Inhaled granulocyte-macrophage colony stimulating factor for mild-to-moderate autoimmune pulmonary alveolar proteinosis - a phase II randomized study for 6 months and follow-up to 24 months

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Abstract

Background: Treatment of autoimmune pulmonary alveolar proteinosis (aPAP) by inhaled granulocyte-macrophage colony stimulating factor (GM-CSF) is considered safe and effective. Evidences of benefit from GM-CSG inhalation for mild to moderate aPAP patients are limited.

Methods: In this multicenter, randomized, open-labeled clinical trial, 36 aPAP patients with mild to moderate disease severity were randomized into either GM-CSF treatment group or control group. Inhaled GM-CSF was prescribed for 6 months, and patients were followed-up for another 18 months without treatment. Physiological features of the patients were analyzed.

Results: There were 36 patients (19 in treatment group, 17 in control group) included. No significant difference in primary endpoints measured by the change of alveolar arterial oxygen gradient (A-aDO2) from the baseline value to the values obtained during treatment or during the following 18-month non-treatment observation period [control group vs. treatment group: 0.51±12.09 mmHg vs. -0.35±13.76 mmHg, p=0.848 (3 month); 1.85±11.21 mmHg vs. 7.31±8.81 mmHg, p=0.146 (6 months); 6.05±11.14 mmHg vs. 6.61±10.64mmHg, p=0.899 (24 months)]). Percentage of diffusion capacity predicted (DLCO%) and percentage of total lung capacity predicted (TLC%), however, were significantly improved in the treatment group at the end of the study (P=0.010 and 0.027). St. George Respiratory questionnaire (SGRQ) scores were better after 6 months treatment with GM-CSF than control group, and the benefits of treatment were maintained throughout the observation period. No severe side effects were observed during the study.

Conclusion: Six months of inhaled GM-CSF treatment had no effect on the alveolar–arterial oxygen gradient in patients with mild to moderate pulmonary alveolar proteinosis. There were changes in some clinical or laboratory measures, but no clinically important changes were noted at the end of study.

Background

Autoimmune pulmonary alveolar proteinosis (aPAP, previously known as idiopathic PAP) is a rare interstitial lung disease elicited by the formation of autoantibodies which neutralize the activity of granulocyte-macrophage colony stimulating factor (GM-CSF), consequently decreasing macrophage clearance of surfactant [1]. Currently, the standard treatment strategy for PAP is whole lung lavage (WLL). About 70% patients need another WLL within 3 years due to recurrence [2, 3]. Patients who undergo WLL require general anesthesia and double-lumen endotracheal intubation, which means only hospitals with experienced physicians can perform the procedure. Considering the recurrence rate and the cumbersome procedure of WLL, whether or not patients with mild or moderate disease should obtain WLL is a matter of controversy.

Inhaled GM-CSF therapy has become an alternative option for aPAP patients not only due to its effectiveness and safety [4, 5], but also because it is a convenient treatment method for patients who are reluctant to receive WLL. Previous studies included small sample sizes, and as a result, disease severity has not been stratified. Nevertheless, whether patients with mild or moderate disease will benefit from the GM-CSF treatment over the long term is still unclear.

We prospectively evaluated if inhaled GM-CSF would delay disease progression in patients with mild-to-moderate aPAP over a two-year period. We designed a 6-month treatment and 18-month follow-up observation.

Results

Baseline demographic information

Forty-two aPAP patients were screened and 36 patients were randomized (19 in the treatment group and 17 in the control group). After 24 months of follow up, 26 patients (72.2%, 15 from the treatment group and 11 from the control group) completed the study. The period of recruitment and follow up was from July 20, 2014 to July 6, 2018 after the last enrolled patient completed his 24 months follow up. In the treatment group, one patient deteriorated at 3 months and required rescue therapy (WLL). Another patient lost follow up at 1 month and two more patients withdrew at 6 months. In the control group, 4 patients deteriorated at 3 months and required rescue therapy (one received GM-CSF inhalation, two received WLL and one was prescribed traditional medicine). 2 patients withdrew at 21 months. (Fig. 1)

In 36 patients, the most common presenting symptom was dyspnea (20/36, 55.6%), followed by cough (13/36, 36.1%) and phlegm (6/36, 16.7%) and chest pain (3/36, 8.3%). 4 out of our 36 patients was diagnosed by regular health check-up without any symptoms. The media duration of symptoms is 6 months (inter-quartile range is from 0 to 60 months) in our patients. All of our patients had extent bilateral pulmonary infiltrates confirmed by HRCT.

Demographic information of the 36 patients entered the study is shown in Table 1. There were no significant differences in demographic information between the two groups including age and sex. No significant differences were found in patients' disease severity markers at baseline, including symptoms, ABG, pulmonary function tests, 6 minutes walking distance (6MWD) and anti-GM-CSF antibody levels between the treatment group and the control group.

Table 1
Demographic features of autoimmune pulmonary alveolar proteinosis (aPAP) patients at baseline.

Parameter		Control group (n = 17)	Treatment group (n = 19)	P value
Age (year)		42.88 ± 12.75	43.53 ± 12.89	0.881
Sex (female/male)		4/13	6/13	0.717
Duration of the disease (months)*		6 (6–60)	6 (0.5–60)	0.852
Smoking status	Never	7	7	0.965
	Ex-smoker	5	6	
	Current smoker	5	6	
Disease Severity Score	1	0	0	0.409
	2	13	11	
	3	4	8	
Hb (g/dL)		16.11 ± 1.61	16.00 ± 1.64	0.839
HCT (%)		46.07 ± 3.7	45.74 ± 4.47	0.810
LDH (U/L)		244.06 ± 53.02	233.82 ± 43.43	0.547
CEA (U/L)		5.34 ± 4.96	4.74 ± 3.41	0.678
FEV_1 pred (%)		78.09 ± 14.12	79.16 ± 15.68	0.832
FVC pred (%)		79.14 ± 13.42	79.71 ± 13.70	0.900
TLC pred (%)		74.59 ± 9.65	74.82 ± 10.78	0.946
DLCO pred (%)		69.50 ± 13.94	68.41 ± 16.90	0.835
DLCO/VA pred (%)		98.58 ± 18.45	98.67 ± 24.32	0.990
PaO_2 (mmHg)		77.51 ± 8.53	76.88 ± 11.23	0.854
A-aDO_2 (mmHg)		28.32 ± 9.09	28.74 ± 11.04	0.902
SGRQ symptom		24.06 ± 13.55	29.17 ± 29.75	0.506
SGRQ activity		30.70 ± 18.46	30.33 ± 16.55	0.949
SGRQ effect		24.20 ± 16.10	21.83 ± 21.29	0.712
SGRQ total		26.47 ± 14.74	27.38 ± 19.75	0.878
6MWD		495.25 ± 79.39	477.95 ± 65.68	0.485
SpO_2 at the end of 6MWD		94.87 ± 2.70	94.58 ± 5.20	0.847
Mean lung density		-718.62 ± 82.70	-687.25 ± 68.48	0.315
Total lung volume radiological measurement (ml)		4556.08 ± 841.15	4461.08 ± 1399.60	0.841
GM-CSF antibody (g/ml)		75.86 ± 93.94	73.30 ± 58.65	0.922

