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Promising cellular therapeutics for prevention or management of graft-versus-host disease (a review)

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Abstract

Graft-versus-host disease (GVHD) frequently occurs following allogeneic hematopoietic stem cell transplantation. The primary treatment for GVHD involves immune suppression by glucocorticoids. If patients become refractory to steroids, they have a poor prognosis. Therefore, there is a pressing need for alternative therapies to treat GVHD. Here, we review clinical data which demonstrate that a cellular therapy using mesenchymal stromal cells (MSCs) is safe and effective for GVHD. Since MSCs derived from bone marrow present certain limitations (such as time lag for expansion to clinical dose, expansion failure *in vitro*, painful and invasive bone marrow MSC isolation procedures), alternative sources of MSCs for cellular therapy are being sought. Here, we review data which support the notion that MSCs derived from Wharton's jelly (WJ) may be a safe and effective cellular therapy for GVHD. Many laboratories have investigated the immune properties of these discarded MSCs with an eye towards their potential use in cellular therapy. We also review data which support the notion that the licensing of MSCs (meaning the activation of MSCs by prior exposure to cytokines such as interferon-γ) may enhance their effectiveness for treatment of GVHD. In conclusion, WJCs can be collected safely and painlessly from individuals at birth, similar to the collection of cord blood, and stored cryogenically for later clinical use. Therefore, WJCs should be tested as a second generation, off-the-shelf cell therapy for the prevention or treatment of immune disorders such as GVHD.

Keywords

Mesenchymal stromal cells; Immune modulation; Bone marrow transplantation; Wharton's jelly; Leukemia; Allogeneic transplant

1. Graft-versus-host disease (GVHD) is a significant clinical problem

Allogeneic hematopoietic stem cell transplantation (allo HCT) is increasingly utilized successfully as a potentially curative treatment in the management of hematologic malignancies, bone marrow failure syndromes, and inborn errors of metabolism [1]. In the treatment of hematologic malignancies, a critically important component of the efficacy of

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allo HCT comes from a donor-derived, immunologically driven, graft-versus-tumor effect which lowers risk for relapse when compared to high dose chemo-radio therapy and autologous HCT. Correlated with the positive graft-versus-tumor effect is the occurrence of graft-versus-host disease (GVHD). GVHD is a transplant-related complication mediated by donor-derived T-cells and affects 25–75% of patients receiving allo HCT [2,3,4]. GVHD is a principle contributor to transplant-related non-relapse morbidity and mortality following allo HCT and represents the major non-relapse barrier to the success of this otherwise potentially curative treatment approach [2].

GVHD occurs as an acute (a GVHD) or chronic (cGVHD) clinical syndrome, somewhat arbitrarily defined as occurring prior to or after 100 days post transplant, respectively. Although significant overlap exists, aGVHD and cGVHD have very distinct clinical manifestations, natural histories, treatment responses and prognosis [5,6]. Specifically, aGVHD manifest most commonly as an acute inflammatory process principally involving the integument, intestinal tract and liver, and frequently presents as a maculopapular rash, nausea, vomiting and diarrhea and hepatic cholestasis, respectively. In contrast, cGVHD is a chronic inflammatory process leading to fibrosis of involved organs and frequently presents clinically with SICCA syndrome-like features, scleradermatous-like skin changes, chronic fibrosing pulmonary, hepatic and intestinal manifestations and cytopenias. The severity of aGVHD is determined by a staging/grading system grade I–IV, with higher grades related to a worsening prognosis and likelihood of response to any therapy [7]. Following allo HCT, patients with aGVHD grades I–II experience 5 year leukemia-free survival of 44–51%; in contrast, survival decreases to 26% for patients with grade III and 7% for grade IV aGVHD [8]. Chronic GVHD historically has been determined as limited or extensive; however, a National Institute of Health consensus criteria model has been established recently which will facilitate better prognostication and treatment response determinations [9]. Chronic GVHD represents the leading cause of late treatment related deaths among recipients of allo HCT. Despite its adverse effects, cGVHD is associated with a decreased risk for relapse of hematologic malignancies.

