



Research paper

The efficacy of mesenchymal stem cells in bronchiolitis obliterans syndrome after allogeneic HSCT: A multicenter prospective cohort study

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ABSTRACT

Background: Bronchiolitis obliterans syndrome (BOS) after allo-HSCT is a devastating complication with limited therapeutic options. We aimed to assess the efficacy and safety of mesenchymal stem cells (MSCs) in BOS after allo-HSCT.

Methods: This multicenter prospective cohort study enrolled 81 allo-HSCT recipients whose BOS were diagnosed within 6 months. The choice of prednisone and azithromycin combined with or without MSCs was based on patient preferences (MSC $n = 49$, non-MSC $n = 32$). The primary endpoint was response rate at 3 months, defined as the proportion of patients achieving FEV1 improvement or steroid sparing. The trial was registered at ClinicalTrials.gov (NCT02543073).

Findings: Response rate was 35/49 patients (71%, 95% CI 59 to 84%) and 14/32 (44%, 27 to 61%) in MSC and non-MSC group, respectively ($p = 0.013$). The addition of MSCs was associated with a better difference for change in FEV1 rate of decline, compared to non-MSC group (53 mL/months, 2 to 103; $p = 0.040$). The 3-year overall survival post-diagnosis was 70.6% (55.9 to 85.3%) and 58.2% (36.1 to 78.5%) in MSC and non-MSC group, respectively ($p = 0.21$). Clinical improvement was accompanied by a significant increase of interleukin (IL)-10-producing CD5+B cells. There was no statistical difference in the rates of infections and leukemia relapse between the two groups. MSCs were well-tolerated with no serious adverse events.

Interpretation: MSCs offer an effective and safe therapeutic option for BOS after allo-HSCT. Our study strengthens evidence for clinical use of MSC therapy in BOS. These data also provide novel insight into potential biological mechanisms of MSC treatment and support further investigation in larger randomized controlled trials.

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Research in context

Evidence before this study

This trial was planned based on extensive preclinical and clinical studies of mesenchymal stem cells (MSCs) for graft-versus host disease (GVHD) carried out by our research group and based on a review of publications identified by searches of PubMed from January 1, 2002 to January 1, 2014, using the search terms “(bronchiolitis obliterans syndrome OR bronchiolitis obliterans OR obliterative bronchiolitis OR chronic GVHD) AND (mesenchymal stem cells OR mesenchymal stromal cells)” with no language restrictions. We also reviewed studies of MSCs in humans for other diseases, such as idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and acute myocardial infarction. Animal studies have shown therapeutic promise of MSCs therapy for BOS and chronic GVHD, and human trials suggest that MSCs are well-tolerated even in severe lung dysfunction.

Added value of this study

To our knowledge, this is the first multicenter prospective cohort study of MSCs focused solely on lung complications after allogeneic hematopoietic stem cell transplantation. We found that the response rate was significantly improved in the MSC group. This benefit appeared to be the greatest among patients with mild and moderate BOS, and was accompanied by a significant increase in the frequency of CD5+ regulatory B cells. MSC therapy was well tolerated without increasing the incidences of infection and leukemia relapse. Overall survival did not differ significantly between the two groups.

Implications of all available evidence

Our results demonstrate that MSCs are effective and safe in patients with BOS after allo-HSCT and provide evidence for clinical practice of MSC therapy in BOS. Our data suggest that the biological mechanisms of MSC treatment are associated with CD5+ regulatory B cells. Further larger randomized controlled trials are needed to assess long-term benefits.

1. Introduction

Bronchiolitis obliterans syndrome (BOS), characterized by persistent airflow obstruction, is a devastating complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1,2]. The development of BOS has been considered to be closely associated with chronic graft-versus host disease (GVHD), in which recurrent immune attacks damage the small airways [3]. Although only affecting 4% to 6% of allo-HSCT recipients [4,5], BOS confers high morbidity and mortality, especially in patients presented with a rapid onset of forced expiratory volume in 1 s (FEV1) decline [6,7]. Current treatment of BOS mainly relies on steroid-based immunosuppressive therapy. Apart from poor efficacy, prolonged steroid exposure results in many severe adverse effects, such as infections and malignancy relapse adding to the risk of death in BOS [2,8]. Some studies, including ours, suggested that steroids combined with azithromycin could stabilize or improve lung function in some patients with BOS after allo-HSCT [9–11].

Mesenchymal stem cells (MSCs), which exert a broad spectrum of immunoregulatory effects, have been widely used to treat inflammatory and immune-mediated disorders, most successfully in the field of GVHD [12,13]. In an open-label single-arm study among lung transplant recipients, MSCs infusions have shown potential benefits for BOS [14]. Our group also observed in a small pilot study that MSCs improved steroid-resistant chronic GVHD (cGVHD)

with lung manifestations [15]. Preclinical studies have provided rationales for use of MSCs in BOS [16–18]. Accordingly, in this prospective cohort study, we assessed the efficacy and safety of MSCs combined with azithromycin and prednisone on lung function of patients with HSCT-related BOS.