Abbreviations: aPAP: autoimmune pulmonary alveolar proteinosis; A-aO_2: alveolar arterial oxygen gradient; CEA: carcinoembryonic antigen; DLCO: DLCO: diffusing capacity for carbon monoxide; DLCO/VA: diffusing capacity for carbon monoxide corrected for alveolar volume; FEV1: forced expiratory volume in the first second; FVC: forced vital capacity; GM-CSF: granulocyte macrophage colony stimulating factor; Hb: hemoglobin;HCT: hematocrit; LDH: lactate dehydrogenase; PaO_2: partial pressure of oxygen; SGRQ: St George Respiratory Questionnaire; SpO_2: oxygen saturation in pulse oximetry; TLC: total lung capacity; 6MWD: 6 minutes walking distance (test).

*: median (inter-quartile range)

**: Traditional medicine treatment

Parameter		Control group (n = 17)	Treatment group (n = 19)	P value
Treatment before the trial	Never	13	14	0.489
	WLL	3	5	
	Others**	1	0	

Abbreviations: aPAP: autoimmune pulmonary alveolar proteinosis; A-aO_2: alveolar arterial oxygen gradient; CEA: carcinoembryonic antigen; DLCO: DLCO: diffusing capacity for carbon monoxide; DLCO/VA: diffusing capacity for carbon monoxide corrected for alveolar volume; FEV1: forced expiratory volume in the first second; FVC: forced vital capacity; GM-CSF: granulocyte macrophage colony stimulating factor; Hb: hemoglobin;HCT: hematocrit; LDH: lactate dehydrogenase; PaO_2: partial pressure of oxygen; SGRQ: St George Respiratory Questionnaire; SpO_2: oxygen saturation in pulse oximetry; TLC: total lung capacity; 6MWD: 6 minutes walking distance (test).

*: median (inter-quartile range)

**: Traditional medicine treatment

Primary endpoint: A-aDO_2

There were no significant differences between the treatment group and control group based on primary endpoints measured by the change of A-aDO_2 from baseline to 3 and 6 months treatment and during the following 18 months [control group vs. treatment group: 0.51 ± 12.09 mmHg vs. -0.35 ± 13.76 mmHg, p = 0.848 (3 month); 1.85 ± 11.21 mmHg vs. 7.31 ± 8.81 mmHg, p = 0.146 (6 months); 6.05 ± 11.14 mmHg vs. 6.61 ± 10.64 mmHg, p = 0.899 (24 months)] (Fig. 2A). The change of PaO_2 level from baseline to 3 and 6 months treatment, and during the following 18 months also showed no significant difference between the two groups (Fig. 2B). The actual level of A-aDO_2 and PaO_2 showed no differences during both the treatment period and follow up period as well (Fig. 2C and D) (Tables 2 and 3).

Table 2

The clinical parameter of the effects of inhaled GM-CSF during the 6 months treatment periods.

	3 months			6 months		
	Control group (n = 17)	Treatment group (n = 17)	P value	Control group (n = 13)	Treatment group (n = 17)	P value
ΔA-aDO_2 (mmHg)	0.51 ± 12.09	-0.35 ± 13.76	0.848	1.85 ± 11.21	7.31 ± 8.81	0.146
A-aDO_2 (mmHg)	27.81 ± 11.04	28.79 ± 10.19	0.794	25.69 ± 11.70	22.31 ± 7.45	0.342
ΔPaO_2 (mmHg)	0.92 ± 10.34	0.62 ± 12.07	0.94	2.95 ± 10.34	7.26 ± 10.29	0.267
PaO_2 (mmHg)	78.42 ± 10.74	78.37 ± 10.17	0.988	82.42 ± 9.71	83.84 ± 8.57	0.589
FVC pred (%)	77.24 ± 14.91	78.48 ± 13.72	0.801	80.41 ± 15.60	77.34 ± 23.32	0.688
TLC pred (%)	74.09 ± 11.37	73.12 ± 15.29	0.836	74.28 ± 11.18	78.48 ± 8.88	0.269
DLCO pred (%)	67.12 ± 14.72	69.19 ± 19.83	0.732	70.83 ± 14.62	74.91 ± 14.80	0.465
DLCO/VA pred (%)	95.67 ± 17.32	98.71 ± 21.03	0.641	98.92 ± 12.47	95.93 ± 15.44	0.577
SGRQ symptom	24.84 ± 17.33	24.22 ± 23.32	0.521	29.50 ± 18.61	18.47 ± 19.29	0.097
SGRQ activity	33.45 ± 19.35	24.31 ± 18.92	0.173	28.98 ± 18.78	19.41 ± 17.10	0.149
SGRQ effect	16.38 ± 15.94	17.11 ± 17.86	0.336	21.58 ± 17.60	9.29 ± 10.73	0.023
SGRQ total	14.76 ± 14.52	20.45 ± 17.55	0.285	25.11 ± 16.36	13.88 ± 10.91	0.030
6MWD	494.06 ± 75.43	496.41 ± 75.43	0.926	475.09 ± 85.31	501.13 ± 88.31	0.452
Mean lung density	NA	NA		-739.64 ± 82.70	-733.17 ± 61.41	0.804
Total lung volume (ml)	NA	NA		4485.71 ± 971.37	4365.67 ± 1322.58	0.808
Hb (g/dL)	15.42 ± 1.53	15.34 ± 1.42	0.863	15.65 ± 1.79	15.70 ± 1.60	0.932
HCT (%)	44.35 ± 3.54	44.52 ± 3.48	0.881	45.10 ± 4.04	45.50 ± 4.46	0.792
LDH (U/L)	226.88 ± 46.22	223.86 ± 59.32	0.867	230.36 ± 28.96	203.38 ± 60.36	0.130
CEA (U/L)	3.72 ± 3.31	4.87 ± 3.68	0.619	4.40 ± 2.25	3.14 ± 1.74	0.076

Abbreviations: See Table 1.

