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Mesenchymal Stem Cells as Cellular Immunotherapeutics in Allogeneic Hematopoietic Stem Cell Transplantation

Claudia Papewalis, Daniela Topolar, Barbara Götz, Stefan Schönberger and Dagmar Dilloo

Abstract Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment option in hematopoietic disorders, immunodeficiencies and leukemia. To date graft-versus-host disease (GvHD) represents a life-threatening complication even if associated with beneficial antileukemic reactivity. GvHD is the clinical manifestation of donor cells reacting against host tissue. Because of their ability to facilitate endogenous repair and to attenuate inflammation, MSC have evolved as a highly attractive cellular therapeutic in allo-HSCT. Here we report on the clinical experience in the use of MSC to enhance engraftment and prevent and treat acute and chronic GvHD. In early clinical trials, MSC have shown considerable benefit in the setting of manifest GvHD. These encouraging results warrant further exploration.

Keywords Graft-versus-host disease · Clinical grade MSC · Immunosuppresion

Abbreviations

APC	Antigen presenting cells
BM	Bone marrow
CR	Complete response
DC	Dendritic cell
EBMT	European Group for Blood and Marrow Transplantation
GvHD	Graft-versus-host disease
GvL	Graft-versus-leukemia
(allo-)HSCT	(allogeneic) Hematopoietic stem cell transplantation
HLA(-G)	Human leukocyte antigen(-G)
HSC	Hematopoietic stem cell
IDO	Indoleamine-2,3,-dioxygenase

C. Papewalis · D. Topolar · B. Götz · S. Schönberger · D. Dilloo (✉)
Department of Pediatric Hematology and Oncology, Center for Pediatrics, University Hospital, Friedrich-Wilhelm University, Bonn Germany
e-mail: Dagmar.Dilloo@ukb.uni-bonn.de

ISCT	International Society for Cellular Therapy
IFN	Interferon
IL	Interleukin
MSC	Mesenchymal stem cell
OR	Overall response
PR	Partial response
PBSC	Peripheral blood stem cell
PD-L	Ligand of the programmed death receptor
PGE_2	Prostaglandin E_2
PL	Platelet lysate
TGF	Transforming growth factor
Th	T helper cells
TNF	Tumor necrosis factor
UCB	Umbilical cord blood

Contents

1 Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has evolved as a potentially curative treatment option for patients with malignant and nonmalignant hematological and immunological disorders. In bone marrow failure syndromes and immunodeficiencies, hematopoietic stem cells (HSC) from a healthy donor are transplanted with the intent to reconstitute the patient with a functional hematopoietic and immunological system. In leukemia and other hematological malignancies, the aim is to eliminate residual neoplastic disease in a twofold manner. Thus, treatment with cytotoxic radio-/chemotherapy pre-transplant is consolidated

by antineoplastic immunological attack mediated by donor-derived immune system cells and myeloablative conditioning regimens. The allogeneic HSC-graft also serves to compensate for treatment-related lethal hematopoietic failure. Although the graft-versus-leukemia (GvL) reaction is a critical therapeutic component of allo-HSCT, it is associated with the potentially detrimental effects of graft-versus-host disease (GvHD) [65, 132]. GvHD results from cytotoxic allo-reactivity of grafted immune cells against normal host tissue. Severe donor versus host reactions lead to massive tissue injury and ultimately to impaired immunological recovery. As greater HLA disparity between recipient and donor is associated with an enhanced risk for GvHD, related and unrelated donors are generally chosen by a close degree of human-leukocyte-antigen (HLA) match [95].

Currently diverse sources of allogeneic stem cells, namely bone marrow (BM), cytokine-mobilized peripheral blood stem cells (PBSC) [113, 137], as well as umbilical cord blood (UCB) [9, 115, 127] are in use. In UCB-transplantation, the potential to cross significant HLA-barriers safely due to the relative immaturity of donor T cells in the graft has extended the access to suitable HSC products even in populations with rare tissue phenotypes. Also the possibility of mobilizing HSC to the periphery by growth factor stimulation has opened the avenue to harvest large quantities of HSC that lend themselves to further selection with the aim of enriching the stem cell population and/or depleting potentially allo-reactive T lymphocytes [21, 64, 92]. This has cleared the way for transplantation of HSC from donors with a full HLA-haplo-type mismatch such as patients' parents [27, 105] and has further expanded the use of allogeneic HSCT over the last several years. To date already more than 25,000 patients per year worldwide have been transplanted with allogeneic HSC [39, 108]. Given current trends, the number of transplants from unrelated donors is expected to double within the next five years which will also significantly increase the population of patients at risk for GvHD [28].

With continuous improvement in anti-infectious, particularly antiviral and antimycotic therapy [108, 114, 123] and concepts of reduced intensity conditioning [41, 131, 136], the treatment-related mortality (TRM) of allo-HSCT has decreased considerably compared to its early beginnings [13, 106]. Yet, even with enhanced accuracy in HLA-typing and improved donor selection [96], the various possibilities of graft manipulations, and optimized immunosuppressive prophylaxis and therapy, GvHD remains a therapeutic challenge.

In addition to HSC, bone marrow and umbilical cord blood also harbor a mesenchymal stem cell (MSC) population with self-renewal and multilineage-differentiation ability [16, 109]. MSC further possess immunomodulatory potential that is not constitutive but specifically triggered in an inflammatory milieu. As MSC are able to migrate to sites of cellular injury and inflammation [135] and to exert their immunosuppressive activity in an environment of tissue damage [59, 90], MSC have gained considerable interest as cellular immunotherapeutics in allo-HSCT, particularly in the setting of GvHD.

2 Clinical GvHD

Graft-versus-host disease describes the clinical manifestations of recipient cells under attack by grafted donor immune cells. To date, it is still a life-threatening complication.

Acute GvHD (aGvHD) is defined to occur within the first 100 days after HSCT, and chronic GvHD (cGvHD) thereafter. In principle, the acute and chronic forms of GvHD may have overlapping symptoms and merge into each other. Acute GvHD can also resolve completely and still be followed later by cGvHD [28, 47]. In aGvHD, skin is most commonly affected and is usually the first organ involved. Acute GvHD of the skin often coincides with engraftment of donor cells. The characteristic of skin disease is a pruritic rash that can spread all over the body. In severe cases, the skin may blister and ulcerate. Gastrointestinal tract involvement usually presents as diarrhea combined with vomiting, anorexia, and abdominal pain. Depending on the severity, bloody diarrhea as a result of mucosal ulceration carries a particularly poor prognosis [37]. Liver disease caused by aGvHD may be difficult to distinguish from other causes of liver dysfunction following allo-HSCT such as veno-occlusive disease (VOD), drug toxicity, viral infection, or sepsis [34].

A grading system for aGvHD was introduced in the 1970s by Glucksberg et al. [36]. Today, most institutions use sets of criteria previously established at the Keystone Consensus Conference of 1994 [103] or the consensus criteria issued by the Center for International Blood and Marrow Transplant Research [76]. Scoring aGvHD severity is carried out by first staging the affection of skin, liver, and gastrointestinal tract as a basis of an overall grade that acknowledges both the stage of organ pathology as well as the number of organs involved. These overall grades are classified as I (mild), II (moderate), III (severe), and IV (very severe), or A–D, respectively. Severe aGvHD carries a poor prognosis, with 25 % long-term survival for grade III and 5 % for grade IV [18]. The incidence of aGvHD is related to the degree of mismatch between HLA-proteins and the degree of ex and in vivo graft manipulation [18]. Acute GvHD ranges from 35 to 45 % in BM or PBSC recipients of fully matched siblings to 60–80 % in T-replete $\geq$1 HLA-mismatched unrelated transplant recipients [31, 47, 70, 113, 137]. The same degree of mismatch causes less GvHD using UCB grafts. Thus, the incidence of aGvHD is lower following the transplant of partially matched UCB units and ranges from 25 to 65 % depending on the overall transplant setting such as intensity of conditioning, and in haploidentical PBSC-transplantation also on the extent of graft manipulation [9, 10, 27, 105].

