Placenta-Derived Decidua Stromal Cells for Hemorrhagic Cystitis after Stem Cell Transplantation

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Keywords

Allogeneic stem cell transplantation · Hemorrhagic cystitis · Mesenchymal stromal cells · Placenta-derived decidua stromal cells

Abstract

Background/Aims: Hemorrhagic cystitis (HC) is a serious complication after hematopoietic stem cell transplantation (HSCT). Stromal cells have been tested as therapy for HC. Decidua stromal cells (DSCs) protect the fetus from the mother's immune system. **Methods:** Eleven patients with HC of grades 3–4 were treated with DSCs after HSCT. The median age was 33 years (range 8–50), and the median dose of DSCs was $1.5 \times 10^6/\text{kg}$ (range 0.7–2.5). The patients were given 1 dose (1–4). **Results:** In 5 patients, HC disappeared within 5 days after DSC infusion. Patients who received DSCs within 3 days after the start of HC had a duration of HC of 5 days and a shorter duration of pain than patients who were given DSCs later ($p = 0.02$). Three patients received DSCs prepared

in albumin instead of AB-plasma and tended to have a shorter duration of pain ($p = 0.07$). There was no infusion toxicity. Adverse events were those often seen after HSCT. Nine of the 11 patients (82%) were alive 1 year after HSCT. **Conclusions:** Based on this pilot study, we started a randomized, placebo-controlled double-blind study using 2 doses of 1×10^6 DSCs/kg suspended in albumin for treatment of early HC.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established therapy for the treatment of hematological malignancies, severe aplastic anemia, and certain rare congenital metabolic defects [1, 2]. The side effects of HSCT include toxicity of the conditioning regimen, bacterial, viral, and fungal infections as a result of immunosuppression, and graft-versus-host disease (GVHD) [3].

Hemorrhagic cystitis (HC) may occur after HSCT either as early-onset disease due to conditioning toxicity, or late-onset disease associated with GVHD or viral infections such as BK virus, adenovirus, and cytomegalovirus (CMV) [4–9]. The incidence of HC after HSCT has been reported to range from 10 to 70%. There is no effective treatment, and 1-year mortality in patients with moderate-to-severe HC is 55–70% [8]. Treatments that have been tried for HC include hyperhydration and diuretics, oral prostaglandin E2, catheterization for bladder irrigation and clot removal, cystoscopy to cauterize bleeding points, bladder irrigation, analgesics, antispasmodics, antivirals including vidarabine, ribavirin, and cidofovir, estrogens, and hyperbaric oxygen [10, 11]. Severe cases may be treated with urethral catheterization, nephrostomy, dialysis, and ligation of hypogastric arteries with or without cystectomy [12, 13].

Mesenchymal stromal cells (MSCs) suppress alloantigen-induced T cells in vitro and have immunomodulatory and anti-inflammatory effects combined with low immunogenicity [14–16]. We introduced MSCs as a treatment for severe steroid-refractory acute GVHD and HC [17–19]. Recently, the feasibility of MSCs as a treatment for HC was confirmed by others [20, 21].

The term placenta and its adjacent tissues provide a ready source of stromal cells [22, 23]. We isolated maternal decidua stromal cells (DSCs) from the fetal membranes of term placentas that were obtained after caesarian section [24, 25]. The DSCs are immunosuppressive in vitro, and they express surface markers that are important for homing to inflamed tissues [24, 25]. DSCs differ from bone marrow (BM)-MSCs in several aspects: they are half the size, more immunosuppressive, need direct contact to show the immunomodulatory effect, have a better expansion capacity, and do not differentiate well to cartilage and bone [24–26]. We have used DSCs after HSCT to treat severe acute GVHD, chronic GVHD, and acute respiratory distress syndrome, with positive responses in the vast majority of patients [27–29]. There are also in vitro data to suggest that DSCs promote clotting, which is in line with the fast recovery after postpartum hemorrhaging [26]. In this study, we wanted to find an optimal treatment for HC using DSCs, to be able to continue with a prospective double-blind, placebo-controlled study and test the possible efficacy.

