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LETTER TO THE EDITOR

Additional infusions of mesenchymal stem cells improve response rate in multidrug-resistant GvHD patients

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GvHD is one of the main limitations to the success of allogeneic hematopoietic stem cell transplantation (HSCT) and has a substantial impact on the quality of life and survival of patients. Its incidence varies from 30 to 80% and depends on several factors such as prophylaxis method, donor/recipient matching, intensity of the conditioning regimen and composition of the graft.[1] In GvHD, initial treatment relies on corticosteroids, which produce sustained responses in 50–80% of patients depending on the severity.[2,3] Currently, many drugs are used in patients refractory to steroids, but the majority of these agents are insufficient. In the last few years, studies have been published reporting successful use of mesenchymal stem cells (MSCs) in the treatment of immune-based diseases such as GvHD.[4,5] MSCs have been used in GvHD with a variety of cell doses and infusions with conflicting results due to differences in type and severity of the disease. In this study, we investigated the efficacy of additional infusions of MSCs in patients with partial response (PR) to induction therapy with MSCs.

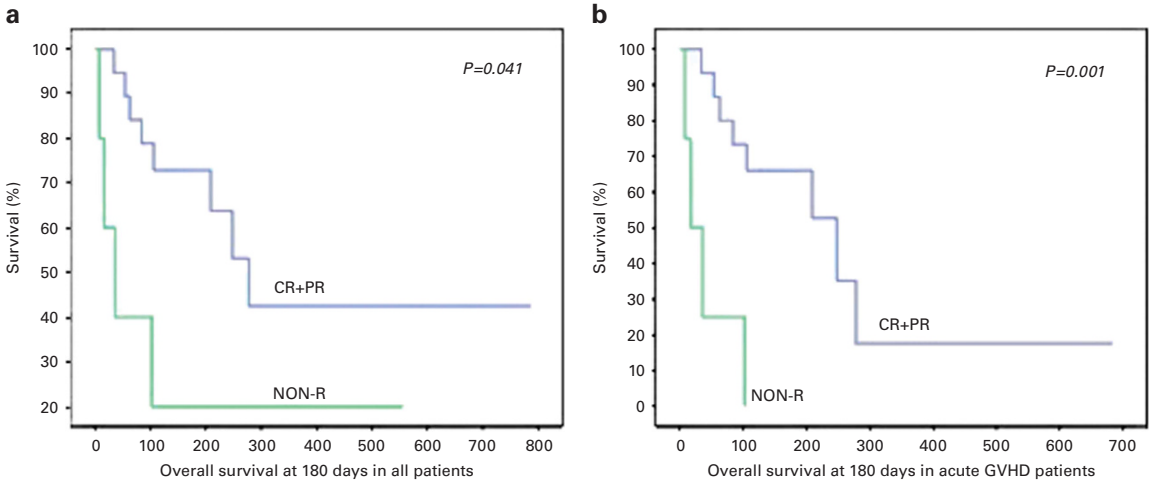
This study was conducted on 22 patients with refractory GvHD (7 females, 15 males) at the Erciyes Transplantation Center in Kayseri, Turkey between March 2013 and August 2015. Patients with acute or chronic GvHD, grade II–IV, resistant to several lines of therapy were eligible for the study. Steroid resistance was defined according to the current literature.[6] All patients received at least two lines of steroid-containing GvHD treatment before MSC therapy. Treatment was continued during MSC therapy. Steroids were gradually stopped before initiation with MSC therapy. In accordance with the current literature MSCs were given in combination with preexisting GvHD treatments.[7,8] The study was approved by the Ethics Committee of Erciyes University and National Stem Cell Council of the Ministry of Health.

MSCs were derived from the bone marrow or adipose tissue of unrelated, HLA-mismatched voluntary adult donors under good manufacture practice (GMP) conditions at the Genome and Stem Cell Center of Erciyes University (Genkok). The median dose of infused MSCs was 1.64×106 cells/kg (range 0.84–2.54). In total, 79 MSC infusions were given, consisting of 21 bone marrow-derived MSCs (18 infusions for acute GvHD and 3 for chronic GvHD) and 58 adipose-derived MSCs (43 infusions for acute GvHD and 15 for chronic GvHD). There was no difference between cell source and

