Biol Blood Marrow Transplant 20 (2014) 375–381

Treatment of Graft versus Host Disease with Mesenchymal Stromal Cells: A Phase I Study on 40 Adult and Pediatric Patients

Martino Introna [1,*], Giovanna Lucchini [2], Erica Dander [3], Stefania Galimberti [4], Attilio Rovelli [2], Adriana Balduzzi [2], Daniela Longoni [2], Fabio Pavan [2], Francesca Masciocchi [2], Alessandra Algarotti [1], Caterina Micò [1], Anna Grassi [1], Sara Deola [5], Irene Cavattoni [5], Giuseppe Gaipa [2], Daniela Belotti [2], Paolo Perseghin [6], Matteo Parma [7], Enrico Pogliani [7], Josee Golay [1], Olga Pedrini [1], Chiara Capelli [1], Sergio Cortelazzo [5], Giovanna D'Amico [3], Andrea Biondi [2], Alessandro Rambaldi [1], Ettore Biagi [2]

[1] *Division of Hematology and Laboratory of Cell Therapy "G. Lanzani", Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy*
[2] *HSCT Pediatric Unit and Laboratory of Cell Therapy "S. Verri", San Gerardo Hospital, Monza, Italy*
[3] *HSCT Pediatric Unit, "M. Tettamanti" Research Centre, San Gerardo Hospital, University of Milano-Bicocca, Monza, Italy*
[4] *Department of Health Sciences, Center of Biostatistics for Clinical Epidemiology, University of Milan-Bicocca, Monza, Italy*
[5] *Divisione di Ematologia e TMO, Ospedale Generale di Bolzano, Bolzano, Italy*
[6] *Immunotransfusion Unit, San Gerardo Hospital, Monza, Italy*
[7] *HSCT Adult Unit, San Gerardo Hospital, Monza, Italy*

Article history:
Received 30 August 2013
Accepted 30 November 2013

Key Words:
Steroid refractory graft-versus-host disease (GVHD)
Mesenchymal stromal cells
Cell therapy
Immunosuppressive treatment
Hematopoietic stem cell transplantation (HSCT)

A B S T R A C T

This phase I multicenter study was aimed at assessing the feasibility and safety of intravenous administration of third party bone marrow–derived mesenchymal stromal cells (MSC) expanded in platelet lysate in 40 patients (15 children and 25 adults), experiencing steroid-resistant grade II to IV graft-versus-host disease (GVHD). Patients received a median of 3 MSC infusions after having failed conventional immunosuppressive therapy. A median cell dose of 1.5×10^6/kg per infusion was administered. No acute toxicity was reported. Overall, 86 adverse events and serious adverse events were reported in the study, most of which (72.1%) were of infectious nature. Overall response rate, measured at 28 days after the last MSC injection, was 67.5%, with 27.5% complete response. The latter was significantly more frequent in patients exhibiting grade II GVHD as compared with higher grades (61.5% versus 11.1%, $P = .002$) and was borderline significant in children as compared with adults (46.7 versus 16.0%, $P = .065$). Overall survival at 1 and 2 years from the first MSC administration was 50.0% and 38.6%, with a median survival time of 1.1 years. In conclusion, MSC can be safely administered on top of conventional immunosuppression for steroid resistant GVHD treatment. Eudract Number 2008-007869-23, NCT01764100.

INTRODUCTION

Graft-versus-host disease (GVHD) is a severe and potentially life-threatening complication after hematopoietic stem cell transplantation (HSCT) [1]. Around 50% of patients exhibiting GVHD are expected not to benefit from conventional treatment with steroids [2]. Although a wide variety of second-line treatments for these patients are available, the prognosis for these patients remains dismal because of higher risk of infectious complications, immunosuppression-mediated toxicity, and often incomplete GVHD remission [3,4]. The development of a better treatment strategy for steroid resistant GVHD, therefore, represents a key factor to achieve improved long-term survival for HSCT recipients. Mesenchymal stromal cells (MSCs), a pluripotent cell population [5], are endowed with broad immunosuppressive activity [6] and have been reported to be effective for GVHD treatment [7-9]. The present prospective, multicenter, phase I study was aimed at assessing the feasibility and safety of platelet lysate (PL)–expanded, third party, bone marrow (BM)–derived MSCs administration for the treatment of steroid resistant GVHD in adult and pediatric patients. Secondary aims were to assess the efficacy of such an approach on top of conventional immunosuppression, the response to treatment, and the overall survival (OS) in the whole cohort.

MATERIALS AND METHODS

Patients

Patients exhibiting acute or chronic, steroid-resistant or -dependent, grade II to IV GVHD were eligible for the study. Histopathology to confirm clinical diagnosis of GVHD was encouraged but not required to enter the study. GVHD was graded according to the Seattle-Glucksberg modified criteria for acute forms [10] and to the National Institutes of Health consensus criteria for chronic cases [11]. In the acute GVHD setting, steroid resistance was defined as lack of clinical improvement after 5 days of

Financial disclosure: See Acknowledgments on page 380.