Treatment of primary aGvHD largely comprises the same agents used for prophylaxis such as calcineurin-inhibitors and mycophenolate mofetil [130] with glucocorticoids representing the backbone of aGvHD treatment [76, 110]. Overall less than 50 % of patients respond to glucocorticoids with slightly higher response rates in children [47].

Chronic GvHD remains the major cause of late nonrelapse death following HSCT [63]. The syndrome has features resembling autoimmune and other immunological disorders such as scleroderma, Sjögren syndrome, primary biliary cirrhosis, wasting syndrome, bronchiolitis obliterans, immune cytopenias, and chronic immunodeficiency. Manifestations of cGvHD may be restricted to a single organ which is classified as limited or mild cGvHD. Chronic GvHD can also be widespread affecting many organ sites and is then termed extended or severe. It can lead to debilitating consequences, for example joint contractures, loss of sight, end-stage lung disease, or mortality due to profound chronic immune suppression with recurrent and ultimately life-threatening infections [29]. aGvHD consensus criteria for grading the severity of cGvHD have been published but are as yet not employed consistently [5].

Treatment of cGvHD follows along the same lines as in aGvHD. Yet the response rate is even lower, with a third of patients [4] not responding to first-line therapy often consisting of corticosteroid and calcineurin inhibitor therapy either alone or in combination [57]. Although for refractory cGvHD a variety of therapies have been evaluated [20, 46, 50, 71], efficacy has been limited. Long-term survival is poor due to toxicity related to profound and prolonged immunosuppression. Thus, treatment of GvHD remains a therapeutic challenge warranting the evaluation of novel treatment options [82, 134]. To date, glucocorticoid-resistant GvHD is among the most challenging complications in allo-HSCT.

3 Pathophysiology of GvHD

The paradigm of GvHD development has been conceptualized as a three-step process [28]. The initiation phase is characterized by tissue damage caused by intensive conditioning therapy pre-transplant. As a result, host antigen-presenting cells (APC) such as dendritic cells (DC) and monocytes become activated: HLA-antigens as well as co-stimulatory and adhesion molecules are up-regulated on their cell surface. In addition pro-inflammatory cytokines such as interleukin (IL)-1β, and tumor necrosis factor (TNF)-α and chemokines are released. Treatment-related mucositis with destruction of the gastrointestinal mucosal barrier results in systemic translocation of inflammatory stimuli derived from microbial products. These pathogen-associated molecular patterns serve as "danger-signals" and further enhance the activation and maturation of host APC [25, 43]. Recipient APC seem to be sufficient to induce GvHD, however, murine models suggest that donor APC may also contribute by indirect antigen presentation [3].

The second phase of GvHD-development is characterized by activation of mature donor T cells recognizing cognate antigens presented by host APC. In response, T cells proliferate and differentiate into activated effector cells within a danger-signal-rich milieu. They contribute to this by release of additional cytokines. Indeed, polymorphisms for critical cytokines such as TNF-α and interferon (IFN)-γ have been implicated as risk factors for GvHD [67]. Most of this process

takes place within secondary lymphoid organs as early as three days after transplant, long before de novo regeneration of donor T cells has ensued [117].

The third phase is the effector phase of GvHD which leads to target organ destruction. Chemokines over-expressed by macrophages direct the migration of donor cells from lymphoid organs to the target tissues. Here cytotoxic cellular mediators, namely donor T and NK cells, and soluble inflammatory factors such as TNF-α, IFN-γ, IL-1β, and nitric oxide synergize and amplify local tissue damage and promote inflammation. In intestinal GvHD, integrins further facilitate homing of donor T cells to Peyer's patches [128]. Ultimately, end organ damage is predominately due to T cell-mediated tissue toxicity, which involves soluble mediators, including TNF-α, perforin, granzymes, Fas, and Fas ligand [7, 17, 40, 49, 77]. As hepatocytes express large amounts of Fas, in liver GvHD cytotoxic T cells preferentially use the Fas/FasL pathway for target cell lysis. In contrast, the perforin/granzyme pathway plays a dominant role in GvHD affecting skin and the gastrointestinal tract [125].

Thus in allo-HSCT, severe donor-versus-host immune reactions can result in massive end-organ injury. Based on the multitude of immunomodulatory activities and their capacity to support the healing process at sites of tissue injury, MSC are deemed highly attractive candidates for mitigation of both acute and chronic GvHD following allo-HSCT.

4 Immunomodulation in GvHD Mediated by MSC

MSC are pluripotent cells characterized by self-renewal and the multilineage differentiation capacity for a variety of cell types such as chondrocytes, adipocytes, and osteoblasts. MSC were originally isolated and characterized as nonhematopoietic multipotent progenitors of adult bone marrow [15, 16] and termed "multipotent stromal cells" [44]. They have been implicated in hematopoietic support [23].

Meanwhile, it is known that MSC can be effectively detected in almost every tissue such as umbilical cord blood, Wharton's jelly, amniotic fluid, adipose tissue, skeletal muscle, liver, brain, hair follicle, and dental pulp [42, 45, 104, 139]. Based on their ability to home to sites of organ injury, to facilitate tissue repair, and to critically modulate immune responses, MSC have generated considerable interest as cellular therapeutics. In an effort to harmonize MSC characterization, the International Society for Cellular Therapy (ISCT) has issued a consensus set of three minimal criteria to define MSC regardless of their tissue of origin: (I) plastic adherence, (II) maintenance of tri-lineage osteogenic, adipocytic, and chondroblastic differentiation potential after in vitro propagation, and (III) lack of the hematopoietic markers CD45, CD34, CD14, CD11b, CD79-α, CD19, and HLA-DR, and simultaneous expression of the surface molecules CD73, CD90, and CD105 on $\geq$95 % of the population [24]. The surface molecule CD73, an ecto-5′-nucleotidase, is involved in cellular crosstalk, migration, and modulation of

adoptive immunity. The interaction between CD73 and adenosine A2A receptor results in the blockade of the adenosine pathway in activated T cells with a subsequent proliferation stop [16, 112]. CD90 (Thy-1) is viewed as a marker of "stemness". Its function on MSC is not entirely resolved but as a GPI-anchor it is known to mediate cell-to-cell interactions as well as monocyte and lymphocyte adhesion. CD105 (endogline) belongs to the TGF receptor family [16].

Of note, homing and immunosuppressive activity of MSC is not a constitutive phenomenon but requires a pro-inflammatory milieu. Expression and release of critical immunosuppressive factors such as prostaglandin E_2 (PGE_2), hepatocyte growth factor (HGF), IL-10, transforming growth factor-β (TGF-β), and leukemia inhibitory factor (LIF), human leukocyte antigen-G (HLA-G), and galectin-1 are dependent on MSC priming by cytokines such as by IFN-γ, TNF-α, and IL-1β [35, 118]. Also the enzyme Indoleamine 2,3-dioxygenase (IDO) is regulated by IFN-γ, IDO catabolizes tryptophan to kynurenine resulting in depletion of the cellular milieu from tryptophan and accumulation of cell-toxic kynurenine metabolites. We and others have previously shown that tryptophan starvation of the microenvironment down-tunes effector cell function such as proliferation, cytotoxicity, and cytokine production in activated T and NK cells [68, 84, 119, 120]. In addition, IFN-γ-dependent up-regulation of STRO-1 and ligand of the programmed death receptor-1 (PD-L1) are among the surface molecules involved in MSC-mediated T cell inhibition in a cell-contact-dependent manner [88, 111]. MSC further modulate the complement activation pathways by constitutive expression of factor H which again may be up-regulated by TNF-α and IFN-γ, key mediators of aGvHD [124].

