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Human Clinical Article

Mesenchymal Stromal Cells for Treating Steroid-Resistant Acute and Chronic Graft Versus Host Disease: A Multicenter Compassionate Use Experience

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Abstract

Graft versus host disease (GVHD) is a severe complication after allogenic hematopoietic cell transplantation (HSCT). Several clinical trials have reported the use of mesenchymal stromal cells (MSCs) for the treatment of GVHD. In March 2008, the Andalusian Health Care System launched a compassionate use program to treat steroid-resistant GVHD with MSC. Clinical-grade MSC were obtained under GMP conditions. MSC therapy was administered intravenously in four separate doses of 1×10^6 cells/kg. Sixty-two patients, 45 males (7 children) and 17 females (2 children), received the treatment. Patients had a median age of 39 years (range: 7–66) at the time of the allogenic HSCT. The overall response was achieved in 58.7% of patients with acute (a)GVHD. Two years' survival for aGVHD responders was 51.85%. The overall response for patients with chronic (c)GVHD was 65.50% and the 2-year survival rate for responders was 70%. Age at the time of HSCT was the only predictor found to be inversely correlated with survival in aGVHD. Regarding safety, four adverse events were reported, all recovered without sequelae. Thus, analysis of this compassionate use experience shows MSC to be an effective and safe therapeutic option for treating refractory GVHD, resulting in a significant proportion of patients responding to the therapy.

Key words: mesenchymal stromal cells; allogenic hematopoietic stem cell transplantation; graft versus host disease; cell therapy

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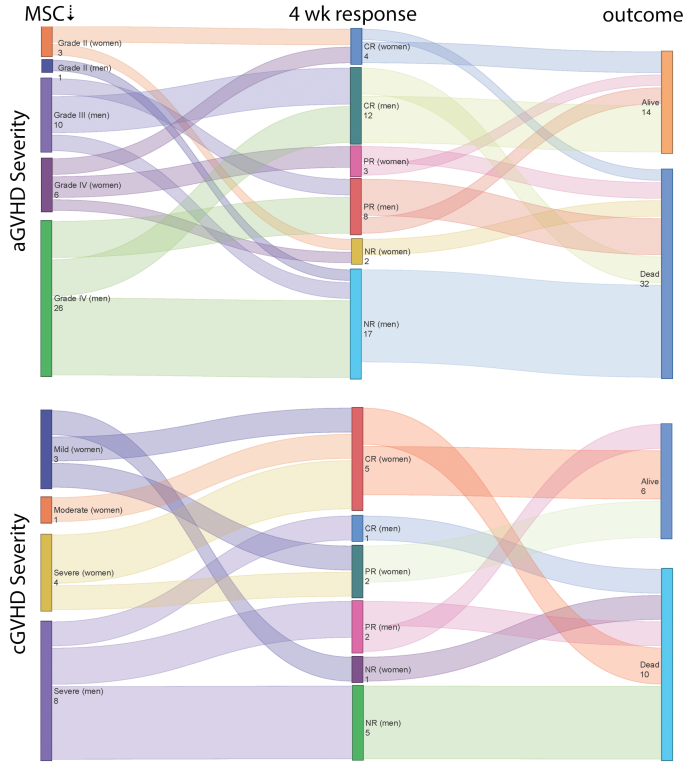
Graphical Abstract



Lessons Learned

- MSC infusions were well tolerated.
- MSC response was 58.7% for patients with aGVHD and 65.50% for patients with cGVHD.
- Pediatric sub-group patients showed 100% overall response for aGVHD and 75% for cGVHD.
- aGVHD responders group showed 51.85% two years' overall survival and cGVHD responders group 70%.
- MSC is a promising therapy in patients with GVHD.

Significance Statement

For some patients with steroid-refractory graft-versus-host disease (GVHD), there is no effective treatment. Several clinical trials have reported the use of mesenchymal stromal cells for the treatment of steroid-refractory GVHD with variable outcomes. In 2008, the Andalusian Health Care System launched a compassionate use program to treat steroid-resistant GVHD with mesenchymal stromal cells. This article reports the outcome of this series of acute and chronic steroid-resistant GVHD patients who received mesenchymal stromal cell therapy in a real-life setting.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is commonly used in clinical practice for the treatment of different hematological diseases.[1] Despite the use of hematopoietic stem cells (HSC) from HLA-matched donors, the success of the HSCT is frequently hampered by the development of graft versus host disease (GVHD).[2,3] Graft versus host disease occurs in around 50% of the patients who underwent HSCT[2,3] and only 30%–50% respond to steroids as first-line of treatment[3-5] and a second-line of treatment is not universally agreed.[4,6] In addition, these patients suffer the consequences of the long-term immunosuppressant therapy.[6-9] Therefore, it is essential to define novel therapeutic protocols more effective and safer for the treatment of steroid-resistant GVHD.

Since 2004,[10] several clinical trials have reported the use of mesenchymal stromal cells (MSC) for the treatment of GVHD. Mesenchymal stromal cells are multipotent progenitor cells; although initially described in bone marrow,[11] MSC are present in other tissues, such as adipose tissue[12] and umbilical cord.[13] Mesenchymal stromal cells possess extensive immunomodulatory properties, such as the capacity to inhibit T- and B-cell activation,[14] to increase the regulatory T-cell population[15] and to induce the release of anti-inflammatory cytokines.[16] Importantly, MSC express low levels of human leukocyte antigen (HLA) major histocompatibility complex (MHC) class I molecules and do not express HLA class II. Thus, they are not immunogenic and can be used in HLA-mismatched receptors.[17] Unfortunately, data available from the different clinical trials have shown heterogeneous

results, related to variability of the doses, sources of MSC, and patient characteristics, among others. Thereby, the reported complete response rates for aGVHD patients treated with MSC vary from 0.08%[18] to 83%[19,20] and for cGVHD from 0.0% to 40%, being the patients with highest response rates those displaying the best overall survival (OS) rates.[21-27]