2. Methods

2.1. Study design and patients

This was a multicenter, open-label, phase I/II, prospective cohort study performed at six centers across China (Nanfang Hospital, Guangzhou First People's Hospital, SUN Yat-Sen Memorial Hospital, Guangzhou Provincial People's Hospital, Zhongshan City People's Hospital, and Third Affiliated Hospital of SUN Yat-Sen University). Consecutive patients were recruited to receive MSCs plus azithromycin and prednisone (the MSC group) or azithromycin and prednisone alone (the non-MSC group) based on patient choice. Eligible patients were allo-HSCT recipients aged at least 18 years, and diagnosed with HSCT-associated BOS for less than 6 months at the time of enrollment. BOS was clinically diagnosed according to modified National Institutes of Health (NIH) criteria: FEV1/vital capacity < 0.7, FEV1 < 75% predicted along with ≥10% decline from the pre-transplantation baseline, and absence of active respiratory infections [3,19]. Exclusion criteria included: 1) previous MSCs therapy for GVHD; 2) prior azithromycin therapy exceeding 1 month during the past 3 months; 3) uncontrolled respiratory infections; 4) baseline post-bronchodilator FEV1 > 75% or less than 20% predicted; 5) contraindications to steroids; 6) abnormal pre-transplant spirometry parameters; 7) leukemia relapse; 8) life expectancy of less than 3 months; 9) any conditions not suitable for the trial as judged by the study investigator.

Eligibility was determined by the local investigator and confirmed by expert review before study entry. The protocol was approved by institutional review boards at each participating center. Written informed consent was obtained from all participants. The study was undertaken in accordance with the Helsinki Declaration.

2.2. Procedures

In the MSC group, MSCs were intravenously given at a median dose of 1×10^6 cells/kg once weekly for 4 consecutive weeks as a cycle. If tolerated, a second cycle was given at a 2-week interval. In both the MSC and the non-MSC group, prednisone was given initially at 1 mg/kg/day for 4 weeks, and then gradually tapered by 10% of the total dose per week. Oral azithromycin was maintained at 250 mg daily in a 4-week cycle for a total of 3 cycles. Patients were withdrawn if leukemia relapsed, or treatment caused uncontrollable infection or toxicity, or FEV1 rapidly lost for more than 10% predicted at 1 month. Patients were also withdrawn based on the clinical judgment of the treating physician. During the treatment period, concomitant use of other immunosuppressive agents, such as calcineurin inhibitors and mycophenolate mofetil, was allowed as deemed necessary for active extra-thoracic GVHD, whereas tyrosine kinase inhibitors or leukotriene antagonists were not. Additional systemic treatment was not allowed during the 3-month treatment period. Patients in whom an infection was identified were given antibiotics treatment. Patients were required to return to the out-patient clinic at follow-up time points and any intervals for reasons such as recurrence or progression of lung manifestations during the follow-up.

2.3. Spirometry

FEV1 was measured at enrollment, 1 and 3 months after treatment according to American Thoracic Society guidelines [20]. FEV1

was used to grade BOS severity according to the NIH Lung Score [4]. If a patient had an episode of infection at the time points, spirometry was postponed for at least 1 month to allow resolution of infection. Predicted values for FEV1 were calculated using published formulae [20]. Pre-transplant FEV1 were collected from the pre-transplant evaluation, which is completed within one month before allo-HSCT. FEV1 data within 9 months prior to enrollment were also collected from electronic medical record.

2.4. MSC manufacture

Human bone marrow (BM) cells were obtained from healthy third-party unrelated donors with written informed consent. Isolation, expansion and characterization of MSCs were performed as described previously under good manufacturing practice (GMP) condition [21]. Release criteria for MSCs included purity, viability, endotoxin and pathogen-free.

2.5. Endpoints

The primary endpoint was the response rate at 3 months after treatment, defined as the proportion of patients who achieved FEV1 improvement (an increase in FEV1) or steroid sparing (at least 50% dose reduction without disease progression). Disease progression was defined as FEV1 decrease from baseline by 10% predicted or more as suggested by the NIH Consensus for cGVHD [22]. Patients with missing primary endpoint data, such as for early withdrawal or death, were regarded as no response at 3 months. Secondary endpoints included the following: the treatment effect on FEV1 decline, the incidence of infection and leukemia relapse, safety, leukemia-free survival and overall survival (OS). Infections were diagnosed by new-onset clinical signs, such as fever, an elevated C reactive protein, positive cultures (upper respiratory tract viral screen, sputum, blood, bronchoalveolar lavage, or another site) or radiology, and the clinically identified need to administer medication. Leukemia relapse was defined according to previously published criteria [21]. Adverse events were graded using the Common Terminology Criteria for Adverse Events (CTCAE version 3.0). OS was defined as the time from BOS diagnosis to death of any cause. Leukemia-free survival was defined as the time in continuous complete remission without leukemia relapse. To evaluate the therapeutic mechanism of MSCs in BOS, peripheral B-cell subsets were detected at enrollment and 3 months in both groups [15].

2.6. Statistical analysis

The sample size was estimated on the basis of the response rate. Based on our previous single-center pilot study [15], 32 patients in each group would be needed to have 80% power to detect a 30% difference in response with a one-side type I error at 0.05. The study finally enrolled 49 patients in the MSC group and 32 in the non-MSC group. A *post-hoc* power analysis indicated that the final sample size would be adequate to detect a 28% difference.