Table 3

The primary end point results in the 18 months observational periods after 6 months inhaled GM-CSF treatment.

	9 months			12 months			18 months			24 months		
	Control group (n = 12)	Treatment group (n = 14)	P value	Control group (n = 13)	Treatment group (n = 14)	P value	Control group (n = 12)	Treatment group (n = 13)	P value	Control group (n = 11)	Treatment group (n = 15)	P value
Δ A-aDO_2 (mmHg)	4.53 ± 7.59	8.11 ± 10.89	0.461	5.19 ± 9.72	7.02 ± 12.35	0.674	8.13 ± 15.02	7.39 ± 8.677	0.579	6.05 ± 11.14	6.61 ± 10.64	0.899
A-aDO_2 (mmHg)	25.24 ± 7.05	22.16 ± 9.09	0.366	22.35 ± 6.54	23.25 ± 8.10	0.756	18.38 ± 10.50	22.24 ± 9.43	0.343	23.72 ± 9.41	23.66 ± 8.96	0.988
Δ PaO_2 (mmHg)	4.29 ± 8.38	7.45 ± 11.82	0.267	2.78 ± 11.41	5.89 ± 11.91	0.496	8.13 ± 15.02	7.71 ± 8.72	0.931	6.16 ± 9.88	4.76 ± 10.72	0.741
PaO_2 (mmHg)	81.08 ± 7.14	84.15 ± 8.93	0.362	81.84 ± 7.13	82.59 ± 7.70	0.794	86.48 ± 10.75	84.99 ± 9.76	0.721	82.95 ± 8.05	81.46 ± 10.84	0.707

Abbreviations: see Table 1.

The diffusion capacity and total lung capacity were improved by the end of study

The DLCO% and TLC% showed significant differences between the treatment group and the control group by the end of the study (Fig. 3A and B). [DLCO% (control group vs. treatment group): 67.12 ± 14.72 vs. 69.19 ± 19.83, p = 0.732 (3 months); 70.83 ± 14.62 vs. 74.91 ± 14.80, p = 0.465 (6 months); 64.67 ± 16.22 vs. 80.87 ± 19.40, p = 0.027 (24 months)]. [TLC% (control group vs. treatment group): 74.09 ± 11.37 vs. 73.12 ± 15.29, p = 0.836 (3 months); 74.28 ± 11.18 vs. 78.48 ± 8.88, p = 0.269 (6 months); 70.97 ± 10.79 vs. 79.77 ± 7.76, p = 0.010 (24 months)]. However, other pulmonary function tests, including FVC, FEV_1 (data not shown) and DLCO/VA, did not show any significant differences between the treatment group and the control group, both during the 6-month treatment period and the 18-month follow-up period. (Fig. 3C and D) (Table 2 and supplemental table 1)

The SGRQ scores increased after 3 months and 6 months of inhaled GM-CSF treatment and 18 months follow-up

Meanwhile, we can find obvious difference in patients' quality of life between the treatment group and control group, as measured by SGRQ. The total SGRQ score was improved after 6 months of GM-CSF treatment compared to the no treatment group, and the benefits were nearly continuously maintained throughout the 18-month observation period. Similar trends can be observed in symptom score, activity score and effect score, but not all time points show significant differences between the two groups. (Fig. 4)

Quantitative CT did not find difference after treatment

There was no significant difference in the total lung volume and mean lung density between the treatment group and control group. (Table 2 and supplemental Table 1)

Time to rescue therapy during the 24-month study was not improved

There was no significant difference in time to rescue therapy between the treatment group and control group. Kaplan-Meier Curve analysis for the two groups was shown in Fig. 5. (P = 0.304)

Safety and tolerability of inhaled GM-CSF

None of our patient dead during the trial. None of the patient complained of fever, wheezing or coughing over the duration of their inhaled GM-CSF treatment in the treatment group.

An increase in transaminase levels during GM-CSF inhalation treatment was observed (P = 0.037). Fortunately, none of the patients required medical intervention. The highest level of transaminase in the GM-CSF treatment group was 123 U/L for glutamic-pyruvic transaminase (ALT) and 63 U/L for cereal grass transaminase (AST), while the highest level in the control group was 95 U/L for ALT and 77 U/L for AST respectively. All parameters remained stable or gradually declined after patients ceased alcohol consumption and stopped taking medications with possible interfering effects. White blood cell and neutrophil levels were not obviously increased within the GM-CSF inhalation group, when compared to the control group (P = 0.429). (Table 4)

Table 4
The side-effect of patients with aPAP during the GM-CSF treatment and follow up period.

	Treatment periods			Follow up periods		
	No treatment group (n = 17)	Treatment group (n = 19)	P value	No treatment group (n = 12)	Treatment group (n = 16)	P value
Leukocytosis	1/17 (5.9%)	1/19 (5.3%)	1.0*	1/12 (8.3%)	0/16 (0)	0.429*
Increase in aminotransferases	4/17(23.5%)	11/19 (57.9%)	0.037	6/12 (50.0%)	4/16 (25.0%)	0.333**
Increase in bilirubin	6/17 (35.3%)	2/19 (10.5%)	0.167**	4/12 (33.3%)	0/16 (0)	0.051**
*: Fisher X^2 test; **: continuous correction X^2 test.						

No other significant safety and tolerability differences were observed between the two groups during the study.

Other details of side effects happened during the study can be found in the supplementary.

Discussion

In the present study, we prospectively evaluated the effects of inhaled GM-CSF on mild-to-moderate autoimmune pulmonary alveolar proteinosis (aPAP) patients. In contrast to previous report, no obvious effects were found in our study. During the 6 months treatment and 18 month

subsequent observation, the primary endpoint, A-aDO_2 did not changed. Health-related quality of life as measured as SGRQ improved from 3 months of treatment and maintained to 24 months. Marginal improvement was also noted that TLC and DLCO were improved at the end of the study. This research provides valuable clinical data and experience for inhaled GM-CSF treatment in aPAP patients who do not meet the criteria for WLL.