Most of the previous trials were carried out with bone marrow-derived MSC (BM-MSC) and only a few studies have used MSC derived from adipose tissue (AT-MSC).[20,28,29] Adipose tissue is easier to harvest, it may allow obtaining a higher number of MSC[30,31] and AT-MSC have a higher proliferation capacity than those derived from bone marrow, as shown in some studies.[32,33] In a previous study from our group (NCT01222039) for cGVHD,[34] we reported an 80% complete response rate, and 100% of patients were off steroids at week 56. No suspected unexpected serious adverse reactions occurred during the trial. Neither relapse of underlying disease nor mortality due to infection were observed in this cohort.[34] The results of our recent meta-analysis also indicate that allogenic MSC could be instrumental for the treatment of GVHD.[35] Keeping in mind all these considerations, we decided to compile the information on the compassionate use treatments requested by the hematologists in Andalusia between 2008 and 2018 and analyze the outcome of the patients.

Material and Methods

Andalusian Experience on Compassionate Use of MSC for GVHD

In March 2008, the Andalusian Health Care System launched a compassionate use of MSC program in GVHD promoted and coordinated by the Andalusian Network for the design and translation of Advanced Therapies (ANd&tAT; formerly Andalusian Initiative for Advanced Therapies) after sponsoring several clinical trials with MSC for different conditions, including GVHD. For this purpose, an Independent Data and Safety Committee was created with representatives of five Andalusian hospitals that perform allogeneic HSCT, who assessed, according to available scientific evidence, the indications and conditions in which they considered that the risk–benefit balance was favorable for patients with steroid-resistant GVHD to receive MSC.

Every application for a compassionate use is first evaluated by a member of the Independent Data and Safety Committee. Once the committee gives a written approval, the ANd&tAT, as a sponsor of this advanced therapy treatment, also gives written approval for manufacturing the MSC product. At this point, written approval by the competent national Authority (Spanish Medicine Agency—AEMPS) is requested.

Patients

Population included in the analysis comprises patients (adults and children), suffering steroid-resistant plus ≥1 additional lines of immunosuppressive treatment both in aGVHD and in cGVHD. Patients received MSC between March 2008 and August 2018 within this regional compassionate use program. A total of 62 patients (Table 1) of all ages were included in the program and treated in the Hospital Regional (Málaga) (n = 39), Hospital Universitario Reina Sofia (Córdoba) (n = 10), Hospital Universitario Virgen de las Nieves (Granada) (n = 6), Hospital Virgen del Rocío (Sevilla) (n = 4), and Hospital de Jerez (Cádiz) (n = 3).

Graft versus host disease was classified into acute (aGVHD, occurring within 100 days of the HSCT), late acute (laGVHD, more than 100 days post-HCT but with features of aGVHD) or chronic (cGVHD occurring after 100 days post-HCT) and graded according to international criteria.[36] Whenever possible, diagnosis was confirmed with a biopsy.

The following variables were analyzed: sex, age, disease, HCT-CI, donor sex, donor age, transplant source, donor relation, HLA matching, conditioning and GVHD prophylaxis, days from HSCT to GVHD and days from GVHD to MSC. Immunosuppressive lines of therapy before receiving MSC and affected organs are included in Supplementary Tables S1 and S2.

MSC Preparation and Infusion

Clinical-grade MSC were obtained under GMP conditions from bone marrow and adipose tissue from mismatched third-party donors. After the informed consent was signed, tissues were obtained in aseptic conditions fulfilling the provisions of the Spanish legislation on cell and tissue donation. Bone-marrow mononuclear cells were separated by the Cell Therapy Unit of Hospital Reina Sofía (Córdoba) by density gradient centrifugation. A total of 5×10^6 washed mononuclear cells were plated in 75 cm^2 with MEM supplemented with 15% of FBS, 1 ng/mL of basic FGF, 0.1 mg/mL of streptomycin, and 5 µg/mL of gentamycin and maintained at 37 °C in a humidified incubator with 5% of CO_2. Media was changed every 2 days until the cells reached 85%–90% of confluence. Then, BM-MSC were detached by trypsin and re-plated twice. After expansion, BM-MSC were frozen. When a patient needed to be treated, BM-MSC were thawed and expanded for 1 week. The finished product was a cell suspension containing allogeneic expanded BM-MSC at a concentration of 5×10^6 cells/mL in Ringer's lactate solution containing 1% human albumin. The volume was adjusted according to the patient's weight and packaged in sterile syringes preserved at 4 °C to 22 °C until their intravenous infusion at a dose of 1×10^6 AT-MSC per kilogram of body weight.

Adipose tissue was obtained through surgical exeresis or liposuction from healthy donors. Fat was washed at the Cell Production and Tissue Engineering Unit of Hospital Universitario Virgen de las Nieves (Granada) using DPBS with antibiotics (penicillin 1.200 UI/mL, vancomycin 20 µg/mL, and gentamycin 160 µg/mL) and vessels and connective tissue were removed with sterile surgical tools. Then, a mechanical disintegration of the tissue was performed followed by enzymatic digestion with collagenase type A. The cell fraction was separated by centrifugation and seeded in plates and, after two culture-expansion passages, AT-MSC were isolated. The formulation of the medium of AT-MSC expansion was as follows.

Dulbecco's modified Eagle's medium with 10% of fetal bovine serum, 2% of L-alanine and L-glutamine, 0.1 mg/mL of gentamycin, and 100 UI/mL of penicillin. After the expansion, AT-MSC were frozen. When a patient needed to be treated by compassionate use, AT-MSC were thawed and expanded for 1 week. The finished product was a cell suspension containing allogeneic expanded AT-MSC at a concentration of 2×10^6 cells/mL in Ringer's lactate solution containing 1% human albumin. The volume was adjusted according to the patient's weight and packaged in sterile bags preserved at 2 °C to 8 °C until their intravenous infusion at a dose of 1×10^6 AT-MSC/kg of body weight. Mesenchymal stromal

Table 1. Patient characteristics.