Thanks to the plethora of immunosuppressive effects exerted on APC as well as effector cells, MSC are potentially capable of intercepting each of the individual stages in GvHD development (Fig. 1). In the first phase of GvHD, damage of the host leads to the accumulation of an array of chemokines such as CCL2, CCL5, CCL22, and CXCL12. The respective chemokine and growth factor receptors are expressed on MSC. They become up-regulated on TNF-α primed cells, thereby further enhancing their homing efficiency. All together, the migratory capacity of MSC is under the control of a large range of receptor tyrosine kinases, growth factors, and CC and CXC chemokines [100].

LPS, TNF-α, IL-1, and IL-6 are released at sites of injury. These cytokines stimulate maturation of host antigen presenting cells (APC) such as dendritic cells (DC) critical for subsequent activation of allo-reactive T lymphocytes. Here, MSC provide counter-regulatory signals, namely PGE_2, IL-6, and M-CSF that depress DC surface expression of HLA-DR and CD1a as well as of the co-stimulatory molecules CD80 and CD86 [90]. Also DC-expression of TNF-α, IL-12 is decreased whereas IL-10 release is up-regulated shifting the dendritic surface marker and cytokine profile towards a tolerogenic state. Here, the soluble factors IL-6 and M-CSF have been implicated not only in induction but also maintenance of the immature DC phenotype [89, 135]. In addition, MSC intervene with the effector phase of GvHD by inhibiting expansion of the effector cell pool and down-modulating cytokine production in T and NK cells. Suppression of NK cell

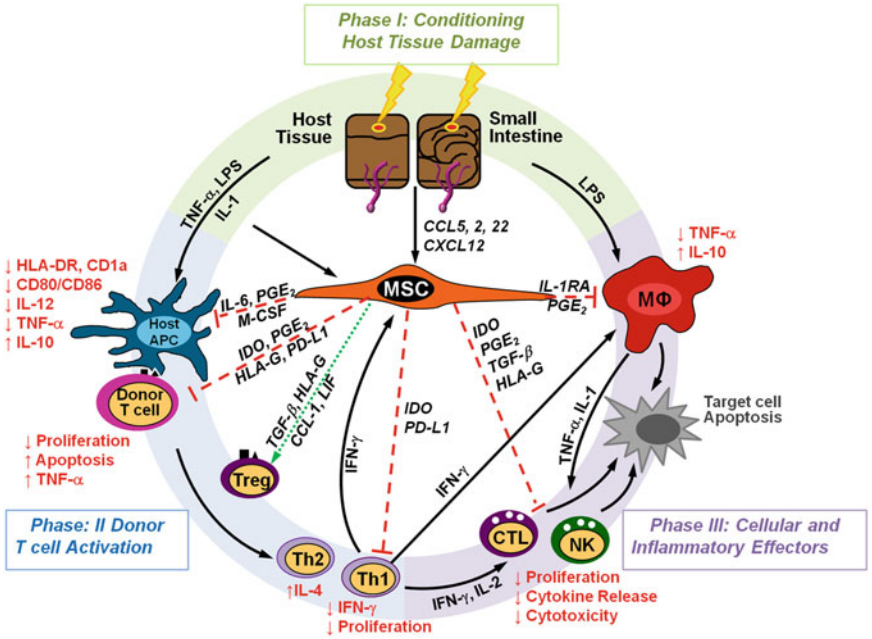

Fig. 1 Scheme of MSC attenuating all three phases of GvHD development. The tri-phasic model of acute GvHD evolution and maintenance is depicted as a self-perpetuating cycle of inflammation resulting in target organ damage mediated by allo-reactive effector cell responses (adapted from [43]). MSC are attracted by the pro-inflammatory milieu to sites of tissue damage. Once licensed by inflammatory cytokines such as IFN-γ and TNF-α, MSC actively modulate each phase of the immune response. *Black arrows* indicate the mode of interaction between different cellular players of the "GvHD-cycle." *Red lines* refer to MSC-mediated attenuating effects and the *green line* implies a supporting role of MSC. The resulting changes in effector functions are printed in *red* or *green*, respectively (Color figure online)

cytotoxicity is also due to MSC-mediated down-regulation of the activating NK receptors NKp 30, NKp 44, and NKD2D [90].

It is important to note that MSC dampen the self-perpetuating inflammatory mechanisms in GvHD by blocking the release of the critical cytokines TNF-α and IFN-γ. Thus, secretion of TNF-α by monocytes is suppressed by MSC-secretion of the IL-1a receptor antagonist (IL-1RA) [90]. Also, the T cell-dependent feedback loop of TNF-α production is intercepted by MSC. Here, TNF-α-induced PGE_2 expression in MSC not only down-modulates T cell proliferation but also T cell cytokine release including TNF-α [135]. Similarly, MSC deflect the IFN-dependent feedback mechanism, as IFN-γ-induced expression of IDO and PD-L1 in MSC [135] in turn reduces IFN production in Th1 cells and up-regulates IL-4 production in Th2 lymphocytes. This creates a tolerogenic milieu not only locally but also systemically tipping the balance towards an anti-inflammatory Th2 response [2]. Regulatory T (Treg) cells also contribute to this MSC-induced local and systemic network. MSC facilitate Treg-induction and expansion by release of

HLA-G, LIF, and CCL1 [90]. In addition, interaction between the surface molecules CD58 and CD52 expressed on MSC with CD2 and CD11a on T cells generates a FOXp3-negative CD4/CD8 double positive Treg population that has been found to be one hundredfold more T cell suppressive than FOXp3-positive CD4/CD25 double positive Treg [102].

Thus, MSC potentially interact with almost every immune cell population involved in GvHD initiation and perpetuation in an attenuating manner. At the same time, the need for so-called "licensing" by pro-inflammatory signals to trigger immunosuppressive activity renders MSC particularly attractive for cell therapy. In the absence of inflammation, MSC stay immunologically inert and thus do not contribute to generalized immune suppression as many pharmacological agents such as steroids do [82]. Moreover the MSC-mediated T cell inhibitory function is differentially directed against allo-specific T cell activity and does not attenuate antiviral recall responses [52]. MSC themselves exhibit profound antiviral and antimicrobial activity. Indeed IDO, one of the key IFN-γ dependent T cell inhibitory mechanisms formerly identified by us, also dampens the amplification of cytomegalovirus and toxoplasmosis, two highly critical infectious agents in allo-HSCT [22, 73, 86]. One of the major issues when introducing novel immunomodulatory cell therapeutics in clinical allo-HSCT is the increased risk of infection. The above-described pre-clinical insights partially address these concerns.

5 MSC for Clinical Application in Allo-HSCT

To date, GvHD remains a significant cause of nonrelapse morbidity and mortality following allo-HSCT. During the last decade, BM-derived MSC have been employed in a series of studies for prevention and treatment of GvHD in the allo-HSCT setting.

Initially, MSC were predominantly isolated from siblings or related haploidentical donors. Separation of MSC from the bone marrow was performed by density gradient centrifugation of the mononuclear cell fraction and subsequent in vitro propagation of the plastic adherent cell fraction over 4–6 passages. Later, particularly in those studies employing MSC products provided by Osiris Therapeutics, Inc. (Prochymal®), MSC were obtained from unrelated healthy third-party volunteer donors with variable degrees of HLA-matching depending on the recipient's phenotype. With a frequency of 0.01–0.001 % mesenchymal progenitors in the BM, a 10-ml aspirate is generally sufficient to yield 50–300 $\times$ 10^6 MSC without loss of multidifferentiation potential [97]. In the earlier investigator-initiated trials (IIT), MSC were often used directly from the culture. Industrially prepared MSC are generally cryopreserved, off-the-shelf products that need to be defrosted prior to use. Also in some preliminary studies, other sources of MSC have been explored such as adipose tissue [26] and cord blood [62, 129].