Current therapy for PAP patients involves the physical removal of surfactant using a procedure in which the lungs are repeatedly filled with saline and emptied–WLL–which is invasive, inefficient, and is not widely available. Some authors reported that fever, hypoxemia, fluid leakage and other complications occurred in patients treated with WLL [6]. Meanwhile, the media time to next WLL is around 15 months[7], and about 30%-57.6% of patients requiring further therapy after the first WLL [8, 9]. Though no consensus of the indication for WLL in treating PAP, most physicians believe that patients with PaO_2 of less than 70 mmHg on room air or an alveolar-arterial [A-a] oxygen gradient of more than 40 mmHg, or patients with disease progression should receive WLL as treatment [6]. In a cohort study from our center, 33% patients are stable or experience spontaneous remission [9], and the spontaneous remission rate varies from 8–18% in different reports [7, 9–11]. Considering the rate of spontaneous remission, rate of recurrence and the cumbersome procedure of WLL, it becomes a critical question whether GM-CSF inhalation could become a primary treatment for mild to moderate aPAP patients.

After GM-CSF was confirmed to play an important role in the disease mechanism of aPAP, the efficacy of exogenous GM-CSF replacement was assessed in previous report. The response rate to this treatment varied and the efficacy rate was 62–100% when using inhaled GM-CSF [5, 11, 12] while the efficacy rate was 43–75% when using subcutaneously administered GM-CSF [13, 14]. Because of better responsiveness and tolerance, the use of inhaled GM-CSF is generally recommended [4] .

In previous studies, inhaled GM-CSF treatment was prescribed in patients with moderate to severe disease [15–19], and the mean PaO_2 level in a large prospective study of inhaled GM-CSF treatment on aPAP patients is 61.7 ± 1.4 mmHg [11]. During preparation of our manuscript, a randomized placebo-controlled study of inhaled GM-CSF was published, A-aDO_2 and CT density quantitative measurement were significantly improved though they concluded that clinical benefits were not significant [20]. The major differences in design between our study and Tazawa et al's study are two points: (1) Tazawa et al recruited patients with PaO_2 less than 70 mmHg (or less than 75 mmHg with symptoms), the average PaO_2 was 66.4±8.66 mmHg and 68.8±8.96 mmHg in GM-CSF group and control group. We recruited patient with DSS 1–3, 11/19 from GM-CSF group and 13/17 from control group had PaO_2 over 70 mmHg, with average PaO_2 77.51±8.53 mmHg in GM-CSF group and 76.88 ± 11.23 mmHg in control group. (2) Both trials use 6-month treatment. Tazawa et al used 125 μg bid continuously and we used 150 μg bid for 3 months and then 150 μg qd for 3 months. The reasons for the no or marginal response rate in our group may be related to the relatively good baseline oxygen content making changes of this indicator less obvious. Based on our study and previous report, the beneficial effects of GM-CSF treatment for aPAP with PaO_2 over 70 mmHg (DSS 1 and 2) may be very limited. There were no enough DSS3 cases (PaO_2 60–70 mmHg) in our study for subgroup analysis. However, Tazawa, et al has answered this question with a randomized placebo-controlled study. We believe GM-CSF could be beneficial for those with PaO_2 less than 70 mmHg.

We found that inhaled GM-CSF therapy is a well-tolerated choice for aPAP patients as previous studies showed [11, 15–17]. Though more than half of our patients in the GM-CSF group were found have slight increases in amino-transferase levels, and a number of abnormal liver function results were observed in the GM-CSF treatment group, the elevation of transaminase levels were all slight and no medical intervention was needed for all patients. All patients remained stable or gradually improved after the cessation of alcohol and stopping intake of possible related combination medicines. Therefore, inhaled GM-CSF therapy is a safe and convenient choice for patients.

Our research has some limitations. Firstly, the sample size of the study was small, and there was no enough DSS3 patients for analysis. Our estimated target sample size was based on the prior results of patients population with more disease severity [11], which may underestimate the sample size actually needed. Secondly, more patients from the control group dropped out of the study during the observation period, which might affect the evaluation of effectiveness for comparing these two groups. Thirdly, the patients in our study did not receive a tailored dosage of GM-CSF treatment, nor did they receive prolonged therapy after the 6 months of treatment, which may make some latent responders, requiring higher dosages or longer treatment time for a positive response, remain hidden.

Conclusions

Six months of inhaled GM-CSF treatment had no effects on the alveolar–arterial oxygen gradient in patients with mild to moderate pulmonary alveolar proteinosis. At the dose we used, there were changes in some clinical or laboratory measures, but no clinically important changes were noted at the end of study. Our study is an important complement for efficacy in aPAP patients with mild to moderate disease severity.

Methods

Participants

Patients with mild or moderate aPAP, aged between 18 and 80, were enrolled at two hospitals, including Peking Union Medical College Hospital (PUMCH) and The Affiliated Drum Tower Hospital of Nanjing University Medical School in China.

The inclusion criteria included: (1) patients had a clinical diagnosis of PAP by high-resolution computed tomography (HRCT) and was further confirmed pathologically by testing for amorphous periodic Acid-Schiff (PAS)-positive granules, found either in milky broncho-alveolar lavage fluid (BALF) or in alveolar structures of lung biopsy tissues. In details, transbronchial lung biopsy (TBLB) (n=12), percutaneous lung puncture biopsy (n=3), surgical lung biopsy (n=1), cytological findings of bronchial lavage fluid (BAL) (n=25) or both TBLB and BAL diagnosis of PAP; and (2) Positive serum GM-CSF antibody test which indicated an elevated serum GM-CSF antibody level. The GM-CSF antibody test was followed the method Uchida et al established [21, 22] and revised the cutoff point (above 2.39 μg/ml) performed by our hospital [23].

Disease severity was assessed with a disease severity score (DSS), with patients having a DSS between 1 and 3 being included in our study. DSS scores were defined based on a previous study as follows [24]: Grade 1: No symptoms and an arterial oxygen partial pressure (PaO_2) ≥ 70 mmHg; Grade 2: PaO_2≥ 70 mmHg with symptoms; Grade 3: PaO_2 between 60–70 mmHg; Grade 4: PaO_2 between 50–60 mmHg; and Grade 5: PaO_2 below 50 mmHg.

