     |
| GVHD localization (gut and other/only liver) | 17/0          | 21/0            | 1.00    |
| Fungal prophylaxis (yes/no)      | 17/0            | 21/0            | 1.00    |
| CMV (double-neg./any pos.)       | 3/14            | 7/14            | .46     |
| GVHD after DLI (yes/no)          | 0/17            | 2/19            | .49     |
| HSC/TLDI steroids, days (range)  | 59 (10–375)     | 64 (5–265)      | .97     |
| Days with steroids median (range) | 13 (1–37)       | 7 (0–35)        | .09     |
| Number of infusions (range)      | 1 (1–5)         | 2 (1–6)         | .002    |
| Cell dose (range)                | 2.0 (0.9–2.8)   | 1.2 (0.9–2.9)   | <.001   |
| Cell passage (range)             | 2 (2–4)         | 4 (2–4)         | <.001   |
| Viability, % (range)             | 90 (70–97)      | 95 (69–100)     | <.001   |

Abbreviations: ATG, antithymocyte globulin; BM, bone marrow; CB, cord blood; CMV, cytomegalovirus; CsA, cyclosporine A; Cy, cyclophosphamide; DLI, donor lymphocyte infusion; DSCs, decidua stromal cells; F, female; GVHD, graft-versus-host disease; HSC/TLDI, allogeneic hematopoietic stem cell transplantation; M, male; MAC, myeloablative conditioning; MTX, methotrexate; MUD, matched unrelated donor; neg., negative; PBSCs, peripheral blood stem cells; pos., positive; RIC, reduced-intensity conditioning; SIB, sibling donor; SIR, sirolimus; TAC, tacrolimus.
As GVHD prophylaxis, most patients received cyclosporine combined with four doses of intravenous methotrexate. In addition, three patients were treated with two doses of post-transplantation cyclophosphamide (100 mg/kg). Nine patients were treated with tacrolimus and sirolimus as part of a randomized trial [22]. Twenty-four recipients of hematopoietic stem cells from unrelated donors were treated with antithymocyte globulin. Patients were treated in reversed isolation, or at home during the pancytopenic phase if they lived close to the hospital [23]. Patient care procedures and the transplantation procedures have been published previously in detail [23].

Acute GVHD was graded according to the Seattle criteria [3]. All patients had gastrointestinal GVHD, and the diagnosis was confirmed by histological analysis of biopsies taken during colonoscopy or gastroscopy prior to therapy. No post-DSC biopsies were performed. Six patients developed acute GVHD after donor lymphocyte infusion. Steroid-refractory acute GVHD was defined as disease progressive after 3 days despite prednisolone 1 or 2 mg/kg/day or lack of response after 7 days. Some patients were included with lack of response of steroids after 3 days due to high age and/or comorbidities. They were treated with DSCs because they were not considered able to tolerate long-term immunosuppressive therapy with high-dose steroids. The infusion schedule was as follows. Group 1 received one dose. If complete response (CR) was seen, no additional DSC doses were given. Patients in group 2 were scheduled to receive a second dose after 1 week even if complete response was seen. Patients with active acute GVHD got additional weekly DSC doses until complete response or acceptable, stable, or partial response (PR) was achieved and the patient could be sent home. Fungal prophylaxis with posaconazole was given to all patients.

All patients received first-line treatment of acute GVHD consisting of oral or intravenous corticosteroids in prednisolone doses of 2 mg/kg/day, which was later changed to 1 mg/kg/day [24]. The controls received median 2 mg/kg/day prednisolone at the beginning of this treatment, as opposed to 1.85 mg/kg/day in group 1 and 1.60 mg/kg/day (p < .001 vs. controls) in group 2. In the latter two groups, some patients got 2 mg/kg/day and others got 1 mg/kg/day. In addition, oral budesonide and a calcineurin inhibitor were given to all patients. No other immunosuppressive therapy was given.

Response to the treatment was evaluated 4 weeks after intervention. CR was defined as disappearance of all symptoms of acute GVHD; PR was defined as improvement by at least one organ-specific grade; and no response was defined as no improvement of GVHD symptoms. Transplantation-related mortality (TRM) included all deaths associated with transplantation of hematopoietic cells, except for those related to recurrence of underlying disease. Data on corticosteroid treatment were obtained from the patients’ charts. GVHD-related mortality was defined as the presence of GVHD symptoms at the time of death.