* Correspondence and reprint requests: Martino Introna, MD, Laboratory of Cell Therapy "Lanzani", c/o Presido Matteo Rota, Ospedale Papa Giovanni XXIII, Via Garibaldi 11/13, 24122 Bergamo, Italy.
E-mail address: mintrona@hpg23.it (M. Introna).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.11.033

treatment (for instance, methylprednisolone 2 mg/kg daily) or GVHD progression of at least 1 grade within 3 days from steroid onset. For chronic GVHD, steroid resistance was defined as absence of clinical improvement after 30 days of treatment and steroid dependence as more than 2 episodes of GVHD reflaring after steroid tapering (less than 1 mg/kg daily).

This protocol has been authorized by the National Authorities for Phase I trials (Istituto Superiore di Sanità, ISS) with protocol number 70524 (08)-PRE.21-959 (Eudract Number 2008-007869-23) and approved by local ethical committees or institutional review boards of the participating centers. Donors and patients, or their legal guardians, gave written informed consent. The study was registered at the official NIH site www.clinicaltrials.gov under the number NCT01764100.

MSC Production

MSCs were derived from BM of unrelated, third party, HLA-mismatched donors. MSCs were produced starting from the washouts of discarded BM bags and expanded with PL, as already described [12]. Briefly, sealed bags and filters from BM harvests were washed with sterile solution to recover cells. MSCs were isolated and ex vivo expanded in the presence of Modified Eagles Medium supplemented with 5% human PL, according to identical standard operative procedures at the 2 production centers. The 2 MSC producing cell factories (Laboratorio di Terapia Cellulare "G. Lanzani" in Bergamo and Laboratorio di Terapia Cellulare "S. Verri" in Monza) had received formal approval by Agenzia Italiana del Farmaco (AIFA, Rome, Italy) to operate according to European good manufacturing practice regulations for the production of sterile, injectable drugs of small volumes. These 2 cell factories provided MSCs for all centers taking part in the study. The delivery system was organized in such a way that each participating center could receive MSCs within 48 to 72 hours from request.

Before distribution, the MSC bags had to satisfy all the release criteria, including absence of gram positive and negative bacteria, absence of fungi and mycoplasma, endotoxin level below < 5 EU/kg, absence of spontaneous growth in semisolid media, absence of cytogenetic lesions in more than 20 metaphases, as well as $> 80\%$ viability and $> 70\%$ positivity for CD73, CD90, and CD105, and $< 10\%$ contamination by CD14, CD34, and CD45 hematopoietic cells. The final product fulfilled the international recognized criteria to be declared "bona fide" MSC [5].

MSC Administration

MSCs were injected intravenously through a central line. The cells were thawed immediately before infusion, paracetamol and antihistamine were pre-emptively administered to avoid acute reactions, and patients were monitored for 2 hours after receiving MSCs. Hence, MSC administration did not require admittance to the transplantation unit; thus, improving the patients quality of life and reducing the treatment costs. MSCs were infused on top of the ongoing immune suppression therapy given for GVHD.

A minimum of 2 MSC infusions was recommended with about 5 to 7 days of interval between them. Each MSC infusion aimed at reaching $1 \pm .5 \times 10^6$ cells/kg of recipient body weight. Further MSC administrations could be provided upon request of the treating physician. The tapering or increase of conventional immunosuppressive therapy after MSC administration was also left to the clinical judgment of the treating teams.

Response to treatment was evaluated on day +28 after the last MSC infusion or at date of death if earlier than day +28. Complete response (CR) was defined as absence of signs and symptoms of GVHD, partial response (PR) as GVHD decrease of at least 1 grade as compared with day 0, and no response as no change in GVHD scoring. Patients were considered to have responded to treatment if exhibiting either PR or CR. Transplantation-related mortality (TRM) included all deaths associated with HSCT except those related to original disease recurrence.

MSC safety was assessed by monitoring the infusion tolerability, by analyzing complete blood count and biochemistry for the entire week after MSC administration, and by recording adverse events (AE) and serious adverse events (SAE) occurring up to 30 days after the last MSC infusion through specific case report forms. AE and SAE were graded according to the Common Terminology Criteria for Adverse Events, Version 4.

Statistical Analysis

The comparison between treatment response rates in different groups was performed by means of the Fisher's exact test. Results are expressed as proportion of success, and the corresponding 95% confidence intervals (CI) were calculated using the Wilson method. The incidence of GVHD recurrence in patients with response was estimated from the date of response assessment, accounting for death as competing event.

Survival was defined as the time from the date of first MSC infusion to death from any cause. Survival curves were estimated with the Kaplan-Meier method, whereas the log-rank test was applied to compare the survival of different groups. The incidence of TRM was also estimated, considering deaths due to disease progression as competing events. Follow-up was updated as of October 2013.

The analyses were carried out using SAS 9.3, all the tests were performed 2-sided with a significance level of .05.