Several well-defined risk factors associated with the development and severity of GVHD include human leukocyte antigen (HLA) mismatching between donor and recipient, sex mismatching, advanced recipient and/or donor age, stem cell source, and methodology of GVHD prophylaxis [10,11]. Despite optimal HLA matching, GVHD commonly occurs and this fact is attributed to the likely presence of donor-recipient mismatching of minor histocompatability antigens not currently accounted for in routine HLA typing [12,13]. Further, given that only approximately 25% of patients in need of an allo HCT will have an HLAmatched sibling donor, alternative graft sources have increasingly been utilized. The use of new conditioning regimens has resulted in a remarkable growth in the use of HLAmatched and mismatched unrelated adult and cord blood stem cell sources, as well as haploidentical related donors, especially in older patients. The increased use of these alternative donor stem cell sources in allo HCT is accompanied by increased transplant-related complications, including GVHD [14]. The long time-course of GVHD which may follow allo HCT produces a huge financial burden to our health care system and a significant time demand upon the health care team.

2. Standard care for GVHD patients

Two principal approaches to the management of GVHD include prevention and treatment. The most commonly employed strategies to prevent GVHD include optimal HLA matching at MHC class I and II loci between donor and recipient, and blocking T-cell antigen recognition and resultant proliferation during the early initiating phases of GVHD through pharmacologic prophylaxis, most commonly consistent of a calcineurin inhibitor in

combination with methotrexate or mycophenolate mofetil or an MTOR inhibitor such as rapamycin. Less common, but increasingly utilized approaches include graft manipulation through *in vivo* or *ex vivo* T-cell depletion strategies and limiting tissue damage caused by the preparative regimen. Once an inflammatorycascade is triggered and donor T-cells begin destroying host tissues, the treatment regimens for GVHD ensue. GVHD treatment involves various immunosuppressive therapies. The standard initial treatment is steroid therapy. However, a significant percentage of patients will prove to be resistant to steroid therapy and will subsequently be treated with second-line immunosuppressive agents [5,6]. Steroid-resistant aGVHD portends a very poor prognosis, second-line agents frequently prove ineffective and as a result survival is <10% at 5 years. Therefore, alternative therapies are needed to prevent and treat GVHD following allo HCT.

3. MSCs for treating GVHD

One promising treatment for GVHD involves the infusion of third party, HLA-disparate, unrelated bone marrow derived mesenchymal stromal cells (BM-MSC). The *in vivo* and *in vitro* properties of BM-MSC suggest their potential use in a broad range of inflammatory and immune-mediated conditions, such as GVHD. BM-MSC are a population of undifferentiated multipotent mesenchymal stromal cells which express HLA class I and do not express HLA class II or costimulatory molecules CD40, CD80 or CD86 [15–19] and have been demonstrated to modulate immune and inflammatory response in animal models of inflammatory disease including GVHD [19–23], and to facilitate repair of connective tissues [24–27].

MSCs inhibit the activation of and proliferation of activated T-cells that have been induced by a variety of stimuli [18,28] and down-regulate inflammatory cytokine expression such as tumor necrosis factor (TNF)-α, IL2R-α, elafin, and interferon-γ (IFN-γ) [29,30]. Dander et al. investigated the effects of MSC infusion on lymphocyte counts in transplanted patients with steroid-refractory GVHD [30]. Interestingly, $CD4^+$ T-cell subsets changed significantly after MSC infusion. Specifically, Tregs increased and Th1 and Th17 populations decreased significantly in patients whose symptoms improved. Le Blanc et al. [31] reported the first case of successful treatment of severe refractory aGVHD using *ex vivo* expanded haploidentical MSCs. In a subsequent report, these investigators demonstrated a positive therapeutic effect with allogeneic MSCs in patients experiencing steroid-refractory aGVHD with no significant adverse events attributed to the cells [32].