Data were analyzed on December 31, 2018. The primary endpoint was analyzed using χ^2 test. Odds ratio (OR) and 95% confidence intervals (CI) were calculated and adjusted for factors that reported to influence FEV1 and prognosis, including timing of BOS onset (BOS within 1 year of HSCT or not), BOS severity at enrollment, and the baseline rate of FEV1 decline [7,8,23]. The rate of FEV1 decline was estimated by regression line using months and FEV1 values (FEV1 data in the 9 months preceding enrollment were used to estimate baseline values) and expressed in% predicted values and mL/month. Univariate and multivariate linear regression analyses were performed to estimate treatment effect on FEV1 decline. Wilcoxon signed-rank test was used to compare the data obtained before and after treatment. Differences between

groups were compared using χ^2 or Fisher's exact tests for proportions and Student's *t*-tests or Mann-Whitney U tests for continuous variables. Kaplan-Meier analysis with the log-rank test was used to compare survival. The linear mixed effect model were performed using SAS/STAT version 9.4 software. All the other tests were performed using the SPSS version 19.0. A *p* value <0.05 was deemed statistically significant.

This study was registered at ClinicalTrials.gov (NCT02543073).

3. Results

3.1. Patient characteristics

Between September 2014 and December 2017, 94 allo-HSCT recipients with BOS diagnosed within 6 months were screened and 81 of them were enrolled in this study, including 49 in the MSC group, 32 in the non-MSC group (Fig. 1). Thirty-nine patients underwent all-HSCT for myelogenous leukemia and 42 for lymphocytic leukemia. The demographic, baseline and transplantation characteristics of study population were similar between the two groups (Table 1). Most participants exhibited moderate to severe BOS and had a rapid decline of lung function (Table 1 and 2). The median FEV1% predicted at enrollment were similar in the MSC and the non-MSC group, (42.3% [range 20.3–64.1] and 44.1% [range 23.5–66.1], respectively, *p*=0.52) (Table 1). All the patients received prednisone at the study onset, with a median dose of 0.85 mg/kg and 0.82 mg/kg daily in the MSC group and the non-MSC group, respectively (*p*=0.60). Thirty-six (73%) patients in the MSC group and 22 (69%) in the non-MSC group received prednisone as a new medication or experienced a dose increase. The median time to diagnosis of BOS from transplantation was 13.9 months (range 5.1 to 76.5 months) in the MSC group and 12.9 months (range 5.8–44.9 months) in the non-MSC group (*p*=0.69).

3.2. Treatment response

Totally 73 participants completed treatment at 3 months, including 45/49 (92%) in the MSC group and 28/32 (88%) in the non-MSC group (*p*=0.71). A total of 379 doses of MSCs were administered to 49 patients. Four patients in each group discontinued treatment, mainly due to rapid loss of FEV1. One patient in the non-MSC group died of pneumomediastinum associated with BOS progression (Fig. 1).

After 3 months of treatment, the proportion of patients who had achieved response was 35/49 (71%, 95% CI 59–84%) in the MSC group (FEV1 improvement, *n*=10; steroid sparing, *n*=25), compared with 14/32 (44%, 95%CI 27–61%) in the non-MSC group (FEV1 improvement, *n*=3; steroid sparing, *n*=11) (*p*=0.013). The adjusted OR of response in the MSC group compared with the non-MSC group was 3.32 (95%CI 1.21–9.11; *p*=0.020). FEV1 stabilization and improvement occurred in 41 (84%) patients in the MSC group and 23 (72%) in the non-MSC group (*p*=0.20). Among the evaluable patients, steroid reduction by at least 50% was superior in the MSC group (34/45 [76%]) than in the non-MSC group (13/28 [46%]) (*p*=0.012). Response was then compared based on BOS severity at enrollment.

For mild and moderate BOS, response was significantly higher in the MSC group than in the non-MSC group (18/26 [69%, 95%CI 51–87%] vs. 7/21 [33%, 95%CI 13–53%], OR 4.50 [95%CI 1.31–15.42]). But there was no statistical difference between the two groups for severe BOS (17/23 [74%, 95%CI 56–92%] vs. 7/11 [64%, 95%CI 35–92%], OR 1.62 [95%CI 0.35–7.56]).