Table 1
Patients Characteristics

Patients Characteristics	Adults n = 25	Children n = 15	Overall n = 40
Age at MSC, median (range), yr	40.5 (19-65)	4.6 (1-18)	27.8 (1-65)
Sex			
Male	16 (64)	11 (73)	27 (68)
Female	9 (36)	4 (27)	13 (33)
Disease type			
Malignant	23 (92)	13 (87)	36 (90)
Nonmalignant	2 (8)	2 (13)	4 (10)
Remission state at SCT*			
Complete remission	12 (52)	9 (69)	21 (58)
Partial nonremission	11 (48)	4 (31)	15 (42)
Conditioning regimen			
Reduced intensity	12 (60)	5 (33)	17 (43)
Fully myeloablative	8 (40)	10 (67)	18 (45)
Donors			
MFD	7 (28)	1 (7)	8 (20)
MUD	11 (44)	10 (67)	21 (53)
MMD	7 (28)	4 (27)	11 (28)
Stem cells source			
PB	18 (72)	2 (13)	20 (50)
BM	5 (20)	10 (67)	15 (38)
CB	2 (8)	3 (20)	5 (12)
GVHD prophylaxis			
CSA+MTX	4 (16)	0 (0)	4 (10)
CSA+MTX+ATG	12 (48)	10 (67)	22 (55)
Other	9 (36)	5 (33)	14 (35)
GVHD grading			
Acute	19 (76)	12 (80)	31 (77)
Grade II	2 (8)	9 (60)	11 (27)
Grade III-IV	17 (68)	3 (20)	20 (50)
Chronic (severe)	2 (8)	1 (7)	3 (8)
Overlap	4 (16)	2 (13)	6 (15)
Organ involvement			
Single organ	9 (36)	7 (47)	16 (40)
Multi organ	16 (64)	8 (53)	24 (60)

MSC indicates mesenchymal stromal cells; SCT, stem cell transplantation; MFD, matched familiar donor; MUD, matched unrelated donor; MMD, mismatched donor; PB, peripheral blood; BM, bone marrow; CB, cord blood; GVHD, graft-versus-host disease; CSA, cyclosporine A; MTX, methotrexate; ATG, antithymocyte globulin.
Data presented are n (%) unless otherwise indicated.
* For malignancies only.

RESULTS

Patients' Characteristics

Between August 2009 and April 2012, 40 patients (25 adults, 15 children) were enrolled and treated according to the present protocol in 5 Italian centers: 17 adults at the USC Hematology of the "Azienda Ospedaliera Papa Giovanni XXIII", Bergamo, 14 children at the Pediatric Department of the "Ospedale San Gerardo dei Tintori" in Monza, 6 at the Adult Hematology Division of the "Ospedale San Gerardo dei Tintori" in Monza, 2 adults at the Hematology Unit of the "Ospedale Generale" in Bolzano, and 1 child at the Pediatric Department of "Ospedale Regionale" in Padova. Only 1 patient received MSC for an acute GVHD (aGVHD) defined as progressive in the first 3 days of steroid treatment, 35 patients exhibited GVHD not responding to steroid treatment after at least 5 days of administration, and 4 patients were defined as affected by steroid-dependent GVHD.

Patients' characteristics as well as transplantation and GVHD details are summarized in Table 1. Of note, the incidence of acute mild GVHD (ie, grade II) at study entry was

Table 2
Safety of MSC Treatment in Adults and Children

Characteristic	Adults n = 25	Children n = 15	Overall n = 40
Patients with			
No events	3 (12.0)	3 (20.0)	6 (15.0)
At least an AE	13 (52.0)	5 (33.3)	18 (45.0)
At least a SAE	9 (36.0)	7 (47.7)	16 (40.0)
Total no. of events	51	35	86
Type			
AE	38 (74.5)	26 (74.3)	64 (74.4)
SAE	13 (25.5)	9 (25.7)	22 (25.6)
Grade			
II	24 (47.0)	22 (62.9)	46 (53.5)
III-IV	27 (53.0)	13 (37.1)	40 (46.5)
Nature			
Infections	37 (72.5)	25 (71.4)	62 (72.1)
Other	14 (27.5)	10 (28.6)	24 (27.9)

MSC indicates mesenchymal stromal cells; AE, adverse event; SAE, serious adverse event.
Data presented are n (%).
Events are graded according to the Common Terminology Criteria for Adverse Events, Version 4.0.

significantly higher in children as compared with adults (60.0% versus 8.0%, $P = .0001$), the latter exhibiting an acute grade III to IV GVHD in 68.0% of the cases. The distribution between single organ or multiple organ GVHD involvement was similar between children and adults; multiple organ presentation being predominant overall (60.0% versus 40.0% single organ presentation, $P = .61$). In the pediatric population, skin was the most commonly affected organ (14 of 15 patients), whereas adults mostly exhibited gut involvement either alone (7 of 25 patients) or in combination with skin or liver involvement (18 of 25 patients) (Table S1).

All patients underwent steroid treatment as first line of immunosuppression against GVHD. Children received MSC as second line of treatment directly after steroids more often than adults (80.0% versus 20.0%, $P = .0003$). GVHD in adults was resistant to 2 to 6 lines of immunosuppression before receiving MSC in 80.0% of the cases (data not shown).

Feasibility and Toxicity

A total of 158 MSC infusions were administered to 40 patients; each patient receiving a median number of 3 infusions (ranges, 2 to 7 for children and 2 to 11 for adults). Patients received a median of 1.5×10^6/kg (range, .8 to 3.1) MSCs per dose, with no difference between adults and children, thus the target cell dose was achieved in all patients (data not shown). Median time of MSC administration was 13 days (range, 4 to 277) and 35 (range, 5 to 1535) from GVHD diagnosis, for children and adults respectively, and median treatment duration was 14 days for adults (range, 3 to 206) and 15 for children (range, 4 to 28), respectively (data not shown). No side effects/adverse reactions were documented during and immediately after MSC infusion.