Additional studies have reported very encouraging clinical results and confirmed the safety of MSCs in the treatment of steroid-refractory aGVHD [31–45]. Specifically, Kurtzberg et al. presented at the 2010 American Society of Blood and Marrow Transplant meeting [46] that using allogeneic MSCs as a rescue agent for severe treatment resistant aGVHD demonstrated a 64% response rate in 59 children by day 28, and that response to MSCs correlated with improved overall survival at 100 days. This work suggests that MSC therapy has an excellent risk/benefit profile [46]. Martin et al. presented at the same meeting the results of a randomized, placebo-controlled, multi-center phase 3 trial of MSCs in the treatment of steroid-refractory aGVHD which involved 244 patients [47]. Although the principle endpoint of durable complete response >28 days was not significantly better in the MSC-treated population, significant differences in response for patients with multi-organ involvement, liver and intestinal involvement were realized for the MSC-treated cohort.

A summary of the published reports describing the clinical outcomes of patients treated with MSCs in the management of both aGVHD and cGVHD with varying results is shown in Table 1 [31–47]. These reports included patients that received a variety of conditioning regimens including myeloablative, or non-myeloablative, or reduced intensity conditioning

(RIC), with no apparent differences in the response to MSC treatment. Furthermore, the patients received MSCs from a variety of sources including HLA identical, haploidentical, or third party, unrelated and unmatched donors. Most clinical data came from MSCs derived from bone marrow, but work from Fang et al. used MSCs derived from adipose tissue [35–37], with no apparent differences in response. Important for the availability of off-the-shelf cell therapy, MSCs from freshly expanded samples or from cryogenically stored/thawed cell preparations have been used with no apparent differences in response. MSCs have been shown to be safe: no ectopic tissue formation has been derived from infused MSCs. Finally, MSCs did no harm: no clearly defined increased incidence of opportunistic infections or relapse of malignancy were reported. In summary, the data support the concept of MSCs as a safe, well-tolerated and variably effective treatment for GVHD. Importantly, MSCs can be cryogenically banked, thawed and given without the need for donor-recipient matching.

4. Improving MSCs for clinical use in GVHD

BM-MSCs have specific problems that limit their usefulness. For example, BM-MSC isolation requiresaspiration from the marrow cavity which is a painful, invasive procedure, with certain risks. Several studies indicate that adult-derived MSCs have limited expansion potential or slower expansion *in vitro* compared to fetal-derived MSCs and that adult MSCs may be less-responsive than fetal or neonatal MSCs in certain applications [48–53]. Therefore, alternative tissue sources, such as discarded tissues resulting from pregnancy, which contain fetal-derived MSCs have been considered as an alternative MSC source. Here, we focus upon the potential of these tissues, with the aim to improve the next iteration of clinical trials. Two notions are presented. First, we review the literature that suggests that MSCs from discarded fetal tissues might be better than BM-MSCs for GVHD therapy. This idea is based upon the fact that BM-MSCs have aforementioned limitations that may be overcome using an alternative MSC source. Second, we review literature that suggests that *in vitro* conditioning by cytokine exposure, called “licensing”, of MSCs during expansion might improve their clinical effect in GVHD. This idea is based upon the relative plasticity of MSCs to culture conditions such as hypoxia, cytokine exposure, etc, that change the physiology of MSCs and may improve their clinical effect.

It is well-understood that BM-MSCs have limitations that may affect their clinical potency and impact. For example, BM-MSCs have a limited expansion potential and grow relatively slowly *in vitro*, and they require a painful and invasive collection procedure, and BM-MSCs from older individuals may not expand to clinically relevant number and may be unsuitable for therapy. To address these limitations, individuals at the EMBO workshop: “From fetomaternal tolerance to immunomodulatory properties of placenta-derived cells in cell therapy” First Bi-annual Meeting of the International Placenta Stem Cell Society (IPLASS) held in Brescia, Italy focused upon the deciduous tissues associated with fetal life, e.g., amnion, placenta and umbilical cord. Our thesis is that MSC derived from umbilical cord may be a effective, safely and painlessly collected alternative source of MSC, and replace BM-MSC, for GVHD prevention or treatment.