Overall, there are 27 reports describing MSC application in allo-HSCT. In addition to a few case reports or case series [60, 87, 107, 129], the studies published are predominately pilot/phase I trial. There are few prospective phase II studies [53, 61, 69]; two of these are randomized open label studies [53, 69] and only one randomized phase III trial [81] which unfortunately has thus far only been published in an abstract format. About a third of the studies evaluate safety and feasibility of MSC transfusion in conjunction with transplantation of allogeneic HSC with the secondary aim to enhance engraftment and potentially prevent GvHD (Table 1). Another third of the studies assess MSC treatment for steroid-resistant aGvHD (Table 2) and only two studies focus entirely on MSC application for treatment of refractory and severe cGvHD [133, 140] (Table 3). Of note, there is one prospective phase II study employing MSC for first-line treatment of aGvHD [53] (Table 2b).

Two landmark reports introduced the medical community to the potential of MSC administration in allo-HSCT. A large multicenter feasibility study [56] documented that MSC expansion from BM to clinically relevant quantities was feasible within one month. A year before, Le Blanc's group from the Karolinska Institute, Stockholm, had reported on the first successful treatment of steroid-refractory severe acute GvHD in a nine-year-old boy with BM-derived MSC from his mother [60].

6 MSC for Enhanced Engraftment and Prevention of GvHD

Feasibility and safety of MSC/HSC co-transplantation was evaluated in two phase I studies [56, 91, 138] and one randomized phase II study following myeloablative conditioning in the context of HLA-matched sibling transplants. Secondary study endpoints assessed the kinetics of HSC engraftment and GvHD incidence. In all three studies, HSCT was performed for adult patients with high risk or relapsed hematological malignancies. GvHD prophylaxis comprised cyclosporine and MTX in all three studies. In the largest of these studies [56], patients were recruited in a multicenter study effort across the United States. For these 56 patients, BM-derived MSC were sampled from the respective HSC donors and prepared by Osiris Therapeutics Inc., Baltimore. Adequate expansion proved feasible in 91 % of sibling donors (51/56 donors) up to a dose of 2.5×10^6/kg within 30 days, even though only 46 patients were eventually transplanted with a combination of HSC and MSC.

Likewise in one of the two Chinese feasibility studies [138], MSC preparation up to a dose of 2×10^6/kg recipient body weight were obtained in 86 % of cases (12/14 donors). Yet, in the only randomized study [91], this target dose was not achieved, with only three patients transplanted with $\geq 1.0 \times 10^6$/kg. The MSC doses infused in this randomized open-label trial were considerably lower than in the other studies with $0.03–1.53 \times 10^6$/kg (median 0.34×10^6/kg). Moreover 5/15 patients of the "intend-to-treat" cohort had to be excluded from further

Table 1 MSC for enhanced engraftment and prevention of GvHD

First author, year Study type	pt/co (*n*)	Child/adult (*n*)	HSCT condi-tioning (*n*)	HSCT source (*n*)	MSC source MSC donor sib/haplo/MM (*n*)	MSC dose ($n \times 10^6$/kg)	Engraft-ment (%)	aGvHD [grade] (*n*/%)	cGvHD (*n*/%)	Relapse (*n*/%)	Infection (*n*)	OS/FU (%/months)
Lazarus, 2005 Phase I study, IIT	46/–	–/46	MAC	BM (19) PBSC (27)	Same as HSC donor 46/–/–	37pt: 1–2.5 5pt: 5	100	[I] 23/50 [II–IV] 13/28	ltd. 14/30 ext. 8/17	12/26	n.i.	53/24
Ning, 2008 Rand. phase II study, IIT	10/15	–/10	MAC	BM (4) PBSC (4) BM & PBSC (2)	Same as HSC donor 10/–/–	0.03–1.5	pt: 90 co: 100	pt: [II–IV] 1/10 co: [II–IV] 8/53	pt: 1/10 co: 4/26	pt: 6/60 co: 3/20	pt: CMV (2) Bacterial and/or fungal pneumonia (2) co: CMV (2) Pneumonia (3)	pt: 40/36 co: 67/36
Zhang, 2009 Pilot/phase I study, IIT	12/–	–/12	MAC	PBSC	Same as HSC donor 12/–/–	1.3–2.2	100	[I] 7/58 [II–III] 2/17	ltd. 2/17 ext. 2/17	4/33	CMV-viremia (4) Hepatitis B (1) Lung infection (1)	58/58
Gonzalo-Daganzo, 2009 Phase I/II study, IIT	9/46	–/9	MAC	UCB & PBSC	Same as PBSC donor –/7/2	1–2.2	pt: 100 co: 93	pt: [I] 1/11 [II] 4/44 co: [I] 18/39 [II] 5/11 [III–IV] 3/6.5	pt: ltd. 1/11 co: ltd. 8/17 ext. 3/7	pt: 1/11 co: 6/13	pt: CMV	pt: 89/22 co: 53/60
MacMillan, 2009 Phase I/II study	8/23	8/–	NMAC	UCB	–/8/– Plasme Lyte®	1pt: 0.9–5 3pt: 0.06–10 (2x)	pt: 100 co: 100	pt: [II] 3/38 co: [II–IV] 5/22	pt: – co: 4/17	pt: 1/13	pt: Aspergillosis (2) Clost. diff. colitis (3) Bacteremia (2) CMV (1) Sinusitis (1) Shingles (1)	pt: 75/12 co: 62/76–94

(continued)

Table 1 (continued)

First author, year Study type	pt/co (*n*)	Child/adult (*n*)	HSCT conditioning (*n*)	HSCT source (*n*)	MSC source MSC donor sib/haplo/MM (*n*)	MSC dose ($n \times 10^6$/kg)	Engraftment (%)	aGvHD [grade] (*n*/%)	cGvHD (*n*/%)	Relapse (*n*/%)	Infection (*n*)	OS/FU (%/months)
Lee, 2011(oral) Pilot study, IIT	7/22	7/–	n.i.	UCB	UCB pt: –/–/7	4pt: 1 (1x) 3pt: 1 (5x)	pt: 100 co: 86	pt: [III–IV] 1/14 co: [III–IV] 1/5	pt: ext. 1/14 co: ext. 4/19	n.i.	n.i.	pt: 75/24
Ball, 2007 Phase I/II study, IIT	14/47	14/–	MAC	sPBSC Haplo	As HSC donor 1/13/–	1–5	pt: 100 co: 85	pt: [I–II] 2/14 co: [I–II] 12/26 [III–IV] 2/4	pt: ltd. 1/7 co: ltd. 4/9 ext. 2/4	pt: 3/18 co: 12/26	pt: viral (7) Sepsis (1) ADV hepatitis (1) co: viral (5) ADV hepatitis (2)	pt: 72/3–28 co: n.i.
Baron, 2010 Pilot study, IIT	20/16	–/20	NMAC	PBSC MM	–/–/20	1	pt: 95 co: 100	pt: [II–III] 7/35 [IV] 2/10 co: [II–III] 6/32 [IV] 3/19	pt: n.i. co: 23/65	pt: 6/30 co: 4/25	pt: cerebral toxoplasmosis (1) Encephalopathy (1)	pt: 80/12 co: 44/12
Liu, 2011 Rand. phase II study, IIT	27/28	n.i./n.i.	MAC	BM & PBSC haplo	MM from rel. or unrel. donors –/4/23	0.3–0.5	pt: 100 co: 96	pt: [I–II] 16/70 co: [I–II] 15/53 [III] 1/4	pt: ltd. 9/33 ext. 4/15 co: ltd. 11/39 ext. 4/14	pt: 2/7 co: 1/4	pt: CMV-viremia (18) Pneumonia (3) EBV-PTLD (2) co: CMV-viremia (18) Pneumonia (3)	pt: 70/24 co: 64/24