Complete blood counts as well as standard biochemistry (serum creatinine, bilirubin, aspartate aminotransferase, albumin, lactate dehydrogenase and glucose), as analyzed for 7 consecutive days after MSC infusion for the first 32 enrolled patients, did not show relevant changes (Table S2).

Thirty-four out of 40 patients (22 adults and 12 children) showed a total of 64 reported AE and 22 SAE after 74.7 person-months of observation, with a rate of AE and SAE of .9 and .3 per person-month, as described in Table 2 and detailed in Table S3. The seriousness for adverse events was assigned in 13 cases to life-threatening situations, and in 9 cases, to the need for hospitalization. Of all reported events, 72.1% were due to viral, bacterial, or fungal infections, followed by multiple organ failures due to GVHD progression (6.9%) (Table S2). AE and SAE were equally distributed among patients independently from age and GVHD characteristics at study entry. Of the reported events, 46.5% were grades III to IV in severity. Seventy percent of all events resolved without sequelae.

Response to MSC Administration

Overall, 27 out of 40 patients (67.5%) showed a GVHD response 28 days after the last MSC infusion: 11 (27.5%) exhibiting a CR (4 adults and 7 children) and 16 (40.0%) exhibiting a PR (13 adults and 3 children) to treatment (Table 3). No response was observed in 3 cases of chronic classic severe GVHD, whereas 2 CR were observed in 2 children (grade II) and 2 PR were observed in 4 adults (grade III) with overlapping syndrome (Table 3).

The results of the univariate analysis evaluating the association between patient characteristics at study entry and treatment response, assessed both as CR and CR+PR, are illustrated in Table 4. Grade II GVHD, as compared with higher grading, showed a statistically significant better chance of achieving a CR (61.5% versus 11.1%, $P = .002$). Overall response rates did not differ between adults and children (68.0% versus 66.7%, $P = 1.00$), even if the rate of CR was higher in children as compared with adults (46.7% versus 16.0%, $P = .065$), probably due to the different severity of the clinical presentations in these 2 groups.

In addition, we found that patients responding to MSC were not different in terms of underlying disease (malignant 66.7% versus nonmalignant 75%, $P = .60$), conditioning regimen (myeloablative 66.7% versus reduced-intensity 69.2%, $P = 1.00$), donor type (matched 72.4% versus mismatched 60%, $P = .67$), and stem cell source (peripheral blood

Table 3
Treatment Response of Adults and Children According to GVHD Type and Grading

GVHD	Adults		Children		Overall	
	CR	CR+PR	CR	CR+PR	CR	CR+PR
Acute						
II	2 of 2 (100)	2 of 2 (100)	4 of 9 (44.4)	7 of 9 (77.7)	6 of 11 (54.5)	9 of 11 (81.2)
III-IV	2 of 17 (11.8)	13 of 17 (76.5)	1 of 3 (33.3)	1 of 3 (33.3)	3 of 20 (15)	14 of 20 (70)
Chronic severe*	0 of 2 (0)	0 of 2 (0)	0 of 1 (0)	0 of 1 (0)	0 of 3 (0)	0 of 3 (0)
Overlap†	0 of 4 (0)	2 of 4 (50)	2 of 2 (100)	2 of 2 (100)	2 of 6 (33.3)	4 of 6 (66.7)
Total	4 of 25 (16.0)	17 of 25 (68.0)	7 of 15 (46.7)	10 of 15 (66.7)	11 of 40 (27.5)	27 of 40 (67.5)

GVHD indicates graft-versus-host disease; CR, complete response; PR, partial response.
Data presented are n (%).
* The 3 chronic severe GVHD were all grade III.
† The 6 overlap were grade II (2 children) and grade III (4 adults).

Table 4
Univariate Analysis of Treatment Response

	CR n (%)	95% CI	*P* Value	CR+PR n (%)	95% CI	*P* Value
Overall	11 of 40 (27.5)	16.1-42.8		27 of 40 (67.5)	52.0-79.9	
MSC doses			1.000			.305
2-3	7 of 24 (29.2)	14.9-49.2		18 of 24 (75.0)	55.1-88.0	
>3	4 of 16 (25.0)	10.2-49.5		9 of 16 (56.3)	33.2-76.9	
Patient type			.065			1.000
Children	7 of 15 (46.7)	24.8-69.9		10 of 15 (66.7)	41.7-84.8	
Adults	4 of 25 (16.0)	6.4-34.6		17 of 25 (68.0)	48.4-82.8	
GVHD type			1.000			.120
Acute	9 of 31 (29.0)	16.1-46.6		23 of 31 (74.2)	56.8-86.3	
Chronic-overlap	2 of 9 (22.2)	6.3-54.7		4 of 9 (44.4)	18.9-73.3	
GVHD grade			.002			.157
II	8 of 13 (61.5)	35.5-82.3		11 of 13 (84.6)	57.8-95.7	
III-IV	3 of 27 (11.1)	3.9-28.1		16 of 27 (59.3)	40.7-75.5	
No. organs involved			.473			.177
1	3 of 16 (18.8)	6.6-43.0		13 of 16 (81.2)	57.0-93.4	
≥2	8 of 24 (33.3)	18.0-53.3		14 of 24 (58.3)	38.8-75.5	
IS Lines			.153			.333
MSC as second line	7 of 17 (41.2)	21.6-64.0		13 of 17 (76.5)	52.7-90.4	
MSC > second line	4 of 23 (17.4)	7.0-37.1		14 of 23 (60.9)	40.8-77.8	
Days from GVHD onset to MSC*			.709			.157
<30 d	7 of 23 (30.4)	15.6-50.9		18 of 23 (78.3)	58.1-90.3	
>30 d	3 of 15 (20)	7.0-45.2		8 of 15 (53.3)	30.1-75.2	

