

# Chapter 54

## Mesenchymal Stromal Cells: Impact on Hematopoietic Cell Transplantation



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### Introduction

Mesenchymal stromal cells (MSCs) were originally characterized as stromal components of the bone marrow microenvironment that could support hematopoiesis. The original investigations were based on the seminal work of Friedenstein et al. [1] who used the colony-forming unit-fibroblast (CFU-F) assay to detect and quantify the influence of these cells. Subsequently, the critical dependence of normal hematopoiesis upon bone marrow stroma was confirmed in mouse models of bone marrow failure. Coculture crossover assays of marrow cells obtained from the mutated  $W/W^v$  and  $Sl/Sl^d$  mice were studied [2]. Both murine strains were fated to have limited survival but in vitro combination of the two cells provided interesting findings. Specifically, adherent marrow microenvironment cells obtained from the  $Sl/Sl^d$  mice, when cultured with bone marrow from  $W/W^v$  strains, did not restore defective hematopoiesis. On the other hand, the reverse combination contributed to sustained cell proliferation of multiple lineages of hematopoietic cells and growth in colony assays. Subsequently, the molecular basis of the bone marrow failure was identified involving stem cell factor (SCF; mutated in the  $Sl/Sl^d$  mice locus [3]) which binds to the c-KIT receptor (CD 117; mutated in  $W/W^v$  mice [4]). These and other studies illustrated how marrow stromal cells are critical for the support of marrow hematopoietic cells.

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Such seminal studies in murine mouse models led to human investigations. The original observations of Caplan et al. [5] identified MSCs as having stem cell characteristics as they exhibited a multilineage differentiation capacity. Further, MSCs could contribute to downstream lineage differentiation pathways of mesodermal cells that differentiated into striated and cardiac muscle, connective tissue, bone, adipose tissue, and marrow stroma. Finally, these cells could undergo self-renewal as well as be transplanted successfully. Immediately thereafter, interesting studies were reported of patients who experienced damage to the bone marrow microenvironment secondary to cytotoxic agent treatment with either radiation or alkylating agents. The stroma from those individuals could not support the growth of healthy hematopoietic stem cells (HSC) [6]. These observations ultimately led to some of the first clinical human MSC treatment studies with the original goals to improve engraftment and support hematopoiesis by regeneration of the marrow microenvironment, specifically the stromal cell compartment.

## Biological Properties of MSCs and Impact on Hematopoiesis

MSCs can be isolated from a wide variety of tissue sources including adipose tissue, dental pulp, mobilized bone marrow cells, placenta, and umbilical cord blood (UCB) cells [5, 7]. Importantly, MSC can be expanded many log-fold in vitro. The biologic behavior of MSCs differs according to the tissue of origin. For example, MSCs derived from bone marrow are twice the size, differentiate into bone, fat, and cartilage, are less immune suppressive in vitro and in vivo, and do not need direct cell–cell contact for immune suppression when compared to MSCs derived from placental decidua. Furthermore, marrow-derived MSCs have lower expression of PD-L1, PD-L2, and CD49d, have less procoagulant activity, and less hemostatic properties as opposed to those obtained from placental decidua.

MSCs exert their immune-modulating effects via paracrine secretion of many cytokines and molecules. MSCs polarize the immune system toward type II inflammatory response and inhibit type I response [8–10]. Mediators include prostaglandin E2, indoleamine-2,3-dioxygenase (IDO), nitric oxide (NO), galectins, HLA-G5, and other factors. MSCs can stimulate Tregs (directly or indirectly) and inhibit Th17 differentiation of naïve CD4+ cells. Additionally, MSCs can increase IL-10 producing CD5+ regulatory B cells. Other MSCs' actions are mediated by cell–cell contact and the induction of effector T-cell apoptosis via the PD-1 and Fas-FasL pathways resulting in the inhibition of effector T-cell proliferation.

Of interest, MSC appear to be immunologically privileged and exhibit an immune sanctuary due to minimal expression of MHC class I and no expression of MHC class II molecules. Further, MSCs have very limited ligand expression for adhesion molecules expressed by T cells, thus making them nearly ideal for use as both selective immune-suppressive agents and a product to enhance or regenerate tissue repair.

