Placenta derived decidua stromal cells - a new frontier in the therapy of acute graft-versus-host disease

Olle Ringdén, Behnam Sadeghi
Placenta derived decidua stromal cells - a new frontier in the therapy of acute graft-versus-host disease

Olle Ringdén¹ and Behnam Sadeghi¹

¹ Translational cell therapy research (TCR), Division of Pediatrics, Department of Clinical science, Intervention and Technology (CLINTEC), Karolinska Institutet, Huddinge.

Author contribution:
O.R.: concept and design, financial support, provision of study material and patients, collection and assembly of data, data analysis and interpretation, writing the first manuscript, editing and approval of manuscript.
B.S.: concept and design, financial support, provision of study material and patients, collection and assembly of data, data analysis and interpretation, writing and revision of manuscript, editing and approval of manuscript.

Correspondence: Olle Ringdén, M.D., Ph.D., Karolinska Institutet, Translational Cell Therapy Research (TCR), Kliniskt Forskningscentrum (KFC), Novum, Hälsovägen 7-9, 141 57 Huddinge, Sweden. Tel. +46858582616; e-mail: olle.ringden@ki.se

Significant Statement
Graft-versus-host disease (GVHD) is a serious complication after allogeneic hematopoietic cell transplantation. If patients don’t respond to corticosteroids, it’s fatal with slim chances of survival. Numerous treatments have been tried but often abandoned due to ineffectiveness. Mesenchymal stem cell therapy was introduced as a biological treatment. This article reviews existing treatments and presents the successful approach using placenta-derived mesenchymal cells (DSCs). We discussed animal studies and clinical trials using DSCs.
Abstract

Acute graft-versus-host disease (GVHD) is a frequent and potentially life-threatening complication following allogeneic hematopoietic cell transplantation (HCT). Mesenchymal stromal cells (MSCs), rare precursors found in all body tissues, possess immunosuppressive properties and can inhibit alloreactivity both in vitro and in vivo. Two decades ago, we introduced bone marrow-derived (BM) MSCs as a novel therapy for acute GVHD. While some patients responded to BM-MSCs, the response was not universal. Commercially available BM-MSCs are now used for acute GVHD treatment in Canada, Japan, and New Zealand. The fetus is protected from the mother’s immune system by the placenta, and our research found that placenta-derived decidua stromal cells (DSCs) offer a stronger immunosuppressive effect than other sources of stromal cells. Safety studies in rabbits, rats, mice, and humans have shown negligible or no side effects from BM-MSCs or DSCs. In a phase I/II trial for severe acute GVHD, we treated 21 patients (median age, 49 years; range 1.6-72 years) with severe biopsy-proven gastrointestinal acute GVHD. The median cell dose of DSCs was 1.2 x10^6 (range 0.9-2.9) cells/kg body weight, with a median of 2 (range 1-6) infusions given one week apart. DSCs cell viability was 93% (range, 69-100%), and the median cell passage number was 4 (range, 2-4). All patients responded, with a complete response of acute GVHD in 11 patients and partial response in 10 and one-year survival of 81%. Randomized trials are needed to prove the superiority of DSCs compared to Ruxolitinib and/or other novel immunosuppressive therapies.

Key words. Desidua stromal cells, Acute graft-versus-host disease, Allogeneic hematopoietic cell transplantation, Mesenchymal stromal cells and Cell therapy
Introduction

Allogeneic hematopoietic cell transplantation (HCT) is a recognized therapy for advanced hematological malignancies, primarily leukemias, aplastic anemia, and metabolic diseases [1-3]. Graft-versus-host disease (GVHD), first described in mouse models and initially termed secondary disease [4], is a significant complication of HCT. Conditioning prior to HCT, which includes total body irradiation and cytotoxic drugs, causes tissue damage, leading to the release of proinflammatory cytokines. Subsequently, alloreactive donor T-cells, activated by recipient and/or donor antigen-presenting cells (APCs), induce GVHD and proliferate. APCs present a variety of histocompatibility class I and class II antigens, viral antigens, or minor antigenic peptides to the donor’s alloreactive T cells. CD4+ T cells recognize antigens in association with HLA class II molecules. Cytokines stimulate CD4+ T cells, which release IL-2, activating cytotoxic CD8+ T cells that react with HLA class I positive targets [5, 6]. The primary target organs of acute GVHD are the skin, gastrointestinal tract, and liver. However, virtually all organs may be involved in GVHD, including the urinary tract, lungs, and brain [7, 8]. The pathogenesis of GVHD is also influenced by the release of danger-associated molecular patterns and tissue damage caused by granulocytes [9, 10].