CR indicates complete response; PR, partial response; MSC, mesenchymal stem cell; IS, immunosuppression; CI; confidence interval; GVHD, graft-versus-host disease.

* Two patients have date of GVHD onset missing.

55% versus bone marrow 80%, $P = .2$). Similarly, the number of previous immune suppression treatments and the number of MSC infusions did not influence the overall and CR rate in our population. A trend was seen in relation to timing between GVHD onset and MSC administration; overall response being more favorable for patients receiving MSC within 30 days from GVHD onset (78.3% versus 53.3%, $P = .157$) (data not shown).

The subsequent analysis on the 27 responders (Supplementary Table 1) indicates that most of the overall and complete responders were found in the subgroups of patients showing isolated skin involvement (CR+PR, 87.5%), or isolated gut involvement (CR+PR, 85.7%), whereas the liver involvement was linked to a worst percentage of complete (21.4%) and partial (50%) responses.

The ongoing immune suppression treatments of these patients, on top of which MSCs were given, are detailed in Table 5. In particular, in 14 patients, the dose of steroids remained unchanged and in 10 it was tapered, whereas in 6 patients other drugs were added. In 3 patients, all immune suppressants were stopped before response evaluation.

Finally, we investigated the evolution of GVHD in the 27 responding patients. We found that chronic GVHD developed in 11 (7 PR and 4 CR, 8 adults and 3 children) of the 27 responders. Taking into account death as a competing event, the cumulative incidence of chronic GVHD, was 37% at six months with only 1 event developing after 1 year from response (Figure 1).

Survival

With a median follow-up of 2.8 years from the first MSC infusion and of 3.7 from SCT, the 1 and 2-year overall survival rates of this cohort of patients were 50 and 38%, respectively, whereas the median survival time was of 1.1 years. Nine patients died before reaching the time of clinical response assessment after a median of 28 days from the first MSC. At last follow-up, 16 out of 40 patients were alive with no evidence of GVHD in 12 of them. Of the 2 components of mortality, TRM was the most relevant, as shown in Figure 2A. Eighteen patients died from TRM: GVHD itself (n = 11), infections (n = 5), organ failure (n = 1), and hemorrhage (n = 1). Six patients died after relapsing from their original malignant disease. A better survival was seen in patients with grade II GVHD ($P = .0482$) and a trend for a better outcome was seen in patients younger than 18 years ($P = .2035$) (Figure 2B,C).

DISCUSSION

The present prospective study underlines the feasibility of a cell therapy–based treatment of pediatric and adult patients with steroid resistant GVHD using third party, PL-expanded BM-derived MSCs. The study was conducted within the context of academic institutions to provide direct evidence of at least a similar clinical effect as compared to what was reported from 2004 onward by other groups of investigators using MSCs expanded using fetal calf serum [9,13-17]. The primary objective of our study was to assess the safety of PL-MSC infusions given on the top of conventional immunosuppression therapy. Only 1 report on a very small group of patients has been published so far on PL-expanded BM-derived MSCs [18] and, therefore, our study is the first extended phase I toxicity study performed with cells expanded in the absence of animal-derived reagents. As in previous reports with MSCs [19], in our study no immediate or late clinical or laboratory relevant toxic events could be attributed to MSC infusions. Therefore, we can confirm that PL-MSCs can be administered safely in both adults and children.

The relationship between MSC infusion and infections was a matter of concern since von Bahr et al. reported an increased number of infectious complications in the first 2 years after MSC administration, with an infection related mortality as high as 54% [20]. In our study, we also report a high rate of infectious episodes, but this is likely not to be different when compared with an historical group of control patients treated with conventional immunosuppressive therapy [21]. Remarkably, in our experience, the infection-related mortality affected only 10% of our patients.

