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Clinical Use of Mesenchymal Stromal Cells in the Treatment of Acute Graft-versus-Host Disease

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Keywords

Allogeneic stem cell transplantation · Mesenchymal stromal cells · Steroid-refractory acute graft-versus-host disease

Abstract

Acute graft-versus-host disease (aGvHD) continues to impact morbidity and mortality after allogeneic stem cell transplantation (allo-SCT). First-line therapy for aGvHD still remains the use of high-dose corticosteroids. Unfortunately, 40–60% of patients with aGvHD exhibit steroid resistance, which is associated with a very poor prognosis. As no effective second-line therapy existed, in recent decades various treatment options were considered for the treatment of therapy-refractory GvHD. Based on their in vitro immunomodulatory properties, the use of mesenchymal stromal cells (MSCs) in the treatment of aGvHD has been introduced. However, most of the clinical data are generated from uncontrolled trials and case series, showing clinical responses to MSCs. Clinical results are more consistent in children despite the use of MSC preparations of various provenance and manufacturing protocols. While these data support the therapeutic principle, the great variability of outcomes strongly suggests that not all MSC preparations are equal and that the specific manufacturing protocols influence therapeutic success in vivo.

Introduction

Allogeneic hematopoietic stem cell transplantation is a well-established therapy option for the treatment of malignant and nonmalignant hematological disorders that cannot be cured with conventional treatments. In recent years, developments in donor source, conditioning regimen, high-resolution HLA typing, graft-versus-host disease (GvHD) prophylaxis, and supportive care led constantly to improved transplant outcomes [1]. However, GvHD still remains the most frequent and serious complication following allogenic stem cell transplantation (allo-SCT), with a significant impact on overall survival (OS) [2]. GvHD is a systemic inflammatory condition where donor-derived lymphocytes recognize recipient antigens as foreign. This leads to an immune response with activated T cells attempting to eliminate antigen-bearing cells of the host that can cause severe multiorgan damage. In malignancies of the hematopoietic system, donor lymphocytes attack recipient malignant cells in the direction of a graft-versus-leukemia (GvL) reaction. The challenge of allo-SCT for the treatment of hematological malignancies is to enhance the GvL effect without inducing more GvHD [3]. The main clinical presentations are acute (a)GvHD and chronic GvHD. Despite advances in GvHD prophylaxis and therapy, this life-threatening complication limits the broader application of allo-SCT.

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aGvHD occurs in 30–50% of recipients and 14% of all patients suffer severe aGvHD grades 3–4 [2]. Chronic GvHD affects 30–70% of patients receiving allo-SCT. Several risk factors, including HLA disparity, donor type, female donor for a male recipient, application of peripheral blood stem cells, intensity of conditioning regimen, older donor, and recipient age, have been associated with the development of GvHD [2, 3]. In the management of allo-SCT prevention of GvHD plays a pivotal role. Calcineurin inhibitors (e.g., cyclosporine A, tacrolimus) and methotrexate are used in the majority of patients undergoing SCT as pharmacological GvHD prophylaxis [3]. In spite of prophylaxis, GvHD occurs and the first-line of treatment persists in the administration of corticosteroids [4]. Within a couple of days, 40–60% of the patients respond to treatment [4]. Steroid-refractoriness is defined as a lack of response or progression after 3–7 days of systemic corticosteroid therapy. The long-term prognosis of steroid-refractory GvHD is poor, with an OS rate less than 30% at 1 year, attrition being due either to GvHD directly, or to sequelae of the aggressive immunosuppression to control GvHD, such as latent virus reactivation, sepsis, or relapse [2, 4, 5]. To date, there is no standard approach practiced as second-line therapy. The development of superior treatment strategies is crucial for survival improvement following allo-SCT. A variety of agents are currently used in steroid-resistant GvHD, e.g., mycophenolate mofetil (MMF), mammalian target of rapamycin (mTOR) inhibitor (Sirolimus), JAK inhibitors, proteasome inhibitors, and monoclonal antibodies [4]. A promising alternative to immunosuppressants is mesenchymal stromal cell (MSC) infusion in corticosteroid-resistant GvHD. In 2004, Le Blanc et al. [6] reported transient resolution of severe therapy-refractory aGvHD grade IV of the gut and liver in a 9-year-old boy after MSC infusion. This observation paved the way for use of MSCs in clinical practice. In this review, we provide current insights into use of MSCs in aGvHD therapy.