Given the various tissue sources and some pleomorphic characteristics, the International Society for Cellular Therapy (ISCT) created a consensus definition of

MSC to allow more uniform characterization of cell products, as well as to facilitate comparative studies [11]. The three minimal proposed criteria to define MSC populations include:

1. Plastic adherence
2. Surface expression of CD105, CD73, and CD90 [in the setting of lack of expression of CD45, CD34] and at least 1 of 2 macrophage markers (CD14, CD11b) and B-cell markers (CD79 $\alpha$ , CD19)
3. Capacity for trilineage differentiation into mesodermal tissues (such as osteoblasts, adipocytes, and chondroblasts)

This unique biology of MSCs contributed to the concept that the marrow itself was an organ system comprised of HSC as well as marrow stromal cells. Data supporting this concept include the findings that osteoblast monolayers independently can support granulopoiesis and B-cell lymphopoiesis [12]; osteoprogenitors could contribute to sinusoid assembly which proved to be a critical step in the generation of the molecular environment to support HSC [13]. Stromal cells themselves can construct proangiogenic environments which will recruit and maintain HSC and progenitors near the vascular sinusoids. Notably, marrow damaging therapies ultimately can damage the sinusoids; it has been shown that osteoblasts and stromal cells provide sanctuary for HSC while sinusoids are recreated [14].

Recognizing that MSCs are the progenitor population for osteoblasts, there was interest in determining whether allogeneic hematopoietic cell transplantation (HCT) could provide a source of donor MSCs. Early investigations suggested that after allograft, there was little contribution from a bone marrow graft to the donor MSC compartment. Stromal cells identified in Dexter cultures could become progressively donor in origin over time after transplant [15], but overall, using sex mismatched, human leukocyte antigen (HLA)-matched allografts, it was found that the majority of the stromal cell population was host-derived [16]. Some supporting evidence came from allogeneic HCT in osteogenesis imperfecta, a genetic disease of osteoblasts [17]. In children with this disease who underwent allograft procedures of either bone marrow or MSCs alone, over time, bone mineralization increased, spontaneous fractures decreased, and the subjects experienced enhanced growth. This clinical benefit appeared to be associated with very low MSC chimerism with only 1.5–2% donor osteoblast identified.

Almost 20 years ago, Lazarus and colleagues published the first-in-human-specific MSC clinical trial, a phase I feasibility study trial in 23 hematologic malignancy patients in complete remission [18]. Ten mL bone marrow samples were obtained, and MSCs were ex vivo culture-expanded over 4–7 weeks, then infused IV to ascertain safety. No untoward effects were noted, and subsequently, a successor study was executed to ascertain whether MSC could augment hematopoiesis. Recognizing that allogeneic HCT was much more complicated, the next investigation, completed by this same group, was the first-in-human autologous HCT study to address whether MSC adjunctive grafts could enhance hematopoietic recovery [19]. Twenty-eight advanced breast cancer patients undergoing myeloablative conditioning and autologous mobilized blood cell grafts also received an infusion of

$1\text{--}2.2 \times 10^6$  autologous MSC/kg. This pilot study suggested efficacy with median time to neutrophil recovery documented at 8 days and sustained platelet recovery above 20,000/mcL at 8.5 days after infusion.

Subsequent studies to augment hematopoiesis targeted situations where HCT was predicted to be suboptimal. Animal transplant studies using a limiting number of HSCs demonstrated that MSC infusions resulted in enhancement of granulopoiesis and megakaryocytopoiesis [20]. These and other such studies spurred the undertaking of further human studies to attempt to augment hematopoiesis in poor engraftment states. Examples included:

1. A single case report of family-directed MSC used alone, more than 2 years beyond autologous HCT for acute myeloid leukemia was shown to reverse critical thrombocytopenia and neutropenia [21].
2. 7 patients with either graft failure or suboptimal HSC engraftment underwent transplantation with HLA-matched or haploidentical MSCs; platelet and neutrophil recovery occurred by day 12, suggesting that second transplants after graft failure may be optimized by use of MSC grafts [22].
3. 2 of 6 patients with delayed engraftment (>30 days after transplant, but still platelets less than 50,000/mcL and neutrophils less than 1000/mcL) were treated with haploidentical MSCs and experienced an improvement in hematopoiesis [23].
4. 14 children undergoing a haploidentical transplant, a procedure associated with a historic 15% graft failure rate, additionally received MSC co-transplantation; all successfully recovered without loss of the hematopoietic graft [24].
5. A single case report presented a child affected by Wiskott-Aldrich syndrome. The direct implantation of MSCs into one hemipelvis was ineffective in improving hematopoiesis; however, bilateral marrow biopsies obtained on day 60 showed that the hemipelvis which received with direct implantation of MSC had a markedly improved marrow cellularity with trilineage hematopoiesis [25].
6. De Lima and colleagues studied engraftment results in 31 adult hematologic cancer patients who underwent hematopoietic cell transplantation using two umbilical cord-blood units, one of which was expanded *ex vivo* 14 days in coculture with a commercial allogeneic MSC product. Compared to 80 historic control patients, these patients had significantly faster neutrophil and platelet count recovery [26].

In summary, studies suggest that MSC products can be manipulated to assist hematopoiesis. Overall, however, the benefit was modest but potentially could be targeted to subjects who are predicted or are observed to have suboptimal marrow recovery. The studies above demonstrated that MSC infusion probably provided a transient effect via elaboration of cytokine mediators. MSCs constitutively secrete multiple soluble mediators such as SDF-1, IL-6, IL-7, IL-8, IL-11, IL--12, IL-14, IL-15, M-CSF, FLT3-L, and SCF. Using various stimuli, MSCs have the capacity to produce multiple other cytokines, including IL-1a, LIF, G-CSF, CCL2, CCL4, CCL5, CCL20, among others. Detailed proteomic assessments of the MSC secretome have confirmed these findings as well as documented multiple other soluble

mediators that were capable of inducing angiogenesis and immunosuppression as well as other mechanisms for these effects including cell–cell interaction [27]. Interestingly, trans-well studies were utilized to separate MSC populations from other purified cellular populations (such as T cells) and to assess whether the independent populations could exert influence without direct cell: cell contact. Using these technologies, immunologic cross-talk between MSC and T cells demonstrated both induction and suppression of various tissue-specific gene expression and protein production. The studies also characterized differential expression and variations in the immunologic cross-talk between resting and activated T-cell populations when cocultured in the presence of MSCs [28].

These investigations have generated significant interest in the application of MSC in two major clinical approaches: as an immunosuppressive agent and to assist tissue repair. In the former, Caplan and Correa hypothesized that MSCs work as a drug to deliver multiple soluble factors to suppress an activated immune system [29]. For the latter, that is, regenerative medicine, the goal was to provide trophic factors to enhance tissue repair. Ankrum and Karp [30] and Culme-Seymour et al. [31] have reported the burgeoning use of MSCs in trials all over the world, especially given the significant safety profile as reported by Lalu et al. [32].

## MSC for the Treatment of Acute GvHD

Acute GvHD is a dynamic, inflammatory process that occurs with temporal and spatial boundaries after an allogeneic HCT procedure. Multiple cell populations have been implicated as well as multiple cytokine mediators, but the molecular epicenter of the syndrome is the T-cell receptor: MHC interaction expressed by the donor T cells recognizing the host MHC molecules. GvHD takes time to develop as T cells need to proliferate in the host after antigenic challenge and need to traffic to target tissues where presentation will be at the subclinical or clinical levels. In the setting of clinically active GvHD, MSCs have been extensively studied [33].

The application of MSC for management of acute GvHD is often considered to have its origin with the seminal study of Leblanc et al. [34]. A young male hematopoietic cell transplant patient developed severe steroid-refractory grade 4 gastrointestinal and hepatic acute GvHD. After failing multiple anti-GvHD interventions, he was given a family-related, haploidentical, sex-mismatched MSC infusion ( $2 \times 10^6$  cells/kg) from his mother. Over a 3- to 4-week period, his condition improved but with subsequent immune suppressant taper, symptoms of diarrhea and jaundice recurred. He again had a dramatic response to a second infusion of MSCs ( $1 \times 10^6$  cells/kg) achieving a GvHD-free complete remission. Important correlative science studies demonstrated female cells within the gastrointestinal tract suggesting the MSCs were able to traffic to the GvHD target organ.