Individuals were excluded if they meet following criteria: (1) patients received previous GM-CSF therapy or other forms of cytokine therapy, or had undergone lung lavage therapy within 3 months prior to enrollment; (2) Individuals with PAP resulting from other conditions (e.g. occupational exposure to silica, underlying human immunodeficiency virus infection, respiratory infections, myeloproliferative disorder or leukemia); or (3) Individuals had histories of severe allergic or anaphylactic reactions to humanized or murine monoclonal antibodies; or (4) Individuals with chronic lung diseases or any other serious medical conditions, and (5) Women who were pregnant, lactating or planned to become pregnant during the study period were also excluded.

Study Design

This was a multicenter, randomized, open-label clinical trial (clinical trial number: NCT02243228, Inhalation of granulocyte-macrophage colony stimulating factor for autoimmune pulmonary alveolar proteinosis) comprising three sequential periods: high-dose therapy for 3 months, low-dose therapy for 3 months and observation for 18 months. Study visits during treatment were designed at 0, 1, 3 and 6 months. Thereafter, patients were followed up by visits at 9, 12, 15, 18, 21 and 24 months (Fig. 6). The 1st, 15th and 21th month's visits are phone call contacting patients for safety questionnaire. Before the therapeutic trial, all participants entered an initial 3-month observation period, during which disease severity and progression were evaluated. Participants that had a PaO_2 increase by 10 mmHg or more, or alveolar-arterial oxygen gradient ($A–aDO_2$) decreased by 10 mmHg or more were regarded as having undergone spontaneous improvement and were excluded from enrollment. Additionally, patients eligible for the trial should have progressive or unremitting PAP, defined as worsening or unchanging PaO_2 or $A–aDO_2$ over a 3-month period of observation. It should be noted that if the participant was acquainted to the principal investigator as a patient with a well-documented history showing an unremitting aPAP state, he/she could be enrolled into the study without this observation period. After 3-month observation, all unremitting PAP patients underwent a stratified randomization based on DSS at the time of enrollment to ensure equal representation of patients with various severities in both the treatment group and the placebo group using a random number table. The randomization was blinded to both the patients and the investigators before study started.

Recombinant human GM-CSF (rhGM-CSF) was administered to patients in treatment group by inhalation as previously described [15]. 150 μg of rhGM-CSF was dissolved in 2 ml of sterile saline, and was inhaled as an aqueous aerosol using an LC-PLUS nebulizer with a manual interrupter valve connected to a PARI Turbo BOY compressor (PARI GmbH, Starnberg, Germany) [25]. The drug was donated by North China Pharmaceutical Corporation (NCPC) and nebulizers were bought from PARI. Treatment was designed according to a previous study[11], including 3 months of high-dose GM-CSF administration (150 μg twice daily every other week) and another 3 months of low-dose administration (150 μg once daily every other week), serving as induction and maintenance therapy, respectively.

In the control group, patients did not prescribe any kind of treatment related to PAP (including GM-CSF, WLL or anti-CD20, et al) but have the same follow up plan as treatment group.

In previous published studies, patients inhaling GM-CSF had a mean change in $A–aDO_2$ of 11 mmHg [11]. Thus, the target sample size was 25, chosen to give a detection power of 90%, allowing for a 5% incidence of type-I error. Considering other outcome measurements and participant dropout, the study size was increased to 35–45 patients.

The study was approved by the institutional review board of PUMCH (Approval No. S-717) and reviewed by the institutional review boards at the Affiliated Drum Tower Hospital of Nanjing University Medical School. All participants gave written informed consents prior to enrollment.

Assessments

Clinical information including history, symptoms, serological (including lactate dehydrogenase, carcinoembryonic antigen levels) and radiological features, pulmonary function testing and physical examinations were obtained regularly at each visit during the study. Arterial blood gas analysis (ABG) tests were performed with patients that had been breathing room air for at least 15 minutes. Low dose quantitative computed tomography

of the chest (in PUMCH) or HRCT of the chest (in The Affiliated Drum Tower Hospital of Nanjing University Medical School) was obtained before and after GM-CSF therapy and evaluated in a blinded fashion by a board-certified radiologist. The original CT measurement were collected and the total lung volume and mean lung density were automatically calculated by post-processed with Pulmo 3D (syngo. via, version VA 30, Siemens Healthcare, Germany) for the automatic segmentation of the pulmonary parenchyma by excluding the intrapulmonary vessels following the process published by one of our co-author, Dr. Sui [26].

Intergroup differences in the change of $A–aDO_2$ from baseline to the end of treatment were defined as primary endpoints.

Other data, representing efficacy of GM-CSF inhalation, were also evaluated as secondary endpoints, including pulmonary function test differences between the treatment group and the control group (forced vital capacity [FVC], total lung capacity [TLC], diffusing capacity for carbon monoxide [DLCO] or diffusing capacity for carbon monoxide corrected for alveolar volume [DLCO/VA]), 6 minutes walking distance differences between the groups, and relapse time in the two groups. The definition of relapse was as follow: 1) new requirement for whole lung lavage (WLL) or any other kind of treatment (including traditional medicine, subcutaneous injection or GM-CSF inhalation) due to disease progression; or 2) PAP death; or 3) reduction in PaO_2 of more than 10 mmHg, or increase in $A–aDO_2$ of more than 10 mmHg; or 4) a worsened chest HRCT independently confirmed by two physicians. Adverse events were monitored during the study, including airway hypersensitivity, fever, mylagia, arrhythmia and potential effects on the circulatory system.

All blood tests were performed in the laboratories affiliated with the two hospitals, both of which have the quality management certification of China. Serum levels of GM-CSF antibody were tested in PUMCH.

All the data were collected and stored in the database system founded by Beijing Yikang Healthcare Technology Co.

Statistical analysis

All statistical analyses were performed using a personal computer and SPSS 20.0 software. Numeric results were presented as either the mean ± SD or the median and inter-quartile range. Metric variables were shown as the mean and categorical variables were given in terms of frequencies and percentages. The X^2 test was used to analyze proportions of variables. For group comparisons, the unpaired t tests and Wilcoxon rank-sum test were used to evaluate the differences in normally distributed variables. Kaplan-Meier Curve analysis was used to analyze time for relapse in the two groups. All P values reported were two-sided.

