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Emerging Therapeutic Approaches For Multipotent Mesenchymal Stromal Cells (MSCs)

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Abstract

Purpose—Multipotent mesenchymal stromal cells (MSCs) are rare cells resident in bone marrow and other organs capable of differentiating into mesodermal lineage tissues. MSCs possess immunomodulatory properties and have extensive capacity for *ex vivo* expansion. Early clinical studies demonstrated safety and feasibility of infusing autologous MSCs and suggested a role in enhancing engraftment after hematopoietic cell transplant (HCT). Subsequent pilot studies using allogeneic MSCs showed safety but contradictory results regarding efficacy in treating graft-versus-host disease (GVHD).

Recent findings—Larger, phase II allogeneic MSC infusion studies, including cells obtained from haploidentical and third-party donors, showed efficacy in GVHD treatment; however, recent randomized, placebo-controlled studies failed to corroborate these results. New investigations include MSC infusions in umbilical cord blood transplantation, MSC therapy for tissue regeneration/repair, harvest and use of MSCs from adipose tissue and cell-tracking/imaging studies using radionuclides, gene-and fluorescent dye labeled-MSCs.

Summary—MSCs remain the subject of intense investigation in HCT because of their differentiation potential and immunomodulatory properties. While infusions of autologous, allogeneic and third-party donor MSCs are safe, further research is needed to clarify the optimal methods for harvesting and expansion, optimal timing of administration and efficacy in the setting of HCT.

Keywords

Multipotent mesenchymal stromal cells; hematopoietic stem cells; graft versus-host disease; expansion; imaging

Introduction

In addition to hematopoietic stem cells (HSCs), the adult bone marrow (BM) contains rare, non-hematopoietic multipotent progenitor cells with fibroblast-like appearance[1], termed multipotent mesenchymal stromal cells (MSCs)[2]. MSCs have the capacity to differentiate *in vitro* along mesenchymal lineages and give rise to multiple tissues, including bone, cartilage, adipose tissue and BM stroma; they exhibit intense paracrine activity, secreting bioactive molecules with trophic and immunomodulatory capacity[3]. When infused, MSCs

home to tissue sites of active inflammation[4]. The combination of tissue regenerative potential and immunomodulatory or immunosuppressive activity has prompted therapeutic interest. MSCs can provide stability and restore function in organs such as the heart, gastrointestinal tract and nervous system as well as in hematopoietic cell transplantation (HCT) to prevent and control graft-versus-host disease (GVHD).

Biologic Properties

MSCs were first identified by Friedenstein and colleagues in post-natal BM more than 40 years ago[1, 5]. MSCs can be isolated from other tissues including BM, adipose tissue and umbilical cord blood[6]. BM MSC frequency varies among species representing 0.001–0.01% of nucleated marrow cells. Their number decreases with age from 1 MSC/10,000 in newborns to 1 MSC/250,000 nucleated marrow cells in adults[7]. This paucity in BM and other tissues along with the lack of specific markers makes study of isolated cells difficult and little is known about the primary progenitor cell *in vivo*. MSCs, however, have a remarkable capacity to undergo extensive expansion in *ex vivo* culture settings.

The International Society for Cellular Therapy (ISCT) defined minimal criteria for MSCs[2]. The fibroblast-like plastic-adherent population is heterogeneous and only a small proportion can generate colonies *in vitro*[8]. In 2005 the ISCT clarified the nomenclature to the current “multipotent mesenchymal stromal cells”.

Immunomodulatory Properties

MSCs suppress T cell proliferation and cytokine production in response to alloantigens and nonspecific mitogens[9, 10], a mechanism mediated both by soluble factors[11, 12], and by direct cell-cell interactions[13]. MSCs inhibit function of dendritic cells[14, 15, 16], N_K cells [17] and B cells [18, 19]. MSCs also appear to be immunologically privileged [20] as they do not induce lymphocyte proliferation *in vitro* or *in vivo*[21]; their immunosuppressive effect is independent of MHC compatibility status[10]. Multi-potent adult progenitor cells (MAPC) are a separate MSC subpopulation with potent immunosuppressive properties that are emerging in laboratory and clinical investigations as an important cellular tool[4, 21].