Subsequent small pilot trials were performed as well as large phase 2 studies with key studies including

1. Ringden et al. [35] described 9 patients (8 steroid-refractory acute GvHD; 1 chronic GvHD) of which 8 patients attained GvHD complete remission after receiving either family donor- directed as well as unrelated mismatched donors; this investigation established unrelated donor MSC as a viable therapeutic product.
2. European Society for Blood and Marrow Transplantation (EBMT) registry: [36]. Of 55 patients who had steroid-refractory acute GvHD, 30 attained complete and 9 attained a partial response when treated with related or unrelated donor MSC.
3. Kebriaei and coworkers [37] reported that of 31 patients affected by GvHD, 24 attained complete and 5 a partial GvHD response after therapy with universal donor, unrelated MSCs. 19 of 24 patients complete responders had sustained responses without the need for the addition of second line therapy for 90 days.
4. In a pediatric study, mismatched, third-party donor MSC were administered to 12 patients (median age 6 years) affected by grade III–IV steroid-refractory acute GvHD. Cells were administered twice weekly for 4 weeks followed by a weekly maintenance schedule for subjects who achieved only partial or mixed responses. All patients responded; 7 attained complete response, 2 partial, and 3 a mixed response. Even severe gastrointestinal GvHD appeared to be amenable to such treatment, and complete response was associated with a with a 2-year survival of 68% [38].

These and other studies led to 2 large, industry-sponsored phase 3 randomized (in a 2:1 ratio), placebo-controlled trials using third-party donor mismatch MSC (remestemcel-L; Prochymal®) both as initial therapy for acute GvHD as well as for salvage of steroid-refractory acute GvHD. The primary endpoint was complete remission with 28 days of sustained response without steroid increase and no second-line therapy; and in the case of the new diagnosis acute GvHD study, 90-day survival was the target. To date, both studies have been published only in abstract form [39–41].

1. Steroid-refractory acute GvHD: using an intent-to-treat analysis, no difference was found in the primary endpoint. Placebo exposure was associated with a 30% complete remission rate versus 35% with MSC ( $p = 0.3$ ). However, examining organ-specific responses, there was a 76% MSC response in patients with hepatic disease versus 47% on the placebo arm. In those subjects with gastrointestinal disease, there was an 82% response in the MSC cohort compared to a 68% response with placebo ( $p = 0.03$ ). For patients with three organs affected by acute GvHD, the overall response rate with MSC was 63% versus 0% with placebo. Notably, in the pediatric patients, not only was there an observed higher overall response rate (64% vs. 36%), but the 100-day survival was improved (79 vs. 50%).
2. New diagnosis acute GvHD: Similar to the steroid-refractory acute GvHD, no difference was identified in the primary endpoint when MSCs were added to the standard of care.

Failure to achieve primary endpoints has dampened enthusiasm for pursuing MSCs as GvHD therapy. Multiple post hoc analyses have been performed regarding these results, including evaluations of the trial design, whether the appropriate MSC product was used, and whether the ex vivo culture conditions were appropriate [33]. Further, the use of a cryopreserved rather than a fresh cultured MSC product may have lessened the biologic effect as François and associates have reported a “freezer burn effect” [42]. The complete response to acute GvHD therapy as seen in the studies were far less than in the smaller phase 2 trials performed previously.

In the absence of further phase 3 trial data, meta-analyses suggest that MSC infusions could remain acceptable therapy for patients affected by steroid-refractory acute GvHD for whom no other prior approved agents were available [43]. As such, an open-label pediatric trial examined the use of unrelated MSCs for grade B to D steroid-refractory GvHD in 75 subjects aged 2 months to 17 years. The data reported an overall response rate of 61%; complete responses were noted in 26% of gastrointestinal GvHD patients, 44% in those with cutaneous GvHD, and 33% in patients with hepatic GvHD. Responders had 28-day persistence of response, and 100-day survival was 78% versus 31% for those who failed treatment [44].