### Statistical Analysis

Time to survival and relapse-free survival were determined with the Lifetable method using the log-rank (Mantel-Haenzel) test, taking censored data into account. The incidence of chronic GVHD, GVHD-related mortality, TRM, and hematological relapse were estimated using a nonparametric estimator of cumulative incidence curves taking competing events into consideration. Competing events were death without GVHD (for GVHD), death from other causes (for GVHD-related mortality), relapse (for TRM), and TRM (for relapse). Patients were censored at the time of death, relapse, or at last follow-up. Analyses were performed using the CMPRSK software package (developed by Gray, June 2001), S-
PLUS 6.2 software, and Statistica (StatSoft, Tulsa, OK). The Mann-Whitney U test was used for unrelated continuous variables comparing two groups, and the Kruskal-Wallis test was used for continuous variables comparing three groups, followed by Dunn’s multiple comparisons test. Fisher’s exact test or the chi-square test was used to compare the distribution of categorical variables.

RESULTS

Characteristics of DSC Treatment

The first 17 patients received DSCs that had been thawed and infused in buffer supplemented with AB plasma (group 1), which was the standard protocol that had been used at this center previously [9–13]. The next 21 patients received DSCs that had been thawed and infused in albumin-supplemented buffer (group 2). The albumin-thawed cells had significantly higher viability than the plasma-thawed cells (Table 1). The patients in group 1 received significantly fewer doses, a higher number of cells per dose, and stromal cells from a lower passage number than group 2 (Table 1).

Response and Survival

The GVHD response (no/partial/complete) was 7/5/5 in group 1 and 0/10/11 in group 2 (p = .013). Group 2 had a significantly higher survival (76%; 51–89) at 1 year than group 1 (47%; 23–68; Fig. 1A). The probability of relapse and chronic GVHD was similar in the two groups (Fig. 1B, 1C). The cumulative incidence of chronic GVHD at 1.5 years was 36% (12–61) in group 1 and 31% (12–53) in group 2, respectively (ns). Of 14 patients in group 1 who were alive beyond day 100, 5, 1, and 1 developed mild, moderate, and severe chronic GVHD, respectively. In group 2, of the 21 patients, 6 developed mild chronic GVHD, 2 developed moderate chronic GVHD, and none developed severe chronic GVHD. The death rate from acute GVHD was 41% (95% confidence interval [CI] 18–64) in group 1 and 5% (95% CI 0–20) in group 2 (Fig. 1D; p = .016).

Steroid-Refractory GVHD

The patients with GVHD that was strictly steroid refractory in each group were compared with retrospective controls from our unit, during the period 2000–2010, who had acute steroid-refractory GVHD (Table 2). Patients treated with mesenchymal stromal cells (MSCs 1 × 10^6 MSC/kg, n = 15) were also reported. Compared with the DSC patients, the historic controls not given stromal cells were younger (p = .02), all had had malignant disorders (p = .02), and all had received cyclosporine and methotrexate as GVHD prophylaxis (p = .005); in addition, fewer control patients who were cytomegalovirus (CMV) seronegative had had a CMV-seronegative donor (p = .05). In the MSCs group, 13 of 15 received bone marrow graft, which differed from all other groups (p < .001). The MSCs patients more often had GVHD grade 3 at intervention.
time, which differed from group 2 and historic control \( (p < .05). \)

There were no other significant differences between the groups.

Among the steroid-refractory patients, overall response at 4 weeks after the DSCs intervention was 100% in SR group 2 \( (n = 13) \), 46% in SR group 1 \( (n = 11; ~p = .013) \), and 25% in the controls \( (n = 12; ~p < .001) \).

SR group 2 had a significantly higher survival rate than SR group 1 \( (p < .001; \text{Fig. 2A}) \). SR group 1 also had a higher survival rate than historic controls \( (p < .001; \text{Fig. 2A}) \).