Biological Properties of MSCs

Bone marrow is a complex tissue containing hematopoietic progenitor and stem cells besides primitive MSCs in specialized niche microenvironments. In the late 1960s, Friedenstein et al. [7] discovered MSCs and attracted scientific interest. Currently, numerous clinical trials with MSCs in the treatment of different ailments are ongoing (Table 1).

MSCs represent a multipotent, heterogeneous, and nonhematopoietic cell population that can be derived from bone marrow and expanded ex vivo in order to achieve sufficient numbers of cells for use as clinical cell therapy [8]. More recently, adipose tissue, placenta, umbilical cord, and dental pulp were also recognized as sources of multipotent MSCs [9]. It has been reported that MSCs both in vitro and in vivo are able to differentiate into a variety of cell types, including osteogenic, chondrogenic, myogenic, and adipogenic lineages [8]. However, not all individual cells in tissue culture flasks have the same level of multipotency. Among human bone marrow MSCs, self-renewing progenitors have been identified and it is unknown whether MSCs from other tissues share this property. Therefore, the term "mesenchymal stromal cells," which does not imply stem cell properties, has been proposed instead of "mesenchymal stem cells." Besides multipotency, MSCs exert extensive immunomodulatory and engraftment-promoting potential [10]. After in vivo application, MSCs secrete cytokines and regulatory molecules that promote anti-inflammatory and regenerative effects by promoting endogenous tissue repair or possibly by replacing damaged tissue [8]. Le Blanc et al. [11] showed that allogeneic MSCs might also engraft and differentiate in humans across major histocompatibility barriers even when the recipient is immunocompetent.

Similar to hematopoietic stem cells, MSCs have multi-organ homing and plasticity capacity. In 2006, the International Society for Cellular Therapy defined MSCs as plastic adherent in standard culture conditions, expressing CD73 and CD90 surface molecules (lack of CD45, CD34, CD14, or CD11b, CD79a or CD19 and HLA-DR) and differentiation capacity into chondroblasts, osteoblasts, and adipocytes in vitro [12].

MSCs may directly affect both innate and adoptive immunity by secreting a large number of soluble factors that include indoleamine 2,3-dioxygenase (IDO), prostaglandin 2, interleukin (IL)-10, transforming growth factor-β, nitric oxide, HLA-G5, and the highly anti-inflammatory molecule tumor necrosis factor (TNF)-α-induced gene/protein 6 (TSG-6) [13]. These molecules contribute to the in vitro and in vivo immunomodulatory effects of MSCs, which are beneficial for a number of immune-pathological conditions, such as GvHD and type 1 diabetes [5, 14]. The mechanisms for therapeutic potential still remain largely unclear. Indeed, autoimmunity and alloimmunity are not exclusively driven by adaptive immune responses. Immunomodulatory potential has been reported on humoral and cellular stimuli of the innate and adaptive immune system [14]. Several key cellular interactions have been described in the literature.

The central component of innate immunity is the complement system. C3 and C5 are cleaved extensively to the anaphylatoxins C3a and C5a by convertases at the site of inflammation. MSCs express the receptors for C3a and C3b on the cell surface. By binding to their receptors, pathways in proliferation and apoptosis protection in MSCs become activated. Furthermore, MSCs secrete fac-

Table 1. Selected clinical trials using MSCs (registered through clinicaltrials.gov)

Indication	Study design	Intervention	Location	Status	Trial No.
aGvHD	Phase 1/2	Adult allogeneic MSCs from adipose tissue	Spain	Recruiting	NCT02687646
	Phase 1	MSCTC-0010 dose escalation	USA	Recruiting	NCT03158896
	Phase 3	MSCs versus placebo	USA	Completed	NCT00366145
	Phase 2/3	MSCs	China	Recruiting	NCT03631589
Chronic GvHD	Phase 3	MSCs	China	Recruiting	NCT02291770
Crohn's disease	Phase 2	Prochymal adult human MSCs	USA	Completed	NCT00294112
Alzheimer's disease	Phase 1	Longeveron MSCs versus placebo	USA	Recruiting	NCT02600130
Renovascular hypertension	Phase 1	MSCs	USA	Recruiting	NCT02266394
Myocardial infarction	Phase 1	Provacel	USA	Completed	NCT00114452
Skin ulcer venous stasis chronic	Phase 1/2	Allo-APZ2-CVU	Germany	Recruiting	NCT03257098

tor H that inhibits complement activation by limiting C3 and C5 convertases activity [14]. In mouse models, MSCs were shown to exhibit a proinflammatory phenotype and secrete chemotactic cytokines such as IL-6, IL-8, GM-CSF, and macrophage inhibitory factor [14]. IL-8 is a chemoattractant for neutrophils and mediates leukocyte extravasation. MSCs can suppress mast cell activation and IgE-mediated mast cell degranulation.