List Of Abbreviations

$A\text{-}aDO_2$	alveolar arterial oxygen gradient
ABG	Arterial blood gas analysis
ALT	glutamic-pyruvic transaminase
aPAP	autoimmune pulmonary alveolar proteinosis
AST	cereal grass transaminase
BAL	bronchoalveolar lavage
CT	computed tomography
DLCO	diffusing capacity for carbon monoxide
DLCO/VA	diffusing capacity for carbon monoxide corrected for alveolar volume
DLCO%	percentage of diffusion capacity predicted
DSS	disease severity score
FEV_1	forced expiratory volume in the first second
FVC	forced vital capacity
GGO	ground-glass opacities
GM-CSF	granulocyte macrophage colony stimulating factor
HRCT	high-resolution computed tomography
PaO_2	arterial oxygen partial pressure
PUMCH	Peking Union Medical College Hospital
SGRQ	St Grorge Respiratory questionnaire
TBLB	transbronchial lung biopsy
TLC%	percentage of total lung capacity predicted
WLL	whole lung lavage
6MWD	6 minutes walking distance

Declarations

Ethics approval and consent to participate

The study was approved by the institutional review board of PUMCH (Approval No. S-717) and reviewed by the institutional review boards at the Affiliated Drum Tower Hospital of Nanjing University Medical School. All participants gave written informed consents prior to enrollment.

Consent for publication

Consent for publication was obtained from all participants.

Availability of data and materials

The data of our patients is available in the department of medical records in PUMCH and the Affiliated Drum Tower Hospital of Nanjing University Medical School. This data can be released with the agreement of patients and available from the corresponding author on reasonable request.

Competing interest

This work was supported partly by North China Pharmaceutical Corporation (NCPC) and Beijing Yikang Healthcare Technology Co. Ltd. Both companies didn't join the data analysis and interpretation.

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Authors' contributions

XT and KFX designed, obtained consent, and drafted the manuscript; KFX and YX reviewed the eligibility of the patients' enrollment. XT, YY, LC, WX, XL, JW, YZ, SM and YX followed up patients. XS and WS reviewed the CT scan and did quantity analysis of chest CT for the patients. YY, XG, WX collected data; XT, XG, LL, YS and KFX made data analysis and interpretation. YS and LC drafted the manuscript. All authors read and approved the final manuscript.

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References

1. Trapnell BC, Whitsett JA, Nakata K: **Pulmonary alveolar proteinosis**. *The New England journal of medicine* 2003, **349**(26):2527-2539.
2. Shende RP, Sampat BK, Prabhudesai P, Kulkarni S: **Granulocyte macrophage colony stimulating factor therapy for pulmonary alveolar proteinosis**. *The Journal of the Association of Physicians of India* 2013, **61**(3):209-211.
3. Inoue Y, Trapnell BC, Tazawa R, Arai T, Takada T, Hizawa N, Kasahara Y, Tatsumi K, Hojo M, Ichiwata T *et al*: **Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan**. *American Journal of Respiratory & Critical Care Medicine* 2008, **177**(7):752-762.
4. Khan A, Agarwal R, Aggarwal AN: **Effectiveness of granulocyte-macrophage colony-stimulating factor therapy in autoimmune pulmonary alveolar proteinosis: a meta-analysis of observational studies**. *Chest* 2012, **141**(5):1273-1283.
5. Tazawa R, Inoue Y, Arai T, Takada T, Kasahara Y, Hojo M, Ohkouchi S, Tsuchihashi Y, Yokoba M, Eda R *et al*: **Duration of benefit in patients with autoimmune pulmonary alveolar proteinosis after inhaled granulocyte-macrophage colony-stimulating factor therapy**. *Chest* 2014, **145**(4):729-737.
6. Awab A, Khan MS, Youness HA: **Whole lung lavage-technical details, challenges and management of complications**. *J Thorac Dis* 2017, **9**(6):1697-1706.
7. Seymour JF, Presneill JJ: **Pulmonary alveolar proteinosis: progress in the first 44 years**. *Am J Respir Crit Care Med* 2002, **166**(2):215-235.
8. Gay P, Wallaert B, Nowak S, Yserbyt J, Anevlavis S, Hermant C, Lovis A, Menard O, Maitre B, Vandemoortele T *et al*: **Efficacy of Whole-Lung Lavage in Pulmonary Alveolar Proteinosis: A Multicenter International Study of GELF**. *Respiration* 2017, **93**(3):198-206.
9. Zhao YY, Huang H, Liu YZ, Song XY, Li S, Xu ZJ: **Whole Lung Lavage Treatment of Chinese Patients with Autoimmune Pulmonary Alveolar Proteinosis: A Retrospective Long-term Follow-up Study**. *Chin Med J (Engl)* 2015, **128**(20):2714-2719.
10. Byun MK, Kim DS, Kim YW, Chung MP, Shim JJ, Cha SI, Uh ST, Park CS, Jeong SH, Park YB *et al*: **Clinical features and outcomes of idiopathic pulmonary alveolar proteinosis in Korean population**. *J Korean Med Sci* 2010, **25**(3):393-398.
11. Tazawa R, Trapnell BC, Inoue Y, Arai T, Takada T, Nasuhara Y, Hizawa N, Kasahara Y, Tatsumi K, Hojo M *et al*: **Inhaled granulocyte/macrophage-colony stimulating factor as therapy for pulmonary alveolar proteinosis**. *Am J Respir Crit Care Med* 2010, **181**(12):1345-1354.
12. Papiris SA, Tsirigotis P, Kolilekas L, Papadaki G, Papaioannou AI, Triantafillidou C, Papaporfyriou A, Karakatsani A, Kagouridis K, Griese M *et al*: **Pulmonary alveolar proteinosis: time to shift?** *Expert Rev Respir Med* 2015, **9**(3):337-349.
13. Kavuru MS, Sullivan EJ, Piccin R, Thomassen MJ, Stoller JK: **Exogenous granulocyte-macrophage colony-stimulating factor administration for pulmonary alveolar proteinosis**. *Am J Respir Crit Care Med* 2000, **161**(4 Pt 1):1143-1148.
14. Seymour JF, Presneill JJ, Schoch OD, Downie GH, Moore PE, Doyle IR, Vincent JM, Nakata K, Kitamura T, Langton D *et al*: **Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor in patients with idiopathic acquired alveolar proteinosis**. *Am J Respir Crit Care Med* 2001, **163**(2):524-531.
15. Tazawa R, Hamano E, Arai T, Ohta H, Ishimoto O, Uchida K, Watanabe M, Saito J, Takeshita M, Hirabayashi Y *et al*: **Granulocyte-macrophage colony-stimulating factor and lung immunity in pulmonary alveolar proteinosis**. *Am J Respir Crit Care Med* 2005, **171**(10):1142-1149.
16. Wylam ME, Ten R, Prakash UB, Nadrous HF, Clawson ML, Anderson PM: **Aerosol granulocyte-macrophage colony-stimulating factor for pulmonary alveolar proteinosis**. *Eur Respir J* 2006, **27**(3):585-593.
17. Papiris SA, Tsirigotis P, Kolilekas L, Papadaki G, Papaioannou AI, Triantafillidou C, Papaporfyriou A, Karakatsani A, Kagouridis K, Griese M *et al*: **Long-term inhaled granulocyte macrophage-colony-stimulating factor in autoimmune pulmonary alveolar proteinosis: effectiveness, safety, and lowest effective dose**. *Clin Drug Investig* 2014, **34**(8):553-564.
18. Ohkouchi S, Akasaka K, Ichiwata T, Hisata S, Iijima H, Takada T, Tsukada H, Nakayama H, Machiya JI, Irokawa T *et al*: **Sequential Granulocyte-Macrophage Colony-Stimulating Factor Inhalation after Whole-Lung Lavage for Pulmonary Alveolar Proteinosis. A Report of Five Intractable Cases**. 2017, **14**(8):1298-1304.