Currently, although not approved for use in adults affected by steroid-refractory acute GvHD, the FDA now has accepted the use of remestemcel-L (Ryoncil™) for priority review in steroid-refractory acute GvHD in children. The Biologics License Application currently is under consideration utilizing data from three clinical trials of a combined 309 children with steroid-refractory acute GvHD. Across trials, after a 4-week course of twice-weekly treatment, 66% of patients responded; day 28 responders also had improved survival versus nonresponders (83% versus 38% at day 180). MSC therapy for steroid-refractory acute GvHD in children currently is approved in Canada and New Zealand.

## **MSC for Prophylaxis of Acute GvHD**

MSC have been used for prophylaxis of acute GvHD based on in vitro and preclinical animal studies demonstrating their potent immunosuppressive capacity [33, 45]. Multiple mechanisms for this immune suppression in HCT models have been discussed above.

Lazarus et al. [46] reported the first application of related donor, ex vivo-expanded MSC infusions in the myeloablative allogeneic HCT setting. They demonstrated the feasibility and safety of procuring MSCs as well as hematopoietic cells from the sibling-matched donor, successful ex vivo expansion and subsequent infusion of allogeneic MSC into patients undergoing myeloablative and allogeneic HCT. Specifically, 46 subjects received varying doses of MSCs administered for the same hematopoietic cell donor with the infusion given 4 hours prior to the hematopoietic graft infusion. GvHD prophylaxis was limited to two-drug therapy with a calcineurin inhibitor and only 3 days of methotrexate. No accelerated neutrophil or

platelet recovery was seen but notably, there was no increase GvHD seen as a consequence of the MSC infusion despite the 3-day methotrexate exposure only.

Subsequently, a case-match control study compared the data set from this trial with the EBMT database. The analysis suggested a lower incidence of both acute and chronic GvHD in patients treated with MSC, and although only small numbers, there was a survival advantage at 6 months (96% vs. 68%) [47].

Other small studies performed include:

1. Bernardo et al., reported MSC prophylaxis therapy administered to 13 patients undergoing UCB transplantation. The data suggested a lower degree of grade III/IV acute GvHD than anticipated ( $p = 0.05$ ) [48]
2. Ning and coworkers reported in a small, randomized phase 2 study of patients undergoing allogeneic HCT using HLA-matched sibling donors. With the co-transplantation of MSCs, the incidence of grade 2–4 acute GvHD was lessened, but at the cost of a higher degree of relapse in myeloablative conditioning patients ( $n = 10$ ) versus controls ( $N = 15$ ) [49].
3. Baron et al., reported feasibility with an acceptable nonrelapse mortality at 1 year of only 10% in 20 patients who received HLA mismatched mobilized blood allografts with co-transplantation of the HLA mismatched HSC with unrelated third-party MSC in a phase I/II trial [50].
4. Maziarz and associates reported a 36 patient, multi-arm phase I co-transplant study assessing the potential efficacy of an MSC subset, the universal donor multipotent adult progenitor cell (MAPC), as prophylaxis for acute GvHD unrelated donor transplantation. Trial design included a single dose escalation on day 2 or repeat dose escalation over the first 28 days of transplant course. Similar to other studies, no infusional or drug-related toxicity was reported over the first 30 days of treatment. Engraftment was not affected. The overall grade II–IV acute GvHD rate was 38% (grade III/IV 15%). The cohort of interest was identified as the  $1 \times 10^7$  MAPC/kg dose administered on day 2 where an 11% grade II–IV acute GvHD incidence was observed with 0% grade 3 and grade 4 [51].
5. Finally, Kuzmina et al. reported a randomized study comparing 34 patients treated with standard acute GvHD prophylaxis versus 32 subjects receiving MSCs, at the time of blood count recovery. At day 100, a threefold decrease in acute GvHD frequency was seen in the experimental group (9.4% vs. 29.3%;  $p = 0.041$ ). Kaplan–Meier survival curves also suggested clinical benefit ( $p < 0.05$ ) [52].