However, pathogen-associated molecular patterns and other myeloid derived cells are also important [11, 12].

The Janus kinase (JAK) and signal transducers and activators of transcription signaling pathway play a role in immune cell activation and tissue inflammation during acute GVHD [13, 14]. Tissue damage is also driven by inflammatory cytokines.
**Treatment of acute graft-versus host disease**

Over the past two decades, a calcineurin inhibitor combined with methotrexate has been the primary prophylaxis to prevent GVHD [15]. More recently, post-transplant cyclophosphamide was introduced [16, 17].

For several decades, corticosteroids have been the first-line therapy for acute GVHD. Over the years, virtually all immunosuppressive therapies have been used to treat steroid-refractory acute GVHD [18, 19]. These therapies include anti-thymocyte globulin (ATG), extracorporeal photopheresis, mycophenolate mofetil, inolimomab, daclizumab, sirolimus, infliximab, alemtuzumab, methotrexate, tacrolimus, cyclophosphamide, pentostatin, etanercept, and mesenchymal stromal cells (MSCs). Patients treated for steroid-refractory acute GVHD with ATG had poor outcomes [20]. Most secondary therapies for acute GVHD were unsuccessful [18]. For instance, infliximab showed a complete response of 17% in one study and 62% in another, with survival rates of 17% and 38% in the two studies, respectively [21]. Busca and colleagues demonstrated that etanercept had a complete response rate of 31% in 13 patients with steroid-refractory acute GVHD [22]. Extracorporeal photopheresis showed varying responses in different studies treating patients with grade II-IV acute GVHD [23, 24]. Vedolizumab, an anti-α4β7 integrin antibody, showed promise in a few patients [25]. However, vedolizumab was found to be more promising as prophylaxis against GVHD early after transplantation [26].

In recent years, the most promising pharmacological immunosuppressive drug treatment against GVHD has been ruxolitinib, a Jak 1 and 2 inhibitor, which was studied in two prospective randomized trials [27, 28]. Overall responses at day 28 were 62% and 55%, significantly better than other available therapies. Overall survival at 6 months was 46%
and 51% in the two studies, respectively. Side effects due to ruxolitinib were common and included anemia, thrombocytopenia, hypokalemia, neutropenia, edema, and infections. Based on these two studies, ruxolitinib is approved for treatment of corticosteroid-refractory acute GVHD in adults and pediatric patients above 12 years of age by the US Food and Drug Administration (FDA).

**Mesenchymal stromal cells**

Friedenstein et al were the first to describe MSCs [29]. MSCs are very rare precursor cells (<1:10,000 cells) and are found in all tissues of the body [30, 31]. MSCs have potential in regenerative medicine and can differentiate into several cells of mesenchymal cell origin such as bone, cartilage, tendons, cardiomyocytes, and fat [32, 33]. MSCs are positive for CD29, CD73, CD90, CD105, and CD166 [33, 34], but negative for hematopoietic markers. They express HLA class I but not HLA class II [35].

**Immunosuppression by mesenchymal stromal cells**

MSCs inhibit T-cell alloreactivity in mixed lymphocyte reaction (MLR) [36, 37]. The inhibitory effect of MSCs in MLR and suppression of T cells does not depend on HLA-compatibility between the lymphocytes and MSCs [37]. MSCs also inhibited MLR after differentiation to bone, fat, and cartilage [35]. MSCs increase CD4+T cells, CD25+ regulatory T cells (T reg) and IL-10 production [38-41]. MSCs affect dendritic cells and decrease their TNF-α and IL-12 production and promote IL-10 secretion by LPS-stimulated type 2 dendritic cells. MSCs produce several immunoregulatory factors such as HLA-G5 [42], prostaglandin E2 [38], Galectin-1 [43], and indoleamine-2,3-dioxygenase (IDO) [44]. IDO profoundly inhibits T-cells by converting tryptophan to kynurenine [45].
MSCs not only secrete soluble immune modulators, but also have direct contact inhibitory dependent modulatory effects. This includes activation of the PD-1 pathway [46]. MSCs also activate VCAM-1 and ICAM-1 [47]. MSCs induce Fas-mediated T-cell apoptosis [48]. They also suppress activated T cells by upregulating CD39 and adenosine production [49].

