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Clinical and laboratory features of mesenchymal stromal cells in pediatric stem cell transplantation

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Chapter 7

Mesenchymal stem cells for treatment of severe, refractory acute graft-versus host disease

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Summary

Severe graft-versus-host disease (GVHD) is a life-threatening complication after allogeneic transplantation with haemopoietic stem cells. Mesenchymal stem cells modulate immune responses in vitro and in vivo. We aimed to assess whether mesenchymal stem cells could ameliorate GVHD after haemopoietic-stem-cell transplantation. Patients with steroid-resistant, severe, acute GVHD were treated with mesenchymal stem cells, derived with the European Group for Blood and Marrow Transplantation ex-vivo expansion procedure, in a multicentre, phase II experimental study. We recorded response, transplantation-related deaths, and other adverse events for up to 60 months' follow-up from infusion of the cells. Between October, 2001, and January, 2007, 55 patients were treated. The median dose of bone-marrow derived mesenchymal stem cells was 1.4×10^6 (min-max range $0.4-9 \times 10^6$) cells per kg bodyweight. 27 patients received one dose, 22 received two doses, and six three to five doses of cells obtained from HLA-identical sibling donors (n=5), haploidentical donors (n=18), and third-party HLA-mismatched donors (n=69). 30 patients had a complete response and nine showed improvement. No patients had side-effects during or immediately after infusions of mesenchymal stem cells. Response rate was not related to donor HLA-match. Three patients had recurrent malignant disease and one developed de-novo acute myeloid leukaemia of recipient origin. Complete responders had lower transplantation-related mortality 1 year after infusion than did patients with partial or no response (11 [37%] of 30 vs 18 [72%] of 25; $p=0.002$) and higher overall survival 2 years after haemopoietic-stem-cell transplantation (16 [53%] of 30 vs four [16%] of 25; $p=0.018$). Infusion of mesenchymal stem cells expanded in vitro, irrespective of the donor, might be an effective therapy for patients with steroid-resistant, acute GVHD.

Introduction

Allogeneic haemopoietic-stem-cell transplantation is the treatment of choice for many malignant and non-malignant disorders.^{1,2} Severe graft-versus-host disease (GVHD) is a life-threatening complication after this treatment and donor lymphocyte infusion is used for treatment or prevention of relapse of leukaemia.^{3,4} Steroids are still the first-line treatment for established GVHD with a response rate of 30–50%; however, the outcome for patients with severe, steroid-resistant, acute GVHD is poor, and overall survival is low.^{1,3–5}

Mesenchymal stem cells are multipotent bone-marrow cells able to differentiate in vitro and in vivo into tissues of mesenchymal origin.^{6,7} Moreover, these cells provide support for the growth and differentiation of haemopoietic progenitor cells in bone-marrow microenvironments and, in animal models, promote engraftment of haemopoietic cells.⁸ In co-culture experiments with allogeneic lymphocytes, mesenchymal stem cells do not induce lymphocyte proliferation, interferon-gamma production, or up-regulation of activation markers.^{9,10}

Mesenchymal stem cells suppress proliferation of activated lymphocytes in vitro in a dose-dependent, non-HLA-restricted, manner.^{9–11} In a baboon skin-graft model, Bartholomew and co-workers¹¹ showed that infusion of ex-vivo expanded donor-derived or third-party cells prolonged the time to rejection of histo-incompatible skin grafts. Furthermore, infused cells improve the outcome of acute renal, neural, and lung injury, possibly by promoting a shift from production of pro-inflammatory cytokines to anti-inflammatory cytokines at the site of injury.^{12–14}

In phase I and II trials, HLA-identical mesenchymal stem cells expanded ex vivo have been infused to promote haemopoietic recovery after autologous and allogeneic haemopoietic-stem-cell transplantation and to treat patients with osteogenesis imperfecta.^{15–20} So far, neither acute nor long-term adverse events have been reported after infusion of mesenchymal stem cells. Two reports on the use of in-vitro expanded cells for the treatment of severe, acute GVHD have recently been published.^{21,22}

To facilitate large-scale, multicentre trials, the European Group for Blood and Marrow Transplantation Developmental Committee has adopted a common protocol for expansion of mesenchymal stem cells. We report the results of a multicentre, phase

II study of the use of these cells in 55 patients with severe and steroid-resistant, acute GVHD.