## Conclusions

MSCs continue to be evaluated for their immunosuppressive properties. GvHD is a complex syndrome resulting from immunologic interactions developing after tissue damage from transplant conditioning regimens. Further, balancing the benefit of a graft versus leukemia versus GvHD remains an area of study. After 20 years, MSC



therapy has not been confirmed as a standard treatment. However, MSC therapy is an approved therapy for steroid-refractory acute GvHD in the pediatric population in other countries and is currently under consideration in the United States [53].

In the last several years, greater attention for the clinical application of MSC products has focused on regenerative medicine efforts. Multiple phase 2 trials have been undertaken in various areas such as traumatic brain injury, acute lung injury, organ transplantation, myocardial infarction, stroke, autoimmune disorders, inflammatory bowel disease, and multiple sclerosis. The largest use remains in orthopedic clinics where MSC products are grown *ex vivo* for application in degenerative arthritis. These unproven indications remain under FDA scrutiny and await confirmation based on the gold standard of phase 3 randomized, blinded treatment trials.

The MSC remains a provocative pharmaceutical agent with its excellent safety profile, the multitude of growth factors and small molecules that are secreted, or as recently demonstrated, that can be transferred to the target cell by endosomes [54]. However, like all drugs, if MSC is to be considered as an effective pharmaceutical agent, detailed studies still remain necessary to determine optimal timing of application, optimal dose, optimal route of delivery, whether MSC should be administered as fresh or cryopreserved product, and whether MSC biology will be facilitated by simultaneous small molecule co-treatment.

## References

1. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation*. 1968;2:230–47.
2. Dexter TM, Moore MA. In vitro duplication and “cure” of haematopoietic defects in genetically anaemic mice. *Nature*. 1977;269:412–4.
3. Huang E, Nocka K, Beier DR, et al. The hematopoietic growth factor KL is encoded by the Sl locus and is the ligand of the c-kit receptor, the gene product of the W locus. *Cell*. 1990;63:225–33.
4. Nocka K, Tan JC, Chiu E, et al. Molecular bases of dominant negative and loss of function mutations at the murine c-kit/white spotting locus: W37, Wv, W41 and W. *EMBO J*. 1990;(6):1805–13.
5. Pittenger MF, Discher DE, Peault BM, et al. Mesenchymal stem cell perspective: cell biology to clinical progress. *NPJ Regen Med*. 2019;4:22. <https://doi.org/10.1038/s41536-019-0083-6>. eCollection 2019
6. O’Flaherty E, Sparrow R, Szer J. Bone marrow stromal function from patients after bone marrow transplantation. *Bone Marrow Transplant*. 1995;15:207–12.
7. Kern S, Eichler H, Stoeve J, et al. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells*. 2006;24:1294–301.
8. Riquelme P, Haarer J, Kammler A, et al. TIGIT+ iTregs elicited by human regulatory macrophages control T cell immunity. *Nat Commun*. 2018;9:1–18.
9. Zheng G, Huang R, Qiu G, et al. Mesenchymal stromal cell-derived extracellular vesicles: regenerative and immunomodulatory effects and potential applications in sepsis. *Cell Tissue Res*. 2018;374:1–15.
10. Melief SM, Schrama E, Brugman MH, et al. Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. *Stem Cells*. 2013;31:1980–91.

11. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8:315–7.
12. Taichman RS, Emerson SG. Human osteoblasts support hematopoiesis due to production of granulocyte colony stimulating factor. *J Exp Med*. 1994;179:1677–82.
13. Sacchetti B, Funari A, Michienzi S, et al. Self-renewing osteo-progenitors and bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell*. 2007;131:324–36.
14. Garrett RW, Emerson SG. Bone and blood vessels: the heart and the soft of hematopoietic stem cell niches. *Cell Stem Cell*. 2009;4:503–6.
15. Keating A, Singer JW, Killen PD, et al. Donor origin of the in vitro hematopoietic microenvironment after marrow transplantation in man. *Nature*. 1982;298:280–3.
16. Simmons PJ, Przepiorcka D, Thomas ED, Torok-Storb B. Host origin of marrow stromal cells following. Allogeneic bone marrow transplantation. *Nature*. 1987;328:429–32.
17. Horwitz EM, Prockop DJ, Fitzpatrick LA, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med*. 1999;5:309–13.
18. Lazarus H, Haynesworth SE, Gerson SL, et al. *Ex vivo* expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use. *Bone Marrow Transplant*. 1995;16:557–64.
19. Koç ON, Gerson SL, Cooper BW, et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. *J Clin Oncol*. 2000;18:307–16.
20. Angelopoulou M, Novelli E, Grove JE, et al. Cotransplantation of human mesenchymal stem cells enhances human myelopoiesis and megakaryocytopoiesis in NOD/SCID mice. *Exp Hematol*. 2003;31:413–20.
21. Fouillard L, Chapel A, Bories D, et al. Infusion of allogeneic-related HLA mismatched mesenchymal stem cells for the treatment of incomplete engraftment following autologous haematopoietic stem cell transplantation. *Leukemia*. 2007;21:569–70.
22. LeBlanc K, Samuelsson H, Gustafsson B, et al. Transplantation of mesenchymal stem cells to enhance engraftment of hematopoietic stem cells. *Leukemia*. 2007;21:1733–8.
23. Meuleman N, Tondreau T, Ahmad I, et al. Infusion of mesenchymal stromal cells can aid hematopoietic recovery following allogeneic hematopoietic stem cell myeloablative transplant: a pilot study. *Stem Cell Dev*. 2009;18:1249–52.
24. Ball LM, Bernardo ME, Roelofs H, et al. Cotransplantation of ex vivo expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haplo-identical hematopoietic stem-cell transplantation. *Blood*. 2007;110:2764–7.
25. Resnick I, Stepensky P, Elkin G, et al. MSC for the improvement of hematopoietic engraftment. *Bone Marrow Transplant*. 2010;45:605–6.
26. de Lima M, McNiece I, Robinson SN, et al. Ex-vivo co-culture with mesenchymal cells improves cord blood engraftment. *N Engl J Med*. 2012;367:2305–15.
27. Burrows GG, van't Hof W, Newell L, et al. Dissection of the human multipotent adult progenitor cell (MAPC) secretome by proteomic analysis. *Stem Cells Transl Med*. 2013;2:745–57. Epub 2013 Aug
28. Burrows GG, van't Hof W, Reddy AP, et al. Solution phase cross talk and regulatory interactions between multipotent adult progenitor cells and peripheral blood mononuclear cells. *Stem Cell Trans Med*. 2015;4:1–14, Oct 22. pii: sctm.2014–0225. [Epub ahead of print]
29. Caplan AI, Correa D. The MSC: an injury drugstore. *Cell Stem Cell*. 2011;9:11–5.
30. Ankrum J, Karp JM. Mesenchymal stem cell therapy: two steps forward, one step back. *Trends Mol Med*. 2010;16:203–9.
31. Culme-Seymour EJ, Davie NL, Brindley DA, et al. A decade of cell therapy clinical trials (2000–2010). *Regen Med*. 2012;7:455–62.
32. Lalu MM, McIntyre L, Pugliese C, et al. Canadian Critical Care Trials Group: safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One*. 2012;7:e47559.