In mice, direct contact is important for MSCs-induced immunosuppression, and this occurs via concerted action of chemokines and nitric oxide [50]. In mice with GVHD, it was shown that MSCs are actively induced to undergo perforin-dependent apoptosis by recipient cytotoxic T-cells, a process which was essential to induce immunosuppression [51]. Following MSCs infusion, recipient mice phagocytes engulf apoptotic MSCs and produce IDO, which was necessary to induce immune suppression. Furthermore, in nude mice, it was demonstrated that shortly after infusion, MSCs became apoptotic in the lungs and were locally eliminated by phagocytes [52]. de Witte and colleagues also found that MSCs-induced immunomodulation was triggered by phagocytosis of MSCs by monocytes [53]. MSCs are rejected following infusion to MHC mismatched mice [54]. Furthermore, allo-antibodies were formed after infusion of allogeneic MSCs in baboons [55]. Anti-HLA antibodies were not found in patients treated with MSCs for acute GVHD [56].

Following MSCs infusions, the coagulation system is affected, and MSCs are susceptible to complement activation [57]. After infusion, MSCs are killed by complement after contact with serum [58]. MSCs activate coagulation factors after infusion into blood [59]. MSCs also exhibit potent fibrinolytic properties through their expression of a diverse array of matrix metalloproteinases [60].
Various sources of mesenchymal stromal cells for treatment of acute graft-versus-host disease

Koc et al. demonstrated that it was safe to infuse various doses of MSCs to patients undergoing autologous HCT for advanced breast cancer [61]. MSCs were also shown to prolong skin allograft survival in baboons [36]. We found that third-party MSCs inhibited alloreactivity in mixed lymphocyte reactions [37]. Subsequently, we treated a nine-year-old boy with devastating grade IV acute GVHD, who dramatically responded to HLA-haploidentical maternal MSCs. Encouraged by this case, we performed a pilot study in 8 patients with steroid-refractory acute GVHD and noted a significantly better survival compared to retrospective controls treated with pharmaceutical immunosuppressive drugs [62]. This study was followed by a European multicenter study including 55 patients with severe steroid-refractory acute GVHD [63]. The study included 55 patients who received MSCs from various donors. Complete resolution of acute GVHD was seen in 30/55 patients. Among those patients, the two-year survival was 52%, compared to 16% among 21 patients with partial or no response (p=0.02). During subsequent years, there were many small studies, including a total of 190 patients, treated with BM-MSCs in doses ranging from 0.4–9.0x10^6 cells/kg from 1 to 21 doses with a complete response of 52%, partial response 23%, and no response 25% of the patients [64]. Subsequently, Osiris therapeutics performed a prospective double-blind placebo-controlled phase III study, where patients with grade II-IV GVHD were randomized to either Prochymal (Remestem-L) or placebo. Overall complete response at 28 days was 45% in the Prochymal group and 46% in the placebo group [65]. However, among 61 patients with acute GVHD of the liver, complete response was 76% in the Remestem-L group and 47% in the placebo
group (p=0.03). In 71 patients with gastrointestinal acute GVHD, complete response was 88% in the Remestem-L group compared to 64% in the placebo group (p=0.02). There was also a trend for a better outcome in children than in adults. Subsequently, Remestem-L was registered for treatment of severe acute GVHD in pediatric patients in Canada and New Zealand. In Japan, the Pharmaceuticals and Medical Devices Agency approved BM-MSCs (TEMCELLR) for acute GVHD in children and adults.

In a study by Ball et al. using BM-MSCs, the response for steroid-refractory acute GVHD was 65%, and 3 years survival was 57% in 37 pediatric patients [66]. Several Brazilian centers reported responses of 50% and survival of 20% for severe acute GVHD [67]. Platelet lysate-expanded MSCs were used to treat steroid-refractory acute GVHD in children and adults [68]. Responses were seen in 80% of the children compared to 50% in the adults, and survival was 88% and 29% in the two groups, respectively (p=0.003).