Methods

Patients

Between October, 2001, and January, 2007, patients of all ages with grade 2–4 GVHD after haemopoietic-stem-cell transplantation, who did not respond to steroid treatment (≥ 2 mg per kg per day) for at least 7 days, or with progression of at least one grade within 72 h were eligible for the study. 55 patients were treated (table 1); 48 had developed GVHD after transplantation of haemopoietic stem cells and seven after donor lymphocyte infusion.

Most patients had grade 3 or 4 GVHD involving two or three organs, confirmed by biopsy in 36 patients (43 biopsies; table 2).

23 patients were treated at Karolinska University Hospital, Huddinge, Sweden (of whom, eight were previously reported^{21,22}), 14 at Leiden University Medical Center, Leiden, the Netherlands, eight at Ospedale San Martino or Gaslini Institute, Genova, Italy, seven at IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy, and three patients were from Royal Adelaide Hospital, Adelaide, Australia.

This phase II study was a prospective registration study to include all patients consecutively treated with mesenchymal stem cells in the participating centres of the European Group for Blood and Bone Marrow Transplantation mesenchymal stem cell consortium: the study was approved by the local ethics committees or institutional review boards of the participating institutions. Donors and patients, or their legal guardians, gave written informed consent.

Table 1. Characteristics of patients and treatment

	MEASURE
RECIPIENTS	
Recipient age, years	22 (0·5-64)
Male,female	34,21
Child,adult	25,30
DIAGNOSIS	
AML	8
ALL	12
CML	7
CLL	2
JMML	4
Multiple myeloma	2
Myeloproliferative disorder	1
Myelodysplastic syndrome	6
Lymphoma	1
Non malignant disorders	10
Solid tumor	2
Disease early , late*	21,24
DONOR and CELLS	
Female donor to male recipient	10
Male donor to female recipient	9
HLA-identical sibling	19
Unrelated A, B, DRβ1 identical	25
Mismatched donor	6
Unrelated CB (matched/mismatched)	3/2
HLA-identical sibling	19
Stem cell source (BM/PBSC/BM+PBSC/CB)	19/30/1/5
GvHD PROPHYLAXIS	
CsA	4
CsA + MTX	38
CsA + MMF	5
CsA + prednisolone	6
Other	2
ATG/ALG/Campath	30/1/5
DOSE	
Nucleated cell dose x 10 ⁶ /kg	5.9 (0·17-20·6)
CD34+ cell dose x 10 ⁶ /kg	8 (0·15-28)
CYTOMEGALOVIRUS SEROLOGY	
Negative in donor and recipient	14
Positive in donor and recipient	20
Positive in donor or recipient	21

Abbreviations: ALG = Antilymphocyte globulin; ALL = Acute lymphoblastic leukaemia; AML = Acute myeloid leukaemia; ATG = Antithymocyte globulin; BM = Bone Marrow; CB = Cord blood; CML = Chronic myeloid leukaemia; CLL = chronic lymphocytic leukemia; JMML = Juvenile myelomonocytic leukaemia; CsA = Cyclosporine; HLA = Human leukocyte antigen; MTX = Methotrexate; MMF = Mycophenolate mofetil; PBSC = Peripheral blood stem cell.

* Early: non-malignant disease, 1st complete remission, 1st chronic phase; late: beyond these stages at time of transplant

Table 2. GVHD grade and organ involvement

	NUMBER OF PATIENTS
GvHD SEVERITY	
GvHD II/III/IV	5/25/25
ORGAN INVOLVEMENT	
Only one organ S/G/L	3/6/1
Two: G+S/G+L/L+S	15/7/4
Three: G+ S+L	19
GvHD CONFIRMED ON BIOPSY	
S/G/L	10/31/2
GvHD TREATMENT PRIOR TO MSC INFUSION	
Cyclosporine or tacrolimus	55 (53)*
Prednisolone $\geq$ 2 mg/kg	55 (55)*
MMF	10 (10)*
Daclizumab + Infliximab	4 (-)
Daclizumab alone	1 (3)*
Etanercept and PUVA	1 (-)
Extra-corporeal photochemotherapy	10 (8)*
Cyclophosphamide	3
ATG†	2
Rituximab	(1)*
PREVIOUS FAILED THERAPY	
First line	55
Second line	33
Third line	14
Fourth line	4
Fifth line	2