The interaction between natural killer (NK) cells and MSCs is complex and depends on the microenvironment and activation status of the NK cells. MSCs suppress proliferation and cytokine production and can interfere with NK cell cytotoxicity [15]. Additionally, these cells are capable of inhibition of maturation of monocytes and CD34+ precursor cells into dendritic cells and of activation of dendritic cells via cytokines. Therefore, dendritic cells, which are the main type of antigen-presenting cells, cannot effectively induce T-lymphocyte activation due to reduced capacity for antigen presentation [16]. Otherwise, MSCs promote wound repair by the recruitment of monocytes and macrophages through secretion of chemokine ligands [17].

MSCs regulate the adaptive immune system through various redundant pathways. Obviously, these cells suppress the proliferation, interferon-γ (IFNγ) production, and cytotoxicity of CD4+ and CD8+ T lymphocytes. Di Nicola et al. [8] reported in vitro suppression of T-lymphocyte proliferation induced by autologous and allogeneic MSCs. T cells were cultured with dendritic cells or blood lymphocytes in mixed lymphocyte reactions. After the addition of MSCs to the stimulated T lymphocytes, a significant and dose-dependent reduction of T cell proliferation (60 ± 5% to 98 ± 1%) was observed even when the MSCs were added in culture after 5 days. IFNγ and TNF-α are the most important T cell effector cytokines in MSC immunomodulatory effects. Davies et al. [18] demonstrated that MSCs express and secrete programmed death ligands (PD-L) 1 and 2. They reported PD-L1- and PD-L2-mediated suppression of T cell proliferation by MSCs, IL-2 secretion, and induction of an irreversible hyporesponsive state, as well as apoptosis. In vivo observations suggest that MSCs restore the balance between T helper 1 and 2 cells in diseases associated with a shift towards dominance of one of these T cell subpopulations [19]. In vitro models indicate that MSCs induce regulatory T cells and also sustain their survival and suppressive phenotypes [19]. It is documented that MSCs affect the status of T cells and skew them towards a regulatory phenotype [18]. MSCs also interact with B cells by inhibiting B cell responses [19] which results in cell cycle arrest, decreased immunoglobulin production, and impaired chemotaxis. Taken together, immunomodulating properties of MSCs underlie complex regulatory effects and interactions of MSCs with the innate and adaptive immune system.

MSCs in the Treatment of aGvHD

Over the last 2 decades, MSCs have been investigated in a large number of clinical trials as novel cellular therapy in aGvHD (Table 2). The first promising resolution of treatment-refractory aGvHD prompted a pilot study using MSCs to treat severe GvHD grade III–IV after allo-SCT [20]. Eight patients had received MSCs, of which 6 responded completely to treatment. Based on these encouraging initial reports, in a multicenter (5 European centers), phase II experimental study, 55 patients were treated with bone marrow-derived MSCs due to steroid-resistant, severe aGvHD between October 2001 and January 2007 [21]. The patients received 1–5 doses of MSCs obtained from matched sibling donors, haploidentical donors, and third-party HLA-mismatched donors. Complete responses (CR) were achieved by 30 patients and 9 patients showed clinical improvement. No acute side effects were seen and response was not associated with do-