Ex Vivo Expansion of Human MSCs

Clinical use of human MSCs is investigational and regulated in the United States by the FDA as a Human Cell and Tissue Product under section 351 of the Public Health Service Act. MSCs must be manufactured under an Investigational New Drug Application (IND) and be monitored both for potency and consistency. Human MSCs can be passaged only a finite number of times, thereafter experiencing reduced proliferation and differentiation potential. Furthermore, growth characteristics and cell yield of an MSC preparation are dependent on donor age and vary among individuals [22]. The majority of expansion protocols utilize basal medium and low glucose along with 10–20% fetal bovine serum (FBS), although other protein sources such as platelet lysates can be used. Some investigators use additional factors such as recombinant human fibroblastic growth factor (rhFGF) as a culture supplement to enhance proliferation capacity. Our studies showed that the addition of 10ng/ml rhFGF-2 (final concentration) to culture medium starting at first passage resulted in a decreased population doubling time. This maneuver reduced time to reach target cell dose from median 41 days to 24.5 days without altering immunosuppressive function or differentiation potential [23, 24]; 90% of rhFGF-2-containing cultures achieved target cell dose (n=10) compared to only 55% of rhFGF-negative cultures (n=20). Donors whose cultures failed to expand in the absence of rhFGF-2 reached the target dose with BM

re-harvest and culture in the presence of rhFGF-2. Human MSCs are cytogenetically stable under the culture conditions described above, even long-term [25, 26].

Serum-Free Media

FBS may harbor pathogens and MSC recipients may develop anti-FBS antibodies [27], hence the motivation for using serum-free media. MSCs can be grown in serum-free media supplemented with FGF, platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-beta) but there are no consistent strategies. Platelet-rich plasma appears an effective substitute for FBS [27] and a clinical trial using human platelet lysate is in progress (www.clinicaltrials.gov; NCT00827398). Limitations include microbiologic disease transmission, risk of anaphylactoid reactions and blood group-related antibodies. Autologous serum potentially is an alternative, though limited by the large volume necessary to supplement the media.

Early Clinical Studies (Table 1)

There are no adequate preclinical animal models for MSC use in HCT and clinical MSC infusion trials began before completely understanding their *in vivo* properties [31]. Table 1 shows the results of several early trials demonstrating that *ex vivo* expansion and re-infusion of autologous MSCs is safe, feasible and associated with efficacy. Subsequent trials have been undertaken in the allogeneic setting.

GVHD

This syndrome remains a major life-threatening complication of allogeneic HCT, especially if corticosteroid-refractory [32]; additional immunosuppression increases risk of toxicity, infections and relapse. The immunomodulatory properties and capacity to home to sites of inflammation have made MSCs the object of intense research in the HCT setting. *In vitro* and early *in vivo* studies suggested the suppressive effect of MSCs did not affect virus-specific T cell immune responses [33], i.e. treating GVHD without increasing infectious complications.

Treatment of GVHD (Table 2)

The Karolinska University group successfully managed a case of severe corticosteroid-refractory gastro-intestinal acute GVHD using infusion of haploidentical MSCs harvested from the patient's mother[41]. Subsequently, these investigators reported a small series of predominantly corticosteroid-resistant acute GVHD patients [34]. MSC graft sources varied and included HLA identical sibling-, haploidentical- and HLA mismatched-donors. Six of eight patients had significant reduction in acute GVHD after MSC infusions. Muller and colleagues [35] noted improvement of GVHD in two of seven pediatric HCT patients receiving parental haploidentical MSCs. von Bonin and coworkers [36] reported benefit in two of 13 adult acute GVHD patients given third-party MSCs expanded in platelet lysate-containing medium. These less impressive outcomes can be attributed to differences in patient populations, timing of MSC infusion and variations in culture and expansion methods. Fang and coworkers[37] reported no infusion-related side effects and complete remission in five of six corticosteroid-refractory acute GVHD patients given 1×10^6 MSC/kg culture-expanded MSCs obtained from abdominal subcutaneous adipose tissue of third-party donors undergoing lipectomy. These exciting results need to be confirmed in larger studies but demonstrate that adipose tissue-derived MSCs have immunoregulatory properties similar to those derived from BM [42, 43, 44, 45].