Abbreviations:

G = Gut; L = Liver; S = Skin;

ATG=antithymocyte globulin. MMF=mycophenolate mofetil. MSC=mesenchymal stem cell. PUVA=psoralen and ultraviolet-A irradiation.

*Numbers in brackets had immunosuppressive therapy at time of MSC infusion. †One Thymoglobulin, Genzyme, USA; one ATGAM, Upjohn, USA.

Procedures and definitions

Before treatment with haemopoietic stem cells, patients received either myeloablative or reduced-intensity conditioning (figure 1). Conditioning was myeloablative in 37 patients, who were given cyclophosphamide (120 mg per kg) combined mainly with busulfan (16 mg per kg), melphalan, or fractionated whole-body irradiation (≥ 12 Gy). Eighteen patients had low-intensity conditioning regimens with fludarabine phosphate combined with various cytotoxic drugs or 2 Gy whole-body irradiation.

As GVHD prophylaxis, most patients received ciclosporin combined with either four doses of intravenous methotrexate or mycophenolate mofetil. In patients receiving cord-blood transplantation, ciclosporin was combined with prednisolone. Recipients of haemopoietic stem cells from unrelated donors were treated with antithymocyte globulin, anti-lymphocyte globulin, or alemtuzumab. All patients had been treated with prednisolone 2 mg/kg per day or more as first-line immunosuppressive GVHD therapy and were resistant to this treatment.

Thirty three patients (60%) had failed two or more lines of immunosuppressive therapy before receiving mesenchymal stem cells (table 2). All patients continued treatment with steroids and a calcineurin inhibitor (n=53) or mycophenolate mofetil (n=2) at the time of infusion. Twenty patients also continued additional treatments while receiving stem cells (table 2).

We defined resistance to treatment as no improvement in overall grade of GVHD or progression. Acute GVHD was graded according to internationally accepted criteria by physicians at individual centres.²³ When possible, we confirmed diagnosis with biopsy of the involved organs.

We used best response to define the response to treatment: complete response was loss of all symptoms of acute GVHD; partial response was improvement of at least one grade; stable disease was no change in GVHD grade; and progressive disease was worsening of GVHD.

Patients were judged to have responded if they had either complete or partial response. Transplantation-related mortality included all deaths associated with transplantation of haemopoietic stem cells except those related to recurrence of underlying disease.

Laboratory methods

Table 3 shows the characteristics of the donors and grafts. Mesenchymal stem cells were derived from either HLA-identical stem-cell donors, haploidentical family donors, or unrelated HLA-mismatched donors.

Table 3. Mesenchymal-stem-cell donor and graft characteristics

	MEASURE
DONORS	
No. of donors	45
Donor sex (male/female)	25/20
Donor age	36 (1-67)
NUMBER OF INFUSIONS BY DONOR TYPE	
HLA-identical sibling	5
HLA-haploidentical donor	18
Unrelated HLA-mismatched donor	69
Volume of bone marrow harvested (ml)	60 (32-220)
Median MSC cell dose ($\times 10^6/\text{kg}$, range)	1.4 (0.4-9)
CULTURE PASSAGE AT MSC HARVEST	
Passage 1	14
Passage 2/2+3	42/7
Passage 3/3+4	23/2
Passage 4	4
NUMBER OF MSC INFUSIONS	
One	27
Two	22
Three	4
Four	1
Five	1

Data are number or median (min-max range). MSC=mesenchymal stem cell.