Table 2. Overview of studies on therapy-refractory aGvHD

Reference, year	Cohort	Diagnosis (*n*)	GvHD	Dose of MSC, cells/kg body weight	Response rate day +28	Predicted survival
Bader et al. [5], 2017	*n* = 69 ≤18 years: *n* = 51 >18 years: *n* = 18	Malignant (51) Nonmalignant (18)	aGvHD II: *n* = 3 (4%) III: *n* = 25 (36%) IV: *n* = 41 (59%)	1–2 × 10^6	CR = 31.9% PR = 50.7% OR = 82.6%	6-month OS = 71 ± 6%
Salmenniemi et al. [30], 2017	*n* = 30 ≤18 years: *n* = 8 >18 years: *n* = 22	Malignant and nonmalignant	aGvHD II: *n* = 2 (7%) III: *n* = 14 (47%) IV: *n* = 10 (33%) cGvHD: *n* = 4 (13%)	2 × 10^6	CR = 23% VGPR = 13% PR = 17% OS = 53%	6-month OS = 54% 2-year OS = 29%
Dotoli et al. [31], 2017	*n* = 46 ≤18 years: *n* = 16 >18 years: *n* = 30	Leukemia (22) MDS (7) Nonmalignant (12) Others (5)	aGvHD III: *n* = 10 (21.7%) IV: *n* = 36 (78.3%)	6.81 × 10^6	CR = 6.5% PR = 43.5% OR = 50%	100-day OS = 34.4% 2-year OS = 17.4%
von Dalowski et al. [32], 2016	58 adults	Leukemia (39) MDS (5) Others (14)	aGvHD I: *n* = 1 (2%) II: *n* = 3 (5%) III: *n* = 8 (14%) IV: *n* = 46 (79%)	0.99 × 10^6	CR = 9% PR = 38% OR = 47%	100-day OS = 34.5% 2-year OS = 16.6%
Kurtzberg et al. [29], 2014	75 children	Leukemia (35) MDS (7) Genetic disease (16) Others (17)	aGvHD B: *n* = 9 (12%) C: *n* = 21 (28%) D: *n* = 45 (60%)	2 × 10^6	OR = 61.3%	100-day OS = 57.3%
Sánchez-Guijo et al. [26], 2014	25 adults	AML (6) MDS (7) Others (12)	aGvHD II: *n* = 7 (28%) III: *n* = 13 (60%) IV: *n* = 3 (12%)	1.1 × 10^6	CR = 44% PR = 27% OR = 71%	44% (11/25) alive after 12 months
Ball et al. [23], 2013	37 children	Leukemia (21) MDS (7) Nonmalignant (9)	aGvHD III-IV: *n* = 37	1–2 × 10^6	CR = 65% PR = 21.5% OR = 86.5%	6-year OS = 37%
Introna et al. [28], 2014	*n* = 40 ≤18 years: *n* = 15 >18 years: *n* = 25	Malignant (36) Nonmalignant (4)	aGvHD II: *n* = 11 (27%) III–IV: *n* = 20 (50%) cGvHD: *n* = 3 (8%) overlap: *n* = 6 (15%)	1.5 × 10^6	CR = 27.5% PR = 40% OR = 67.5%	1-year OS = 50% 2-year OS = 38.6%
Resnick et al. [22], 2013	*n* = 50 ≤18 years: *n* = 25 >18 years: *n* = 25	Malignant (43) Nonmalignant (7)	aGvHD II–III: *n* = 8 (16%) IV: *n* = 42 (84%)	1.14 ± 0.47 × 10^6	CR = 34% OR = 66%	3.6-year DFS = 56%
Hermann et al. [34], 2012	19 adults	Leukemia (15) Other malignancies (4)	aGvHD: *n* = 12 (63%) cGvHD: *n* = 7 (37%)	1.7–2.3 × 10^6	CR = 47.4% PR = 31.6% OR = 79%	30-month OS 55%
Prasad et al. [35], 2011	12 children	Malignant (7) Nonmalignant (5)	aGvHD III: *n* = 5 (42%) IV: *n* = 7 (58%)	*n* = 10: 2 × 10^6 *n* = 2: 8 × 10^6	CR = 17% PR = 50% OR = 67%	2-year OS 40%
Pérez-Simon et al. [36], 2011	18 adults	n/a	aGvHD II: *n* = 3 (17%) III–IV: *n* = 7 (39%) cGvHD: *n* = 8 (44%)	1–2 × 10^6	CR = 11% PR = 50% OR = 61%	33% (6/18) alive at last follow-up
Lucchini et al. [27], 2010	11 children	Leukemia (8) Nonmalignant (3)	aGvHD I–II: *n* = 4 (36.4%) III–IV: *n* = 4 (36.4%) cGvHD: *n* = 3 (27.2%)	1.2 × 10^6	CR = 23.8% PR = 47.6% OS = 71.4%	73% (8/11) alive after 8 months
von Bonin et al. [37], 2009	13 adults	Malignant (12) SAA (1)	aGvHD III: *n* = 11 (85%) IV: *n* = 2 (15%)	0.9 × 10^6	OR = 45%	45% (5/13) alive after 257 days
Le Blanc et al. [21], 2008	*n* = 55 ≤18 years: *n* = 25 >18 years: *n* = 30	Leukemia (33) MDS (6) Nonmalignant (10) Others (6)	aGvHD II: *n* = 5 (9%) III: *n* = 25 (45.45%) IV: *n* = 25 (45.45%)	1.4 × 10^6	CR = 54.5% PR = 16% OR =70.5%	2-year OS = 35%