A systematic review and meta-analysis to evaluate response to and survival after MSCs treatment in patients with steroid-refractory acute GVHD was performed after a search in Medline, and Cochrane databases on published studies [69]. The study included 336 patients after the exclusion of 610 reports. Survival at 6 months after MSCs treatment was 63%. Survival did not differ with respect to age, culture medium, or dose of MSCs. A Cochrane Library analysis was performed to evaluate MSCs as treatment and prophylaxis for acute or chronic GVHD [70]. The conclusion was that to date there is no evidence to support the conclusion that BM-MSCs are an effective therapy.

Bonig et al. used pooled BM-MSCs, from multiple donors, and achieved an overall response of 82% and a 6 months survival of 64% [71]. A Turkish study found that MSCs induced a complete response in 54% of the children and a partial response of 21% and
a two-year survival of 75% among responders [72]. MSCs were given to 58 adults with steroid-refractory acute GVHD [73]. Response was seen in 47%, 100 days’ survival was 35%, and 2 years’ survival was 17%. Kurtzberg and co-workers used Remestemcel-L in 241 pediatric patients with steroid-refractory acute GVHD [74]. Overall response at day +28 was 65%. Survival at 100 days was 82% among responders and 39% among non-responders (p<0.001). Children treated with Remestemcel-L were compared to matched patients from the Mount Sinai Acute GVHD International Consortium (Magic) [75]. Children with high levels of biomarkers Reg 3a and ST2 suggesting severe GVHD were compared. The MSCs group had a 6 months’ survival of 64% compared to 10% for children treated with best available therapy (p=0.01). Best available therapy included extracorporeal photopheresis, etanercept, infliximab, ruxolitinib, ATG, MMF, alemtuzumab, basiliximab, and tocilizumab.

Gregoire and co-workers compared MSCs from different organs for treatment of GVHD in a humanized mouse model [76]. They compared BM-, umbilical cord (UC), and adipose-MSCs regarding T cell function in vitro and for treatment of acute GVHD in NOD SCID mice. The various sources of MSCs had different effects on immune cells in vitro and in vivo. However, BM-, UC-, or adipose-derived MSCs did not significantly prevent death from GVHD.

**Anti-inflammatory and immunosuppressive effects by placenta-derived decidua stromal cells (DSCs)**

The fetus is protected from the mother’s HLA haploidentical immune system by the placenta. The placenta has been successfully used to treat burn injuries in Africa for more than one hundred years and has shown a potent anti-inflammatory effect [77]. The
placenta also provides a readily available source of stromal cells without any invasive procedures like BM-MSCs with no or limited ethical consideration [31, 78, 79]. In vitro studies showed that stromal cells from the fetal membrane (DSCs) had a stronger inhibition on lymphocyte proliferation in MLR assay compared to BM-MSCs and stromal cells from the cord or placenta tissue [80].

DSCs induce FoxP3-positive regulatory T cells and inhibit alloreactivity in vivo in a contact-dependent manner as well as by soluble factors like BM-MSCs [81]. DSCs are half the size of MSCs and do not differentiate well to chondrocytes and osteocytes [82, 83]. In MLR, DSCs promoted an anti-inflammatory cytokine-profile [81, 84]. DSCs also have stronger hemostatic properties than MSCs. DSCs had a higher expression level of programmed cell death ligand-1 (PD) L1, PD-L2, and CD49d (markers for homing to inflamed tissue) than BM-MSCs [85]. Other differences between BM-MSCs and DSCs are that the latter are more dependent on cell-to-cell contact for immunosuppression [81]. Blocking experiments suggest that interferon-γ, prostaglandin E2, IDO, and PD-L1 are involved in the immunosuppressive mechanism of DSCs.

**Safety of bone-marrow derived mesenchymal stromal cells and placenta-derived decidua stromal cells (DSCs)**

In contrast to pharmaceutical immunosuppressive agents, MSCs seem safe and there are only a few side effects reported in meta-analyses including more than 1000 patients [86-88]. Lalu et al. reported transient fever as the only side effect of MSCs treatment [87]. In the most recent study, more than 3000 patients from 62 randomized clinical trials, treated with MSCs injections for various disorders were included [88]. The conclusion was
that MSC administration was safe in different patient populations compared with other placebo modalities.