Materials and Methods

Study Design

This was a prospective pilot study investigating safety and efficacy. The primary endpoint was the disappearance of macro-

scopic hematuria. Secondary endpoints included infusion toxicity, side effects, time to disappearance of pain, infections, causes of death, and acute and chronic GVHD.

Grading of HC

HC was graded from 0 to 4 according to the scale proposed by Droller et al. [30]: 0 meant that there was no hematuria, 1 that there was microscopic hematuria, 2 that there was macroscopic hematuria, 3 that there was macroscopic hematuria with clots, and 4 that there was obstructive clotting perhaps requiring invasive clot evacuation. The patient's pain was graded from 0 to 3 according to the level of analgesia required: 0 required no analgesics, 1 needed oral administration of paracetamol, 2 required oral administration of opiates, and 3 needed intravenous administration of opiates and/or epidural administration of analgesics. The pain level reported in this study was the highest level of pain experienced by the patient during active HC.

Patients

Three patients had myelodysplastic syndrome and 1 had also developed severe aplastic anemia. Six had acute myeloid leukemia, 1 had acute lymphoblastic leukemia, and 1 had chronic myeloid leukemia. Eight patients with grade 3 HC and 3 patients with grade 4 HC were treated with DSCs. The patient characteristics are detailed in Table 1. The patients signed written informed consent to receive DSCs for HC. All patients failed treatment with oral prostaglandin E2 and hyperhydration with diuretics.

Ethical Approval and Regulatory Issues

Ethical approval was provided by the Ethics Committee, Karolinska Institutet, to collect stromal cells from placenta after caesarian sections (2009/418-31/4), and to use DSCs clinically for tissue toxicity, hemorrhages, and GVHD (2010/452-31/4). Approval was also given to perform a randomized study for HC to compare DSCs with placebo (2013/2147-31/3). The placenta donor signed a written informed consent form. According to agreement from the Swedish Food and Drug Agency (because it was an academic study), the trial was started while working on approval for chemistry, manufacturing, and control.

From 2012 to 2014, DCSs were used under the so-called hospital exemption according to the declaration of Helsinki and approved by the Swedish Drug Agency as a pilot study. The GMP laboratories at the Blood Bank, Karolinska University Hospital, Huddinge, used for culturing of DSCs is licensed as a tissue establishment by the Health and Social Care Inspectorate of Sweden. They also stated that DSCs fulfil their criteria of quality and safety (6.1.3.42994/2013). During recent years we have been working on obtaining approval for the production of DSCs according to the EU regulation (1394/2007). The trial is registered at Clinical Trials NCT02172963.

Transplantation Procedure

The patients with myeloid malignancies were treated with busulfan (16 mg/kg) and cyclophosphamide (Cy; 120 mg/kg; Table 1) [31]. The patient with acute lymphoblastic leukemia was treated with Cy and fractionated total body irradiation (12 Gy). The MDS patient who developed aplastic anemia was treated with reduced-intensity conditioning consisting of fludarabine (30 mg/m²) for 6 days followed by Cy (120 mg/kg) [32]. Grafts were from 2 HLA-identical siblings, 6 matched unrelated donors, 2 HLA-mismatched unrelated donors, and 1 unrelated cord blood transplant. The re-