aGvHD, acute graft-versus-host disease; cGvHD, chronic graft-versus-host disease; MSC, mesenchymal stromal cell; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; SAA, severe aplastic anemia; OS, overall survival; DFS, disease-free survival; CR, complete response; VGPR, very good partial response; PR, partial response; OR, overall response; n/a, not available.

nor major histocompatibility matching. Lower transplantation-related mortality (TRM) 1 year after infusion (37 vs. 72%; $p = 0.002$) and higher OS 2 years after allo-SCT (53 vs. 16%; $p = 0.018$) were detected in complete responders to MSC infusions versus nonresponders [21].

The majority of clinical trials confirmed the safety of MSCs in steroid-refractory aGvHD in the pediatric as well as adult patient population with variable success rates [5, 22–27]. However, children showed a trend towards better CR compared with adults [25, 28]. Certain factors, including skin involvement and lower aGvHD grade, yielded a higher response rate to MSC application [25]. Possibly, inconsistent results of clinical studies are attributable to different or inconsistent pharmacological quality of MSCs resulting from the lack of a standardized methodology for MSC generation, dosing, and interdonor heterogeneity [5, 24]. Kuçi et al. [24] developed a novel approach for MSC generation from pooled bone marrow mononuclear cells of 8 allogeneic, third-party donors to overcome donor-to-donor variability. Generated MSCs were frozen in 209 vials and labeled as an MSC bank. This novel manufacturing protocol is characterized by high potency and near-identity of individual doses, termed MSC-Frankfurt am Main (MSC-FFM) [24]. Recently, Bader et al. [5] reported outcomes of 69 adult and pediatric patients who received a total of 212 doses of MSC-FFM for therapy-refractory aGvHD in a routine clinical setting in 23 allogeneic transplant centers from 6 countries. The patients were either steroid-refractory (29%) or refractory to steroids plus 1–5 additional immunosuppressive agents (71%). Except for 3, all of the patients suffered from severe aGvHD grade III (36%) and IV (59%). The recommended dose and dosing schedule of MSC-FFM is $1–2 \times 10^6$ MSCs/kg body weight once weekly for a total of 4 doses, although the majority of patients did not tightly adhere to these recommendations. The average dose was 1.4×10^6 MSCs/kg. On average, a total of 3 doses were administered, with approximately one third each receiving 2 or 4 doses, the remainder anywhere between 1 and 10 doses. Adverse events were reported only in 2 children, 1 suffering from nausea/vomiting and the other from headache, presumably due to DMSO and cold infusion solution. Long-term adverse events in GvHD patients include relapse of the underlying disease and severe infections as sequelae of GvHD treatment. In this cohort, the 6-month predicted mortality rate due to relapse and nonrelapse mortality was 2 and 27%. By day +28, 22 recipients of MSC-FFM (32%) had achieved a CR, 35 (81%) a partial response, and the overall response rate was 83%. At the last follow-up (median follow-up 8.19 months), 61% of patients showed a CR, 25% of patients had a partial response, and 14% of patients were nonresponders. Respectively, the estimated OS at 6 months was 75% for grade III and 67% for grade IV aGvHD after MSC-FFM treatment. Interestingly, in contrast to all the other clinical studies performed thus far, in that cohort clinical responsiveness did not differ between children and adults. The overall response status at the last follow-up after the first administration of MSC-FFM among children and adults was 89 and 84%, respectively.