	Adults	Children	Overall
	N (53)	*N* (9)	*N* (total = 62)
Sex (f/m)	15/38 (28.30/15.09)	2/7 (22.22/77.77)	17/45 (27.42/72.58)
Age, years, median (range)	43 (19–66)	14 (7–18)	41 (7–66)
Indication for HSCT			
AML	16 (30.19)	3 (33.33)	19 (30.65)
ALL	3 (5.66)	4 (44.44)	7 (11.29)
MDS	12 (22.64)	1 (11.11)	13 (20.97)
NHL	8 (15.10)	0 (0.0)	8 (12.90)
HL	5 (9.43)	0 (0.0)	5 (8.06)
CLL	2 (3.77)	0 (0.0)	2 (3.23)
CML	2 (3.77)	0 (0.0)	2 (3.23)
MM	2 (3.77)	0 (0.0)	2 (3.23)
Others	3 (5.66)	1 (11.11)	4 (6.45)
HCT-CI			
0	31 (54.50)	7 (77.77)	38 (61.29)
1	4 (7.55)	1 (11.11)	5 (8.06)
2	7 (13.21)	1 (11.11)	8 (12.90)
3	5 (9.43)	0 (0.0)	5 (8.06)
4	4 (7.55)	0 (0.0)	4 (6.45)
5	2 (3.77)	0 (0.0)	2 (3.23)
Donor			
Sex (f/m/unk)	21/31/1 (39.62/54.59/1.88)	6/3/0 (66.66/33.33)	27/34/1 (44/55/2)
Age (median (range))	43 (19–70)	14 (7–18)	38 (7–70)
Transplant source			
Bone marrow	11 (20.75)	3 (33.33)	14 (22.58)
Peripheral blood stem cells	42 (79.25)	6 (66.66)	48 (77.42)
Related/unrelated donor	42/11 (79.25/20.75)	5/4 (55.55/44.44)	47/15 (76/24)
HLA matching			
8/8 or 10/10	33 (62.26)	5 (55.55)	38 (61.29)
7/8	2 (3.77)	3 (33.33)	5 (8.06)
Haploidentical	18 (33.96)	1 (11.11)	19 (30.65)
Conditioning			
Ablative	19 (35.85)	8 (88.89)	27 (43.55)
Non-ablative	7 (13.21)	0 (0.0)	7 (11.29)
Reduced-intensity	27 (50.94)	1 (11.11)	28 (45.16)
GVHD prophylaxis			
CsA/MTX	7 (35.85)	5 (55.55)	12 (19.35)
Tacrolimus/SRL	9 (16.98)	1 (11.11)	10 (16.13)
Tacrolimus	7 (13.21)	1 (11.11)	8 (12.90)
CsA/MMF	5 (9.43)	0 (0.0)	5 (8.06)
Tacrolimus/MTX	5 (9.43)	0 (0.0)	5 (8.06)
Tacrolimus/MMF	3 (5.66)	0 (0.0)	3 (4.84)
CsA/Pred	1 (1.89)	1 (11.11)	2 (3.23)
Other	16 (30.18)	1 (11.11)	17 (27.42)
aGVHD + laGVHD	41 (77.35)	5 (55.55)	46 (74.19)
aGVHD	34 (64.15)	4 (44.44)	38 (61.29)
laGVHD	7 (13.21)	1 (11.11)	8 (12.90)
cGVHD (n=16)	12 (22.64)	4 (44.44)	16 (25.80)
aGVHD grade			
Grade II	4 (7.55)	0 (0.0)	4 (8.69)
Grade III	9 (16.98)	1 (11.11)	10 (21.73)
Grade IV	28 (52.83)	4 (44.44)	32 (69.56)

Table 1. Continued

	Adults	Children	Overall
	N (53)	*N* (9)	*N* (total = 62)
cGVHD grade			
Low	2 (3.77)	0 (0.0)	2 (12.5)
Moderate	0 (0.00)	1 (11.11)	1 (6.25)
Severe	10 (18.87)	3 (33.33)	13 (81.25)
Days from HSCT to GVHD (median (range))			
aGVHD + laGHVD (*n* = 46)	54 (7–395)	27 (8–157)	52 (7–395)
aGVHD (*n* = 38)	42 (7–95)	21 (8–37)	37 (7–95)
laGHVD (*n* = 8)	147 (87–395)	157	152 (87–395)
cGVHD (*n* = 16)	163 (98–524)	300 (224–866)	220 (98–524)
Days from GVHD to MSC infusion (Median (range))			
aGVHD+ laGVHD (*n* = 46)	40 (19–1205))	57 (22–132)	39 (19–1205)
aGVHD (*n* = 38)	41 (20–1205))	86 (22–132)	44 (20–1205)
laGVHD (*n* = 8)	40 (19–322)	25	34 (19–322)
cGVHD (*n* = 16)	102 (16–1932)	175 (12–338)	118 (12–1932)

Data are *n* (%) or median (range).
Abbreviations: *F*, female; *M*, male; *UNK*, unknown; *HSCT*, hematopoietic stem cell transplantation; *AML*, acute myeloid leukemia; *ALL*, acute lymphoblastic leukemia; *MDS*, myelodysplastic syndrome; *NHL*, non-Hodgkin's lymphoma; *HL*, Hodgkin's lymphoma; *CLL*, chronic lymphocytic leukemia; *CML*, chronic myeloid leukemia; *CsA*, cyclosporin A; *MM*, multiple myeloma; *HCT-CI*, hematopoietic cell transplantation comorbidity index; *CsA*, cyclosporin A; *MTX*, methotrexate; *SRL*, sirolimus; *MMF*, mycophenolate mofetil; *Pred*, prednisolone; *aGVHD*, acute graft versus host disease; *laGVHD*, late acute graft versus host disease; *MSC*, mesenchymal stem cells.

cells were intravenously infused according to local protocols. Premedication was administered at medical discretion.