Table 1. Patient characteristics before therapy with MSC

Patient			*Transplantation*			*GvHD*			
n *(#)*	*Sex/age (years)*	*Diagnosis*	*Conditioning regimens*	*Stem cell sources*	*GvHD prophylaxis*	*GvHD type*	*GvHD grade*	*Involved organ*	*Before treatments (drugs)*
1	F/26	AML	Bu/Thio/Flu/ATG	UCB	CsA/MTX/ATG	Acute	II	GI/S	CsA/MP/MMF/P
2	M/24	AML	Bu/Cy	MFD	CsA/MTX	Chronic	Extensive	L/S	CsA/MP/MMF
3	M/21	ALL	Mel/Thio/Flu/ATG/TBI	Haplo	-	Acute	III	GI/L/S	CsA/MP/MMF/P
4	F/22	AML	Mel/Thio/Flu/ATG	Haplo	-	Acute	II	GI/L	CsA/MP
5	F/26	AML	Bu/Cy	MFD	CsA/MTX	Acute	III	GI/S	CsA/MP/MMF
6	M/21	ALL	Bu/Flu	MFD	CsA/MTX	Acute	II	L	CsA/MP/MMF
7	M/50	AML	Bu/Cy	MFD	CsA/MTX	Acute	IV	L	CsA/MP/MMF/P
8	M/25	MDS	Flu/TBI	MUD	CsA/MTX	Chronic	Limited	GI/S	CsA/MP/MMF
9	F/34	AML	Bu/Cy	MFD	CsA/MTX	Chronic	Extensive	S /Art.	CsA/MP/MMF/I
10	M/29	AML	Bu/Cy	MFD	CsA/MTX	Acute	IV	GI/S/lung	CsA/MP/MMF/P
11	M/43	AML	Bu/Cy	MFD	CsA/MTX	Chronic	Limited	S/lung	CsA/MP/MMF
12	M/43	ALL	Cy/TBI	MFD	CsA/MTX	Acute	IV	GI/L	CsA/MP/MMF/P
13a	M/51	AML	Mel/Thio/Flu/ATG	Haplo	CSA	Acute	IV	L/S	CsA/MP/MMF/P
13b	M/51	AML	Mel/Thio/Flu/ATG	Haplo	CsA/MMF	Acute	IV	L/S	CsA/MP/MMF
14	F/50	MM	Flu/Mel	MFD	CsA/MTX	Acute	IV	L	CsA/MP/MMF/P
15	M/49	MDS	Bu/Cy	MFD	CsA/MTX	Acute	III	L	CsA/MP/MMF
16	F/36	AML	Bu/Cy	MFD	CsA/MTX	Acute	II	L/S	CsA/MP/MMF
17a	M/29	ALL	Cy/TBI	MFD	CsA/MTX	Acute	III	S	CsA/MP
17b	M/29	ALL	Cy/TBI	MFD	CsA/MTX	Acute	III	S	CsA/MP
18	M/46	MDS	Bu/Cy	MFD	CsA/MTX	Acute	II	GI/L	CsA/MP/MMF
19	F/37	AML	Bu/Cy	MFD	CsA/MTX	Acute	III	L/S	CsA/MP
20	M/25	AML	Bu/Cy	MFD	CsA/MTX	Acute	II	L	CsA/MP/MMF/P
21	M/47	CML	Bu/Flu/ATG	MFD	CsA/MTX	Chronic	Extensive	S /Art	CsA/MP/P/I
22	M/28	AML	Mel/Thio/Flu/ATG	Haplo	-	Acute	II	S/renal	MMF/MP

Abbreviations: Art = arterial; Bu = busulfan; CsA = cyclosporine A; Cy = cyclophosphamide; Flu = fludarabine; GI = gastrointestinal; Haplo = alpha/beta T-cell depleted haploidentical transplantation; I = imatinib mesylate; L = liver; MDS = myelodysplastic syndrome; Mel = mephalan; MFD = matched familiar donor; MMF = mycophenolate mofetil; MM = multiple myeloma; MTX = methotrexate; MUD = matched unrelated donor; P = photopheresis; S = skin; Thio = thiotepa; UCB = umbilical cord blood transplantation. a and b indicates first and second attack of same patient.



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Response type	Acute (*n*=19)	Chronic (*n*=5)	Total (*n*=24)	Skin (*n*=16)	Liver (*n*=14)	GIS (*n*=8)
Complete	9 (47.4%)	2 (40%)	11 (45.8%)	12 (75%)	7 (50%)	7 (87.5%)
Partial	6 (31.6%)	2 (40%)	8 (33.3%)	3 (18.8%)	4 (28.6%)	0 (0.0%)
Stable	2 (10.5%)	1 (20%)	3 (12.5%)	1 (6.3%)	1 (7.1%)	0 (0.0%)
Death (progress)	2 (10.5%)	0 (0.0%)	2 (8.3%)	0 (0.0%)	2 (14.3%)	*1 (12.5%)*

Response to MSCs according to type of GVHD and organ involvement

Figure 1. Response and survival rate in patients after therapy with MSCs. (**a**) Overall survival at 180 days in all patients. (**b**) Overall survival at 180 days in acute GvHD patients. (**c**) Response to MSCs according to type of GvHD and organ involvement.

clinical response ($P > 0.05$). In patients with acute GvHD, response to MSCs was evaluated after two MSC infusions with a 7–14-day interval. In patients with chronic GvHD, the response was evaluated after three MSC infusions. For acute GvHD, complete response (CR) was defined as the absence of GvHD signs. PR was defined as at least 1 grade decrease in GvHD when compared with day 0. No response (NR) was defined as no change in GvHD grade.[8] For chronic GvHD, response is defined according to the 2014 National Institutes of Health Response for Chronic Graft-Versus-Host Disease.[9] In both acute and chronic GvHD, patients who achieved a CR were followed up for 6 months. When patients received only a PR two additional infusions were administered. In non-responders MSC therapy was stopped. Patients were evaluated for safety until death, withdrawal or 180 days after the first infusion of MSCs.