An open-label, single-arm, prospective multicenter study evaluated the risk and benefit profile of Prochymal [29]. Prochymal was derived from human bone marrow of 7 different donors. Seventy-five children with aGvHD grade B–D failing steroids and/or other immunosuppressants were enrolled. For 4 weeks, infusions of 2×10^6 MSCs/kg were administered biweekly, with additional 4-weekly infusions after day +28 for patients with a partial response. At the time of treatment, 88% of the patients suffered from severe aGvHD grade C or D. The overall response rate at day +28 was 76.2% for aGvHD grade C, 53.3% for grade D, and 61.3% for the entire cohort. Unsurprisingly, the estimated OS at day +100 was higher in patients who responded to treatment (78.1%) compared with nonresponders (31%; $p < 0.001$). However, the long-term outcome for adult recipients of Prochymal was no better than for the untreated control group. Le Blanc et al. [21] had previously reported similar findings in a phase II study.

In 2013, Introna et al. [28] presented a phase I multicenter study, in which the administration of MSCs expanded in platelet lysate was assessed in 15 children and 25 adults with aGvHD grade II to IV. Following the failure of conventional immunosuppressants, a median of 3 MSC infusions and a median cell dose of 1.5×10^6/kg were applied. Acute toxicity was not documented. Of the 86 adverse events, 72.1% were of an infectious nature. The overall response rate at day +28 was 67.5%, of which 27.5% showed CR. The median survival time was 1.1 years and OS rates at 1 and 2 years were 50 and 38.6%.

Salmenniemi et al. [30] reported the outcome of 22 adults and 8 children treated with third party bone marrow-derived, platelet-lysate-expanded MSCs as salvage therapy for steroid-refractory GvHD between January 2013 and August 2015. Six doses of MSCs were administered to each patient with a target dose of 2×10^6/kg recipient body weight per infusion bi- or once weekly. Four adult patients were treated due to chronic GvHD, whereas 80% of the patients suffered from aGvHD grade III–IV. The day +28 assessment revealed an overall response rate of 62% in aGvHD patients. The overall response rate did not differ statistically significantly between adults (50%) and children (88%), respectively ($p = 0.099$). At the median follow-up of 767 days, 42% of the patients were alive. OS in pediatric patients (88%) was significantly higher compared with adults (22%; $p = 0.003$).

In a multicenter, retrospective study of 3 Brazilian public hospitals, 46 patients were treated with MSCs between October 2007 and March 2015 [31]. Sixteen chil-

dren and 30 adults with steroid-refractory aGvHD grade III (21.7%) and grade IV (78.3%) were included. The median cumulative MSC dose was 6.81×10^6/kg body weight in a median of 3 infusions. Response to MCSs occurred in 23 patients (50%), of whom only 3 (6.5%) had CR. Respectively, the estimated probability of OS at day +100, 1 year, and 2 years were 34.4, 19.56, and 17.4%. Seven patients, including 3 adults and 4 children, who were responders to MSC treatment were alive at the last follow-up. No late or severe side effects were reported due to MSC infusion.

Dalowski et al. [32] reported similar outcomes in adult patients (median age 55 years) with steroid-refractory GvHD. On average, each patient received 2 MSC infusions with a median dose of 0.99×10^6/kg body weight. Of 58 patients, 79% suffered from aGvHD grade IV. The overall response rate was 47% ($n = 27$), including a CR in 9% ($n = 5$). The estimated 1-year OS was 19% and did not differ significantly from a historical control group who received alternative salvage therapy.

Ball et al. [23] analyzed a cohort of 37 children who received multiple MSC infusions. CR occurred in 24 patients (65%), 8 showed a partial response and 5 patients were nonresponders. In contrast to patients who reached CR, the cumulative incidence of TRM was significantly higher in patients who did not achieve CR (17 and 69%; $p = 0.001$). In patients who achieved a CR, OS was 65% compared with patients who did not experience CR (0%; $p = 0.001$). The OS of the whole cohort was 37% after a median follow-up of 2.9 years.

Based on the assumption that, despite strong data towards the opposite, different MSC preparations are mostly pharmacologically similar, Thielen et al. [33] attempted to construct treatment algorithms. Data from 327 patients documented in 14 phase II trials were extracted to estimate long-term outcomes and the natural history of disease. Prior to MSC treatment, all patients had aGvHD grade II–IV. Within the first 28 days the CR probability was 43.4, while 43.7% showed no CR, and 12.8% of patients died during this period. The median survival for complete responders and noncomplete responders was 3.2 and 0.5 years.