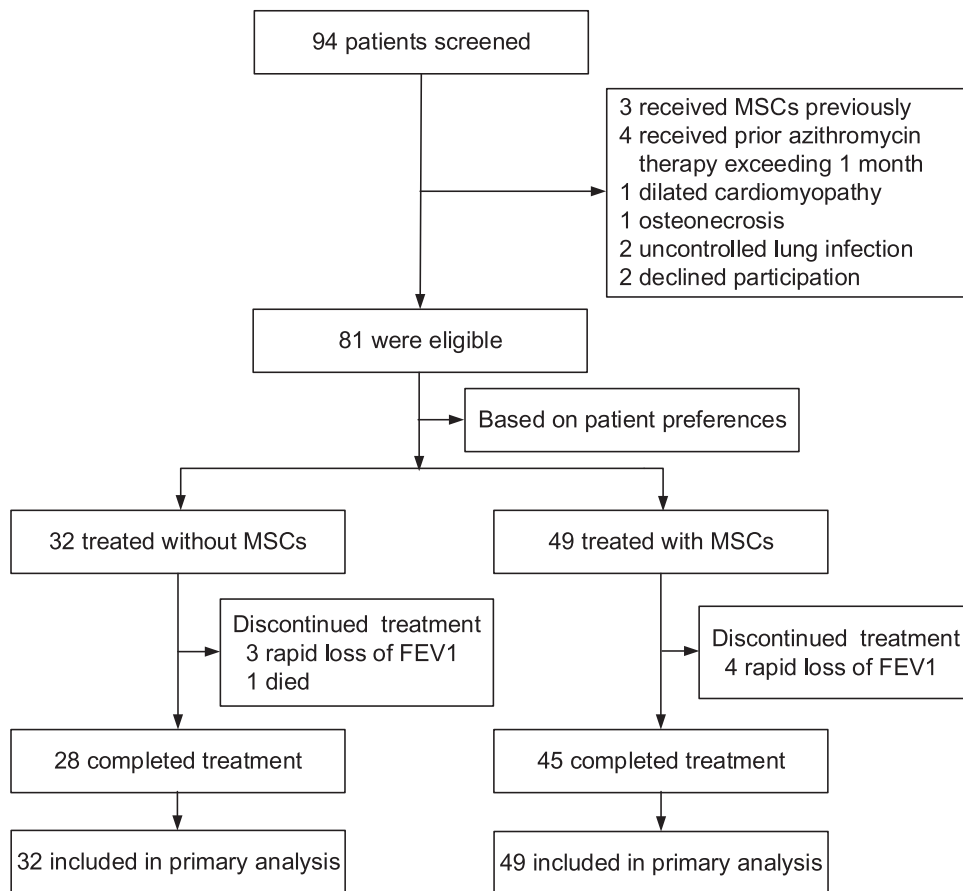


Fig. 1. Trial profile.

3.3. FEV1 evolution

The baseline rates of FEV1 decline were similar between the two groups (MSC 169 vs. non-MSC 156 mL/month, $p = 0.569$). After 3 months of treatment, the rates of FEV1 decline were statistically lower in the MSC group than in the non-MSC group (27 vs. 48 mL/month, $p = 0.035$) (Fig. 2 and Table 3). Univariate regression analysis showed that MSCs were associated with a better difference for change from baseline in FEV1 rate of decline at 3 months versus no MSC treatment (53 mL/months, 95% CI 2–103; $p = 0.040$). This result also did not change in multivariate analysis with adjustment for timing of BOS onset and BOS severity at enrollment (51 mL/months, 0–102; $p = 0.050$).

Besides the results using simple regression analysis shown above, a linear mixed effect model was used to determine whether the changes from baseline values (enrollment) between groups were significantly different. As shown in Table 3, the estimated difference in rate of FEV1 decline between the two groups was -0.076 ($p = 0.048$), which means that the rate of decline in FEV1 for the non-MSC group was significantly greater than that for the MSC group.

3.4. B-cell subsets

Our previous study suggested that amelioration of refractory cGVHD by MSCs was associated with changes in peripheral B-cell subsets [15]. Thus, flow cytometry was performed to detect B-cell subsets in the two groups before and after treatment. There were no significant changes in the number and frequency of CD19+ B cells in either the MSC or the non-MSC group ($p > 0.05$; Fig. 3A and B). The frequencies of CD5+ B cells and interleukin (IL)-10-producing CD5+ regulatory B cells (Bregs) in CD19+ B cells were significantly increased after MSC treatment at 3 months ($p < 0.01$; Fig. 3C and D), whereas no such change was observed in non-MSC group.

3.5. Survival

In the MSC group, 15 (31%) patients died during a median follow-up duration of 22.9 months (IQR, 11.9–32.0); the causes of death included respiratory infection ($n = 4$), respiratory failure associated with BOS progression ($n = 6$) and nonpulmonary causes ($n = 5$). In the non-MSC group, 13 (41%) patients died during a median follow-up duration of 19.8 months (IQR, 9.8–26.8); the

Table 2

FEV1 rate of decline at baseline and 3 months reported as median values with interquartile range (IQR).

	Pre-treatment median (IQR)		Post-treatment median (IQR)	
	MSC ($n = 48$)	Non-MSC ($n = 32$)	MSC ($n = 45$)	Non-MSC ($n = 28$)
ml/month	169 (110, 235)	156 (118, 213)	27 (3, 55)	48 (28, 86)
% predicted	4.79 (3.51, 7.56)	4.36 (3.25, 6.18)	0.83 (0.03, 1.66)	1.17 (0.79, 2.03)

Table 1
Demographic, baseline and transplantation characteristics of the study population.

Variables	MSC (n = 49)	Non-MSC (n = 32)	p value
Age at enrollment, yr, median (range)	32(18–59)	25(18–52)	0.76
Gender, n (%)			0.70
Female	22(45)	13(41)	
Male	27(55)	19(59)	
Primary disease, n (%)			0.27
Myelogenous leukemia	26(47)	13(41)	
Lymphocytic leukemia	23(53)	19(59)	
Disease status at transplantation, n (%)			0.38
CR	38(78)	22(69)	
NR	11(22)	10(31)	
Conditioning regimens, n (%)			0.20
TBI	25(51)	21(66)	
Non-TBI	24(49)	11(34)	
GVHD prophylaxis, n (%)			0.65
ATG	13(27)	10(31)	
Non-ATG	36(73)	22(69)	
Stem cell origin, n (%)			1.0
PBSCs	42(86)	28(87.5)	
PBSCs+BM	7(14)	4 (12.5)	
HLA typing, n (%)			0.87
Match	39(80)	25(78)	
Mismatch	10(20)	7(22)	
BOS severity, n (%)			0.55
Mild	4(8)	3(9)	
Moderate	22(45)	18(56)	
Severe	23(47)	11(34)	
Extrathoracic cGVHD, n (%)			0.45
0	10(20.4)	4(12.5)	
1 Organ	11(22.4)	12(37.5)	
2 Organs	19(38.8)	12(37.5)	
3+ Organs	9(18.4)	4(12.5)	
Other sites involved in cGVHD, n (%)			0.87
Skin	29(59)	18(56)	
Eyes	9(18)	7(22)	
Oral	20(41)	15(47)	
Liver	16(33)	6(19)	
Joint	1(2)	1(3)	
Gut	2(4)	2(6)	
Vagina	1(2)	0(0)	
Steroid dose, mg/kg, median (IQR)	0.82 (0.72–1.0)	0.85 (0.75–1.0)	0.60
Steroid given as a new medication or increased, n (%)	36 (73%)	25 (78%)	0.64
Time to BOS from transplantation, months, median (range)	13.3 (5.1–76.5)	12.9 (5.8–44.9)	0.69
FEV1 at the time of HSCT, L, mean (SD)	3.25 (0.70)	3.30 (0.67)	0.74
% predicted, median (range)	95.9 (80.6–137.9)	95.3 (80.3–149.1)	0.49
FEV1 at enrollment, L, mean (SD)	1.40 (0.52)	1.48 (0.54)	0.56
% predicted, median (range)	42.3 (20.3–64.1)	44.1 (23.5–66.1)	0.52