DSCs were investigated for safety in rabbits, rats, and mice [89, 90]. In the rabbit, DSCs were infused i.a. to small arteries without adverse events [89]. Secondly, this was administrated to Sprague-Dawley rats i.a. or i.v without any negative effects on body weight, body temperature, activity, motility, or histological effects on internal organs. Subsequently, DSCs were given i.v. at doses ranging from 4-40x10⁶ cells/kg to 120 mice with no immediate or long-term side effects [90]. None of the mice died from acute toxicity or adverse reactions more than 3 and 30 days after DSCs infusion. Murine blood biochemistry profiles were normal. Compared to BM-MSCs, the DSCs have better viability, are half the size, and had a stronger clotting in human blood and plasma [83].

To conclude from these safety studies, DSCs infusions are safe with almost no side effects in doses up to 40 times higher than used clinically.

The safety studies in humans included 44 patients treated with DSCs and 40 controls [91, 92]. A median DSCs dose infused was 1.5 (range 0.9–2.9 x10⁶ cells/kg). The patients were given a median of 2 (1-5) injections [91]. Three patients had transient reactions during DSCs infusion. One of them had septicemia and fever and had transient chills during DSCs infusion. Another patient experienced transient vertigo. One patient experienced headache and dyspnea during the fourth DSCs infusion, reversed by oxygen support. The laboratory values, hemorrhages, causes of death, infections, and infusions were similar between the patients treated with DSCs and the controls.

To conclude from safety analysis in experimental animals and in humans, DSCs seem safe even using high doses. DSCs are only half the size of BM-MSCs [83]. To conclude
from the safety studies, both BM-MSCs and DSCs seem safe and with no side effects. This is in contrast to Ruxolitinib where patients often have thrombocytopenia, anemia, neutropenia, and infections [27, 28].

**Decidua stromal cells for treatment of acute graft-versus-host disease**

In initial studies, DSCs were called fetal membrane cells (FMCs), because at that time we didn’t know that they were of maternal origin [85]. Initially, DSCs were dissolved in AB plasma before i.v. infusion in patients with severe acute GVHD. We later found that DSCs dissolved in albumin had a significantly better viability in median 95% as compared to DSCs dissolved in AB plasma where it was 90% (p=0.001) [92]. Patients who were treated for severe acute GVHD with DSCs dissolved in albumin had significantly better response compared to patients given DSCs dissolved in AB plasma (p=0.01). In the former groups, 11 had complete response and 10 had partial response compared to 5 patients with partial response; 5 with complete response and 7 with no response treated with DSCs dissolved in AB plasma. One year survival was 76% and 47% in the two groups, respectively (p=0.01). Among patients with defined steroid refractory acute GVHD, one year survival in patients treated with DSCs dissolved in albumin was 73%. This was significantly better than 20% in patients treated with BM-MSCs (p=0.0015), (Fig. 1).

Three patients succumbed approximately 4 months after undergoing DSC therapy. Each of these patients had achieved complete responses in their acute GVHD cases. A 42-year-old woman diagnosed with pre-B-ALL passed away due to an invasive zygomycetes infection. A 48-year-old woman with myelodysplastic syndrome who had received reduced intensity conditioning (RIC) unfortunately experienced relapse and did not
survive. Lastly, a 49-year-old female diagnosed with myeloma, also treated with RIC, ultimately passed away due to recurrent disease.

Subsequently, we did a long-term follow-up of the 21 patients treated with DSCs dissolved in albumin [93]. The cumulative incidence of chronic GVHD was 52%. This was mild in 6 patients, moderate in 4, and severe in 1 patient based on National Institute of Health chronic GVHD severity scoring [94]. 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. At one year, survival was 81%. At 4 years, transplantation-related mortality was 28.6% and overall survival was 57%. Survival was similar to that for all 293 patients who underwent HCT during the same period 2012-2015 at our unit, with 66% overall survival (p=0.33), (Fig. 2).