Clinical Outcome

Treatment response was classified as follows: complete response (CR); resolution of all signs and symptoms of GVHD; partial response (PR), a decrease of at least one GVHD grade in one organ as a minimum, without worsening in any other organ system; no response (NR), no change in any organ system or worsening in one or more organ system without improvement in any other organ system.[37] Overall response was considered in patients who achieved a complete or partial response. Clinical response was evaluated 4 weeks after receiving the first infusion. Survival time was considered from the date of the first MSC infusion to the date of death or last follow-up.

Statistical Analysis

Categorical variables were compared between responders and non-responders using Pearson χ^2 or Fisher's exact tests. Quantitative variables were compared using Mann-Whitney nonparametric test.

The Kaplan-Meier method and log-rank test were used to estimate and compare OS rates on responders and nonresponders. Univariate Cox proportional hazards regression models were used to obtain adjusted estimates for predictors of OS, including MSC treatment response, age, sex, and therapy lines prior to MSC.

Results

Patients' Characteristics

The baseline characteristics of the 62 patients (53 adults, 9 children) are shown in Table 1. Patients [45 males (7 children), 17 females (2 children)] had a median age of 41 years (range: 7–66) at the time of the allogenic HSCT. The most common indication for HSCT was acute myeloid leukemia (AML, 30.65%) followed by myelodysplastic syndrome (MDS, 20.97%). The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI)[38] ranged from 0 (61.29%) to 5 (3.23%) in these patients. Seventy-six percent of the patients received the HSCT from compatible familiar donors. Before allogeneic HSCT, patients received either myeloablative (43.55%), non-myeloablative (11.29%), or reduced-intensity (45.16%) conditioning. As GVHD prophylaxis, most patients were treated with tacrolimus alone or in combination with sirolimus, methotrexate, or mycophenolate mofetil (Table 1).

GVHD Characteristics

Sixteen patients received MSC for chronic GVHD (cGVHD) and 46 patients received for acute GVHD (aGVHD), including 8 for late acute GVHD (laGVHD). The median time of presentation of the GVHD after the HSCT was 37 days for the acute cases, 152 for acute cases of late onset, and 220 for chronic presentations (Table 1).

MSC Therapy

A total of 215 infusions were administered to 62 patients; each patient received a median number of 4 infusions (range: 1–12) (Figure 1). Mesenchymal stromal cells were infused at a median dose of 1×10^6 cells/kg (range: 0.52–2.10). Fifty-three patients (85.48%) were treated with AT-MSC, 8 patients (12.90%) were treated with BM-MSC, and 1 patient (1.61%) was treated with BM-MSC and AT-MSC.

The overall response for aGVHD was 58.69% (27 of 46 patients) and 62.50% (10 of 16 patients) for cGVHD (Figure 2). All patients who did not have a response died with a median of 81 days after the first infusion (range: 3–1813). In

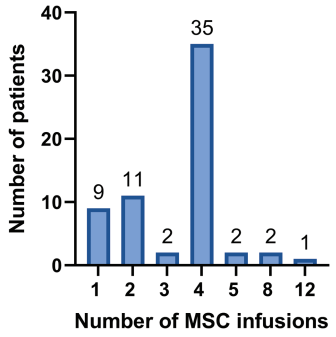


Figure 1. Median number of doses of MSC was 3 (range 1–12), with the majority receiving four doses.

addition, around 30% of the patients progressed to cGVHD (Supplementary Table S3). In this series, the time from GVHD presentation to MSC infusion was significantly higher in aGVHD responders (med = 48 days) than in nonresponders (med = 27 days ($P < .001$) but not in cGVHD responders with a median of 70 days against 364 days in nonresponders ($P = .073$). To determine whether the supportive treatment given to aGVHD responders before MSC could have a beneficial interaction with the response, we further analyzed the treatments these patients received before MSC infusion. The overall response was achieved in 100% of patients who received etanercept (two of two) and sirolimus (two of two), 77.78% (seven of nine) for patients receiving kinase inhibitors (imatinib, ruxolitinib), 66.66% (8 of 12) for patients receiving basiliximab, 60% (three of five) for patients receiving photopheresis, 50% (three of six) for patients receiving MMF, 33.3% (one of three) for patients receiving tacrolimus and 20% (one of five) for patients receiving ATG (Supplementary Figure S1). The number of days between the allogenic HSCT and GVHD onset was not a predictor of the clinical response neither for aGVHD nor for cGVHD. We did not find any association among the clinical response to MSC therapy and several clinical, overlapping therapies, demographic or laboratory parameters including GVHD grade or previous comorbidity. Thus, the number of previous immune suppression treatments did not influence the clinical response (Table 2).

Survival Analysis

The follow-up time from the first MSC infusion was 152 days (range: 3–2969). At the end of the follow-up, 20 patients were alive and 42 had died due to GVHD ($n = 27$), disease relapse ($n = 6$), infection ($n = 5$), or other causes ($n = 4$).

In patients with aGVHD, the median survival time was higher in the responder group (608 days) than in the nonresponder group (25 days) ($P < .001$) (Figure 3a). Patients in the complete responder subgroup were tapered off of supportive treatment in 6–8 weeks. Acute GVHD OS at 2 years was 51.85% for responders (14 of 27 patients remained alive) and 0% (0 of 20) for nonresponders ($P < .001$). Regarding cGVHD, the median survival time in the responder group was 1039 days and 127 days in the nonresponder group ($P \leq .01$) (Figure 3b). In the complete responder subgroup, the tapering off of supportive treatment was done in the 6 months following MSC response evaluation. Chronic GVHD OS for responders was 70% (7 of 10 patients remained alive) at 2 years and 16.67% (1 of 6 patients) for nonresponders ($P = .008$). We found that patients aged 18 years and below presented a better OS at 2 years (55.55%, 5 of 9 patients) than those over 18 years of age (32.07%, 17 of 53 patients) ($P = .045$).