A multicenter phase II trial reported by Le Blanc and co-workers [38](**) assessed the efficacy of multiple MSC infusions for corticosteroid-refractory acute GVHD after allogeneic HCT (n=48) and after donor lymphocyte infusion (n=7). Thirty patients (54.5%) had a complete response after one or more MSC infusions. Of note, third-party MSCs appeared to be as effective as HLA identical or haploidentical MSC infusions, supporting the concept that third-party MSCs can be banked in advance for prompt GVHD therapy [38, 46].

Keabraei and colleagues[39](*) administered two different Prochymal® (Osiris Therapeutics, Columbia, MD) cell doses as initial therapy for acute GVHD. This commercial preparation of HLA unrelated, culture-expanded human MSCs was given at either low (2×10^6 MSCs/kg) or high (8×10^6 MSCs/kg) dose plus high-dose corticosteroids within 24–48 hours of diagnosis; a second dose was given 3 days later. No acute infusional toxicity was observed. Complete responses were observed in 24 patients (77%) while 5 patients (16%) had a partial response; response rates did not correlate with MSC dose infused. All 13 patients with skin-only involvement responded (11 complete) and nine of 11 patients with gastrointestinal tract GVHD responded (eight complete). All seven multi-organ GVHD subjects responded (five complete)[39]. Efficacy, however, was difficult to interpret as all patients also received high-dose corticosteroid therapy quickly after the onset of GVHD.

These results contrast with the preliminary findings of a recent prospective, double-blind, randomized clinical trial comparing the addition of Prochymal® versus placebo to existing therapy for corticosteroid-refractory acute GVHD [40](**). This large, multi-center trial enrolled 244 patients, 163 randomly assigned to receive 8 infusions of 2×10^6 MSC/kg over a 4-week period versus 81 to receive placebo. Primary study endpoint was durable complete response (DCR), defined as maintained complete response at 28 days after initiating therapy without additional therapy and survival to 90 days. Using an intention-to-treat analysis, DCR rate was 35% for Prochymal® vs. 30% in the placebo group, ($p=0.3$). Overall response rate at 100 days did not differ statistically, Prochymal® 82% vs placebo 73% ($p=0.12$).

Another phase III randomized, placebo-controlled trial (N=192) compared corticosteroids plus either placebo or Prochymal® in first-line treatment of newly-diagnosed acute GVHD also failed to demonstrate a significant benefit for this MSC preparation (90-day survival 45% versus 46%)[47]. The disparity in these Prochymal® trials could reflect different primary study outcome measures. The initial phase II protocol [47] assessed the proportion of patients that achieved complete remission while the primary outcome measure for the phase III studies was proportion of patients who achieved DCR[47].

Prophylaxis of GVHD

In 2005, Lazarus and coworkers[6] reported a multicenter, cotransplantation study using HSCs and BM-derived MSCs, both products obtained from HLA identical sibling donors. MSC culture-expansion was feasible in 51 donors (91%) and MSC dose was escalated as 1.0, 2.5 or 5.0×10^6 MSCs/kg in 3 cohorts. Forty-six patients received MSCs 4 hours before HSCs without adverse infusion reactions. Hematopoietic engraftment was prompt; acute and chronic GVHD rates were comparable to those observed in other studies of HLA matched sibling allogeneic HCT. This phase I study demonstrated safety and feasibility of cotransplantation of a single MSC infusion during HCT. Subsequent phase II/III studies are required to demonstrate efficacy of this prophylaxis strategy.