(continued)

Table 1 (continued)

First author, year Study type	pt/co (*n*)	Child/adult (*n*)	HSCT conditioning (*n*)	HSCT source (*n*)	MSC source MSC donor sib/haplo/MM (*n*)	MSC dose ($n \times 10^6$/kg)	Engraftment (%)	aGvHD [grade] (*n*/%)	cGvHD (*n*/%)	Relapse (*n*/%)	Infection (*n*)	OS/FU (%/months)
Wang, 2012 Case series in SAA, IIT	6/–	6/–	MAC	BM & PBSC (haplo) (3)/PBSC (MUD)(3)	–/1/5 1x BM 5x UCB	0.85–2.5	100	[I–II] 2/33	–	n.i	–	100/6–29
LeBlanc, 2007 Pilot study, IIT	7/–	4/3	MAC (3) NMAC (4)	BM (2) PBSC (4) UCB (1)	3/4/–	1	100	pt:[I–II] 6/85	1/14	n.i.	Bacterial (2) Aspergillosis (1)	86/≥1 12
Meuleman, 2009 Pilot/phase I study, IIT	6/–	–/6	MAC	PBSC	As HSC donor 3/3/–	1	33	n.i	n.i.	1/17	Pulmonary Aspergillosis (2) Retinitis Encephalitis CMV (1)	33/12 17/158
MSC transfusion at time of leukocyte recovery for prevention of GvHD												
Kuzmina, 2012 Pilot/phase I study, IIT	19/18	–/19	MAC (14) NMAC (4)	n.i.	As HSC donor Related donors **PL**	0.9–1.3	n.i.	pt: [I–II] 6/32 co: [I–II] 9/50 [III–IV] 1/5,5	pt: 5/26 co: 6/33	pt: 4/21 co: 5/28	n.i.	pt: 95/3 co: 78/3

Note With few exceptions, patients were transplanted for malignant hematological diseases. Controls were matched to patients co-transplanted with MSC for age, conditioning, HSC source, donors, and dose. MSC were BM-derived, unless otherwise indicated. Except for randomized studies, historical controls were used

Abbreviations: *ADV* adenovirus, *aGvHD* acute graft-versus-host-disease, *AS* autologous serum, *BM* bone marrow, *CB* cord blood, *cGvHD* chronic graft-versus-host-disease, *Clost. diff. colitis Clostridium difficile* colitis, *co* controls, *com* commercial, *CR* complete response, *EBV-PLTD* Epstein–Barr virus-related post-transplant lymphoproliferative disorder, *ext* extensive, *FCS* fetal calf serum, *FU* follow up, *GI* gastro-intestinal tract, *Haplo* HLA haploidentical related, *HD* high dose, *HM* hematological malignancies, *hist* historical, *HSCT* hematopoietic stem cell transplantation, *IIT* investigator initiated trial, *Inf* infection, *LD* low dose, *ltd* limited, *MAC* myeloablative chemotherapy, *Med* median, *MM* mismatched unrelated, *MOF* multiple organ failure, *MR* mixed response, *MSC* mesenchymal stem cells, *MUD* matched unrelated donor, *n* number, *n.i.* not indicated, *NMAC* nonmyeloablative chemotherapy, *OR* overall response: *sum of CR + PR*, *oral* oral presentation, *OS* overall survival, *PBSC* peripheral blood stem cells, *PL* platelet lysate, *PFS* progression free survival, *PR* partial response, *pt* patient, *rand* randomized, *rel.* related, *SAA* severe aplastic anemia, *SCT* stem cell transplantation, *Sib* HLA-identical sibling donor, *TBI* total body irradiation, *UCBT* umbilical cord blood transplantation, *VOD* veno-occlusive disease

Table 2 MSC for treatment of (a) steroid-resistant and (b) steroid-resistant or de novo* acute GvHD

First author, year Study type	pt/co (*n*)	aGvHD/ cGvHD (*n*)	Child/adult (*n*)	MSC donor Sib/Haplo/MM (*n*) Supplement	MSC application		aGvHD pre MSC I/II/III/IV (*n*) I/II/III/IV (%)	Response to MSC CR/PR/MR/OR (*n*) CR/PR/MR/OR (%)	Infection (*n*)	OS/FU (%/months)
					Infusions (*n*)	Dose ($\times 10^6$/kg)				
(a) *Bone marrow-derived MSC expanded with FCS*										
LeBlanc, 2004 Case report	1/–	1/–	1/–	–/1/–	1pt: 2	2–1	–/–/–/1 –/–/–/100	1/–/–/**1** 100/–/–/**100**	Repeated bacterial, viral, and invasive fungal infections	100/12
Ringden, 2006 Pilot/phase I study, IIT (pt incl. in LeBlanc, 2008)	9/16	8/1	2/7	4/3/1	5pt: 1 3pt: 2 1pt: 1	0.7–2.0 0.7–2.0 9.0	–/2/5/1 –/25/62/13	6/1/1/**7** 75/13/12/**88**	CMV (2) EBV-PTLD (1) ADV (1) Aspergillosis (1)	56/48
Müller, 2008 Pilot study, IIT	7/–	2/5	7/–	–/–/2	4pt: 2 2pt: 1 1pt: 3	0.4–3	–/1/1/– –/50/50/–	1/–/–/**1** 50/–/–/**50**	–	50/60
Fang, 2007 Phase I study, IIT	6/–	6/–	2/6	MSC source: adipose tissue –/2/4	6pt: 1–2	1–2	–/–/2/4 –/–/33/66 (children: n.i.)	5/–/–/**5** 83/–/–/**83**	n.i.	In phase I study 83/12
LeBlanc, 2008 Prospective multicenter phase II study, IIT	55/–	55/–	25/30	5/18/69	27pt: 1 22pt: 2 6pt: 3–5	0.6–9	–/5/25/25 –/9/45,5/45,5	30/9/–/**39** 55/16/–/**71**	CMV (4) EBV (3)-LPD (1) Aspergillosis (5) Septicemia (6) Inf.(viral + bact.) (9)	38/3–60
Prasad, 2011 Compassionate use multicenter study	12/–	12/–	12/–	–/–/12 Prochymal ® Formulated in Plasme Lyte ®	1pt: 3 8pt: 7–12 1pt: 2 2pt: 21	2 2 8 2–8	–/–/8/4 –/–/67/33	7/2/3/**9** 58/17/25/**75**	EBV-LPD (1) Resp. failure (6) Fusarium infection (2)	42/20
Kurtzberg, 2010[b] FDA expanded access program	59/–	59/–	59/–	–/–/59 Prochymal ® Formulated in Plasme Lyte ®	All pt: 8 (+4)	2	–/6/20/33 –/10/34/56	n.i./n.i./n.i./**38** n.i./n.i./n.i./**64**	n.i.	62/3

(continued)

Table 2 (continued)