To obtain adjusted estimates, we fitted a Cox model for aGVHD and cGVHD (Table 3) and found that, in aGVHD, the response improved OS after adjusting for age, and sex (HR = 0.12, 95% CI: 0.05-0.32, $P \leq .001$). We also found that age and survival time were inversely associated in this model, as the predictor increases the hazard of death (HR = 1.04, 95% CI: 1.01-1.07, $P \leq .001$).

Pediatric Patient's Description

Seven male and 2 female patients in pediatric care with a median age of 14 years (range: 7–18) at the time of the allogenic HSCT were included in the study. The indications for HSCT were acute lymphocytic leukemia (ALL, 4) followed by acute myeloid leukemia (AML, 3) medullary aplasia (1), and myelodysplastic syndrome (1). The Hematopoietic Cell Transplant-Co-morbidity Index (HCT-CI) ranged from 0 (77.77%) to 2 (11.11%). Four patients received MSC for chronic GVHD (cGVHD), 4 patients for acute GVHD (aGVHD), and 1 for late acute GVHD (laGVHD). The median time of presentation of the GVHD after the HSCT was 21 days for the acute cases, 157 for acute cases of late-onset, and 300 for chronic presentations. A total of 27 infusions were administered to 9 patients; patients received 4 doses (median: 4, range: 1–5). Most of the patients received 1×10^6 cells/kg (median: 1×10^6 cells/kg, range: 0.52–2.10 1×10^6). The MSC were derived from adipose tissue (77.77%) and bone marrow (22.22%). The results of patients in pediatric care are summarized in Table 2. The overall response for aGVHD was 100% (5 of 5 patients) and 75% (3 of 4 patients) for cGVHD. We did not find evidence of an association between the response to MSC therapy and any clinical, demographic, or laboratory parameters, including previous comorbidities. The median follow-up time from the first MSC infusion was 956 days (range: 213-1951). At the end of the follow-up, 5 patients were alive and 4 had died due to GVHD ($n = 3$) and disease relapse ($n = 1$).

Safety and Tolerability

Mesenchymal stromal cell therapy was administered intravenously. There was one case of cardiac arrest during the first infusion, recovered without sequelae after ardiopulmonary resuscitation maneuvers and intravenous adrenaline. This patient received 3 more doses of MSC without any adverse event. One patient had a syncope during the MSC infusion that recovered without sequelae; a cranial CT scan was normal. Another patient who received 4 doses of MSC developed thoracic pain and tachycardia during the third infusion; the patient received dexchlorpheniramine and recovered without sequelae. There was one case of induration and pain at the infusion site. No other adverse effects were reported. None of the adverse events occurred in the pediatric population. Thus, MSC infusions were overall well tolerated. Bacterial, viral, and fungal infections were common among treated patients, as expected for the pathology, but with no apparent relationship with the MSC, affecting 31 of them (50%).

Discussion

We describe the results of one of the largest series of patients receiving MSC to treat refractory GVHD in adults and children secondary to allogenic HSCT in different hospitals