Our group works with MSCs that are derived from umbilical cord stroma, also known as Wharton's jelly (WJ, or WJCs below). WJ is a primitive, loose connective tissue that is rich in hyaluronan, and supports and cushions the umbilical vessels. WJ's contains an MSC population that is easily isolated following birth from the discarded umbilical cord after umbilical cord blood has been collected. WJCs grow more quickly and produce more cells during expansion *in vitro* compared with BM-MSCs [54,55] and, they have immune properties similar to adult-derived MSCs from bone marrow and adipose tissue [51,54,56–58]. It is their immune properties that make MSCs attractive for immunological disorders. These immune properties are: 1) low immunogenicity and naïve MSCs do not strongly

stimulate allogeneic T-lymphocyte proliferation; 2) MSCs suppress the proliferation of activated T lymphocytes, 3) increased production of regulatory T-cells, and 4) a shift in the immune response towards tolerance or anergy since MSCs do not stimulate B cells and prevent B cells from becoming stimulated.

The mechanisms of MSC immune suppression have been reviewed elsewhere (see [29,59,60]). GVHD may be modeled *in vitro* since treatments which impact on the inflammatory response are reflected by assays of the suppression of mitogen-activated or allo-antigen activated T-cell proliferation, and the expansion of regulatory T-cells, which would reflect a critical component of tolerance induction. The mechanisms used by MSCs are under debate. Evidence exists to support both a direct, contact-dependent mechanism that is mediated at least in part by MSC expression of the cell death ligand, B7-H1 [61], and an indirect, contact-independent mechanism mediated by various cytokines and growth factors such as prostaglandin E2 (PGE2), cyclooxygenase (COX) 1 and 2, hepatocyte growth factor (HGF), transforming growth factor-β, interleukin 10, human leukocyte antigens G5 and E, leukemia inhibitor factor, indoleamine 2,3-dioxygenase (IDO), and others [28,29,56,62–64]. As seen below, the mechanisms are not fully determined and the literature is filled with example and counterexample.

Several studies compared the immune properties of BM-MSCs, WJCs and MSCs derived from adipose tissues [51,54,56,57,65]. Najar et al. reported that adipose-derived MSCs and WJCs had similar *in vitro* immunosuppressive effects for lymphocyte proliferation, compared to BM-MSCs; that MSCs target CD4+ and CD8+ T-cells for immune suppression equally; adipose-derived and WJCs inhibit T-cell activation, and that MSCs were immunosuppressive regardless of the type of stimuli used to activate the lymphocytes [57]. In their hands, MSC immune suppression was mediated by COX 1 and 2 enzymes and by the production of PGE2 and did not involve HGF. In agreement with Najar et al.'s findings, Chen et al. found that PGE2 synthesis, mediated by COX2, produces the majority of WJCs' suppressive effects on T-cell proliferation and on IFN-γ secretion [65]. PGE2 expression by WJCs was stimulated by inflammatory signals IFN-γ or interleukin-1β produced by peripheral blood mononuclear cells following mitogen or allogeneic stimulation. Critically, they found that WJCs cultured with unstimulated (naïve) T-cells do not secrete much PGE2, however, following co-culture with stimulated T-cells, WJCs excreted more PGE2. This finding fits with our own [58], and other labs' findings [23,51,54,56,61,66,67]: MSCs have little effect on unstimulated T-cells and exposure to activated T-cells or inflammatory cytokines changes MSCs so they display immunosuppressive behavior.This has been termed as licensing or priming of MSCs. Chen et al. found that IDO and TGF-β played little role in MSC's suppression of the T-cell proliferation. As a counterexample to Chen et al.'s finding that IDO had little role, Yoo et al. had diametrically different findings when they compared the immunoregulatory properties of adipose-derived, umbilical cord blood-derived MSCs, WJCs and BM-MSC [51]. They found that MSCs from all four tissue sources responded to either IFN-γ or tumor necrosis factor-α (TNF-α) secreted from activated T-cells by inducing IDO secretion, and the released IDO from MSCs suppressed T-cell proliferation, and led to decreases in TNF-α and IFN-γ. Yoo et al. reported that, while MSCs responded to IFN-γ or TNF-α exposure to upregulate IDO expression, they did not increase expression of HGF, Cox 1 and 2, interleukin-10, and transforming growth factor-β. Prasanna et al. also examined the immune properties of MSCs from BM and from WJ, and the effect of IFN-γ and TNF-α exposure on these properties [56]. They found that IFN-γ or TNF-α stimulation produced subtly different responses between BM-MSCs and WJCs. For example, IFN-γ or TNF-α exposure increased the expression of the immune-adhesive ligand, CD54 in both BM-MSCs and WJCs. However, IFN-γ increased expression of HLA class 2 in BM-MSCs and not in WJCs. Prasanna et al. also reported that IFN-γ exposure did not strongly affect the immunogenicity of MSCs in their *in vitro* proliferation assays [56]. In contrast to these