Table 5
Details for Concomitant Treatments in Responder Patients

UPN	GVHD Grading at Enrollment	Ongoing Therapy at Enrollment	Ongoing Therapy at Response Evaluation	Response (GVHD Grading)
101	II	Steroid	Steroid tapering	Complete (0)
102	II	Steroid	Mycophenolate mofetil Steroid tapering	Complete (0)
103	II	Steroid	Steroid tapering	Partial (I)
105	II	Steroid	Steroid tapering	Partial (I)
106	III	Steroid Mycophenolate mofetil	Steroid tapering Mycophenolate mofetil Etanercept	Partial (II)
107	II	Steroid	Steroid tapering	Complete (0)
108	III	Steroid Etanercept	Steroid unchanged	Partial (II)
109	II	Steroid	Steroid Mycophenolate mofetil	Partial (I)
112	II	Steroid	Steroid tapering	Complete (0)
113	II	Steroid	Steroid tapering Mycophenolate mofetil	Complete (0)
114	II	Steroid	Steroid tapering	Complete (0)
116	III	Steroid Etanercept	Steroid tapering	Complete (0)
201	III	Steroid Etanercept	Steroid unchanged Etanercept Mycophenolate mofetil	Partial (II)
202	III	Steroid	Steroid unchanged	Partial (II)
203	III	Steroid Mycophenolate mofetil	Steroid unchanged	Partial (II)
204	II	Steroid Mycophenolate mofetil	Steroid unchanged Mycophenolate mofetil	Complete (0)
306	IV	Steroid	None	Partial (III)
307	III	Steroid	Steroid unchanged Pentostatin	Partial (II)
308	III	Steroid Pentostatin	None	Partial (II)
310	III	Steroid Pentostatin	Steroid unchanged	Partial (II)
312	III	Steroid Pentostatin	Steroid unchanged	Partial (II)
313	IV	Steroid Pentostatin	Steroid unchanged	Partial (I)
314	III	Steroid	Steroid unchanged	Complete (0)
315	II	Steroid Cyclosporine A	None	Complete (0)
316	III	Steroid Pentostatin	Steroid unchanged	Complete (0)
317	III	Steroid Pentostatin	Steroid unchanged	Partial (II)
501	IV	Steroid Pentostatin	Steroid unchanged	Partial (III)

GVHD indicates graft-versus-host disease; UPN, unique patient number.

Therefore, we believe that such a pattern of infections could be observed in steroid refractory aGVHD with any different second line treatment, as recently confirmed by Alousi et al., who showed a range from 44% to 62% of severe infectious

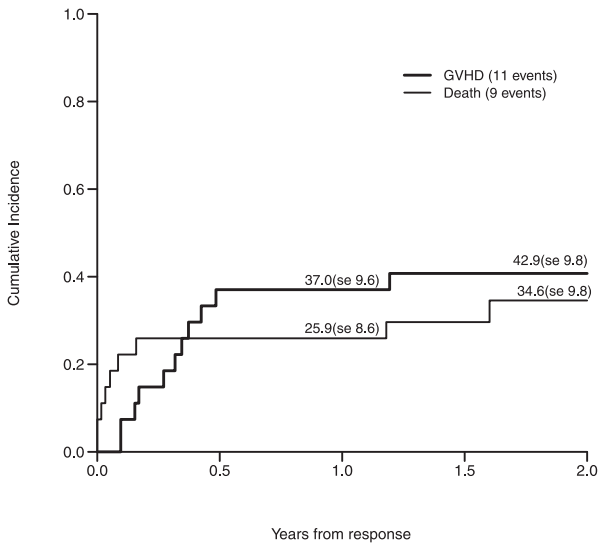

Figure 1. Cumulative incidence of GVHD and death post response, for the 27 patients who responded to MSC. The estimates are reported at 1 and 2 years from response assessment with standard errors (se).

complications observed after 4 different second lines of treatment for aGVHD [22]. Another possible drawback of an excessive immunosuppression might have been an increased rate of leukemia relapse [23]. In contrast to this possibility, in our study, the underlying disease recurred only in 6 patients, who were all at high risk of disease relapse because of poor remission status at transplantation. Finally, no evidence of heterotopic tissue formation was documented, confirming what observed in all the other clinical reports with MSCs [7,8,24,25].

Evaluation of efficacy of PL-MSC was the secondary objective of this study, but it is worth mentioning that, in more than 60% of our patients, a PR or CR was observed. Although other studies reported a higher CR rate, we provide evidence that 38% of our patients were alive at 2 years from the first MSC administration. These encouraging results obtained in mostly adult patients and with an appropriate prolonged period of follow-up are indeed at least comparable with those reported by the Swedish group [26].

When comparing responders (CR + PR) versus non-responders, a significant difference was found in measured plasma levels of IL2R-alpha (lower in responders) (Figure S1), suggesting the possible role of this test as a marker for MSC response evaluation, as already previously investigated by our group [27]. This, however, still remains to be validated in future prospective studies.

Beyond all these positive results, our study highlights some possible limits of this therapeutic approach. In our