Clinical-grade mesenchymal stem cells were generated under good manufacturing practice conditions according to a common protocol devised by the European Group for Blood and Bone Marrow Transplantation developmental committee and approved by the medicinal-product agencies in the respective countries. Bone-marrow mononuclear cells were separated by density gradient centrifugation as previously described.^{7,8,10}

Washed cells were re-suspended in Dulbecco's modified Eagle's medium–low glucose (Life Technologies, Gaithersburg, MD, USA, or Paisley, UK) supplemented with 10% fetal bovine serum (National Veterinary Institute, Uppsala, Sweden, or HyClone, Logan, UT, USA) and plated at a density of 160 000 cells per cm². Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂ in 175 cm² flasks (Falcon, Franklin Lakes, New Jersey, USA, or Greiner Bio-One, Frickenhausen, Germany). When the cultures were near confluence (>80%), the cells were detached by treatment with trypsin and EDTA (Invitrogen, Grand Island, NY, USA, or Lonza Verviers, Verviers, Belgium) and re-plated at a density of 4000 cells per cm². When 2×10⁶ cells or more were obtained, they were harvested and either cryopreserved in 10% dimethyl sulphoxide (Research Industries, Salt Lake City, UT, USA, or Leiden University Medical Centre Pharmacy, Netherlands) or washed repeatedly and re-suspended to a final concentration of 2×10⁶ cells per ml in saline solution according to local guidelines.

Criteria for release of mesenchymal stem cells for clinical use included absence of visible clumps, spindle-shape morphology, absence of contamination by pathogens (as documented by aerobic and anaerobic cultures before release), viability greater than 95%, and immune phenotyping proving expression of CD73, CD90, and CD105 surface molecules (>90%) and absence of CD34, CD45, CD14, and CD3.²⁴ Cells were given as intravenous infusions. Cells for 11 infusions were harvested fresh from culture and given to the patients. In all other cases, frozen cells were thawed and infused.

Statistical analysis

Data were analysed as of last data collection in March, 2007. We estimated the probability of survival with the Kaplan-Meier method and significance of differences with the log-rank test (Mantel-Cox). Transplantation-related mortality was estimated non-parametrically. Patients were censored at the time of death or last follow-up.

Because relapse and non-relapse mortality are competing events, we estimated their incidence with a non-parametric estimator of cumulative incidence curves.^{25,26} All results were expressed as 2-year probability of survival or 1-year cumulative incidence (%) of transplantation-related mortality and 95% CI. We used Fisher's exact test to compare distribution of categorical variables. Analyses were done with the cmprsk package (developed by Gray, June, 2001), Splus 6.2 software, and Statistica software.

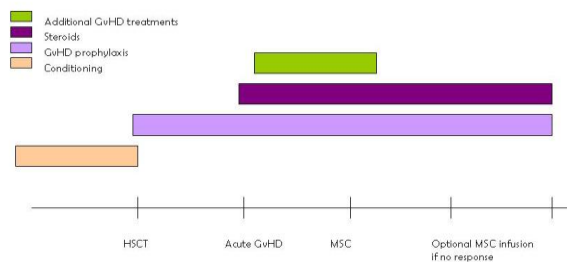
Role of the funding source

The sponsors of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

Results

Ninety-two infusions of mesenchymal stem cells were given; 27 patients had one infusion, while 28 had two or more (figure 1, table 3). Of the 28 patients treated with multiple infusions, 15 received cells derived from two or more donors. No patients had acute side-effects either during or after infusion; and none have had late side-effects so far. Median time from transplantation of haemopoietic stem cells to infusion of mesenchymal stem cells was 103 days (min–max range 27–533).

Figure 1. Scheme of mesenchymal stem-cell therapy



Just over half the patients had a complete response, and about a fifth had a partial response; most of the remainder had worsening disease (table 4). A greater proportion of children responded than did adults ($p=0.07$). Median time from first infusion of mesenchymal stem cells to complete response was 18 days (min–max range 3–63). Thirty patients achieving complete response were available for assessment at 6 weeks after infusion. Of these, four had died, 19 still had complete response, one had grade 1 and six had grade 2 acute GVHD.

Table 4. GvHD response and outcome

	CHILDREN	ADULTS	ALL PATIENTS
	n = 25	n = 30	n = 55
RESPONSE			
Complete	17 (64%)	13 (47%)	30 (55%)
Partial	4	5	9
Stable disease	2	1	3
Progressive disease	2	11	13
Overall response	21 (80%)	18 (60%)	39 (69%)
SURVIVAL *	13	8	21
CHRONIC GvHD			
Limited	2	0	2
Extensive	4	2	6

*At last data collection, March, 2007.