First author, year Study type	pt/co (*n*)	aGvHD/ cGvHD (*n*)	Child/adult (*n*)	MSC donor Sib/Haplo/MM (*n*) Supplement	MSC application		aGvHD pre MSC I/II/III/IV (*n*) I/II/III/IV (%)	Response to MSC CR/PR/MR/OR (*n*) CR/PR/MR/OR (%)	Infection (*n*)	OS/FU (%/ months)
					Infusions (*n*)	Dose ($\times 10^6$/kg)				
Martin et al., 2010[b] Rand. placebo-controlled multicenter phase III study	163/81	163/–	–/163	–/–/163 Prochymal ® Formulated in Plasme Lyte ®	all pt: 8 (+4)	2	–/36/83/44 –/22/51/27	65/n.i./n.i./**103** 40/n.i./n.i./**63**	n.i.	n.i.
(b) *MSC expanded with platelet lysate (PL) or human autologous serum (AS)*										
Lucchini, 2010 Pilot study, IIT	11/–	8/3	11/–	–/–/7 **PL**	4pt: 1 4pt: 2	0.7–3.7 1–1.6	3/1/–/4 37/13/–/36	3/2/–/**5** 38/25/–/**63**	Pneumonia (1) Lung aspergillosis (1) Sepsis (1)	63/11
von Bonin, 2009 Pilot/phase I study, IIT	13/–	13/–	–/13	4/–/9 **PL**	13pt: 2	0.6–1.1	–/–/2/11 –/–/15/85	1/1/–/**2** 8/8/–/**16**	Infection (3)	69/12
Perez-Simon, 2011 Phase I/II study, IIT	18/–	10/8	–/18	–/8/2 **AS**	18 pt: 1–4	0.2–2.9	–/3/3/4 –/30/30/40	1/5/–/**6** 10/50/–/**60**	Sepsis (2)[a] Infection (3)[a]	10/11
Kebriaei, 2009 Rand. multicenter phase II study, IIT*	31/–	31/–	–/31	Prochymal ® **PL**	HD: 15pt: 1 LD: 16pt: 1	8 2	All: –/21/7/3 –/67/22/10 HD: –/10/4/1 –/66/26/6 LD: –/11/3/2 –/69/19/12	All: 24/7/–/**31** 77/22/–/**99** HD: 10/5/–/**15** 66/33/–/**99** LD: 14/2/–/**16** 87/13/–/**100**	BK(2) CMV(5) ADV (1) Bacteremia (4) Meningitis (1) Aspergillosis (1) Pneumonia (1)	71/3

Note With few exceptions, patients were transplanted for malignant hematological diseases except indicated patients who received myeloablative conditioning (MAC) and were transplanted with BM-derived MSC. MSC were cultured in FCS-supplemented medium unless stated otherwise. For abbreviations refer footnote in Table 1

[a] Mortality

[b] Short publication

Table 3 MSC for treatment of chronic GvHD

First author, year Study type	pt cGvHD/total (*n*)	Child/adult (*n*)	MSC Donor sib/haplo/MM (*n*) Supplement	MSC application		Effect CR/PR/MR/OR (*n*) CR/PR/MR/OR (%)	Infection (*n*)	OS/FU (%/months)
				Infusions (*n*)	Dose ($\times 10^6$/kg)			
Ringden, 2006 Pilot/phase I study, IIT	1/9	–/1	–/1/–	All pt: 1	1	–/–/1/– –/–/11/–	EBV-PLTD	–
Müller, 2008 Pilot study, IIT	3/7	3/–	–/2/1	All pt: 1	1.4–3	–/–/1/– –/–/33/–	EBV-PLTD (1)	33/48
Lucchini, 2010 Pilot study, IIT	5/11 (2/5 progr. aGvHD)	11/–	–/–/5	1pt: 4 4pt: 1–2	0.7–1.4 1–1.2	1/3/–/**4** 20/60/–/**80**	–	100/10
Perez-Simon, 2011 Phase I/II study, IIT	8/18	–/18	–/5/3 **AS**	7pt: 1–2 1pt: 4	0.2–1.2 0.8–1	1/3/–/**4** 5/16/–/**21**	Infection (1)	63/≥5
Zhou, 2010 Pilot/phase I study, IIT	4/4 Sclerodermatous cGvHD	–/4	–/–/4	All pts: 4–8	0.1–0.3	gradual response	n.i.	100/5–23
Weng, 2010 Pilot/phase I Study, IIT	19/19	–/19	–/–/19	All pts: 1–5	0.2–1.4	4/10/3/**14** 21/53/16/**74**	Infection (2)	100/3 77/≥24

Note With few exceptions, patients were transplanted for malignant hematological diseases, except indicated patients who received myeloablative conditioning (MAC) and were transplanted with BM-derived MSC. MSC were cultured in FCS-supplemented medium unless stated otherwise. For abbreviations refer footnote in Table 1

comparison because MSC preparations failed. In all three studies, no immediate side effects from MSC infusion and no ectopic tissue formation were observed.

Time to platelet and neutrophil engraftment was as expected for this type of transplant and did not differ significantly from the control group in the study by Ningh et al. Acute GvHD was low in all three studies with aGvHD II–IV in 24 % (16/68) of patients to whom MSC were administered. Overall 40 % (27/68) of patients were affected by cGvHD. Of these, about half suffered from the extensive form of the disease. In the three studies, relapse occurred in 35 % (24/68) of MSC/HSC co-transplanted patients. In the open-label randomized trial by Ning et al., however, there was a particularly high relapse rate with 60 % in the MSC group which was significantly different from the controls with only 20 %. Consequently, the three-year overall survival (OS) also differed significantly with 40 % in patients co-transplanted with MSC and 67 % for controls.

The study was closed early based on a potentially increased relapse risk associated with MSC. However, a generalized conclusion correlating relapse and MSC co-transplantation cannot be drawn, due to small patient numbers, the exclusion of five patients from the "intent-to-treat" population, and the use of historic controls. Accordingly, this study has caused considerable controversy [12]. It is valid to ask whether beyond the feasibility issue of timely large-scale MSC-preparation in patients, post-sibling donor HSCT with a low risk of graft failure and GvHD, MSC co-infusion as a prophylactic measure can be expected to provide any clinical benefit.

Over the last decade, transplant procedures have evolved that predominately rely on the GvL effect for elimination of malignant disease [131]. Intensity of pre-transplant radio-/chemotherapy has been significantly reduced to minimize conditioning-related toxicity. Following such nonmyeloablative conditioning, the risk of graft rejection is overcome by transplantation of large numbers of donor HSC. Still, in mismatched or haploidentical allo-HSCT the risk of graft failure has been higher than in HLA-matched transplants following myeloablative conditioning. Nonengraftment is also a concern in UCB transplantation, particularly in adults in whom adequate cell doses are not always readily available.

The notion that MSC might be employed to support hematopoietic engraftment in allo-HSCT is based on the longstanding concept that bone marrow stromal cells represent the key structural and regulatory components of the hematopoietic niche [16, 78]. This model has meanwhile been extended to include osteoblasts lining the bone surface, marrow endothelial cells, and primitive mesenchymal cells including CXCL12-abundant reticular and Nestin-expressing cells as HSC-niche forming cell populations [59, 122]. Yet, transplantation efficiency of stromal bone marrow cells has been a matter of longstanding debate [66, 93, 116]. The difficulty of detecting donor stromal cells may well be a result of different transplanted cellular doses and sensitivity of detection techniques. One recent study formally reported on 36 % donor stromal cell chimerism following HSCT from sibling donors. Donor stromal cell engraftment occurred in 3/8 BMT patients and in 5/18 patients transplanted with growth factor-mobilized PBSC [99]. This is in line with previous observations that MSC are also contained in peripheral blood [32]. Following

MSC transplantation the group from the Karolinska University Hospital, Stockholm, describes the autopsy results obtained from 18 patients. This includes 108 tissue samples analyzed by PCR for detection of donor DNA. Donor MSC engraftment was inversely correlated with the time from MSC infusion with 50 days seemingly a cut-off for donor MSC persistence. MSC distribution was limited to lung, lymph nodes, and intestine. In the BM, donor MSC were detected only in one patient in keeping with the results of Gonzalo-Daganzo et al. who after HSC/MSC co-transplantation submitted patients to serial bone marrow biopsies for chimerism analysis and found no MSC engraftment [38].