Despite the considerable number of failed trials, especially in adults, the upshot from these studies is guarded optimism with regard to the potential of bone marrow-derived MSCs as second-line agents for steroid-refractory aGvHD. Reflecting this, national marketing authorizations have been obtained in a small number of countries for Prochymal in pediatric GvHD and for MSC-FFM in adult and pediatric GvHD. Also, both the FDA and EMA have conferred orphan designation to these MSC preparations in the respective territories

Discussion and Perspective

MSCs are one of the latest therapeutic options for aGvHD. A small number of case series report favorable outcomes; however, the larger series as well as the controlled trials would support safety, but not necessarily efficacy in the treatment of therapy-refractory GvHD. Despite general agreement with safety and the overall impression of efficacy of MSCs, there is wide variability in the outcome of GvHD patients after MSC treatment [37]. The present systematic review emphasizes the heterogeneity of outcomes of clinical trials using different MSC products.

Herein, we presented several studies with the aim of assessing the feasibility and efficacy of MSC treatment. Even with MSCs, in some studies the mortality rate was relatively high [30–32, 36]. Ball et al. [23] demonstrated a better OS (56%) and lower TRM (17%) in patients who received MSCs between days +5 and +12 after steroid initiation compared with treatment between days +13 and +85 (25 and 53%; $p = 0.22$ and $p = 0.06$). Dotoli et al. [31], Dalowski et al. [32], and also Salmenniemi et al. [30] reported considerably lower response rates and OS. Bader et al. [5] reported a large cohort of patients with refractory aGvHD who received the completely new product "MSC-FFM." The overall response rate at day +28 as an outcome parameter was 83%. Indeed, these results are very encouraging compared with other aGvHD studies [21, 28, 29, 31, 32]. To our knowledge, so far MSC-FFM offered the best response rates of all MSC preparations in children and especially in adults. The authors attribute this superiority to the MSC preparation procedure. Kuçi et al. [24] demonstrated that generation of MSCs from pooled bone marrow mononuclear cells of multiple donors seems to be more efficient than pooling MSCs from various donors in that it yields cells with significantly higher allosuppressive potential. In vitro, all tested MSC end products showed an equivalent allosuppressive effect after thawing, therefore every patient received the same, standardized product.

In the majority of studies human bone marrow-derived MSCs were administered. The number of MSC infusions as well as the MSC doses varied greatly across the trials. Trento et al. [38] analyzed data from 17 European Group for Blood and Marrow Transplantation (EBMT) centers via questionnaire, especially focused on MSC manufacturing. Eighty-eight percent of centers manufactured bone marrow-derived MSCs, while only 2 centers produced from umbilical cord. Release criteria differed largely among centers. The authors hypothesized that discrepancies may impact on MSC therapeutic activity and clinical outcomes. The questionnaire results highlighted heterogeneity and identified a need for harmonization of MSC manufacturing.

No limiting acute and late toxicity or side effects were reported due to MSC infusion in numerous trials [5, 22, 32, 35]. However, the benefit obtained with MSCs is partly offset by mortality due to infectious complications. von Bahr et al. [39] evaluated the outcome of 31 patients who had been treated with MSCs. They revealed that after recovery from aGvHD, 54% of patients died of infectious complications, which occurred between 4 months and 2 years after MSC therapy. Patients with therapy-resistant GvHD are heavily immunocompromised and susceptible to infections. Effective infectious prophylaxis appears to be essential with regard to improved outcomes. The available data reveal antimicrobial activity of MSCs; nevertheless, there is a paucity of in vivo and in vitro studies evaluating the antiviral and antifungal effects of MSCs [40]. Future surveys should focus on in vivo interactions between pathogens and MSCs in the setting of allo-SCT.

The available body of data seems to send two salient messages. First, MSCs possess immunosuppressive potential, which can be harnessed to treat inflammatory conditions like steroid-refractory aGvHD. Second, clearly not all MSC preparations are equal in potency. Even though at this point of time it is impossible to pinpoint the relevant quality attributes, which can be gauged in vitro as predicators of in vivo effectiveness, to be used as release criteria and for guided optimization of manufacturing processes, it is becoming clear that careful attention to manufacturing protocols can result in reproducibly efficacious MSC-based medicines.

In conclusion, MSCs are emerging as a promising alternative to second-line immunosuppressants and can be safely administered for steroid-refractory aGvHD therapy.

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Disclosure Statement

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