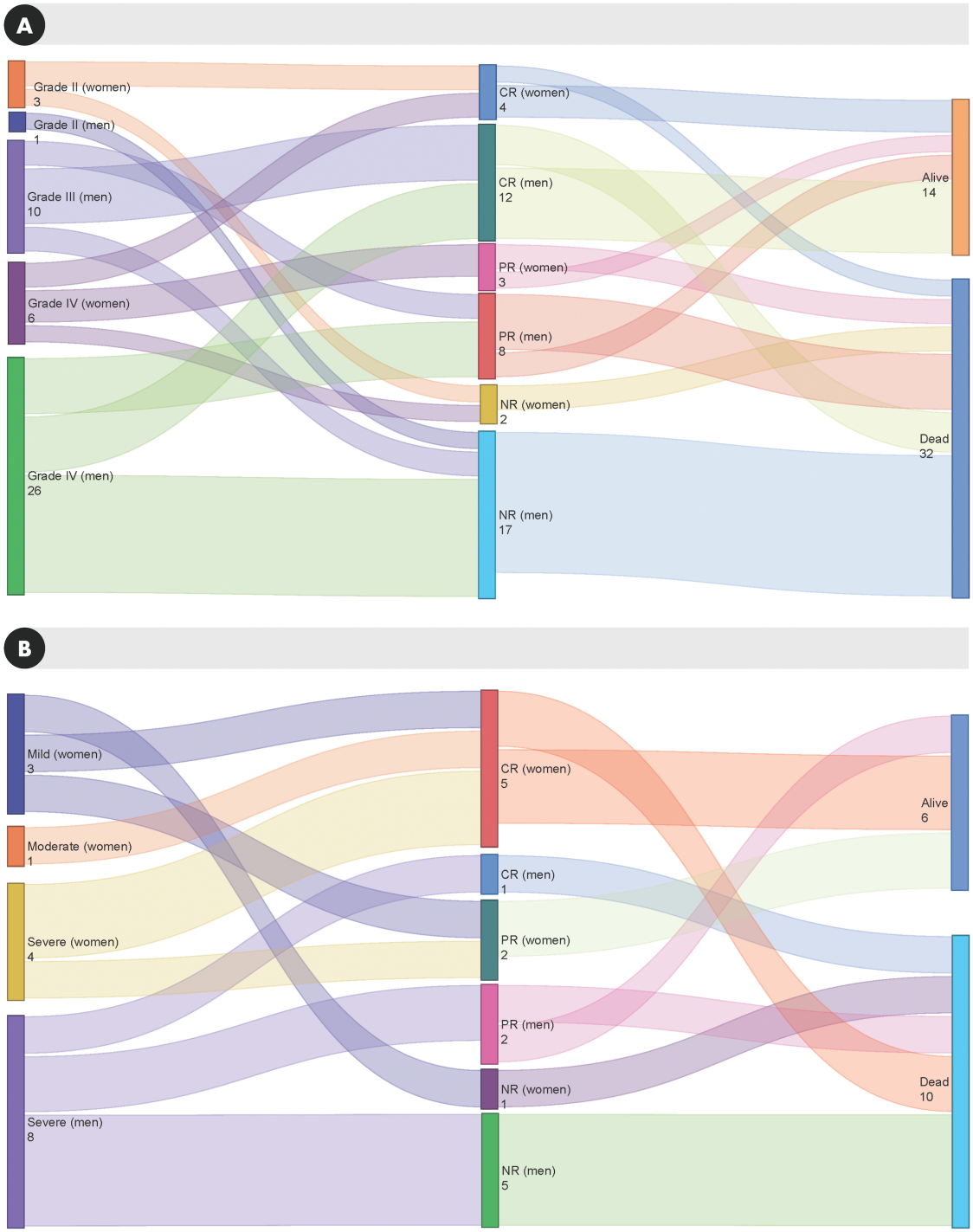


Figure 2. Sankey diagram of overall response and survival by sex and severity over time; the first column represents disease severity; the second column represents treatment response and the third column represents the status at last follow-up; the width of each bar represents their relative frequency within the cohort. (A) aGVHD patients with grades II, III, and IV. (B) cGVHD patients with grades, mild, moderate and severe (NIH severity scoring), and sex.

within a regional compassionate use program in Andalusia. Having sponsored clinical trials on cGVHD[34] that demonstrated the safety of the procedure with variable efficacy, we launched this compassionate use program to provide an alternative to patients with GVHD steroid-refractory. The findings of this study have to be seen in light of some inherent limitations because it is a case series and there is no control group. Nevertheless, it can be viewed as a more realistic scenario to assess the therapeutic effect without the constraints of stringent inclusion and exclusion criteria in clinical trials.

In this compassionate use experience, MSC therapy was overall well tolerated, with only 4 adverse events occurring in the adult cohort. In this series, 58.7% of patients with aGVHD and 62.5% of patients with cGVHD responded to MSC therapy, being within the response rates collected

Table 2. Univariate analysis of treatment response in aGVHD and cGHVD for adults, children and overall according to sex, HCT-CI, GVHD prophylaxis, conditioning, HLA matching, aGVHD grade, cGVHD severity.

A. aGVHD

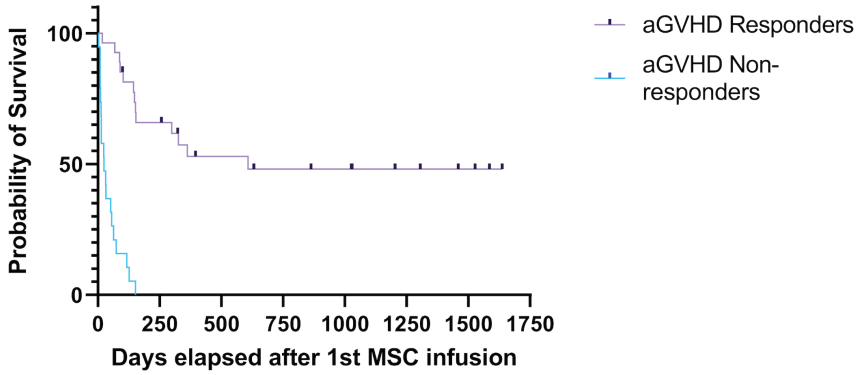
	Adults			Children			Overall		
	Responders *n* = 22	Nonresponders *n* = 19	*P* value	Responders *n* = 5	Nonresponder *n* = 0	*p* value	Responders *n* = 27	Nonresponders *n* = 19	*P* value
Sex			.661			>.999			.270
Male	16 (48.48)	17 (51.52)		4 (85.71)	0 (0.00)		20 (54.05)	17 (49.95)	
Female	4 (66.67)	2 (33.33)		1(100)	0 (0.00)		7 (77.78)	2 (22.22)	
HCT-CI			.493			>.999			.190
0–1	17 (58.62)	12 (41.38)		5 (100)	(0.00)		22 (64.71)	12 (35.29)	
>1	5 (41.67)	7 (58.33)		0 (0.00)	(0.00)		5(41.67)	7 (58.33)	
GVHD prophylaxis			.846			-			.869
CsA/MTX	1 (33.33)	2 (66.67)		2 (100.00)	0 (0.00)		3 (60.00)	2 (40.00)	
Tacrolimus/SRL	4 (66.67)	2 (33.33)		1 (100.00)	0 (0.00)		1 (25.00)	3 (75.00)	
Tacrolimus	2 (50.00)	2 (50.00)		1 (100.00)	0 (0.00)		3 (60.00)	2 (40.00)	
CsA/MMF	1 (25.00)	3 (75.00)		0 (0.00)	0 (0.00)		3 (60.00)	2 (40.00)	
Tacrolimus/MTX	3 (60.00)	2 (40.00)		0(0.00)	0 (0.00)		2 (66.67)	1 (33.33)	
Tacrolimus/MMF	2 (66.67)	1 (33.33)		0(0.00)	0 (0.00)		5 (75.43)	2 (28.57)	
CsA/Pred	1 (100.00)	0 (0.00)		0(0.00)	(0.00)		1 (100)	0 (0)	
Other	8 (53.33)	7 (46.67)		1 (100.00)	0 (0.00)		9 (56.25)	7 (43.75)	
Conditioning			.177			>.999			.194
Ablative	11 (68.42)	6 (31.58)		4 (100.00)	0 (0.00)		15 (71.43)	6 (28.57)	
No- ablative	2 (28.57)	4 (71.43)		0 (0.00)	0 (0.00)		2 (33.33)	4 (66.67)	
Reduced intensity	9 (51.85)	9 (48.15)		1 (100.00)	0 (0.00)		10 (52.63)	9 (47.37)	
HLA matching			.826						.674
Identical	12 (50.00)	12 (50.00)		2 (80.00)	0 (0.00)		14 (53.85)	12 (46.15)	
Haploidentical	9 (60.00)	6 (40.005)		1 (100.00)	0 (0.00)		10 (62.50)	6(37.50)	
Others	1 (50.00)	1 (50.00)		2 (100.00)	0 (0.00)		3 (75.00)	1 (25.00)	
aGVHD grade	22	19	.257	5	0	>.999	27	19	.300 (Low grade vs High grade 0 = >.999
I	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
II	2 (50.00)	2 (50.00)		0 (0.00)	0 (0.00)		2 (50)	2 (50)	
III	7 (77.78)	2 (22.22)		1 (100.00)	0 (0.00)		8 (80)	2 (20)	
IV	13 (46.43)	15 (53.57)		4 (100.00)	0 (0.00)		17 (53.13)	15 (46.88)	
IS Lines			.490						.504
MSC as second and third line	20 (51.28)	19 (48.72)		5 (100)	0 (0.00)		25 (56.82)	19 (43.18)	