Table 3
Test of fixed effect in the linear mixed effect model (using random slope or intercept).

Fixed Effects Effect	MSC	Estimate	Standard error	degree of freedom	t value	p
Intercept		1.42	0.078	75	18.22	<0.0001
group	0	0.057	0.12	75	0.46	0.64
group	1	0				
time		−0.096	0.023	71	−4.10	0.0001
time*group	0	−0.076	0.038	71	−2.01	0.048
time*group	1	0				

Group 0 = non-MSC group and group 1 = MSC group.

causes of death included respiratory infection ($n=5$), respiratory failure associated with BOS progression ($n=5$) and nonpulmonary causes ($n=3$). Causes of death were comparable between the two groups ($p=0.89$). OS was not significantly different between the two groups (hazards ratio [HR] 0.62, 95% CI 0.30–1.31; log-rank $p=0.21$), with an estimated 3-year post-diagnosis OS of 70.6% (95%CI 55.9–85.3%) for the MSC group and 58.2% (95%CI 36.1–78.5%) for the non-MSC group (Fig. 4A). The leukemia-free survival was also similar between the two groups (HR 0.63, 95% CI 0.30–1.34; log-rank $p=0.23$) (Fig. 4B).

3.6. Infections and leukemia relapse

During the 3-month treatment period, 13 (27%) patients in the MSC group developed 19 episodes of infections, including bacterial infection ($n=6$), fungal infection ($n=2$), viral infection ($n=4$), mixed bacterial and fungal infection ($n=2$) and infection of unknown etiology ($n=5$). Ten (31%) patients developed 15 episodes of infections in the non-MSC group, including bacterial infection ($n=4$), fungal infection ($n=3$), viral infection ($n=2$), mixed bacterial and fungal infection ($n=2$), and infection of unknown eti-