findings, Cho et al. reported that IFN-γ exposure induced expression of MHC class II in swine and human WJCs, and that IFN-γ stimulate WJCS produced an antibody response following subcutaneous or intravenous injection of allogeneic WJCs faster than when unlicensed WJCs were used in a swine model [66]. One unexpected finding in the Prasanna et al. report was the importance of MSC proliferation (possibly) on immune suppressive properties: both BM-MSCs and WJCs that had been mitotically inactivated lost their immune suppressive effect. To our knowledge, this was the first report to correlate MSC proliferation with suppression of lymphocyte proliferation. If MSC proliferation is critical for immune modulation, this would significantly impact upon how MSCs are derived for therapeutic use. The differences between Cho et al. and Prasanna et al. on changes in HLA expression are explained by work from Deuse et al., who compared the immunogenicity of allogeneic BM-MSCs and WJCs both *in vitro* and *in vivo* following exposure to different doses of IFN-γ [54]. At doses of IFN-γ below 50 ng/ml, IFN-γ upregulated HLA-DR and doses from 100 to 500 ng/ml of IFN-γ down-regulated HLA-DR. Interestingly, in all cases, WJCs had lower expression of HLA-I and HLA-DR compared to BM-MSCs, WJCs had weaker allogeneic T-cell stimulation compared to BM-MSCs, and WJCs had longer survival following allogeneic transplantation in immunocompetent Balb/c mice. To summarize these studies, MSCs from Wharton's jelly, adipose tissue and bone marrow can potently suppress T-cell activation, and suppress both CD4+ and CD8+ T-cell proliferation induced by mitogen or allogeneic stimulation. Both soluble factors and direct contact are important for full effect of MSC_s on immune cells. These studies did not consistently identify soluble factors involved; rather indicate a role for PGE2, IDO, COX 1 and 2, and other factors. The reason for these differences is unknown. Several studies indicate that differences exist between MSC sources, but the physiology that accounts for these differences is not understood, currently. For example, does MSC proliferation, or some other attribute, limit MSC immune suppression [56]? MSCs from adipose, BM and WJ have similar *in vitro* and *in vivo* immune properties. The advantages of WJCs, e.g., their lower immunogenicity, less immune activation and slower rejection compared to BM-MSCs, would not be apparent without direct comparisons (as was conducted in these studies). While some studies found that WJCs and adipose MSCs have equal or superior suppression of activated T-cells proliferation compared to BM-MSCs, in other studies the differences were less apparent. Finally, consistently, adipose and Wharton's jelly MSCs have superior *in vitro* expansion properties compared to BM-MSCs.

As mentioned above, MSCs' immune properties, specifically their immunogenicity, their ability to suppress T-cell activation and their immune suppression of activated T-cell and B cell proliferation, can be modified by manipulating their environment. This has been called licensing or priming. Thus, the therapeutic effect of MSCs may be “tuned” to improve performance for a particular therapeutic application by appropriate priming. Several studies that address this hypothesis are discussed briefly below [23,56,61,66,67]. Cho et al. showed that exposure of WJCs to IFN-γ increases the expression of MHC class I and induces the expression of MHC class II [66]. This was accompanied by increases in the immunogenicity of WJCs in an allogeneic swine model. Similar and different findings were reported by Prasanna et al., as was discussed above [56]. Again the theme is that IFN-γ exposure modifies MSC effect on immune properties including expression of IDO, HLA class I and class II surface marker expression, etc. Tipnis et al. reported that IFN-γ caused WJCs to upregulate the expression of cell death ligand B7-H1, in addition to confirming that IFN-γ stimulates increased expression of IDO, and induces HLA class II expression [61]. Valencic et al. evaluated two variables: the priming effect of IFN-γ exposure on WJCs and the timing of lymphocyte exposure to WJCs [67]. They found that the timing of WJC priming was critical to reveal their immune suppressive effects on lymphocytes and priming WJCs increased their immune suppressive action in both contact and non-contact settings. In contrast, if pre-stimulated lymphocytes were added to non-primed WJCs, the lymphocytes