Table 1. Patient characteristics

Patient No.	Gender/age, years	Diagnosis	Donor	Conditioning	Immuno-suppression	CD34+ cell dose, $\times 10^6/\text{kg}$	Days to engraftment (after HSCT)	
							ANC $>0.5 \times 10^9/\text{L}$	platelets $>30 \times 10^9/\text{L}$
1	M/32	AML CR1	MUD	Bu+Cy	CsA+Mtx	7.9	16	13
2	M/49	AML CR1	Sib	Bu+Cy	CsA+Mtx	3.9	14	14
3	F/8	CML 2CP	MMCB	Bu+Cy	CsA+Pred	0.04	Rejection	Rejection
4	F/49	AML CR1	MMUD	Bu+Cy	CsA+Mtx	5.1	12	12
5	F/40	MDS	MMUD	Bu+Cy	CsA+Mtx	7.4	16	17
6	F/23	AML CR2	MUD	Bu+Cy	CsA+Mtx	8.5	13	13
7	F/43	AML CR1	MUD	Bu+Cy	CsA+Mtx	9.7	12	14
8	M/44	MDS	MUD	Bu+Cy	CsA+Mtx	8.8	19	18
9	M/36	ALL CR1	MUD	fTBI+Cy	CsA+Mtx	8.4	20	20
10	M/50	MDS, SAA	Sib	Flu+Cy	CsA+Mtx	0.7	20	20
11	F/47	AML CR1	MUD	Bu+Cy	CsA+Mtx	2.0	19	18

M, male; F, female; AML, acute myeloid leukemia; MUD, matched unrelated donor; Bu, busulfan; Cy, cyclophosphamide; CsA, cyclosporine; Mtx, methotrexate; Sib, HLA-identical sibling; CML, chronic myeloid leukemia; MNCB, HLA-mismatched cord blood; MMUD, HLA-mismatched unrelated donor; Pred, prednisolone; MDS, myelodysplastic syndrome; SAA, severe aplastic anemia; fTBI, fractionated total body irradiation; Flu, fludarabine.

recipient of the cord blood graft had a rejection and was regrafted with her mother's haploidentical graft. Antithymocyte globulin was given to 10 patients before transplant [33]. GVHD prophylaxis consisted of cyclosporine and short-term methotrexate [34]. The recipient of the cord blood graft received cyclosporine and prednisolone. The patients were treated in reverse isolation or at home during the pancytopenic phase if they lived within 2-h driving distance from the hospital [35]. Acute GVHD was graded according to the Seattle criteria [36]. Chronic GVHD was graded as mild, moderate, or severe [37, 38].

Isolation and Preparation of DSCs

DSCs were isolated from the fetal membranes of term placentas that were obtained after caesarian section, as described in detail previously [25, 28]. Briefly, human term placentas were obtained from healthy mothers during elective caesarean section births, after obtaining informed consent. All donors were seronegative for HIV, hepatitis A and B, and syphilis. CMV was not checked for because it is not carried by stromal cells [39]. The placenta was put in a sterile container and immediately transferred to the laboratory for processing. The fetal membrane was carefully dissected from the placenta in a class II biosafety flow-cabinet and transferred to a sterile beaker for extensive washing in Hyclone phosphate-buffered saline (PBS; Thermo Fisher Scientific, Waltham, MA, USA; <http://www.thermofisher.com>). The tissue was cut into smaller pieces and transferred to 50-mL Falcon tubes (BD Biosciences, San Jose, CA, USA; <http://www.bdbiosciences.com>). An equal volume of trypsin/EDTA (Thermo Fisher Scientific) was added to the tissue for 10 min at 37°C and was then discarded. The tissue was then incubated twice in trypsin/EDTA for 40 min at 37°C, and the trypsin digests were pooled and washed in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific) containing 10% fetal calf serum (Thermo Fisher Scientific) and penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA; <http://www.invitrogen.com>).

The digested cells were counted and seeded at 2.9×10^5 cells per cm^2 in Nunc T175 flasks (Nunc A/S, Roskilde, Denmark) using complete DMEM. The remains of the fetal membrane were washed in complete DMEM and cut into 3- to 4- cm^2 pieces, and these were spread out and incubated in T175 flasks. The tissue explants were removed from the flasks when colonies of fibroblast-like cells appeared after approximately 1 week of culture. When the cells from the trypsin-digested suspension and from the tissue explants were about 90–95% confluent, the cells were harvested with trypsin/EDTA, washed in complete DMEM, and seeded in new T175 flasks at 2.9×10^3 cells per cm^2 in complete DMEM. The cells were cultured to passage 2 or 3 and frozen slowly in complete DMEM containing 10% dimethyl sulfoxide (WAK-Chemie Medical GmbH, Steinbach, Germany). DSCs were expanded and cultured under good manufacturing practice conditions using a room with reversed isolation, a sterile bench, and a separate incubator for cells from each donor. In all cases, DSCs were evaluated for mycoplasma or bacterial infection before infusion.