**Discussion**

There is now 20 years of experience treating severe acute GVHD using MSCs. The by far most commonly used source of MSCs has been from BM which is ready-available by aspiration from healthy donors. From meta analysis there is no clear cut advantage using BM-MSCs compared to controls treated with other therapies for severe acute GVHD [70]. Although the vast majority of patients were treated with BM-MSC, other sources such as MSCs from adipose tissue, umbilical cord, crude placenta, DSCs and more were also evaluated [95]. Most of the cells used did not only differ by source of stromal cells, viability and cell dose. In addition both human and host factors also have to be considered [96]. Compared to all these other studies, DSCs dissolved in albumin seem to have a most
promising outcome for severe acute GVHD in 21 patients with a one year survival of 81% [93].

Only three out of the 11 patients who had steroid-refractory acute GVHD succumbed to the condition. One of these patients fell victim to an invasive fungal infection (IFI). IFIs are a prevalent occurrence post HCT, particularly among individuals grappling with severe acute GVHD [97]. Our observation revealed a notable incidence of IFIs in patients subjected to BM-MSC-based treatment for acute GVHD [98].

Two patients experienced mortality due to recurrence of their malignant diseases. This is noteworthy despite the well-documented graft-vs-leukemia effect and the anti-cancer influence associated with HCT-induced GVHD [99-101]. Furthermore, survivors of acute GVHD display a diminished likelihood of relapse [102]. Other recognized complications that emerge post HCT encompass viral and bacterial infections, along with subsequent occurrences of secondary malignancies as time progresses [103].

To comprehensively assess the potential elevation in the risk of IFIs, other infections, and disease recurrence attributed to DSCs, a substantial cohort of patients would be imperative. Similarly, the assessment of whether DSCs contribute to heightened instances of secondary malignancies would necessitate a considerable patient cohort and an extended follow-up period, extending beyond five to ten years.
Survival also compared favourably with ruxolitinib where 6 months’ survival was around 50% [27, 28]. To be convincing for treatment of severe acute GVHD, DSCs need to be compared to ruxolitinib or best available therapy in a prospective randomized trial. Such a prospective study requires quite a lot of financial support. This may be difficult in an academic setting due to the new laws introduced in Europe, and previously in the USA, where cell therapy is regarded as a pharmacological drug. DSCs or MSCs need to be cultured in GMP laboratories under rigorous restricted rules. The law was introduced in Europe, because the big pharmaceutical companies wanted to make it very expensive with cellular therapy for small pharmaceutical companies (Åkerblom, Swedish Drug Agency, personal communication). Before the introduction of this new law we gave thousands of cell products including HCT, BM-MSCs cells in laboratories with reversed isolation using a sterile bench and a laminar air flow hood without having any infectious complications. The only septisemia that occurred following cell infusion was when we gave NK-cells prepared in our GMP-laboratory [104]. There has been no prospective trial comparing MSCs manufactured in a previously conventional labroom with a sterile bench and reversed isolation compared to an advanced and highly expensive GMP laboratory.

MSCs have only been approved for the treatment of acute GVHD in three countries, while their exploration is ongoing in several European countries. The restricted usage can be attributed to the outcome of a significant prospective randomized trial involving BM-MSCs, which did not achieve the primary endpoint of complete response by day 28 [65]. Currently, there is insufficient convincing data to establish MSCs as a standard therapy for acute GVHD [70].
To improve on efficacy of various MSCs products, several priming approaches to enhance efficacy were used [105]. Priming of MSCs was attempted with cytokines and growth factors mainly with interferon-γ (IFN-γ) [106, 107]. Priming of various sources of MSCs such as umbilical cord, Wharton’s jelly, BM, placenta, adipose tissue and cord blood were attempted by priming with hypoxia [105]. Priming was also tried with pharmacological drugs in various disease models such as Huntington’s disease, myocardial infarction model, stroke model, excisional wounds model, emphysema model and Ankylosing Spondylitis model. Priming of MSCs using biomaterials or different culture conditions was used in several studies and using various sources of MSCs for different diseases. Regarding DSCs as simple as changing dissolution from plasma to albumin, significantly improved cell viability and also outcome in steroid refractory acute GVHD [92]. Similar improvements in culture conditionings or priming also may have improved treatment of acute GVHD using other sources such as BM-MSCs and with MSCs from adipose, cord blood, cord, crude, placenta or fat. DSCs from the placenta also have other advantages compared to other sources of MSCs. DSCs are ready available in large numbers, because the placenta is thrown away after delivery. Furthermore, there is an unlimited supply and there is no invasive procedure. This is in contrast to bone marrow aspiration, even if this may not be very painful.