MSC Facilitation of Engraftment (Table 3)

Some allogeneic HCT and MSC infusion studies were designed to correct or prevent engraftment failure. The Karolinska University investigators [48] reported 7 patients who

underwent cotransplantation (including use of haploidentical MSCs), three for a previous graft failure/rejection and four to enhance hematopoietic engraftment. Despite remarkable variability in the patient population, sources of HSCs and MSCs, and HLA compatibility, all patients engrafted HSCs.

Ball and colleagues[49] cotransplanted MSCs and HSCs obtained from haploidentical related donors into 14 pediatric hematologic malignancy and immune deficiency/non-malignant disorder patients, all of whom demonstrated HSC engraftment. Compared to historic controls (n = 47), the 14 study patients had comparable platelet and neutrophil recovery yet faster attainment of a total WBC count $> 1 \times 10^9/L$. More recently, Macmillan and colleagues [50] reported eight children with hematologic malignancies given umbilical cord blood (UCB) grafts plus haploidentical MSCs. Although MSC infusions were safe, tempo to recovery of neutrophil and platelet engraftment as well as rates of acute GVHD were similar to historic controls.

A phase I/II study by Gonzalo-Daganzo et al [51] examined nine patients given third-party MSCs after coinfusion of UCB and third party HSCs. Median MSC dose was 1.18 (range 1.04–2.22) $\times 10^6$ MSC/Kg and without adverse effects. Hematopoietic engraftment and severe acute GVHD rates were comparable to controls who did not receive MSCs. Two patients given subsequent MSC infusions for corticosteroid-refractory GVHD attained complete GVHD response. Despite the small study size, the authors concluded that use of MSCs should be reserved for early treatment of acute GVHD rather than as cotransplantation.

MSC infusions potentially could be used for treatment of primary graft failure after HCT. Fouillard and colleagues[52] reported a 40-year-old woman with AML in complete remission who developed primary graft failure after autologous HCT that was only partially responsive to G-CSF and erythropoietin therapy. Culture-expanded MSC infusion ($2.78 \times 10^6/kg$) from her HLA mismatched brother three years after HCT resulted in rapid and sustained recovery of neutrophil and platelet counts without evidence of GVHD. Male DNA transiently was identified one month after infusion but was undetectable one year later.

Negative Effects of MSCs

In an open label, randomized study, Ning and colleagues[53](*) compared cotransplantation of MSCs plus HSCs (N=10) versus HSCs alone (N=15). Hematopoietic engraftment rates did not differ but grade II – IV acute GVHD incidence was reduced for those receiving MSCs. Relapse rates, however, were significantly higher in MSC recipients (60% versus 20%, log rank $p=0.02$); correspondingly, 3-year disease free survival rates were lower at 30% for the MSC group compared to 66.7% in the non-MSC group[53]. Other risk factors for disease relapse were not evaluated[54]. Larger randomized studies are needed to confirm these findings.

An additional concern related to infusion of culture-expanded MSCs is the potential for malignant transformation. While development of malignant tumors has not been reported in human studies, Tolar and colleagues[55] noted sarcomas in lungs and extremities of mice injected with *ex vivo* expanded MSCs. The authors hypothesized that genetic instability and resultant cytogenetic abnormalities occurred as a result of prolonged *in vitro* expansion, a phenomenon seen in murine cells. Human MSC cultures, therefore, should be monitored for genetic integrity.

Tissue Repair

MSCs have tissue-repair potential and have been infused in a variety of clinical circumstances. Uses have included post-myocardial infarction and after HCT for pneumomediastinum, severe hemorrhagic cystitis and colonic perforation[6]

Imaging and Tracking of MSCs

Tracking MSCs after an allogeneic HCT may be important but extremely few cells are transferred [6]. MSCs home to damaged tissues via the utilization of selectins, integrins and chemokines expressed on the cell surface, in a manner similar to leukocytes [56]. Most cellular tracking methodologies, however, cannot quantitatively, longitudinally and non-invasively track MSCs in a living animal and reveal their spatial, temporal and intensity of expression patterns. Recently, non-invasive imaging-based monitoring modalities have been used to track cells via direct labeling with radionuclides, ferromagnetic particles, or indirect labeling with reporter genes for PET or SPECT imaging[57, 58, 59, 60, 61]. Imaging using reporter gene-labeled MSCs revealed that IV injection led to entrapment of most MSCs in the lung[60], impeding migration to target organs. Such imaging techniques have demonstrated that routes such as intra-arterial infusion of MSCs resulted in superior biodistribution compared to the IV route[62, 63, 64, 65, 66].