Table 2. Continued

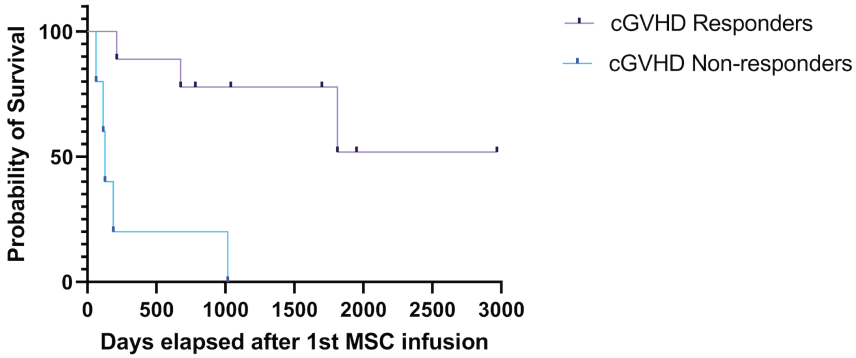
B. cGVHD

	Adults			Children			Overall		
	Responders *n* = 7	Nonresponders *n* = 5	*P* value	Responders *n* = 3	Nonresponder *n* = 1	*P* value	Responders *n* = 10	Nonresponders *n* = 6	P value
Sex			.072			>.999			.119
Male	1 (20.00)	4 (80.00)		2 (66.67)	1 (33.33)		3 (37.50)	5 (62.50)	
Female	6 (85.71)	1 (14.29)		1 (100)	0 (0.00)		7 (87.50)	1 (12.50)	
HCT-CI			>.999			>.999			>.999
0-1	4 (66.67)	2 (33.33)		2 (66.67)	1 (33.33)		6 (66.67)	3 (33.33)	
>1	3 (50.00)	3 (50.00)		1 (100.00)	0 (0.00)		4 (57.14)	3 (42.86)	
GVHD prophylaxis			.489			.250			.216
CsA/MTX	2 (66.67)	1 (33.33)		3 (100.00)	0 (0.00)		5 (71.43)	2 (28.57)	
CsA/MMF	3 (75.00)	1 (25.00)	0 (.00)	0 (0.00)					
Tacrolimus/SRL	2 (66.67)	1 (33.33)		0 (0.00)	0 (0.00)		0 (0.00)	1 (100.00)	
Tacrolimus	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)		3 (100)	0 (0.00)	
Tacrolimus/MMF	0 (00.00)	0 (0.00)		0(0.00)	0 (0.00)		2 (66.67)	1 (33.33)	
CsA/Pred	0 (0.00)	1 (100.00)		0(0.00)	1(100.00)		0 (0.00)	1 (100.00)	
Other	0 (0.00)	1 (100.00)		0 (0.00)	0 (0.00)		0 (0.00)	1 (100.00)	
Conditioning			.239			>.999			.227
Ablative	2 (58.82)	0 (41.18)		3 (75.00)	1 (25.00)		5 (83.33)	1 (16.67)	
No- ablative	0 (0.00)	1 (100.00		0 (0.00)	0 (0.00)		0 (0.00)	1 (100.000)	
Reduced intensity	5 (100.00)	4 (0.00)		0 (0.00)	0 (0.00)		5 (55.56)	4 (44.44)	
HLA matching			>.999			–			.711
Identical	5 (55.56)	4 (44.44)		2 (66.67)	1 (33.33)		7 (58.33)	5 (41.67)	
Haploidentical	2 (66.67)	1 (33.33)		0 (0.00)	0 (0.00)		2 (75.00)	1(25.00)	
Others	0 (0.00)	0 (0.00)		1 (100.00)	0 (0.00)		1 (100.00)	0 (0.00)	
cGVHD severity	7	4	.658	3	1	>.999	10	5	.692
Low	1 ()	0 (0.00)		1 (100.00)	0 (0.00)		1 (100.00)	0 (0.00)	
Moderate	1 ()	1 (50.00)		0 (0.00)	0 (0.00)		1 (50.00)	1 (50.00)	
Severe	5 ()	3 (33.33)		2 (66.66)	1 (33.33)		8 (66.67)	4 (33.33)	
IS Lines			.151			>.999			.604
MSC as second and third line	7 (70.00)	3 (30.00)		1 (50.00)	1 (50.00)		8 (66.67)	4 (33.33)	
MSC as fourth and fifth line	0 (0.00)	2 (100.00)		2 (100.00)	0 (0.009)		2 (50.00)	2 (50.00)	