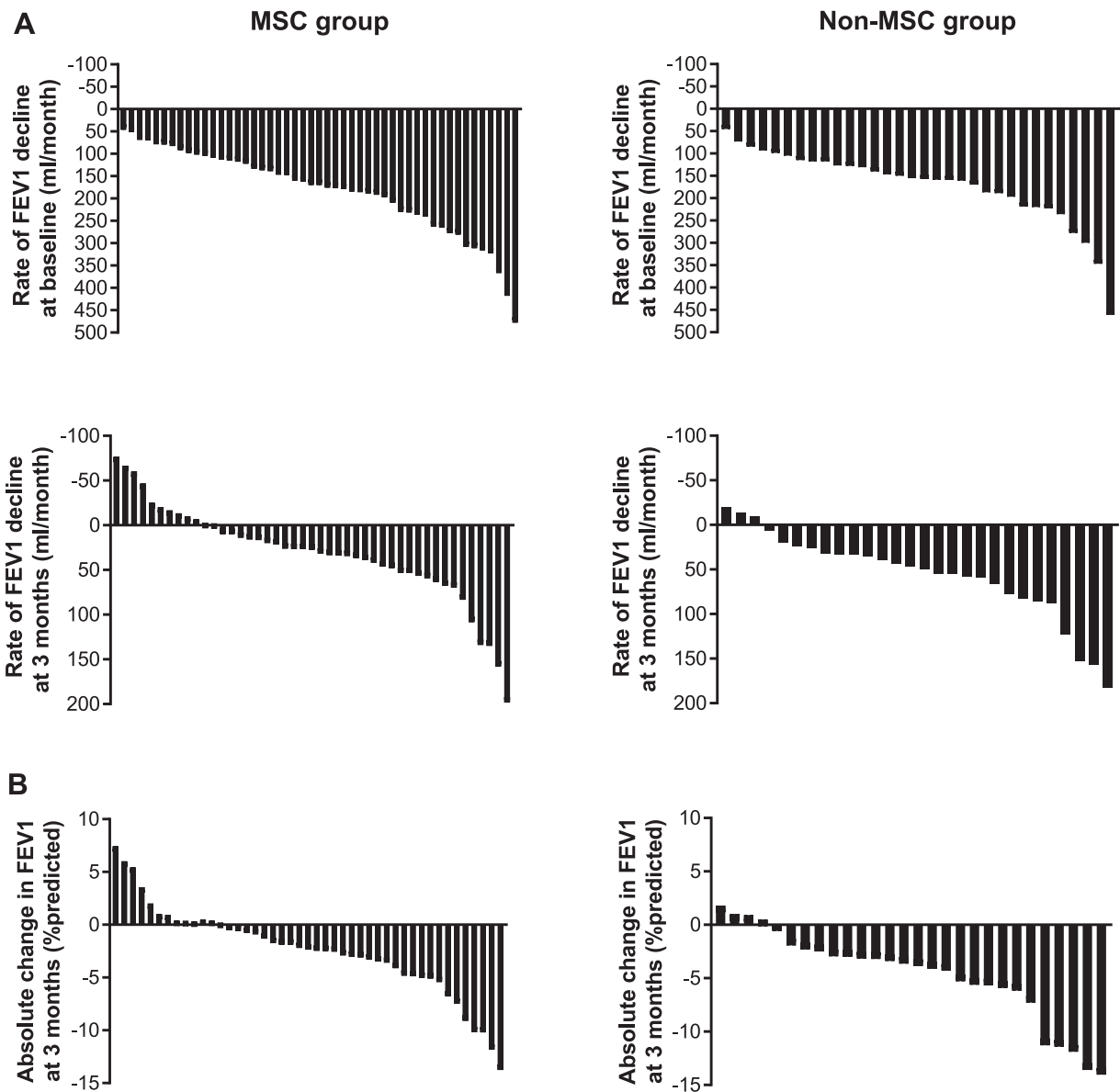


Fig. 2. Individual distribution of each evaluable patient's FEV1 evolution before and after 3-month treatment in each group. FEV1 evolution expressed both in the rate of FEV1 decline (mL/month; A) and absolute change in FEV1 (% predicted; B) compared to baseline at enrollment.

ology ($n=4$). The incidence of infection episodes was not significantly different between the groups during the treatment period ($p=0.34$).

During the long-term follow-up, one patient in the MSC group experienced a relapse of primary malignancy. The time to relapse was 23.1 months post-transplantation and 5.7 months after MSC initiation. This patient subsequently died of leukemia.

3.7. Safety

Multiple infusions of MSCs were well-tolerated with no acute infusional toxicity. Forty-two patients experienced at least one treatment-emergent adverse event (TEAE): 27 (55%) in the MSCs group and 15 (47%) in the non-MSCs group. The most common TEAEs were related to infections and grade 1–2 liver dysfunction and renal impairment. No grade 3/4 adverse event and secondary tumors were observed in the MSC group.

4. Discussion

In this study, we prospectively investigated the efficacy and safety of MSCs as an alternative treatment for BOS. Our study demonstrated that MSC infusion was associated with a significantly increased response in BOS patients after allo-HSCT, including FEV1 improvement and steroid sparing, compared with non-MSC treatment at 3 months. Multiple infusions of MSCs were well-tolerated without increasing the incidences of infections and leukemia relapse. To the best of our knowledge, this is the first prospective study of MSCs focusing solely on patients with BOS following allo-HSCT.

The pathogenesis of BOS after allo-HSCT is still unclear. Some studies suggested that allo- and auto-immune attacks to host bronchioles, leading to airflow obstruction [1,2]. MSCs have potent immunomodulatory effects on both innate and adaptive immune cells, and have shown promise in preclinical and clinical therapies for a variety of inflammatory and immune diseases [12,13,24].