In spite of these incongruent results, MSC are deemed useful in the setting of UCB transplantation (UCBT) based on their graft-promoting effects. This hypothesis has also found support in a murine study [54]. Three small trials with 7–9 patients each were conducted to evaluate efficacy of MSC administration for improved engraftment and GvHD prophylaxis in UCBT (Table 1) [38, 62, 74]. In one study, patients received a transplant consisting of three cellular components, namely UCB, PBSC, and MSC [38]. In all studies, transplants were performed for high risk or relapsed hematological malignancies. Matched historic controls were provided for comparison of the outcome parameters in all three studies. Yet, no statistically significant difference in engraftment and acute and chronic GvHD was observed between UCB/MSC co-transplanted patients (pts.) and controls. Still it is noteworthy that in these three studies, only a single patient (1/24 pts.; 4 %) developed severe aGvHD III–IV in the MSC co-transplanted groups compared to the controls (9/91 pts.; 9 %). Likewise, only one patient suffered from limited and one from extensive cGvHD (2/24 pts.; 8 %) in the MSC cohorts. In the controls, the incidence of cGvHD was slightly higher (17/91 pts.; 18 %). In view of the favorable results in both the MSC and control groups, Gonzales-Daganzo et al. closed their study early based on the lack of evidence that MSC transplants are of benefit in UCBT in which hematopoietic engraftment is already bridged by co-transplantation of PBSC.

The MSC co-transplantation approach was further evaluated in mismatched/haploidentical HSCT [8, 11, 69, 129] with enhanced engraftment and GvHD prophylaxis as primary endpoints. In the three studies assessing this approach in the haploidentical setting, patients received myeloablative therapy prior to transplant. In the fourth study patients were transplanted with nonselected PBSC from $\geq$1 antigen-mismatched donor following reduced intensity conditioning. In none of these studies accelerated formal neutrophil or platelet engraftment was noted. Liu et al. observed, however, that in the MSC group platelets reached the 50×10^9/l threshold faster (22 days; range 12–58 days) than in the controls (28 days; range 10–99 days). In one trial, due to the typical NK cell surge, leukocyte counts rose to 10×10^6/l three days faster in the MSC co-transplantation group [8]. Acute GvHD in the two haploidentical transplant trials [8, 69] was generally low grade, both in the MSC co-transplant cohorts with no aGvHD III–IV and 43 % of aGvHD I–II (20/47 pts.) compared to the controls with only three patients with aGvHD III and aGvHD I–II in 36 % (27/75 pts.). Also in the study by Baron et al., severe aGvHD was comparable following mismatched unrelated

HSCT with aGvHD II–III in 35 % (7/20 pts.) and aGvHD IV in 10 % (2/20 pts.) following MSC co-transplantation versus aGvHD II–III in 32 % (6/16 pts.) and aGvHD IV in 19 % (3/16 pts.) of the controls. Yet, with 31 % the one-year probability of "dying from GvHD or infection while on GvHD therapy" was significantly higher in the controls compared to 10 % of patients co-transplanted with MSC. This translates into 37 % nonrelapse mortality in the controls compared to only 10 % in the MSC-transplanted patients at one-year post HSCT.

In summary, generation of clinical-scale quantities of MSC was feasible even when the HSC and MSC were harvested from the same donors. There were also no immediate side effects from MSC infusion and no evidence of ectopic bone formation over time. Yet, the other endpoints were not successfully met. Thus, HSC/MSC co-transplantation was not associated with accelerated engraftment. So far only in the setting of poor hematopoietic recovery, has salvage from graft failure been reported in individual patients [61, 85, 87].

Likewise, no significant difference in the incidence of acute or chronic GvHD was observed following HSC/MSC co-infusion which may well be explained by lack of appropriate inflammatory signals in the immediate post-transplant period. Indeed, in the absence of inflammation MSC are not capable of preventing or ameliorating GvHD as shown in a murine IFN-γ knockout model. Also immediately after HSCT levels of IFN-γ and TNF-α, both critical triggers of MSC activity, are low [98]. Pre-incubation of MSC with IFN-γ can compensate for this deficiency in the early transplant period. Thus, timing of MSC administration seems to be the key. Indeed, in several murine studies [98, 121], HSC/MSC co-transplantation failed to prevent GvHD whereas delayed MSC infusion seemed to effectively elicit the immunosuppressive properties of MSC [98]. Also manifest GvHD was mitigated by MSC application in a dose-dependent manner [51]. Although one needs to keep in mind the distinct immunoinhibitory mechanisms of MSC in mouse and man [83], these models do suggest that MSC might prove more useful for treatment of overt GvHD than for prevention.

7 MSC for Treatment of Steroid-Refractory Acute GvHD

For evaluation of response to MSC administration in steroid-resistant GvHD, four studies (Table 2) with a total of 289 patients and severe aGvHD III/IV in 84 % (242/289) of cases can be submitted to aggregated analyses based on the focus on aGvHD and standardized regimens for MSC-preparation from bone marrow and expansion [55, 58, 81, 101]. Although a multicenter phase II study, the European Group of Blood and Marrow Transplantation employed a consensus protocol for FCS-supported MSC-generation [58]. The three other studies employed industrially manufactured MSC. The general challenge in comparing efficacy of GvHD therapies between studies resided in the variability of endpoint definitions with regard to the scoring of clinical benefit as well as choice of timepoints for such an assessment. Also durability of response is not uniformly addressed [75, 79, 80].

In GvHD, a complete response (CR) is defined by disappearance of all symptoms. Yet, partial response (PR) may simply indicate an improvement from baseline but not necessarily a clinically meaningful benefit.

A consensus statement [80] demands that PR should signify a difference by two grades, however, this recommendation is not consistently followed or even specified. In the above studies, PR thus refers to improvement by at least one GvHD grade, mixed response (MR) describes reduction in severity of symptoms at a minimum of one affected site, and overall response (OR) summarizes the frequency of complete and partial responses. Few studies provide prospective timeframes for response evaluation and duration of follow-up, yet in most studies, best responses are documented. Aggregated calculation of OR is 65.4 % (189/289 pts.) of the above 289 high-risk patients with 47 % aGvHD grade III and 37 % grade IV [55, 58, 81, 101]. Complete responses are presented for 230 patients in three of the four studies with an aggregated CR of 44 % (101/230 pts.) [58, 81, 101]. This is a noteworthy result that compares well with other forms of second-line immunomodulatory interventions for refractory aGvHD such as treatment with TNF-α and IL-2 antibodies [4, 47, 48, 57].

One of the reasons for this favorable outcome might be the fact that in addition to 193 adults, 96 children are included in these studies. The European Group of Blood and Marrow Transplantation (EBMT) multicenter effort is the only prospective trial that includes equal numbers of children ($n = 25$) and adults ($n = 30$) clinically matched for aGvHD grade to allow for prospective comparison of age-dependent benefit from MSC within one study. In the EBMT trial 84 % OR and 68 % CR in children versus 60 % OR and 43 % CR in adults and a superior two-year overall survival in children with 45 % versus 26 % in adults ($p = 0.06$) confirms a more favorable outcome in the younger patient cohort.

One of the unresolved issues to date is the question of how many applications of MSC are required to maintain a durable response in aGvHD. Among the four studies described above, three consistently administer a minimum of eight infusions of 2×10^6 MSC/kg [55, 81, 101]. In these studies employing the commercially prepared MSC product Prochymal® an OR of 64 % is achieved compared to an OR of 71 % in the EBMT study limiting MSC application to $1–2 \times 0.6–2.0 \times 10^6$ MSC/kg in 89 % (49/55 pts.) of patients. In children, multiple infusions of Prochymal® resulted in 66 % OR compared to a considerably higher OR of 84 % in children treated in the EBMT study.