Severe Adverse Events and Causes of Death

Severe adverse events in the DSC patients \( (n = 38) \) and in the control group \( (n = 32) \) included the following: relapse \( (8/10) \), pneumonia \( (5/9) \), proven or probable invasive fungal infection \( (6/5) \), bacterial infection \( (2/6) \), graft failure \( (3/3) \), multiple organ failure \( (1/5) \), viral infection \( (2/3) \), central nervous system complication \( (2/3) \), septicemia \( (2/2) \), skin squamous cell carcinoma \( (2/0) \), interstitial pneumonitis \( (0/1) \), acute pancreatitis \( (1/0) \), and cardiac failure \( (0/1) \). Adverse events in DSC patients and laboratory values are reported in detail in a separate article \([25]\).

Causes of death (in DSC-treated patients/controls) were acute GVHD \( (9/18) \), relapse \( (2/4) \), bacterial infection \( (2/6) \), multiple organ failure \( (0/1) \), viral infection \( (0/1) \), invasive fungal infection \( (1/1) \), liver failure \( (1/0) \), hemorrhaging \( (1/0) \), and secondary malignancy \( (1/0) \).

A 64-year-old woman with myelodysplastic syndrome died from squamous cell carcinoma 4 years after HSCT complicated by GVHD, which had been treated with two doses of DSCs \([18]\). She was a heavy smoker and had sun-tanned extensively.

A 67-year-old man with chronic lymphocytic leukemia was treated with four doses of DSCs for acute GVHD. One year after HSCT, he was operated on for squamous cell carcinoma and basal cell carcinoma at the site of a pretransplant actinic keratosis in the face. Two years after HSCT, a malignant melanoma was removed radically from his back.

**DISCUSSION**

Here we present data on 38 patients with severe acute gastrointestinal GVHD treated with placenta-derived DSCs. The treatment protocol was changed after the first 17 patients, because the

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### Table 2. Patient characteristics for all steroid-refractory DSC-treated patients and controls

| Characteristics | SR group 1, n = 13 | SR group 2, n = 11 | SR MSC, n = 15 | SR controls, n = 32 |
|-----------------|-------------------|-------------------|----------------|---------------------|
| Sex (M/F)       | 6/7               | 7/4               | 11/4           | 18/14               |
| Age at GVHD, years, median (range) | 54.8 (16.4–64.4) | 42.4 (1.6–53.9) | 57 (34–65) | 40.65 (3.7–67.7) |
| Diagnosis (malignant/nonmalignant) | 11/2 | 8/3 | 15/0 | 32/0 |
| Disease status (high risk/low risk) | 8/5 | 6/5 | 6/7 | 17/12 |
| Conditioning (MAC/RIC) | 7/6 | 3/8 | 8/7 | 20/12 |
| ATG (yes/no) | 6/7 | 7/4 | 9/6 | 20/12 |
| GVHD prophylaxis | | | | |
| CsA/MTX | 10 | 6 | 14 | 25 |
| CsA/MMF | 0 | 0 | 1 | 7 |
| TAC/SIR | 2 | 3 | 0 | 0 |
| CsA/MTX/Cy | 1 | 2 | 0 | 0 |
| Donor SIB/MUD/CB/haplo | 6/7/0/0 | 4/6/0/1 | 9/5/1/0 | 11/19/2/0 |
| Graft source (PBSCs/BM/CB) | 11/2/0 | 8/3/0/1 | 1/13/1 | 25/5/2 |
| GVHD grade at time of intervention (2/3) | 2/11 | 4/7 | 0/15 | 9/23 |
| GVHD localization (gut and other/only liver) | 13/0 | 11/0 | 15/0 | 27/5 |
| CMV (double-neg./any pos.) | 2/11 | 4/7 | 1/14 | 2/30 |
| GVHD after DLI (yes/no) | 0/13 | 1/10 | 5/10 | 5/27 |
| HSCT/DLI steroids, days (range) | 33 (10–375) | 27 (5–200) | 28 (11–94) | 25 (8–171) |
| Steroids DSCs, days (range) | 18 (7–37) | 7 (3–23) | 23 (3–90) | N/A |
| Number of infusions (range) | 1 (1–3) | 3 (2–6) | 1 (1–3) | N/A |
| Cell dose (range) | 2.0 (0.9–2.8) | 1.2 (1.0–2.9) | 1.5(0.7–2.0) | N/A |
| Cell passage (range) | 2 (2–3) | 4 (2–4) | 3 (2–3) | N/A |
| Viability, % (range) | 90 (70–97) | 94 (69–100) | >95 | N/A |