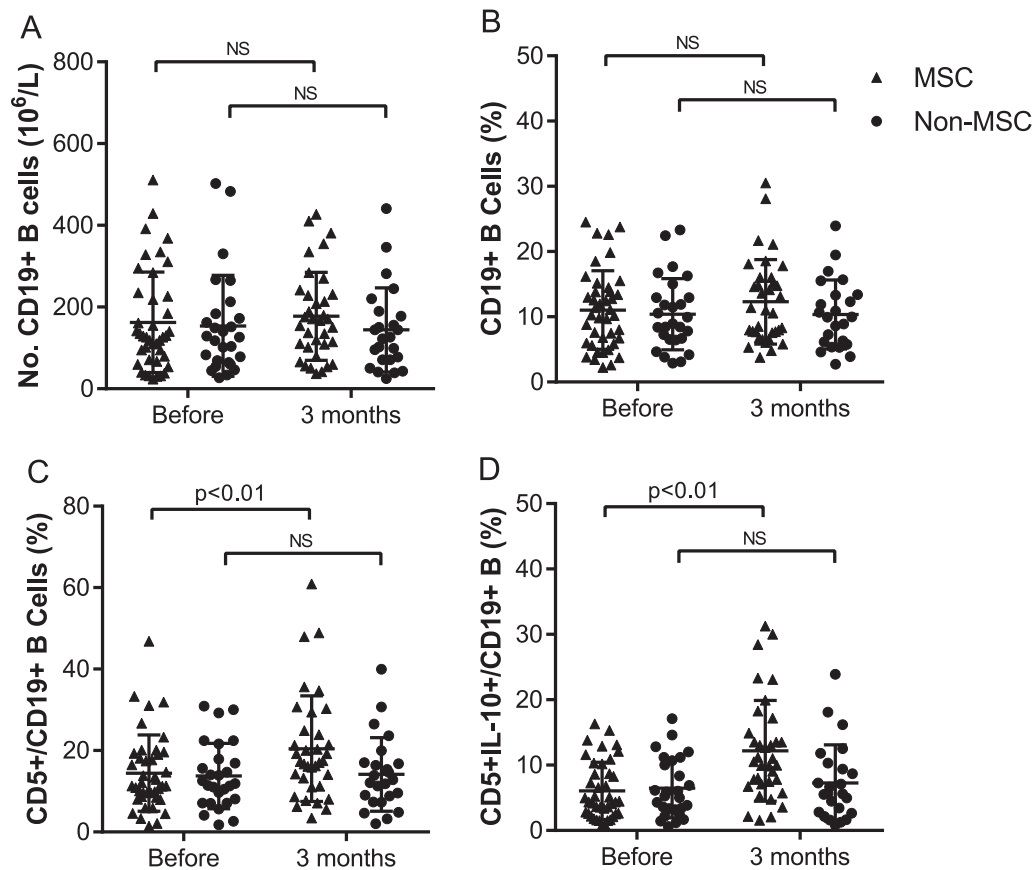


Fig. 3. The increased frequencies of CD5+ and CD5+IL-10+ regulatory B cells after MSC treatment.

The number (A) and frequency (B) of CD19+ B cells, the frequencies of CD5+ B cells (C) and CD5+IL-10+ regulatory B cells (D) were detected before (MSC $n=41$; non-MSc $n=27$) and after 3-month treatment (MSC $n=35$; non-MSc $n=24$). The data are expressed as mean \pm SD. NS, not significant.

In this study, we compared the outcomes of two cohorts of BOS patients after allo-HSCT, who received steroids and azithromycin alone or combined with MSCs. We found that the efficacy of steroids and azithromycin combined with MSCs was significantly superior to that of steroids and azithromycin alone, with 71% of patients achieving response in the MSC group versus 44% in the non-MSc group. MSC group showed a significant improvement in FEV1 rate of decline at 3 months from baseline (53 mL/months, 2–103; $p=0.040$) relative to the non-MSc group even adjusted for timing of BOS onset and BOS severity at enrollment (51 mL/months, 0–102; $p=0.050$). Linear mixed model analysis also showed that MSC treatment was associated with a significantly change in FEV1 decline rate from enrollment between groups, suggesting that MSCs could lead to a better control of progression.

Unfortunately, we did not observe the ability of MSCs to reverse the natural history of BOS in the majority of patients in the MSC group, for only 3/49 (6%) MSC-treated patients had a 5% improvement in FEV1 at 3 month. This rate is lower compared with a recent prospective single-arm study of Fluticasone, Azithromycin, and Montelukast (FAM) treatment, which showed that 13/36 (36%) patients with newly diagnosed BOS had a 5% improvement in FEV1 at 3 months [25]. Some factors may have influenced potential MSC effects on reversal of BOS in the current study. For example, the dosing and treatment interval used were empirically based on data from MSC studies in GVHD and may not be appropriate to achieve the maximum clinical benefit in BOS patients. Concomitant use of azithromycin may also interfere the effects of MSCs or their derivative cellular or molecular product, because azithromycin has

pleiotropic but poorly characterized effects on the immune system. In addition, the full spectrum of pathophysiology that contributes to lung function decline in BOS may not all amenable to MSC treatment. Furthermore, we cannot exclude the possibility that differences exist between our study and the FAM study, such as the heterogeneous pathological condition of BOS patients and additional systemic treatment. A considerable amount of research are warranted concerning the cellular and molecular pathogenesis of BOS as well as the mechanisms MSCs utilize to impact BOS development.

In addition, our study showed that there was no significant difference in the response rate between the MSC group and the non-MSc group in patients with severe BOS (74% vs. 64%) and patients with severe BOS in both groups had a higher response compared to mild and moderate patients (74% vs 69% in the MSC group; 64% vs 33% in the non-MSc group). One reasonable explanation is the natural course of FEV1 evolution in BOS, which is known to sometimes taper as disease progresses [26]. FEV1 evolution may have already leveled off for the majority of severe patients who were diagnosed at a late stage of the disease.

With improvements in supportive care and clinical recognition, the mortality in BOS patients after allo-HSCT has decreased, with 2- to 3-year OS of 60% to 75% [1,23]. In this study, the 3-year OS of the MSC group was numerically but not statistically significantly higher than that of the non-MSc group (70.6% vs. 58.2%), which may be due to the small size of the patient population and the limited follow-up duration. Moreover, it should be noted that we only observed FEV1 evolution for a relatively short period and no