19. Ding J, Xiao Y, Dai J, Miao L, Qiu Y, Chen L, Jiang H, Cai H: **[Effectiveness and safety of inhaled granulocyte-macrophage colony-stimulating factor therapy in idiopathic pulmonary alveolar proteinosis]**. *Zhonghua Yi Xue Za Zhi* 2015, **95**(34):2766-2770.
20. Tazawa R, Ueda T, Abe M, Tatsumi K, Eda R, Kondoh S, Morimoto K, Tanaka T, Yamaguchi E, Takahashi A *et al*: **Inhaled GM-CSF for Pulmonary Alveolar Proteinosis**. *The New England journal of medicine* 2019, **381**(10):923-932.
21. Uchida K, Nakata K, Carey B, Chalk C, Suzuki T, Sakagami T, Koch DE, Stevens C, Inoue Y, Yamada Y *et al*: **Standardized serum GM-CSF autoantibody testing for the routine clinical diagnosis of autoimmune pulmonary alveolar proteinosis**. *J Immunol Methods* 2014, **402**(1-2):57-70.
22. Uchida K, Nakata K, Trapnell BC, Terakawa T, Hamano E, Mikami A, Matsushita I, Seymour JF, Oh-Eda M, Ishige I *et al*: **High-affinity autoantibodies specifically eliminate granulocyte-macrophage colony-stimulating factor activity in the lungs of patients with idiopathic pulmonary alveolar proteinosis**. *Blood* 2004, **103**(3):1089-1098.
23. Li Y, Tian X, Gui Y, Ma A, Li X, Zeng N, Zhang P, Li G, Xu K: **[Serum markers in patients with idiopathic pulmonary alveolar proteinosis]**. *Zhonghua Jie He He Hu Xi Za Zhi* 2014, **37**(7):497-501.
24. Inoue Y, Trapnell BC, Tazawa R, Arai T, Takada T, Hizawa N, Kasahara Y, Tatsumi K, Hojo M, Ichiwata T *et al*: **Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan**. *Am J Respir Crit Care Med* 2008, **177**(7):752-762.
25. Coates AL, Dinh L, MacNeish CF, Rollin T, Gagnon S, Ho SL, Lands LC: **Accounting for radioactivity before and after nebulization of tobramycin to insure accuracy of quantification of lung deposition**. *J Aerosol Med* 2000, **13**(3):169-178.
26. Sui X, Du Q, Xu KF, Tian X, Song L, Wang X, Xu X, Wang Z, Wang Y, Gu J *et al*: **Quantitative assessment of Pulmonary Alveolar Proteinosis (PAP) with ultra-dose CT and correlation with Pulmonary Function Tests (PFTs)**. *PLoS One* 2017, **12**(3):e0172958.