Karyotype Analysis of DSCs

Karyotype analysis with conventional CTG-banding of DSC cultures was performed using standard cytogenetic procedures. At least 11 metaphase nuclei from each sample were analyzed.

Characterization and Administration of DSCs

The DSCs expressed CD29, CD44, CD49d, CD73, CD90, CD105, CD54, HLA class I, programmed death ligand-1 (PD-L1), and PD-L2 [28]. The details of expression and variability were previously published in detail [28]. The cells were negative for the hematopoietic markers CD45 and CD34, and for epithelial (EpCAM) and endothelial (CD31) markers. They showed a poor differentiation capacity to bone, fat, and cartilage [24]. Before infusion, the DSCs given to 8 patients were thawed in PBS supplemented with 10% AB plasma. Three patients were given DSCs dissolved

Table 2. DSC data and clinical presentation of the study patients

Case No.	Hemorrhagic cystitis		Days to DSC infusion after HC	Pas-sage	DSC cell dose, $\times 10^6/\text{kg}$	Viability, %	Solution	DSC infusions, <i>n</i>	Days to resolution of macroscopic hematuria after DSC treatment	GVHD		Outcome
	day of onset	grade								acute grade	chronic	
1	58	3	9	2	1.5	83	AB	1	2	III	Mild	MOF, sepsis, died on day 581
2	28	4	6	3	1.6	89	AB	1	No resolution	III	Severe	Died on day 389, infections, cardiac failure
3	42	4	51	3	2.0	86	AB	1	3, transient disappearance of HC	No	No	Rejected CB graft; re-transplanted with a haploidentical maternal graft; alive and well after 3 years and 6 months, but HC data are unavailable
4	35	3	19	3	1.3	92	AB	1	5	I	Mild	Alive and well after 3 years and 7 months
5	56	3	7	3	2.5	75	AB	1	24	II	No	Alive and well after 3 years and 5 months
6	56	3	10	3	1.4	91	AB	1	Intermittent to day 65	No	Mild	Died from relapse after 363 days
7	27	3	8	3	2.1	86	AB	1	11	I	No	Alive and well after 3 years and 7 months
8	41	3	1	4	2.5	94	AB	1	5	II	Obstructive lung disease?	Alive after 3 years and 4 months
9	32	3	0	4	1.5	95	Alb	1	5	III	–	Died from sepsis and pneumonia on day 411
10	13	4	6	3–4	1.2 (0.7–1.7)	91–96	Alb	4	7, intermittent effect	No	No	CMV, EBV infections, graft failure; died from <i>Klebsiella</i> sepsis with alveolar hemorrhage on day 153
11	41	3	2	3	1.6, 1.0	95–99	Alb	2	5	No	No	Alive and well after 2 years and 5 months

HC, hemorrhagic cystitis; DSC, decidua stromal cell; GVHD, graft-versus-host disease, CB, cord blood; CMV, cytomegalovirus; EBV, Epstein-Barr virus; MOF, multiple organ failure; AB, DSCs suspended in saline and 10% AB plasma; Alb, DSCs suspended in saline and 5% albumin.

in PBS and 5% albumin. The DSCs were infused through a central venous line. For adults, the line was flushed with 5 mL of saline with 50 IU heparin/mL both before and after the infusion of DSCs. Children were given lower doses of heparin.

Infections

Viruses such as adenovirus, Epstein-Barr virus (EBV), and CMV were detected using PCR. CMV reactivation was assumed if there were >2,000 copies detected in blood by PCR and, combined with this, CMV disease was defined as a symptomatic infection [40]. Superficial, cutaneous, and oral mycoses were not taken into account.