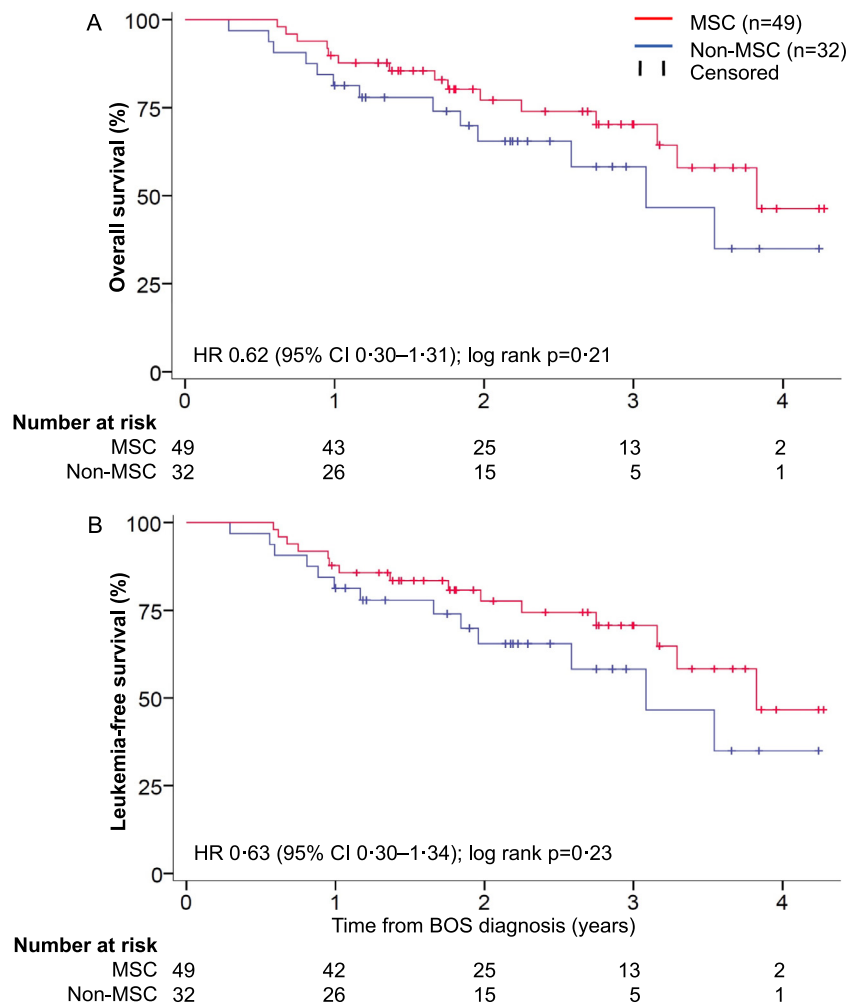


Fig. 4. Survival analysis for both the MSC and the non-MSC group after BOS diagnosis. Kaplan-Meier curves for overall survival (A) and leukemia-free survival (B).

further MSC infusions were given thereafter. The risk of death from respiratory failure in this study was comparable between the two groups, also suggesting that the treatment effects of MSCs might not be sustained in the longer term. Like other chronic airway diseases, respiratory infection is another critical factor associated with disease progression and subsequent mortality in BOS patients. However, this result needs further confirmation in larger randomized controlled trials.

The role of B cells in cGVHD pathogenesis has been receiving increasing attention over the past decade [27]. Patients who develop cGVHD have an obvious deficiency of CD5+ B-cell reconstitution, whereas non-GVHD patients exhibit a more rapid recovery [28]. CD5+ Bregs secreting anti-inflammatory cytokine IL-10 are important for controlling allergic and autoimmune diseases in humans as well as cGVHD in animal [29,30]. We have previously demonstrated that the treatment effects of MSCs on refractory cGVHD were associated with an increase of in the frequency of CD5+ Bregs [15]. Consistent with this observation, we also observed similar tendency in the frequency of Bregs in the MSC treatment group. The role of CD5+ Bregs in mediating the biologic effects of MSCs needs to be further clarified.

It remains a controversial issue whether MSCs increase the incidences of infections and leukemia relapse. Some studies reported that MSCs led to a higher risk of infection and relapse [31,32].

However, other studies, including ours, found that MSCs might have several antimicrobial effects both *in vitro* and *in vivo* [12,33–35]. In the present study, MSC treatment was not associated with increased incidences of infections and relapse, consistent with our previous reports [18,36,37].

This study has several important limitations. First, the randomization, double-blind and placebo study design were not implemented in our study for ethical, feasible and economic reasons, although all consecutive BOS patients after allo-HSCT were included. Second, comparisons were only made at 3 months and the duration of response of MSCs were not assessed. Because the additional modifications in immunosuppressive therapy were allowed after 3 months, only 3-month results were selected as the primary endpoint to minimize confounding factors. As a consequence, our results should be interpreted with caution and need to be confirmed in a larger randomization study evaluating the long-term efficacy. Furthermore, although the influence of possible confounding factors was adjusted into the analysis of the primary outcome, including timing of BOS onset, BOS severity at enrollment, and the baseline rate of FEV1 decline, residual confounding may persist and findings need to be interpreted cautiously.

In conclusion, we demonstrate that MSCs may be a safe and effective therapy for BOS patients after allo-HSCT. Our study

strengthens evidence for clinical practice of MSC therapy in BOS patients.

5. Contributors

QFL and APX designed the study and developed the protocol.

QFL, APX, SC, KZ, RL, and SQW analyzed and interpreted the data, and wrote the paper.

SC, KZ, RL, SQW, ZPF, FH, XYC, DNN, XD, ZWG, DJL, LX, NX, and JS were responsible for enrollment and data collection.

QFL and APX contributed to study supervision.

All authors read and approved the final version before submission.

Declaration of Competing Interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Shan Chen: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Data curation. **Ke Zhao:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Data curation. **Ren Lin:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Data curation. **Shunqing Wang:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Data curation. **Zhiping Fan:** Data curation. **Fen Huang:** Data curation. **Xiaoyong Chen:** Data curation. **Danian Nie:** Data curation. **Xin Du:** Data curation. **Ziwen Guo:** Data curation. **Dongjun Lin:** Data curation. **Li Xuan:** Data curation. **Na Xu:** Data curation. **Jing Sun:** Data curation. **Andy Peng Xiang:** Conceptualization, Formal analysis, Supervision, Writing - review & editing. **Qifa Liu:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ebiom.2019.09.039](https://doi.org/10.1016/j.ebiom.2019.09.039).

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