This technology, however, allows only short-term imaging due to the short half-life of radionuclides, and reporter-gene labeling faces major regulatory (FDA) barriers. Exogenous fluorescent markers such as indocyanine green (ICG), an FDA approved dye, have been used to label MSCs for imaging and may help advance the field[67]**. Newer preclinical approaches also are showing promise in unequivocally identifying donor cells without reliance upon cell surface protein expression[68].

Conclusions

Exploitation of the unique properties of MSCs and MAPCs is only just beginning. Multiple small pilot clinical trials have demonstrated safety of infusion in a variety of settings that has lead to several readily available commercial but investigational products. Unresolved questions include: defining the optimal tissue source and expansion method; cell dose, therapy duration, timing and route of administration; monitoring procedures to track cell fate. The answers ultimately will determine what role these cells will have in the therapeutic armamentarium.

Abbreviations

aGVHD	acute graft versus host disease
AML	Acute myeloid leukemia
BM	Bone marrow
cGVHD	chronic graft versus host disease
DCR	Durable complete response
DNA	Deoxyribonucleic acid
FBS	Fetal bovine serum
FDA	Food and Drugs Administration
G-CSF	Granulocyte colony-stimulating factor

GVHD	Graft versus-host disease
HCT	Hematopoietic stem cell transplantation
HLA	Human leukocyte antigen
HSCs	Hematopoietic stem cells
IND	Investigational New Drug Application
ISCT	International Society for Cellular Therapy
MAPC	Multi-potent adult progenitor cells
MMUD	mismatched unrelated donor
MSCs	Multipotent mesenchymal stromal cells
MUD	matched unrelated donor
PDGF	Platelet-derived growth factor
rhFGF	Recombinant human fibroblastic growth factor
TGF-beta	Transforming growth factor beta
UCB	Umbilical cord blood
WBC	White blood cell
PET	Positron emission tomography
SPECT	Single photon emission computed tomography
IV	Intravenous
ICG	Indocyanine green

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Table 1

Early clinical studies of MSC infusion

Publication: First Author, Year	Type of Study/Design	Patient Characteristics	MSC Source	MSC Dose	Regimen	Results
Lazarus, 1995 [28]	Phase I: feasibility for autologous MSC expansion & infusion (no cytotoxic agents involved)	n = 23 Ages 18–68 yr	Autologous bone marrow	Three groups • 1×10^6 MSCs • 5×10^6 MSCs • 10×10^6 MSCs	None	<ul style="list-style-type: none"> Time collection to infusion 28–49 days 15 patients completed ex vivo MSC expansion No adverse reactions related to MSC infusion
Koc, 2000 [29]	Phase I-II: autologous HSC & MSC cotransplantation in breast cancer patients	n = 32 Median (range) age 47 (37–57) yr	Autologous bone marrow	$1-22 \times 10^6$ MSC/kg	Autologous transplant: cyclophosphamide, thiotepea, carboplatin	<ul style="list-style-type: none"> No adverse events attributable to MSC infusion Rapid hematopoietic engraftment Median (range) time to ANC $> 0.5 \times 10^9/L$ = 8 days (6–11 days) Median (range) time to platelets $> 20 \times 10^9/L$ = 8.5 days (4–19 days)
Frassoni, 2002 [30]	Matched-pair analysis of HLA identical MSC and HSC cotransplantation	n = 31 Median (range) age 43 (19–56) yr	HLA identical sibling bone marrow	$1-2 \times 10^6$ MSC/kg	Myeloablative allogeneic transplant: HLA identical donors	<ul style="list-style-type: none"> Hematopoietic engraftment in all patients Lower GVHD incidence in study patients 6-month survival higher in study patients