After one dose, 27 patients had complete response, two had partial response, and 26 did not respond. Of patients who responded to one dose, two were treated with HLA-identical mesenchymal stem cells, three with haploidentical cells, and 24 with third-party cells. The median dose given to patients who responded to the first dose was 1.4×10^6 cells per kg (min–max range 0.8×10^6 to 9×10^6 cells per kg), which was similar to that given to non-responding patients (1.4×10^6 cells per kg; 0.6×10^6 to 1.9×10^6 cells per kg). Six children and one adult who responded to the first infusion were given a second infusion to prevent GVHD recurrence when immunosuppressive drug treatment was reduced.

Seventeen of the patients who did not have sustained complete response after the first dose were treated with subsequent doses. Five patients had complete response

but received several doses of mesenchymal stem cells because of GVHD recurrence. Five patients had partial responses and were given multiple doses. One patient did not respond to 0.6×10^6 cells per kg but responded to a second dose of 2×10^6 cells per kg. Twelve patients did not respond despite several infusions.

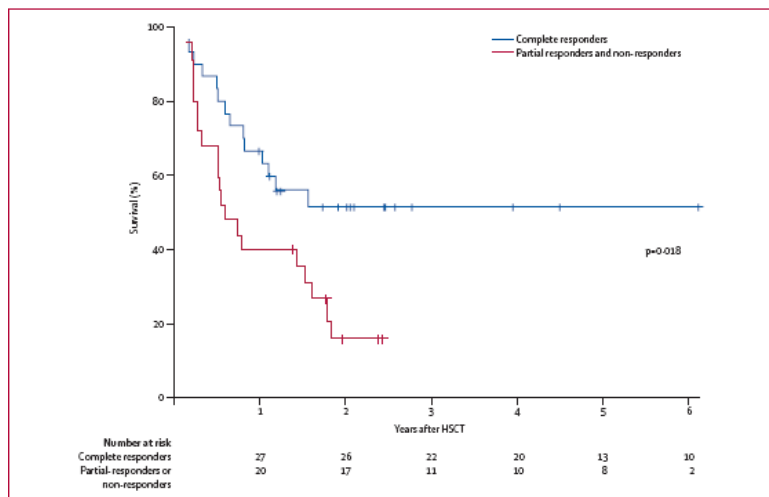
Twenty-two of 30 patients with grade 2 or 3 acute GVHD responded, compared with 17 of 25 with grade 4 disease ($p=0.77$). 28 (78%) of 36 patients with involvement of one or two organs had a response compared with 11 (58%) of 19 patients with involvement of three organs ($p=0.21$).

The age or HLA-match of the mesenchymal-stem-cell donor had no effect on response rate after infusion (data not shown). In patients receiving HLA-identical cells, two of five responded to first dose, compared with nine of 13 patients given haploidentical cells and 27 of 37 receiving unrelated cells. Median time from acute GVHD onset to treatment with mesenchymal stem cells was 25 days (min–max range 3–114) in complete responders, compared with 29 (3–116) days among all other patients ($p=0.48$). We noted variable responses when cells expanded from one donor were given to several recipients. There was no relation between the treatment given before infusion of mesenchymal stem cells and response.

Twenty-one patients were alive at the time of analysis (March, 2007) with a median follow-up of 16 months (min–max range 1.5–64 months) after infusion of mesenchymal stem cells (table 4). The estimated probability of survival 2 years after haematopoietic-stem-cell transplantation for the entire cohort of patients was 35% (95% CI 22–38%); in adults, 2-year survival was 26% (10–42%) compared with 45% (23–67%) in children ($p=0.06$). The 2-year probability of survival in complete responders (52%, 34–70%) was significantly better than that in the patients with partial or no response (16%, 0–32%, $p=0.018$; figure 2).

Figure 2. Survival from time of haemopoietic-stem-cell transplantation in patients given mesenchymal stem cells

Survival at the end of follow-up was 52% (95% CI 34–70%) for the 30 complete responders and 16% (0–32%) for the 25 partial responders or non-responders.