**Abbreviations:** ATG, antithymocyte globulin; BM, bone marrow; CB, cord blood; CMV, cytomegalovirus; CsA, cyclosporine A; Cy, cyclophosphamide; DLI, donor lymphocyte infusion; DSCs, decidua stromal cells; f, female; GVHD, graft-versus-host disease; HSCT, allogeneic hematopoietic stem cell transplantation; M, male; MAC, myeloablative conditioning; MTX, methotrexate; MUD, matched unrelated donor; N/A, not applicable; neg., negative; PBSCs, peripheral blood stem cells; pos., positive; RIC, reduced-intensity conditioning; SIB, sibling donor; SIR, sirolimus; SR, steroid refractory; TAC, tacrolimus.
cell-handling procedures were optimized during the intervention. With our current protocol (corresponding to group 2), the survival of patients with steroid-refractory acute GVHD was similar to that in all patients who underwent HSCT at our center in the last 5 years. It should be noted that patients who survived severe acute GVHD, treated with conventional immunosuppressive therapy, have a significantly worse outcome than all other HSCT patients [26]. In contrast, patients who were treated with DSCs for severe acute GVHD had much better survival. These promising results are based only on 11 patients. If confirmed in a larger prospective trial in future, this will be a breakthrough in the treatment of severe acute GVHD.

The use of active human AB plasma as a supplement in the thawing and infusion solution for DSCs resulted in a significantly lower viability of the cells compared with the use of albumin. Group 1 received almost twice as many cells, so the amounts of infused, viable DSCs might be expected to have been comparable in the two groups. Thus, the improved survival for the patients in group 2 was probably not because of a higher number of viable cells. It is tempting to speculate that active human AB plasma contains functional complement factors that might bind to the DSCs during thawing, thus priming them for lysis during intravenous infusion. The role of complement in lysing MSCs was previously investigated by Li et al. [27]. The MSCs were washed and dissolved in AB plasma, and it cannot be excluded that MSC therapy may also be improved by being dissolved in albumin.

A previous study from our center showed that patients who received MSCs at a lower passage number had a better survival than those who received MSCs at a higher passage number [13]. Despite the higher passage number of DSCs used in group 2, the clinical outcome was better than in group 1. This suggests that passage number may be less important for DSCs than for MSCs regarding treatment of GVHD. All of the steroid-refractory patients in group 2 responded to DSC treatment, and 7 of 11 patients had a complete response after 4 weeks. In group 1, response rates and survival were comparable to the MSCs group in this study and what has been shown previously using MSCs [10–14, 28]. In the controls, one fourth of the patients with steroid-refractory acute GVHD showed a response to steroids after 4 weeks (Fig. 2). However, these responses did not result in improved survival (Fig. 3A). In patients with severe acute GVHD who were treated with MSCs, the survival of those with a partial response was not improved [11]. This is in contrast to group 2, in which survival was improved in those with a partial response.

Based on the findings in this study and the safety report, it appears that treatment with DSCs is safe [25]. The causes of SAEs and deaths were infections, relapse, and other common complications seen among patients undergoing HSCT, especially those with severe acute GVHD. Two patients had squamous cell carcinoma—one of whom died. Squamous cell carcinoma and other skin malignancies are common secondary malignancies in patients who have undergone HSCT or organ transplantation [29]. In addition to transplantation, these two patients had risk factors for squamous cell carcinoma. Three patients had graft failure, which is relatively common after HSCT in patients receiving reduced-intensity conditioning [30]. Six patients had invasive fungal infections despite prophylaxis. Given the nature of GVHD and the different immunosuppressive therapies used, patients with acute GVHD can be
expected to be heavily immunocompromised. Therapies that have
immunomodulatory effects—including DSCs and MSCs—can be
expected to give an increased risk of infections. We also saw a
high frequency of invasive fungal infection in the controls and in
patients treated with MSCs [12]. In a safety study, we found that
the side effects and causes of death were similar in patients with
GVHD and hemorrhagic cystitis who were treated with DSCs and
in controls treated with other therapies [25]. Because the patients
treated with DSCs have survived longer than expected, they have
had more time to experience severe adverse events after HSCT
complicated by acute GVHD and heavy immunosuppressive treat-
ment. Many more long-term survivors of acute GVHD will be
required to determine whether any particular causes of death and
severe adverse events are associated with stromal cell therapy.