showed normal or enhanced proliferation. Deuse et al. examined the dose-dependent effects of IFN-γ on BM-MSCs and WJCs and found that higher levels of IFN-γ stimulation produce a stronger effect of WJCs on immune suppression [54]. The *in vitro* work suggests that primed MSCs would be more effective at treating chronic GVHD, where they are placed into an environment which will rapidly license them to begin immune suppression, which fits with animal model and human clinical observations [23,40]. It also suggests that unprimed MSCs given together with hematopoietic stem cells during allo HCT would be ineffective at preventing GVHD, which again is supported by animal GVHD model work by Polchert et al. [23], and such speculation might be retrospectively confirmed from clinical data. Additionally, the *in vitro* work suggests that IFN-γ-priming would improve MSCs' therapeutic effect when given together with hematopoietic stem cells before GVHD has developed; which has been confirmed in a GVHD mouse model [23]. While Polchert's work fits with *in vitro* work that indicates that IFN-γ priming will have beneficial effects in GVHD, primed MSCs have not yet been tested in clinical use. In that regard, the clinical findings reported by Dander et al. [30] fit with what we might predict MSCs might do based upon our basic understanding of their immunophysiology. Currently, there is no reason to believe that primed MSCs would not be safe and effective for clinical use. In fact, the *in vitro* and animal model data suggest the primed MSCs would have more potent therapeutic effect than naïve MSCs. We further speculate that hindsight will clarify the target tissue effects reported for MSCs in GVHD [47] once the interactions of MSCs with Tregs, Th1, Th17 and Th2-cell subsets are resolved. Unfortunately, there is not space to discuss this critically important topic.

5. Summary and conclusions

In summary, MSCs appear to be safe and well-tolerated, and they offer a hope for treatment of steroid-refractory GVHD patients. The clinical outcomes to date are good, and there is room for improvement. Most clinical trials have used BM-MSCs; adipose-derived MSCs were used in a few trials for GVHD, and WJCs have not yet been tested clinically for GVHD. As discussed above, in vitro testing of MSCs suggests that off-the-shelf, unmatched cryo-preserved MSCs derived from either adipose or WJ may be a second-generation of MSC-based cell therapy for GVHD. Finally, we must expand our understanding of the concept of priming MSCs since it improves effectiveness in an animal GVHD model and in pertinent *in vitro* assays. In conclusion, new information about MSC biology should be translated rapidly to clinical evaluation for safety and efficacy for therapy in steroid-resistant GVHD.

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Table 1

Review of clinical reports and trials that evaluated mesenchymal stromal cells (MSCs) to treat graft versus host disease (GVHD). This table is an update from the table that appeared in Toubai T et al., Curr Stem Cell Res & Ther, 2009.