Statistics

Continuous variables were calculated using the Mann-Whitney U test due to the small number of patients and their nonnor-

mal distribution. Any *p* values <0.05 were considered significant. Analysis was performed using Statistica 12 software (StatSoft®, Tulsa, MD, USA).

Results

The median time from HSCT to HC was 41 days (13–58). The overall median duration of HC was 22 days (5–361). The first 8 patients were given 1 dose ranging from 1.3 to 2.5×10^6 DSCs/kg. The last 3 patients were given 1, 4, and 2 doses of DSCs, respectively. The doses were between 0.7 and $1.7 \times 10^6/\text{kg}$. The median viabilities of the

Fig. 1. **a** Duration of macroscopic hematuria in patients who were given DSCs within 3 days (early DSC treatment), or later after the start of HC ($p = 0.02$). **b** Duration of pain after the start of HC in patients who were treated with DSCs within 3 days (early DSC treatment) or later ($p = 0.02$). **c** Duration of pain after the start of HC in patients who were treated with DSCs suspended in saline with 10% AB plasma ($n = 8$) or in saline with 5% albumin ($n = 3$).

DSCs in PBS with AB or albumin supplementation were 87% (75–94) and 95% (91–99), respectively ($p < 0.01$). All cultured DSCs were negative for bacterial or mycoplasma infection; moreover, no karyotype abnormality was detected in cultured DSCs.

In 5 of the patients who were treated with DSCs, HC defined as macroscopic hematuria disappeared within 5 days after infusion with DSCs (patients 1, 4, 8, 9, and 11; Table 2). Patient 1 had recurrent HC of grade 3, which was treated with DSCs after 9 days. He had clots, pain requiring opiates, and 4+ hematuria at the time of infusion with DSCs. Macroscopic hematuria did not recur after infusion and the pain disappeared after 17 days. In 3 patients (No. 5–7), macroscopic hematuria disappeared on day 11, 24, and 65 after DSC infusion. One patient (No. 3) improved clinically and had less pain, but there was no improvement in the grade of HC. No further data regarding HC status were retrieved since she was sent to her home country. She is, however, still alive and well at the time of writing. Two patients (No. 2 and 10) died from causes other than HC, but they had macroscopic hematuria at the time of death.

We compared 3 patients who received early DSC intervention less than 3 days after the appearance of HC with the remaining patients ($n = 7$). In all 3 patients, macroscopic hematuria disappeared after 5 days, as compared to a median time of 31 days (8–361) in those who had a later intervention ($p = 0.02$; Fig. 1a). Two of the patients are alive, but 1 died a year later due to pneumonia.

At the time of treatment, 3 patients had grade 2 pain and 8 patients had grade 3 pain. The median duration of

pain was 21 days (4–135). The patients who had a DSC infusion within 3 days of the appearance of HC ($n = 3$) were free of pain after a median time interval of 6 days (4–9), as compared to the other patients in the study ($n = 8$) who were pain-free 31 days (14–135) after the appearance of HC ($p = 0.02$; Fig. 1b).

The 3 patients who received DSCs in albumin-supplemented PBS rather than AB plasma-supplemented PBS tended to have a shorter duration of pain than the other patients in the study group (Fig. 1c; $p = 0.07$). The patients spent a median of 14 days (2–141) in hospital after treatment with DSCs for HC.

Infections

Infections were compared before and after the infusion of DSCs. Bacteremia were seen in 5 patients before DSC infusion and in 3 also after DSC infusion. Candidemia was diagnosed in 1 patient (No. 1) before infusion with DSCs, but in none after infusion. Herpes simplex virus infection was seen in 3 patients before infusion and in none after infusion. CMV reactivation was diagnosed in 5 patients before infusion, but in none after infusion. CMV disease was diagnosed in 1 patient before DSC infusion and in another after infusion. Adenovirus was diagnosed in 3 patients before infusion and in 4 after infusion. BK virus was diagnosed in 2 patients before infusion and in 2 after infusion. After infusion with DSCs, the following viruses were diagnosed: varicella-zoster virus ($n = 3$), EBV (2), coronavirus (1), influenza A virus (1), and norovirus (1).