Abbreviations: ANC: absolute neutrophil count; GVHD: graft versus host disease; HSC: hematopoietic stem cell MSC: multipotent mesenchymal stromal cell;

Table 2

MSC infusions for therapy of GVHD

Publication: First Author, Year	Study	Patient Characteristics	MSC Source/Other	MSC Dose	HSC Source	Results
Ringden, 2006 [34]	MSC for treatment of aGVHD and cGVHD after ASCT	n = 9 Median (range) age 56 (8–61) yr	All bone marrow: HLA identical sibling = 2 Haploidentical donor = 6 Third party donors = 4	0.6–9 × 10 ⁶ /kg	HLA Identical sibling = 4 MUD = 2 HLA mismatched related = 1.	<ul style="list-style-type: none"> Overall response of aGVHD 6/8 Improved survival in MSC recipients No response in extensive cGVHD Source of MSCs did not affect outcome
Muller, 2008 [35]	MSC for treatment of engraftment failure, aGVHD, cGVHD and hemophagocytosis in pediatric patients	n = 7 Median (range) age 14 (4–17) yr	All bone marrow: Haploidentical parent	0.4–3 × 10 ⁶ MSC/kg	Haploidentical parent = 5 MUD = 2	<ul style="list-style-type: none"> No adverse reactions related to MSC infusion 1 case of aGVHD did not progress to cGVHD MSC infusion improved 1 case of hemophagocytosis.
Von Bonin, 2009 [36]	MSCs for treatment of corticosteroid refractory aGVHD.	n = 13 Median (range) age 58 (21–69) yr	All bone marrow: Third party mismatched unrelated donors Platelet-containing medium expansion of MSCs	0.9–1.1 × 10 ⁶ MSC/kg	Matched identical sibling = 3 Mismatched sibling = 1 MUD = 7 MMUD = 2	<ul style="list-style-type: none"> No adverse reactions to MSC infusion 2/13 patients did not require further immunosuppression Overall response: 54% 1 complete response; 1 partial response; 5 minimal responses
Fang, 2007 [37]	Adipose tissue-derived MSCs for treatment of corticosteroid refractory aGVHD	n = 6 Median (range) age 40 (22–49) yr	Adipose tissue-derived MSCs Haploidentical = 4 Third party mismatched = 4	MSC dose 1 × 10 ⁶ MSC/kg	Sibling donor = 3 MUD = 3	<ul style="list-style-type: none"> No adverse reactions 5/6 complete responses
Le Blanc, 2008 [38]	MSCs for treatment of corticosteroid refractory aGVHD	n = 55 Median (range) age 22 (0.5–64) yr	All bone marrow: HLA identical Sibling = 5 HLA haploidentical = 18 Unrelated mismatched = 69	0.4–9 × 10 ⁶ MSC/kg 92 MSC infusions 1 MSC infusion = 27 1 MSC infusion = 28	HLA identical sibling = 19 MUD = 25 Mismatched = 6 Matched unrelated UCB = 3 Mismatched unrelated UCB = 2	<ul style="list-style-type: none"> Complete responses = 30 (54.5%) Partial responses = 9 (16.4%) Larger proportion of children responded. Complete responses: 2 yr overall survival 54% (95%CI 34–70%)

Publication: First Author, Year	Study	Patient Characteristics	MSC Source/Other	MSC Dose	HSC Source	Results
Kebiraei, 2009 [39]	Phase II trial of Prochymal® + corticosteroids for initial treatment of aGVHD	n = 31 Median (range) age 52 (34 – 67) yr	Prochymal®: commercial preparation from HLA unrelated donors	2×10^6 MSC/kg = 16 8×10^6 MSC/kg = 15	Matched related donor = 18 MUD = 13	<ul style="list-style-type: none"> MSC source did not affect outcome No infusional toxicity Complete remission = 24 (77%) Partial remission = 5 (16%) MSC dose did not correlate with response Skin GVHD complete responses 11/13 GI GVHD complete responses 8/11 Multi-organ GVHD complete responses 5/7
Martin, 2010 [40]	Phase III trial of Prochymal® vs placebo for corticosteroid refractory aGVHD	n = 244 Median age N/A	Prochymal®: commercial preparation from HLA unrelated donors	2×10^6 MSC/kg	N/A	<ul style="list-style-type: none"> Durable complete responses 35% with Prochymal®, 30% with placebo (p=NS) Improved response rates in liver and gut corticosteroid resistant GVHD