33. Maziarz RT. Mesenchymal stromal cells: potential roles in graft vs host disease prophylaxis and treatment. *Transfusion*. 2016;56:9S–14S. <https://doi.org/10.1111/trf.13563>.
34. Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet*. 2004;363:1439–41.
35. Ringdén O, Uzunel M, Rasmusson I, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation*. 2006;81:1390–7.
36. Le Blanc K, Frassoni F, Ball L, et al. Developmental Committee of the European Group for Blood and Marrow Transplantation. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet*. 2008;371(9624):1579–86.
37. Kebriaei P, Isola L, Bahceci E, et al. Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2009;15:804–11.
38. Prasad VK, Lucas KG, Kleiner GI, et al. Efficacy and safety of ex vivo cultured adult human mesenchymal stem cells (Prochymal™) in pediatric patients with severe refractory acute graft-versus-host disease in a compassionate use study. *Biol Blood Marrow Transplant*. 2011;17:534–41.
39. Osiris Therapeutics Announces Preliminary Results for Prochymal Phase III GVHD Trials. <http://investor.osiris.com/releasedetail.cfm?releaseid=407404>. Last accessed 2 June 2013.
40. Martin PJ, Uberti UB, Soiffer RJ, et al. Prochymal improves response rates in patients with steroid-refractory acute graft versus host disease (SR-GVHD) involving the liver and gut: results of a randomized, placebo-controlled, multicenter phase III trial in GVHD. *Biol Blood Marrow Transplant*. 2010;16:S169–70.
41. Szabolcs P, Visani G, Locatelli F, et al. Treatment of steroid-refractory acute GVHD with mesenchymal stem cells improves outcomes in pediatric patients; results of the pediatric subset in a phase III randomized, placebo-controlled study. *Biol Blood Marrow Transplant*. 2010;16:S298.
42. François M, Copland IB, Yuan S, et al. Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon- $\gamma$  licensing. *Cytotherapy*. 2012;14:147–52.
43. Hashmi S, Ahmmed M, Murad MH, et al. Survival after mesenchymal stromal cell therapy and steroid refractory acute graft-versus-host disease: systematic review and meta-analysis. *Lancet Haematol*. 2016;3:45.
44. Kurtzberg J, Prockop S, Teira P, et al. Allogeneic human mesenchymal stem cell therapy (remestemcel-L, Prochymal) as a rescue agent for severe refractory acute graft-versus-host disease in pediatric patients. *Biol Blood Marrow Transplant*. 2014;20:229–35.
45. Zahid MF, Lazarus HM, Ringdén O, et al. Can we prevent or treat graft-versus-host disease with cellular-therapy? *Blood Rev*. 2020 Feb 4:100669. <https://doi.org/10.1016/j.blre.2020.100669>. [Epub ahead of print] Review
46. Lazarus HM, Koc ON, Devine SM, et al. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. *Biol Blood Marrow Transplant*. 2005;11:389–98.
47. Frassoni F, Labopin M, Bacigalupo A, et al. Expanded mesenchymal stem cells (MSC), co-infused with HLA identical hematopoietic stem cell transplants, reduce acute and chronic graft-versus-host disease: a matched pair analysis. *Bone Marrow Transplant*. 2002;29(suppl 2):S2. abstract
48. Bernardo ME, Ball LM, Cometa AM, et al. Co-infusion of *ex vivo*-expanded, parental MSCs prevents life-threatening acute GVHD, but does not reduce the risk of graft failure in pediatric patients undergoing allogeneic umbilical cord blood transplant. *Bone Marrow Transplant*. 2011;46:200–7.
49. Ning H, Yang F, Jiang M, et al. The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: outcome of a pilot clinical study. *Leukemia*. 2008;22:593–9.
50. Baron F, Lechanteur C, Willems E, et al. Cotransplantation of mesenchymal stem cells might prevent death from graft versus-host disease (GVHD) without abrogating graft versus-tumor

- effects after HLA-mismatched allogeneic transplantation following nonmyeloablative conditioning. *Biol Blood Marrow Transplant.* 2010;16:838–47.
51. Maziarz RT, Devos T, Bachier CR, et al. Single and multiple dose MultiStem (multipotent adult progenitor cell) therapy prophylaxis of acute graft-versus-host disease in myeloablative allogeneic hematopoietic cell transplantation: a phase I trial. *Biol Blood Marrow Transplant.* 2015;21:720–8.
  52. Kuzmina LA, Petinati NA, Shipounova IN, et al. Analysis of multi potent mesenchymal stromal cells used for acute graft-versus-host disease prophylaxis. *Eur J Haematol.* 2016;96:425–34.
  53. <http://www.mesoblast.com/product-candidates/oncology-hematology/acute-graft-versus-host-disease>.
  54. Keshtkar S, Azarpira N, Ghahremani MH. Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. *Stem Cell Res Ther.* 2018;9:63. <https://doi.org/10.1186/s13287-018-0791-7>.