Fig. 2. a Time to and cumulative incidence of TRM in 11 patients who were treated for HC with DSCs (1 year 9%, 2 years 39%). **b** Overall survival time after stem cell transplantation in 11 patients who were treated for HC with DSCs (1 year 82%, 2 years 52%).

Severe Adverse Events and Causes of Death

Infusion toxicity was not seen in any of the patients. Patient 1 developed acute GVHD of grade III 2 months after he was treated for HC. He was given DSCs and improved, but later developed a chemotherapy-induced cardiomyopathy together with liver and kidney failure. He died 181 days after HSCT from septicemia and gastrointestinal hemorrhage. Patient 2 had several bacterial and viral infections, and a suspected Addisonian crisis, and died of cardiac failure 389 days after HSCT while still suffering from macroscopic hematuria. Patient 6 died of leukemic relapse a year after HSCT.

In patient 8, HC symptoms disappeared 5 days after infusion and the pain disappeared after 6 days. However, he has since contracted several viral infections and

is suffering from chronic thrombocytopenia and obstructive lung disease, which may be a sign of chronic GVHD. He has had no other symptoms of chronic GVHD.

Patient 9 had early recovery of hematuria and pain and later developed grade III acute GVHD that was responsive to steroids. He died from adenovirus pneumonia and septicemia 411 days after HSCT.

Patient 10, who had aplastic anemia subsequent to therapy for myelodysplastic syndrome, developed grade 4 HC 13 days after HSCT. He improved temporarily in HC after each DSC infusion and received in total 4 DSC infusions for HC. He needed a boost of donor cells due to graft failure ($ANC < 0.5 \times 10^9/L$) 2 months after his last DSC infusion. He had a difficult course, with CMV reactivation being treated with intravenous ganciclovir, foscarnet, maribavir, and CMV-cytotoxic T cells. EBV reactivation was treated with rituximab. Due to graft failure, he was treated with donor lymphocyte infusions and subsequently with booster marrow that was depleted of α - β -T cells. A month after the last DSC infusion, he had dyspnea and a pulmonary veno-occlusive disease was suspected. He died of *Klebsiella* septicemia and pneumonia 153 days after HSCT. Autopsy and microscopy showed intra-alveolar hemorrhaging and no thromboembolism in the lung tissue.

GVHD and Survival

Five of the patients developed grade II/III acute GVHD (Table 2). Four developed acute GVHD grade II to III before HC and DSC infusion. Patient 1 developed grade III acute GVHD 2 months after the HC and DSC infusion. Three patients developed mild GVHD and 1 developed severe chronic GVHD. One patient had obstructive bronchiolitis.

Transplant-related mortality (TRM) was 9% at 1 year and 39% at 2 years (Fig. 2a). One-year survival was 82% and 2-year survival was 52% (Fig. 2b).

Discussion

This study was the first investigation of the effect of a novel treatment using infusion with DSCs as a therapy for the treatment of HC in HSCT patients. Because DSCs induce coagulation more effectively than BM-MSCs, they were selected for the treatment of HC in this pilot study [26].

Five out of the 11 patients experienced the disappearance of HC symptoms after DSC intervention. In addi-

tion, 3 other patients experienced the transient disappearance of symptoms. The most interesting group was that of the 3 patients who received DSCs within 3 days of the start of HC. These patients were all free from macroscopic hematuria within 5 days and they were pain free within 9 days. In a retrospective survey, Hassan et al. [8] found that the median duration of hematuria in HC of grade 3 or higher, without stromal cell therapy, was 42 days. This was twice as long as the 22 days observed for the 11 patients in the present study.