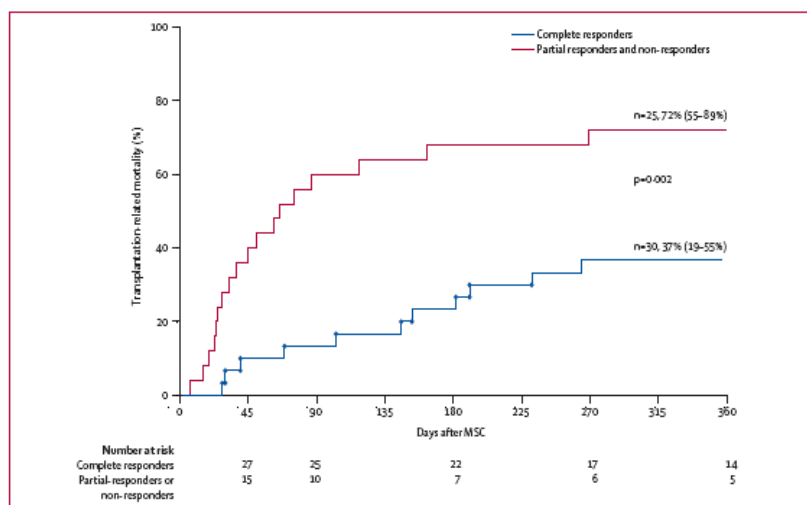


The 100-day transplantation-related mortality from time of infusion of mesenchymal stem cells was 13% (0–26%) for patients with complete response compared with 60% (41–79%) in other patients ($p=0.002$). Transplantation-related mortality 1 year after infusion was 37% (19–55%) in complete responders and 72% (55–89%) in patients with partial or no response ($p=0.002$; figure 3).

Of the survivors, six with complete response developed chronic GVHD, which was limited (mild) in four and extensive (severe or involving several organs) in two patients. Two patients with partial or no response also developed extensive GVHD. At last follow-up (March 1, 2007), eight with complete response had discontinued all immunosuppressive drugs.

Figure 3. 1-year cumulative incidence of transplantation related mortality from time of infusion of mesenchymal stem cells

Transplantation related mortality was 37% (95% CI 19–55%) among the complete responders and 72% (55–89%) among the partial responders or non-responders.



Three patients had recurrence of the original disease one with multiple myeloma, one with acute lymphoblastic leukaemia, and one with acute myeloid leukaemia. One patient with Pearson's disease developed acute myeloid leukaemia de novo originating in endogenous haemopoietic cells. All these patients died. Acute GVHD was the most common cause of death (18 patients), with or without concomitant infection. One patient died from chronic GVHD with obstructive bronchiolitis, and one patient died from multi-organ failure after severe haemorrhagic cystitis. Infections in patients who died to acute GVHD included aspergillosis (five), cytomegalovirus (four), and septicaemia caused by *Enterococci* (four), *Klebsiella* sp (one), *Escherichia coli* (one), and an unidentified pathogen (one). Three patients had infection with Epstein-Barr virus, one of whom developed post-transplantation lymphoproliferative disease related to the virus. Of the patients who responded to mesenchymal stem cells, nine died from infections. Pathogens included *Klebsiella* sp, *E coli*, *Pseudomonas*, adenovirus, and varicella zoster virus infection.

Discussion

Thirty-nine of 55 patients with steroid-resistant, severe, acute GVHD responded to treatment with mesenchymal stem cells. Survival in those with complete response was significantly higher and transplantation-related mortality after infusion was significantly lower than in people with partial or no response. The clinical course of the 13 patients with progressive disease despite treatment may represent the natural progression of severe GVHD resulting in death in most patients. No major toxicities were observed, and treatment with mesenchymal stem cells seemed to be safe.

Although various immunosuppressive treatments have been used, there is no established therapy for steroid-resistant, severe, acute GVHD.^{1,3-5} The outcome for patients who do not respond to corticosteroids is therefore poor, and survival at 2 years is about 10%.^{4,5,27} In this study, survival in people who responded to mesenchymal stem cells was higher (52%) than previously described for patients with a similar grade of acute GVHD.