Abbreviations: aGVHD: acute graft versus host disease; ANC: absolute neutrophil count; cGVHD: chronic graft versus host disease; GVHD: graft versus host disease; HSC: hematopoietic stem cell; MMUD: mismatched unrelated donor; MSC: multipotent mesenchymal stromal cell; MUD: matched unrelated donor; N/A not available, NS: not statistically significant; UCB: umbilical cord blood

Table 3

MSC infusions for facilitation of hematopoietic engraftment

Publication: First Author, Year	Reason For MSC Infusion	Patient Characteristics	MSC Source	MSC Dose	HSC Source	Results
Le Blanc, 2007 [48]	Treatment of graft failure and engraftment enhancement	n = 7 Median (range) age 12 (1–44) yr Graft failure = 2 Graft rejection = 1 Prophylaxis = 4	HLA identical sibling = 3 Haploidentical related donor = 4	1×10^6 MSC/kg	HLA identical Sibling = 3 MUD = 2 MMUD = 2	<ul style="list-style-type: none"> No acute toxicity after MSC infusion All patients achieved hematopoietic engraftment Median (range) time to ANC $> 0.5 \times 10^9/L = 12$ (10–28) days Median (range) time to platelets $> 30 \times 10^9/L = 12$ (8–36) days
Ball, 2007 [49]	Engraftment enhancement in pediatric patients	n = 14 Mean (range) age 8 (1–16) yr	Haploidentical parent	$1-3.3 \times 10^6$ MSC/kg	Haploidentical parent	<ul style="list-style-type: none"> All patients achieved hematopoietic engraftment Neutrophil and platelet engraftment similar to historic Controls
Macmillan, 2009 [50]	Facilitate engraftment of UCB in pediatric patients	n = 8 Median (range) age 7.5 (0.2–16) yr	Haploidentical parent	$0.9-5 \times 10^9$ MSC/kg	UCB	<ul style="list-style-type: none"> All patients achieved neutrophil engraftment Median (range) time to ANC $> 0.5 \times 10^9/L = 19$ (9–28) days 6/8 patients achieved platelet engraftment Median time to platelets $> 50 \times 10^9/L = 53$ (36–98) days Hematopoietic engraftment rates and timing not different from historic controls
Gonzalo-Daganzo, 2009 [51]	Feasibility in setting of combined UCB/HSC cotransplantation	n = 9 Median (range) age 32 (21–48) yr	Haploidentical parent = 1 Other haploidentical relative = 6 No shared haplotype = 2	$1-3.3 \times 10^6$ MSC/kg	UCB (single unit) plus HSC Haploidentical parent = 1 Other haploidentical relative = 6	<ul style="list-style-type: none"> No adverse effects of MSC infusion Median (range) time to ANC $> 0.5 \times 10^9/L = 12$ (10–31) days

Publication: First Author, Year	Reason For MSC Infusion	Patient Characteristics	MSC Source	MSC Dose	HSC Source	Results
					No shared haplotype = 2;	<ul style="list-style-type: none">• Median (range) time to platelets $> 20 \times 10^9/L = 44$ (27–98) days• All patients achieved full UCB chimerism• Median (range) 51 (10–186) days• Hematopoietic engraftment rates and timing not different from historic controls

Abbreviations: ANC: absolute neutrophil count; HSC: hematopoietic stem cell; MMUD: mismatched unrelated donor; MSC: multipotent mesenchymal stromal cell; MUD: matched unrelated donor; UCB: umbilical cord blood;