Previous data indicate that BM-MSCs can elicit a response in the majority of patients who suffer from HC [19, 41]. In one of the studies, macroscopic hematuria disappeared within a median period of 3 days in the responders, who were all treated early after the appearance of HC. Furthermore, in a study by Wang et al. [21], 6 out of 7 patients received MSC infusions within 3 days of the start of HC. In that study, MSCs from Wharton jelly were used, which gave a median time to remission of 18 days (8–60) for HC of grade 3 or 4. In GVHD, younger patients appear to have a better response to MSC therapy than older patients [42]. In the study by Wang et al. [21], only 1 patient was over 30 years of age. In contrast, in the present study, only 2 patients were below 30 years of age.

Stromal cell therapy has so far been reported to be safe, with little infusion toxicity [14–16, 18, 19, 42, 43]. We did not see any infusion toxicity in any of the 17 DSC infusions in this study. Because of the low toxicity, stromal cells seem better than all other therapies used for severe HC, such as bladder irrigation, cystoscopy to cauterize bleeding points, and others [10–13]. DSC is favorable to other sources of stromal cells as no invasive procedure is needed and there is unlimited supply.

It should be noted that HC appears more frequently in high-risk patients who undergo HSCT using myeloablative conditioning [7, 8]. In our previous experience, patients with grade 3 HC have a 1-year TRM of 55%, and those with grade 4 or grade 5 HC have a TRM of 71% [8]. The patients treated with DSCs for HC have a more favorable TRM than those treated conservatively [8]. Regarding toxicity using DSCs, we performed a detailed study including 44 patients treated for acute GVHD or HC and compared them to historical controls [44]. It was a detailed study of safety and side-effects using a novel clinical therapy: DSCs for acute GVHD in 34 patients, and HC in 10 patients. The study also included controls receiving conventional therapy. Severe adverse events and cause of death were similar in the DSC group and the controls. The control patients more often died from acute GVHD.

The present study is the efficacy evaluation using DSCs for HC.

Acute GVHD grade II/III was seen in 5 of the 11 HC patients. There is a correlation between acute GVHD and HC [9]. Four had GVHD before HC and 1 developed it later.

Regarding infections, herpes simplex virus infection and bacteremia were more common before DSC infusion than after. This corresponds to the time of onset of HC, as bacteremia and HSV infection often appears during the pancytopenic phase after HSCT [10]. CMV reactivation was seen in 5 patients and only before infusion with DSCs. An in vitro study showed that MSCs did not impair CMV-cytotoxic T cells, in contrast to their effect on allo-reactive T cells [45]. These observations might at least suggest that, although immunosuppressive, DSCs may not increase the risk of CMV reactivation and infection.

So far, there is limited experience of using human DSCs in experimental animals. A study showed that mice treated with 9-Gy total body irradiation, HSCT, and very high doses of human DSCs (40×10^{-6} /kg) died from pulmonary embolism [46].

The source of MSCs that should be used in the treatment of HC is debatable. Both BM-MSCs and Wharton jelly-derived MSCs have shown promising results [16, 19, 21, 41]. There are several theoretical advantages to using DSCs over BM-MSCs for HC, such as smaller size, better coagulation capacity, less differentiation to cartilage and bone, and an increased expression of integrins that may facilitate migration to inflamed tissue [25, 26, 28].

To conclude, early treatment within 3 days of the start of HC and the use of DSCs thawed in albumin-supplemented saline appear to be beneficial based on the results of this small pilot study. DSCs washed and dissolved in PBS and 5% albumin had a significantly better viability than those dissolved in PBS and 10% AB serum. Patients with acute GVHD treated with DSC dissolved in albumin had a 1-year survival of 76% as opposed to 44% in those receiving DSC dissolved in AB serum [47]. The side effects are those commonly seen in HSCT patients. Based on this pilot study, a randomized placebo-controlled study has been started to evaluate the safety and efficacy of using DSCs for the treatment of HC in patients with grade 3–4 HC and a viral infection found within a minimum of 3 days, and who do not respond to forced diuresis, prostaglandin E2, and antivirals.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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