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

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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

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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