A



B



C

	aGVHD			cGVHD		
	Adults N=41	Children N=5	Overall N=46	Adults N=12	Children N=4	Overall N=16
Complete response	11 (26.82)	5 (100)	16 (34.78)	5 (41.67)	1 (25.00)	6 (37.50)
Partial response	11 (26.82)	0 (0.00)	11 (23.91)	2 (16.67)	2 (50.00)	4 (25.00)
No response	19 (46.34)	0 (0.00)	19 (41.30)	5 (41.67)	1 (25.00)	6 (37.50)
Overall response	22 (53.65)	5 (100)	27 (58.69)	7 (58.34)	3 (75.00)	10 (62.50)

Figure 3. Response and Kaplan–Meier survival analysis. (A) Overall survival estimates for aGVHD patients, for responders (solid purple line), and for non-responders (solid blue line). (B) OS estimates for cGVHD patients, for responders (solid purple line), and for non-responders (solid blue line) (C) aGVHD and cGVHD response for adults, children, and overall.

in our meta-analysis.[35] It is encouraging to see the aGVHD results in patients who were resistant to steroids and other immunosuppressant treatments; these response rates should be understood considering the heterogeneity of the patients and the inherent characteristics of a compassionate use program that includes patients with serious or immediately life-threatening conditions. Confirming previous studies, in our series, children achieved better responsiveness despite having a slightly higher severity than adults (77.78% of the patients of the pediatric sub-group exhibited aGVHD grade IV and severe cGVHD versus 71.70% in adults). Although it was a small number of patients, we found that age at the time of HSCT was inversely correlated with survival in aGVHD. Indeed, it should be pointed out that there are currently two

Table 3. Survival analysis for aGVHD and cGVHD group: Cox regression

Variables	*B*	SE	Wald	Haz. ratio	95% Conf. interval	*P* value
A. aGVHD						
Sex	–0.92	0.53	0.03	0.91	0.32–2.57	.862
Age	0.04	0.01	7.90	1.04	1.01–1.07	.005
Therapy lines prior to MSC	0.49	0.55	0.78	1.63	0.55–4.80	.376
Response	–2.08	0.48	18.90	0.12	0.05–0.32	<.001
B. cGHVD						
Sex	–0.18	1.08	0.30	0.83	0.10–6.92	.865
Age	0.03	0.03	0.01	1.03	0.95–1.05	.919
Therapy lines prior to MSC	0.77	1.07	0.52	2.16	0.26–17.58	.472
Response	–2.56	1.09	5.47	0.07	0.01–0.66	.019

B, regression coefficient; SE, standard error; Wald, Wald statistics; Haz. ratio, hazard ratio; 95% conf. interval, confidence intervals of the hazard ratio; *P*, *P*-value.

marketing authorizations for MSC for treating pediatric aGVHD, Prochymal (remestemcel-L) in Canada and Ryoncil (remestemcel-L) in Australia.

The identification of factors affecting MSC response has remained elusive. There is still a lack of knowledge regarding demographic, clinical, or pharmacological factors that could be used to predict a clinical response. Concerning disease severity, we did not find a better response rate for patients with a low-grade aGVHD when compared with patients with high-grade aGHVD, which, although based on a small number of low-grade cases, is in accordance with previous reports.[17,39] Time elapsed from the aGVHD presentation to the initiation of the MSC therapy has also been explored as a predictor of MSC response. Intriguingly, in this series, we found that in patients with aGVHD achieving a clinical response the time interval to MSC infusion had been significantly longer than in non-responders. In contrast, Introna et al observed an opposite trend, the overall response being better in patients who received the MSC therapy within 30 days from the GVHD presentation.[40] We explored the possibility of an interaction between MSC and the supportive therapy for aGVHD that could explain this somewhat paradoxical response, considering the pharmacodynamics of the different therapies used at the time of MSC administration. For example, complete and consistent blocking of the interleukin-2 receptor is maintained with basiliximab usually up to 4–6 weeks after administration.[41] In contrast, ruxolitinib results in maximal inhibition of STAT3 phosphorylation 2 h after dosing which returns to near baseline by 8 h.[42] As we observed similar response rates in patients receiving either of these drugs, we consider that the reported response to MSC cannot be attributed to a lingering effect of the medication, although with available data we cannot rule out a synergistic effect in some cases. In any event, MSC infusion was indispensable to obtain a clinical improvement in those patients. It would be necessary to determine the optimal period to administer MSC in future studies.

As expected, OS was higher in the group of responders while it was very poor in nonresponders. Acute and chronic GVHD 2-year survival rates for responders (51.85% 14 of 27 and 70% 7 of 10 patients, respectively, remained alive) are somewhat higher in this series than those reported in other studies.[24,40]

Conclusion

Our analysis supports the use of MSC as an effective and safe therapeutic option for treating refractory GVHD, resulting in a large percentage of patients responding to the therapy and in an increase of the probability of survival for patients affected by this severe complication of allogenic HSCT.

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Conflict of Interest

The authors declared no potential conflicts of interest.

Author Contributions

M.M.M.-S.: collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of the manuscript; C.M.-T.: collection and/or assembly of data, manuscript writing, final approval of the manuscript; N.C.: conception and design, data analysis and interpretation, manuscript writing, final approval of the manuscript; A.C.-G.: collection and/or assembly of data, final approval of the manuscript; M.A.C.-C.: provision of study material or patients, collection and/or assembly of data, final approval of the manuscript.; M.J.P.-C., A.P., M.J., C.M.-C., J.A.P.-S.: provision of study material or patients, data analysis and interpretation, final approval of the manuscript; I.E., S.G.: provision of study material or patients, final approval of the manuscript; G.C.-S.: collection and/or assembly of data, final approval of the manuscript; R.M.A.-C.: conception and design, data analysis and interpretation, final approval of the manuscript; R.S.-P.: data analysis and interpretation, manuscript writing, final approval of the manuscript.

Data Availability

Data used to support the results of this study are available from the corresponding author upon request.

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