Citation	MSC Source	MSC Donor	Number of patients/GVHD grade	HCT Conditioning	Dose of MSCs (/kg)	Effect on GVHD	Ref. #
LeBlanc K et al., 2004	BM	Haploidentical (mother)	1 (9 yr boy) Grade 4	Myeloablative	2×10^6 first 1×10^6 second	Improvements	[31]
Ringden O et al., 2006	BM	HLA identical sib $n = 2$ Haploidentical $n = 6$ HLAmismatch $n = 4$	9(12 infusions) steroid refractory aGVHD 8 cGVHD 1	Myeloablative $n = 5$ RIC $n = 3$ ATG only $n = 4$	$0.6–9 \times 10^6$	Complete response 6 Response 1 Slight effect 1 No response 4	[32]
Fang B et al., 2006,2007	Adipose	Unrelated mismatch Haploidnetical Haploidentical Unrelated mismatch $n = 4$	1 (38 yr) 2 steroid refractory 1 chronic hepatic 6-steroid refractory aGVHD	Myeloablative Myeloablative Myeloablative Myeloablative	2×10^6 first 1×10^6 second	Complete response Complete response Complete response Complete response 5/6 complete response	[35–38]
Muller I et al., 2008	BM	Mismatch family $n = 8$ HLA identical $n = 2$ HLA matched unrelated $n = 1$	7 (11 infusions) aGVHD $n = 2$ cGVHD $n = 3$ Hemophagocytosis $n = 1$ Graft rejection prophylaxis $n = 1$	Myeloablative $n = 5$ RIC $n = 2$	$0.4–3 \times 10^6$	aGVHD 1/2 alive and well cGVHD 1/3 slide improvement Hemophagocytosis, good response Graft rejection prophylaxis alive and well	[42]
Le Blanc K et al., 2008	BM	HLA identical sib $n = 5$ Haploidentical $n = 18$ Unrelated mismatch $n = 69$	55 (92 infusions) Grade 2 $n = 5$ Grade 3 $n = 25$ Grade 4 $n = 25$	Unknown	$0.4–9 \times 10^6$	Children 17/25 complete response, 4/25 partial response Adult 13/30 complete response, 5/30 partial response total 30/55 complete response (54%), partial response 9/55 (16%) Overall 2 yr survival 53% for complete response vs 16% for partial or non-response	[40]
von Bonin M et al., 2008	BM	Unrelated mismatched	13 (32 infusions) Grade 3 $n = 2$ Grade 4 $n = 11$	Myeloablative $n = 1$ RIC $n = 12$	0.6×10^6 (0.6–1.1)	2 patients (15%) complete response 5/11 (45%) partial response	[43]
Zhou H et al., 2010	BM	HLAmatched, unrelated	4 cGVHD	Nonmyeloablative $n = 4$	$1–2 \times 10^7$ (4–8 infusions)	4/4 Complete response	[45]
Kebriaei P et al., 2009	BM	Osiris unrelated unmatched $n = 6$	31 (62 infusions) Grade 2 $n = 21$ Grade 3 $n = 1$ Grade 4 $n = 3$	Myeloablative $n = 15$ RIC $n = 8$ Nonmyloablative $n = 4$ DLI $n = 4$	2×10^6 $n = 16$ 8×10^6 $n = 15$	24/31 Complete response, 5 partial response 2 No response	[39]
Arima N et al., 2010	BM	Related, HLA identical $n = 1$ Unknown	3 Grade 3 $n = 3$	RIC $n = 1$ unknown $n = 2$	$0.5–2 \times 10^6$ intra-arterial injection into GVHD sites	1/3 partial response	[33]

Citation	MSC Source	MSC Donor	Number of patients/GVHD grade	HCT Conditioning	Dose of MSCs (/kg)	Effect on GVHD	Ref. #
Baron F et al., 2010	BM	third party, mismatch	20 patients (19 historic controls)	Nonmyeloablative coinfusion w/MSC *n* = 20	Unknown	MSC coinfusion appears safe MSC coinfusion might prevent death from GVHD without impacting GVT	[34]
Lucchini G et al., 2010	BM	Single donor unrelated HLA mismatch	aGVHD Grade 1–4 or cGVHD *n* = 11	Variable: TBI, RIC, etc	$0.7–3.7 \times 10^6$ 1–5 infusions	8/11 Complete (23%) or partial (47%) response 3 No response	[41]
Weng JY et al., 2010	BM	HLAmatched third party, mismatched	cGVHD 73% severe, 26% moderate) *n* = 19	Variable: TBI, RIC, etc	$0.23–1.42 \times 10^6$ 1–5 infusions	14/19 Complete (4) or partial (10) response 5 Died	[44]

Updated from Toubai T, Paczesny S, Shono Y et al. Curr Stem Cell Res & Ther 2009.