The response in patients with grade 2 and 3 acute GVHD was similar to that in patients with grade 4. More patients are needed to investigate responses to the treatment in various subgroups. For example, the difference in response rate between children and adults with acute GVHD of comparable severity was not significant. Because of the low number of patients, the statistical power was insufficient to detect significance for differences the size of that between response rates in adults and in children (estimated power 0.58). At least 80 patients would be needed to detect such a difference with *p* equal to or less than 0.05.

At present, little is known about mechanisms of suppression of GVHD by mesenchymal stem cells. In-vitro, these cells have various effects on immune cells, including T cells, antigen-presenting cells, natural-killer cells, and B cells.²⁸⁻³⁰ The biological relevance of these in-vitro findings is unknown. These cells might suppress donor-T-cell responses to recipient alloantigen. This suppression is probably induced by several mechanisms, including release of soluble factors, induction of regulatory T cells, and repair of damaged target organs.²⁸⁻³⁰

Immunological studies specifically addressing this issue are needed to improve our understanding the treatment of acute GVHD.

Our study was designed to assess safety and efficacy of mesenchymal stem cells for refractory acute GVHD. It was not designed to identify the best dose of mesenchymal stem cells. At present we can note only that, on the one hand, clinically meaningful responses were obtained after infusing a dose as low as 0.8×10^6 cells per kg, whereas on the other, doses as high as 1.9×10^6 cells per kg were not successful in all cases. Thus any conclusion as to relevant dose is premature.

In more than half of patients, a single dose produced a response, whereas in a few patients with partial response or with recurrence of GVHD, several doses were needed to induce a lasting response. The response was not restricted to single organs: skin, gastrointestinal tract, and liver GVHD showed similar responses. How long mesenchymal stem cells survive after injection and to what extent they are able to target tissues are unknown.²²

Thus, whether GVHD multi-organ response to infusion occurs because the cells reach the lymph nodes and inhibit the immunological response that gives rise to GVHD is unclear; they might alternatively or additionally target various organs associated with a tissue-healing effect. Further tracking studies with labelled cells are needed to address these issues.

Third-party mesenchymal stem cells were as effective as HLA-identical or haploidentical cells. This finding has practical implications and suggests that third-party cells can be prepared and stored frozen to be used for GVHD therapy. Little is known about whether HLA disparity determines the response to treatment and survival of cells after systemic administration. Most data derived from animals indicate short survival of mesenchymal stem cells after injection in vivo. Clinical benefit might not require sustained engraftment of many cells but could possibly result from production of growth factors or temporary immunosuppression. With the poor health of patients receiving mesenchymal stem cells, some deaths were expected, despite control of GVHD. Infections are common in severe GVHD because

of the state of immunodeficiency that characterizes these patients. Whether treatment can further aggravate immune incompetence or not is unclear. To properly answer the question of whether treatment increases the risk of infections requires a controlled randomized study.

The survival rate for patients with complete response was significantly better than that for those with partial or no response, suggesting that beneficial effects of mesenchymal stem cells are not overridden by a high number of severe infections. This study was a multicentre collaboration between centres adopting a common protocol for the expansion of mesenchymal stem cells. Because we did not find significant differences in the response rate to treatment in patients in the different participating centres, we conclude that use of a common protocol affords reproducibility of results. Mesenchymal stem cells derived from bone marrow might be a safe and effective treatment for patients with severe, acute GVHD who do not respond to corticosteroids and other immunosuppressive therapies. The number of infusions needed, the best dose of cells in each infusion, and the possible interactions of cells with other drugs for acute GVHD require further investigation. Although the grim outlook for patients who do not respond to treatment suggests that improved survival rate is probably related to infusions of mesenchymal stem cells, randomised clinical studies are needed to compare this treatment with more conventional approaches.

In summary, this study shows that more than half of the patients with steroid-refractory acute GVHD responded to treatment with mesenchymal stem cells. Whether the cell donor was HLA matched or unmatched did not affect the success of treatment. Just over half of patients with a complete response were alive at 2 years.

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