DSCs have a better expansion compared to BM-MSCs [92]. DSCs also seem more robust than other sources of MSCs including BM-MSCs where cryo-preserved cells did not well suppress T cell proliferation in vitro. Another study confirmed that freshly cultured BM-MSCs had a stronger immunosuppressive effect than thawed-frozen cells [106, 108-110].
In contrast to BM-MSCs, DSCs are more robust. Cell survival and immunosuppression by DSCs is not influenced by freeze-thawing [111].

To improve the outcome using BM-MSCs in patients with acute GVHD you may pool MSCs from several donors as was done by Bonig and co-workers [71]. Alternatively, one may select BM-MSCs, which induced cytotoxicity by the recipient immune system [51].

MSCs affect the coagulation system and have been used to treat hemorrhages following HCT [59, 112]. DSCs compared to BM-MSCs have more procoagulant tissue factor and a higher expression of CD55, complement regulatory activity [83]. Before and after infusion of MSCs and DSCs the central venous line is flushed with low dose heparin [92]. The safety studies have not revealed an increased risk of trombosis following infusion of BM-MSCs or DSCs [86, 87, 91, 113]. In mice 40 times higher doses of DSCs than is used clinically did not induce any thrombosis [90].

To conclude, today, in spite of limited experience, DSCs seem so far to be the best therapy for treatment of severe acute GVHD with no side effects and promising responses. Prospective randomized trials are needed to prove efficacy over other immunosuppressive drugs, such as ruxolitimb and are in pipeline.

DSCs also seem superior to BM-MSCs with regard to treatment of other inflammatory disorders such as acute respiratory distress syndrome (ARDS) [114]. DSCs and possibly cord-derived MSC have shown encouraging results for the treatment of Covid-19-induced ARDS [115]. DSCs were also successfully used in small pilot studies and anecdotal cases for hemorrhagic cystitis and polyneuropathies [116, 117]. DSCs may also be tried for many other inflammatory disorder including Crohn’s disease, ulcerative colitis, acute pancreatitis and other inflammatory disorders due to its potent anti-inflammatory effects.
Conclusion: DSCs are ready available following cesarean section from the fetal membrane. DSCs are easy to expand and have a stronger immunosuppressive effect in vitro and in vivo, than other sources of MSCs from the placenta or BM. Like BM-MSCs, DSCs are safe to infuse with no side-effects. Using an optimal protocol, dissolving DSCs in albumin, 21 patients with severe acute GVHD, all responded with a one year survival of 81%. At four years survival was 57%. Outcome for acute GVHD, seems better than other therapies. Prospective randomized trial comparing DSCs with best available immunosuppressive therapy, like Ruxolitinib are needed to prove efficacy. Such studies are planned.
Acknowledgement

The authors thank Gunilla Tillinger for expert typing of the manuscript. We also thank the staff at the Center for Allogeneic Stem Cell Transplantation for their compassionate and competent care of the patients in the study of DSCs. The study was supported by grants from the Swedish Cancer Society (CAN 2018,419) and the Cancer Society in Stockholm (111293). OR was the recipient of a Distinguished Professor Award from Karolinska Institutet.

Disclosure of potential conflict of interest. The authors received honorarium from ASC Therapeutics.

Data Statement. The data underlying this article will be shared on reasonable request to the corresponding author.
Figure legends.

Fig. 1. Kaplan-Meier estimate on overall survival in patients with steroid refractory grades III-IV acute GVHD treated with decidual stromal cells (n=11) compared with retrospective controls treated with BM-MSCs (n=15, p=0.0015) at our institution, Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital.

Fig. 2. Overall survival in all patients who underwent HCT at our institution during 2012-2015 and 21 patients treated with DSCs for severe acute GVHD. Four year survival was 66% and 57% in the two groups, respectively (p=0.33).
References:


Figure 1

SR DSCs n=11, 1y - 73% (37-90%)

SR MSCs n=15, 1y - 20% (5-42%)

Cumulative Proportion Surviving

Time (days) from steroid refractory acute GVHD
Figure 2

4-y OS

All patients 2012-15 n=293, 66%

DSC n=21, 57%

p=0.33