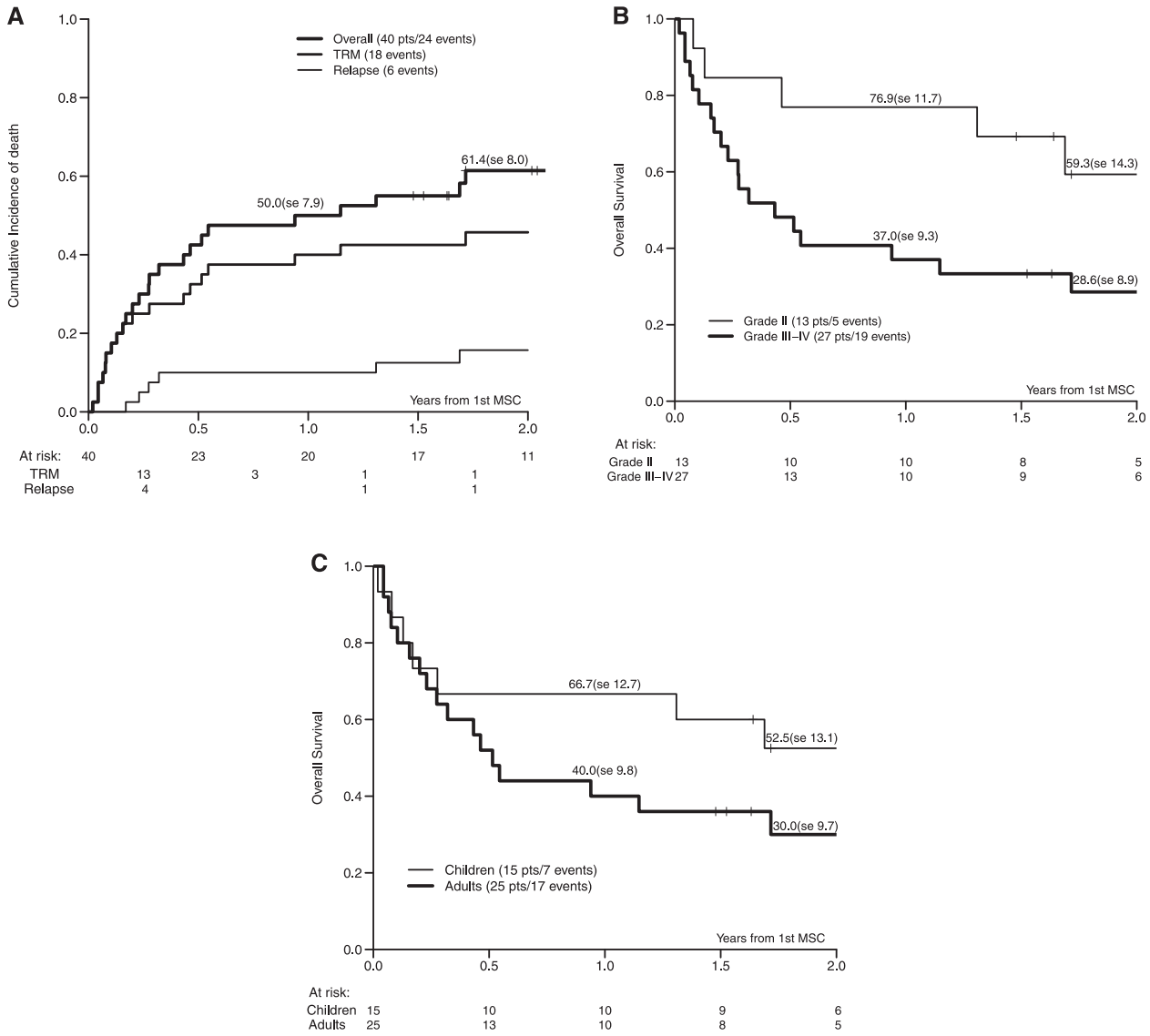

Figure 2. Survival and cumulative incidence of death by cause of death for the 40 treated patients. Cumulative incidence of death by cause, for the 40 treated patients, and overall survival by groups. (2A) Shows cumulative incidence of death for the study cohort (40 patients) is represented. Transplantation-related mortality (TRM) and relapse incidence are detailed as specific cause of death. The estimates are reported at 1 and 2 years with standard errors (se). (2B) Shows overall survival from the first MSC administration is presented for the enrolled patients, as specified for GVHD grading at study entry. The estimates are reported at 1 and 2 years with standard errors (se). (2C) Shows overall survival from the first MSC administration is presented for the enrolled patients, as specified for adult and pediatric patients. The estimates are reported at 1 and 2 years with standard errors (se).

experience, GVHD grading was the only predictor of treatment response and no additional independent factor was detected in the multivariable analysis. Therefore, for most adult patients with the highest grade of GVHD and, therefore, the highest need of an effective therapy, this treatment program was largely unsatisfactory. In addition, our study illustrates that among responders, the risk of developing chronic GVHD remains, suggesting the need of further treatment to avoid this late complication.

All in all, our results indicate that a further optimization of the clinical use of PL-MSC is needed. Possible example of such an optimization could be represented by the use of higher cell doses, the sequential or combined administration of PL-MSC with other second line treatments. Alternatively, the use of MSCs derived from different organs (eg, umbilical cord wall), possibly able to express a higher immune suppressive activity, could also be considered [28].

In conclusion, the present study confirms the feasibility and safety of treatment with third party PL-expanded BM-derived MSCs, which may offer some clinical benefit. Further studies are needed to support this approach as a reliable therapeutic tool and to address the largely unmet clinical needs of patients with steroid refractory severe GVHD.

ACKNOWLEDGMENTS

The authors thank the following funding organizations: Associazione Italiana per la Ricerca contro Cancro, AIRC Molecular Clinical Oncology 5 per mille, “Innate Immunity in Cancer. Molecular targeting and cellular therapy,” AIRC-9962, AIRC-IG 8666 to AB, Ministero della Salute (Bando cellule staminali, Bando Giovani Ricercatori), Associazione Italiana contro le Leucemie (AIL)/Sezione Paolo Belli, Bergamo, Comitato Stefano Verri, and Comitato Maria Letizia Verga, Monza.