Although on first sight this seems to suggest that the commercially prepared third-party donor-derived MSC exhibit a trend towards lower efficacy, there is a variety of confounding factors in study design and endpoint assessment that may have considerable influence on such an interstudy comparison. Still, a closer look at MSC preparations seems justified. In the Prochymal® studies as well as in the EBMT multicenter trial, expansion of MSC did not exceed more than four to six passages. Yet, seeding densities may also play a role. The end product in the EBMT study is characterized according to the ISCT criteria. The Prochymal® studies submit their MSC product to additional functional immunological testing. Cryopreservation prior to infusion is one aspect in MSC preparation that is known

to be critical for vitality but also with regard to the immunosuppressive MSC-mediated activity. It is this distinct difference that could contribute to discrepancies in clinical outcome, as immediately after thawing the immunosuppressive properties of MSC are severely impaired. Thus, defrosted MSC are refractory to IFN-γ which is the key signal for IDO-induction as well as for up-regulation of immunosuppressive cell surface molecules such as PD-L1. The immunoinhibitory activity of defrosted MSC is, however, fully restored if submitted to 24 h of cell culture [33]. This insight might have significant impact on the future design of MSC-facilitated studies.

8 MSC for the Treatment of De Novo Acute GvHD

Kebriaei et al. conducted the first large prospective, open-labeled multicentered phase II study in the United States, Canada, and Australia (Table 2b). Thirty-one adult patients in 16 centers with de novo grade II–IV aGvHD were enrolled, with MSC manufactured by Osiris Therapeutics, Inc., Baltimore, from bone marrow aspirates of six healthy donors. Sixteen patients received low-dose MSC (LD 2×10^6 cells/kg), and 15 received high dose (HD 8×10^6 cells/kg) infusions within 48 h from diagnosis of aGvHD and a second infusion three days later. Of note, only 32 % patients suffered from aGvHD III–IV, considerably fewer than in the studies evaluating MSC efficacy in steroid-resistant aGvHD. MSC infusions proved safe and initial response rate was high, with 24 patients in CR (14 LD-pts., 10 HD-pts.) and 7 in PR (2 LD-pts., 5 HD-pts.). Time to response was also rapid with 42 % patients achieving CR at day 7, 52 % by day 14, and 77 % at day 28. CR was not correlated to donor source, grade, or location of GvHD.

A total of 71 % of patients survived to 90 days with a significantly improved survival of responders (88 % CR vs. 14 % non-CR; $p = 0.0008$). Overall, nine patients died within 13–63 days after MSC-infusion; three patients who had achieved CR died from infections, three nonresponders died from progressive GvHD, and one nonresponding patient from relapsed malignancy or brain bleed. Three patients relapsed within a two-year follow-up period.

9 Alternative Cell Culture Supplements for Clinical-Grade MSC Products

In cell therapy, transmission of prion, viral, and other zoonotic diseases in addition to xenogenic immunization is a concern when preparing clinical-grade products supplemented with FCS. Therefore, alternative sources for expansion and maintenance of MSC have been explored. In vitro platelet lysate (PL) and to a lesser degree autologous serum (AS) proved efficacious, yielding MSC preparations with comparable surface marker profile and tri-lineage differentiation capacity to

FCS-risen MSC. With regard to their influence on T cell effector functions such as cytokine production, cytotoxicity, or proliferation, some variability in the spectrum of immunomodulatory properties and secreted mediators was observed [6, 14, 30]. There is only one study that suggests that overall PL-MSC might be less immunosuppressive than FCS-MSC. In this report PL-MSC had a weaker inhibitory influence on T and NK cell proliferation and NK cell cytotoxicity [1] which was associated with lower constitutive PGE_2-production compared to FCS-MSC.

Yet, clinical experience is sparse with few patients, 8 children and 13 adults, treated in two studies with PL-MSC for GvHD treatment thus far (Table 2b) [72, 126]. Patients enrolled in these pilot/phase I studies suffered from different degrees of steroid-refractory severe aGvHD III–IV ranging from 36 % in children [72] to 100 % in adults [126]. Accordingly in the latter study, OR was only 16 % (2/13) in the adults with steroid-refractory GvHD IV. In contrast, in the trial assessing efficacy in children [72] with slightly less severe GvHD, OR was 63 % (5/8 pts) which is more in line with the FCS-MSC studies described earlier.

In another small study with 10 aGvHD patients human autologous serum was used for MSC culture resulting in a very low CR 10 % and OR 60 % and high early toxicity and mortality (33 %) within the first 100 days post transplant [94]. Thus, these approaches to expand and activate MSC warrant further clinical evaluation. One study has already been in progress in the Netherlands since 2009 employing human plasma compared to platelet lysate for MSC expansion. In this phase I/II study patients with de novo grade II–IV aGvHD and cGvHD are included (http://www.clinicaltrials.gov; identifier: NCT00827398).

10 MSC for Treatment of Refractory Chronic GvHD

Only few studies have been conducted for treatment of cGvHD. In some of the MSC-trials for treatment of steroid-resistant aGvHD, single patients with cGvHD were enrolled (overall 17 pts.) with an aggregated OR of 47 % (8/17) [72, 87, 94, 107] (Table 2). In one small trial, MSC-mediated tissue repair after direct intra-BM injection was assessed in four sclero-dermatuos cGvHD patients. Reversal of the Th1 cells to Th2 cell ratio was observed with reported gradual improvement of symptoms in all four patients [140].

There is, however, one trial with a total of 19 patients focusing entirely on MSC application for refractory cGvHD in patients who failed six months of prior intensive immunosuppressive therapy [133]. This study is noteworthy as it provides clear definitions with regard to indication of MSC infusion, severity of GvHD, and response. Thus, the NIH consensus criteria for organ scoring and global assessment of cGvHD were used. MSC were transfused directly after preparation without intermittent cryopreservation.

As discussed above, this may be one of the reasons why in spite of relatively low MSC doses (median 0.6×10^6; range $0.2–1.4 \times 10^6$/kg) patients still

experienced a considerable clinical benefit. Two of the severely ill patients had organ and four multiorgan disease. Still 14/19 patients (74 %) responded to 1–2 MSC infusions with CR in 4 patients (21 %) and PR in 10 patients (53 %). The highest clinical benefit was observed for cGvHD of the oral mucosa, GI tract, liver, and skin. Concomitantly applied immunosuppressive agent could be tapered in 5 patients and in another 5 patients immunosuppressive therapy could be stopped altogether. These encouraging results commend further evaluation of MSC for the treatment of extended cGvHD. The response profile in this study would also suggest that MSC need to be administered at a timepoint when attenuation of inflammation and tissue repair still hold a chance for facilitating clinical improvement. In contrast end-stage fibrotic disease will no longer benefit from MSC infusion.

Additional prospective studies are under way. Thus a randomized phase I/II study started in Korea in early 2012. Here, umbilical cord blood-derived MSC grown in the presence of FCS (PROMOCHEM^{TM}) are employed for the treatment of steroid-refractory aGvHD and cGvHD (http://www.clinicaltrials.gov; Indent: NCT01549665). Another study is a phase I/II randomized multicenter study in Spain which started recruitment of patients with extensive cGvHD in 2010 for treatment with MSC derived from adipose tissue (http://www.clinicaltrials.gov; Indent: NCT01222039).

Adipose tissue in future might prove to be a highly attractive source for MSC preparation due to its abundant availability and the encouraging results from the one study by Fang et al. in acute GvHD patients grade III–IV disease and a complete response in 83 % of patients (Table 2a).

11 Summary

Overall MSC hold promise in the treatment of acute and chronic GvHD. The application seems to be safe thus far with no evidence of malignant transformation. The influence of different MSC sources and various cell culture supplements in MSC generation on the regenerative and immunomodulatory properties as well as efficacy in the different clinical settings will have to be carefully explored in the future. Also, it would be desirable to accompany the clinical studies with immune-monitoring analyses to better understand the underlying mechanisms in responding and nonresponding patients. This will then provide a basis for further improving MSC therapy.

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