The mechanism by which stromal cells overall exert their
immunosuppressive effects has not yet been fully investigated.
Homing to the spleen and mobilization of macrophages to exert
an anti-inflammatory and immunosuppressive effect is one mech-
anism for bone marrow-derived stromal cells [31]. Similar effects
are possible but not studied using DSCs. DSCs are dependent on
cell-to-cell contact to perform their immunomodulatory function
in vitro [19]. In mixed lymphocyte cultures, regulatory T cells were
increased in the presence of DSCs, a mechanism that was contact
dependent [19]. Blocking experiments suggest that interferon-γ,
prostaglandin E2, indoleamine dioxygenase, and PD-L1 are
involved in the immunosuppressive mechanism of DSCs [19].
DSCs are more advantageous than many other stromal cells, as
the use of term placenta provides an almost unlimited supply of
cells, and there is no need for any invasive procedure for isolation.

MSCs also have functions to differentiate along several mes-
enchymal cell lineages and, in addition, have immunosuppressive
properties. The main function for DSCs seems to be to protect the
fetus from the mother’s HLA-incompatible T cells and have little, if
any, differentiation capacity [21]. Stronger expression of PD-L1,
PD-L2, and CD49d may explain why DSCs are more immunosup-
pressive than MSCs from bone marrow [18].

When a novel therapy is introduced in the clinic, it is often
first tried in end-stage or terminally ill patients, which was the
case when we used MSCs and DSCs [9, 10, 18]. According to the
Declaration of Helsinki, doctors have the possibility to try a ther-
apy that may help a dying patient even though clinical documenta-
tion is missing. When a positive effect is seen with acceptable side
effects, the therapy is subsequently given earlier and earlier,
with successively improved results. Such an effect is probably also
seen using DSCs as well as MSCs. The earlier you treat, even in the
case of severe GVHD, the more likely you are to rescue the
patient. The two protocols of DSC1 versus 2 and the comparison
with MSCs is obscured by the timing of therapy. When prospective
trials are planned, timing must be considered in order to save as
many patients with severe acute GVHD as possible.

Some limitations of the study were, apart from timing, the ret-
rospective approach with a small, heterogeneous patient
population. The controls were historic, and HSCT therapy has
improved in more recent years [32]. The controls were signifi-
cantly younger, and young age is important for survival of severe
acute GVHD [4]. Therefore, the data should be interpreted with
cautions.

**CONCLUSION**

This study has shown promising results in treatment of severe
acute GVHD with DSCs. To further assess safety and efficacy, a
larger, prospective trial will be necessary. If an effective therapy
for severe acute GVHD is indeed found and validated, it will
increase the usefulness of HSCT, with a possible broadening of
indications.

We also used DSCs successfully to treat acute respiratory dis-

**ACKNOWLEDGMENTS**

This study is dedicated to the Seattle team who taught us to
perform HSCT. We thank the staff of the participating depart-
ments for compassionate and competent care of patients. We
want to thank Gunilla Tilling and Inger Holmström for help in
preparing this manuscript. This study was supported by grants
to O.R. from the Swedish Cancer Society (CAN2013/671), the
Swedish Research Council (K2014-64X-05971-34-4), the Swedish
Childhood Cancer Foundation (PR2013-0045), the Cancer Soci-
ety in Stockholm [111293], Stockholm County, and Karolinska
Institutet.

The sponsor of this study had no role in the study design, in
the collection, analysis, and interpretation of data, or in the
writing of the report. The corresponding author and the first
author had full access to all the data and had final responsibility
for the decision to submit for publication.

**AUTHOR CONTRIBUTIONS**

O.R.: initiation of the studies of MSCs and DSCs, study design,
data collection, data analysis, manuscript writing; A.B.: data col-
clection and analysis; M.R.: data collection, statistical analysis;
B.G.: clinical data; B.K.: study performance, analysis, design;
G.M.: data, expertise; L.K.: evaluation of fungal infection; M.W.: or-
ganization and support of the logistics for retrieval of the pla-
centas; B.S.: study performance, analysis, and design. All the
authors contributed to interpretation of the results and to the
final preparation of the article.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

L.K. received honoraria from ABBOTT and Gilead for presentations
at meetings sponsored by the companies. The other authors indi-
cated no potential conflicts of interest.

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