The authors also thank Elisabetta Calore, MD from the department of pediatrics at the University of Padova for her contribution in enrolling one pediatric patient.

Authorship statement: M.I., G.L., A.R., E.B., and S.G. wrote the paper; E.D. and G.D. analyzed the immunobiological data; S.G. performed the statistical analysis of the clinical data; A.R., A.B., D.L., F.P., F.M., A.A., C.M., A.G., S.D., I.C., M.P., E.P., and S.C. enrolled and treated the patients, contributed to data collection, and reviewed data review; G.G., D.B., P.P., C.C., E.G., and J.G. produced and expanded MSCs; M.I., E.B., A.R., and A.B. designed the protocol; and E.B. was the principal investigator of the study, and supervised MSC production and patients enrollment.

Conflict of interest statement: There are no conflicts of interest to report.

Financial disclosure: The authors have nothing to disclose.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2013.11.033.

REFERENCES

1. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. 2009;373:1550-1561.
2. Bacigalupo A. Management of acute graft-versus-host disease. *Br J Haematol*. 2007;137:87-98.
3. Deeg HJ. How I treat refractory acute GVHD. *Blood*. 2007;109:4119-4126.
4. Perez L, Anasetti C, Pidala J. Have we improved in preventing and treating acute graft-versus-host disease? *Curr Opin Hematol*. 2011;18:408-413.
5. 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;8:315-317.
6. Siegel G, Schafer R, Dazzi F. The immunosuppressive properties of mesenchymal stem cells. *Transplantation*. 2009;87(9 Suppl):S45-S49.
7. Baron F, Storb R. Mesenchymal stromal cells: a new tool against graft-versus-host disease? *Biol Blood Marrow Transplant*. 2012;18:822-840.
8. Kebriaei P, Robinson S. Treatment of graft-versus-host-disease with mesenchymal stromal cells. *Cytotherapy*. 2011;13:262-268.
9. Lin Y, Hogan WJ. Clinical application of mesenchymal stem cells in the treatment and prevention of graft-versus-host disease. *Adv Hematol*. 2011;2011:427863.
10. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.
11. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945-956.
12. Capelli C, Domenghini M, Borleri G, et al. Human platelet lysate allows expansion and clinical grade production of mesenchymal stromal cells from small samples of bone marrow aspirates or marrow filter washouts. *Bone Marrow Transplant*. 2007;40:785-791.
13. 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;363:1439-1441.
14. Ringden O, Uzunel M, Rasmusson I, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation*. 2006;81:1390-1397.
15. Herrmann R, Sturm M, Shaw K, et al. Mesenchymal stromal cell therapy for steroid-refractory acute and chronic graft versus host disease: a phase 1 study. *Int J Hematol*. 2011;95:182-188.
16. Muller I, Kordowich S, Holzwarth C, et al. Application of multipotent mesenchymal stromal cells in pediatric patients following allogeneic stem cell transplantation. *Blood Cells Mol Dis*. 2008;40:25-32.
17. Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet*. 2008;371:1579-1586.
18. von Bonin M, Stolzel F, Goedecke A, et al. Treatment of refractory acute GVHD with third-party MSC expanded in platelet lysate-containing medium. *Bone Marrow Transplant*. 2008;43:245-251.
19. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One*. 2012;7:e47559.
20. von Bahr L, Sundberg B, Lonnies L, et al. Long-term complications, immunologic effects, and role of passage for outcome in mesenchymal stromal cell therapy. *Biol Blood Marrow Transplant*. 2011;18:557-564.
21. Lucchini G, Dander E, Pavan F, et al. Mesenchymal stromal cells do not increase the risk of viral reactivation nor the severity of viral events in recipients of allogeneic stem cell transplantation. *Stem Cells Int*. 2012;2012:690236.
22. Alousi AM, Weisdorf DJ, Logan BR, et al. Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the Blood and Marrow Transplant Clinical Trials Network. *Blood*. 2009;114:511-517.
23. 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;22:593-599.
24. von Bahr L, Batsis I, Moll G, et al. Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. *Stem Cells*. 2012;30:1575-1578.
25. Prockop DJ, Brenner M, Fibbe WE, et al. Defining the risks of mesenchymal stromal cell therapy. *Cytotherapy*. 2010;12:576-578.
26. Remberger M, Ringden O. Treatment of severe acute graft-versus-host disease with mesenchymal stromal cells: a comparison with non-MSC treated patients. *Int J Hematol*. 2012;96:822-824.
27. Dander E, Lucchini G, Vinci P, et al. Mesenchymal stromal cells for the treatment of graft-versus-host disease: understanding the in vivo biological effect through patient immune monitoring. *Leukemia*. 2012;26:1681-1684.
28. Capelli C, Gotti E, Morigi M, et al. Minimally manipulated whole human umbilical cord is a rich source of clinical-grade human mesenchymal stromal cells expanded in human platelet lysate. *Cytotherapy*. 2011;13:786-801.