The Kaplan–Meier method was used to estimate survival probabilities for each group. Log-rank test was applied to compare survival probabilities. Analyses were conducted using R 3.2.0 (www.r-project.org) software. A P value less than 5% was considered as statistically significant.

During the 6-month follow-up period, a relapse after CR was observed in two patients with acute GvHD. Overall, 24 GvHD events occurred in 22 patients who were treated with a median of 3.2 doses (range 2–7 doses) of MSCs. Of note, the majority of patients in the study population had acute GvHD (17 of 22 cases). Skin and liver were the most common affected organs; 16 patients had skin involvement and 14 patients had liver involvement. The only adverse event during infusion was arterial vasoconstriction in the using arm, after which infusion was stopped. Death occurred in two patients before response evaluation. Table 1 summarizes patient characteristics as well as details of transplantation and GvHD.

After MSC therapy, overall response rate was 79.1% (19/24). CR occurred in 11 occasions (45.8%) and PR in 8 occasions (33.3%). Five patients (20.9%) were considered unresponsive to MSCs therapy due to stable disease in 3 (12.5%). Death due to progressive disease before first evaluation occurred in two patients (8.3%). Skin was the most common involved organ ($n = 16$) with an OR rate of 93.8%. The overall response rate in patients with liver involvement ($n = 14$) was 78.6%, and that in patients with gastrointestinal tract involvement ($n = 8$) was 87.5%. Figure 1c shows response to MSC therapy according to type of GvHD and organ involvement.

After induction therapy with MSCs, consisting of two infusions for acute GvHD and three infusions for chronic GvHD, CR was achieved in three patients with acute GvHD (15.7%) and one patient with chronic GvHD (20%). However, 7 of the 15 patients (six with acute GvHD and one with chronic GvHD) with only PR after induction therapy achieved CR after additional therapy with MSCs. Patients who survived after 6 months were followed up for a median of 233 days after first MSC infusion (min 182-max 784 days). Fourteen of the 22 patients (63.6%) survived 6 months after the first MSC infusion. Survival rate in patients at 6 months was significantly higher in the responder group (CR+PR $= 17$ patients, survival rate; 76.5%, $P = 0.007$) compared with the non-responder group ($<$ PR $= 5$ patients, survival rate; 20%) (Figure 1a). In patients with acute GvHD, survival rate at 6 months was 69.2% in the responder group (CR+PR, $n = 13$, $P = 0.001$) compared with no survival in the non-responder group ($<$ PR, $n = 4$, 6-month survival rate 0.0%). (Figure 1b). Three patients died due to primary disease relapse, one patient died due to infection in the responder group. In the non-responder group, four patients died due to uncontrolled acute GvHD before evaluation at 6 months. After 6 months follow-up, all patients with chronic GvHD were alive.

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After the first publication by Le Blanc *et al.*[7], many authors reported beneficial effects and high overall response rate of MSC therapy (ranges 61.3–93%) in patients with severe acute GvHD.[3,10] In our study, overall, complete and PR rates for acute GvHD (79.0%, 47.4% and 31.6%, respectively) were in line with the current literature. In 2010, Weng *et al.*[11] evaluated 19 patients with chronic GvHD treated with MSCs and reported a response rate of 73.7%. Lucchini *et al.*[12] reported a limited benefit after only one or two infusions with MSCs in five patients with chronic GvHD (1/5 CR with flare, 2/5 PR 2/5 non-responder). Our results were similar to the mentioned studies with an overall response rate of 80%, and CR in 40% and PR in 80% of the patients with chronic GvHD.

No consensus exists on the timing of induction therapy with MSCs, on the number of infusions and the need for consolidation therapy in the treatment of steroid-resistant GvHD. There is a great variation in intervals between the MSC infusions, ranging from a week to a month. In addition, the number of MSC infusions range from a single administration to 8 infusions with varying cell dosages from 300 000 cells/kg and 3 200 000 cells/kg.[13] In our study, the first infusion of MSCs after diagnosis with acute GvHD ranges from 18 to 179 days with median 47 days and ranges from 190 to 3375 days with a median of 340 days for chronic GvHD. Our study differs because the number of MSC infusions was increased until CR was achieved in patients with PR to induction therapy. After induction therapy, CR was achieved in only three patients with acute GvHD (15.7%) and in one patient with chronic GvHD (20%). Interestingly, consolidation therapy resulted in a CR in 7 of 15 patients with a PR after induction therapy. Our study showed that consolidation therapy in patients with a PR after induction therapy doubled the CR.

In conclusion, MSCs are effective in the treatment of multi-therapy resistant GvHD. Additional infusion of MSCs in will increase CR rate in patients with PR.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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