Figures

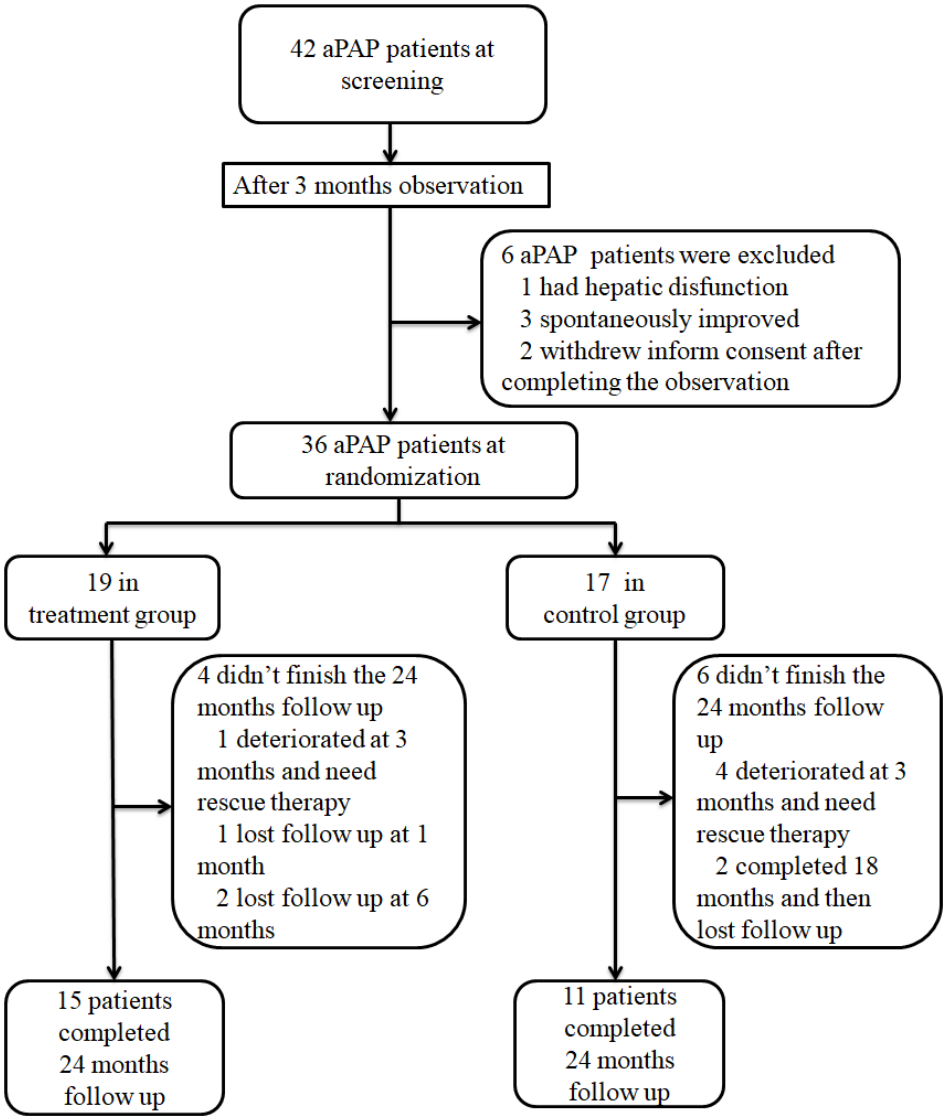

Figure 1

Flow diagram of the study cohort. aPAP: autoimmune pulmonary alveolar proteinosis; GM-CSF: granulocyte-macrophage colony stimulating factor.

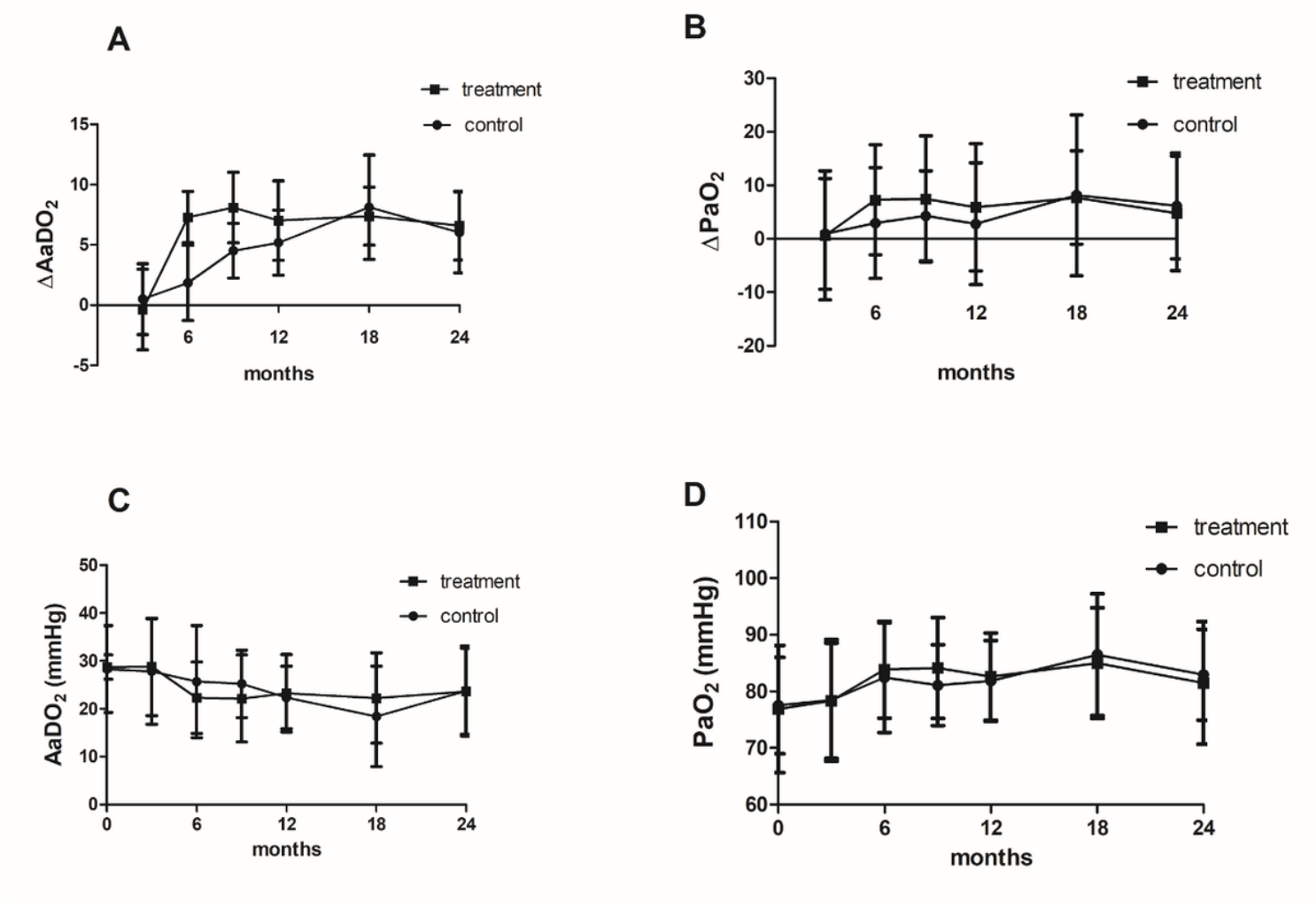

Figure 2

No significant differences were observed between the treatment group and the control group for changes of A-aDO2 and PaO2 from baseline to 3, 6 months treatment and over the following 18 months (A and B). [A-aDO2 levels in control group vs. treatment group: 0.51±12.09 mmHg vs. -0.35±13.76 mmHg, p=0.848 (3 month); 1.85±11.21 mmHg vs. 7.31±8.81 mmHg, p=0.146 (6 months); 6.05±11.14 mmHg vs. 6.61±10.64mmHg, p=0.899 (24 months)]. No significant differences were observed between the treatment group and the control group for the absolute value of A-aDO2 and PaO2 in treatment periods and follow up period as well (C and D).

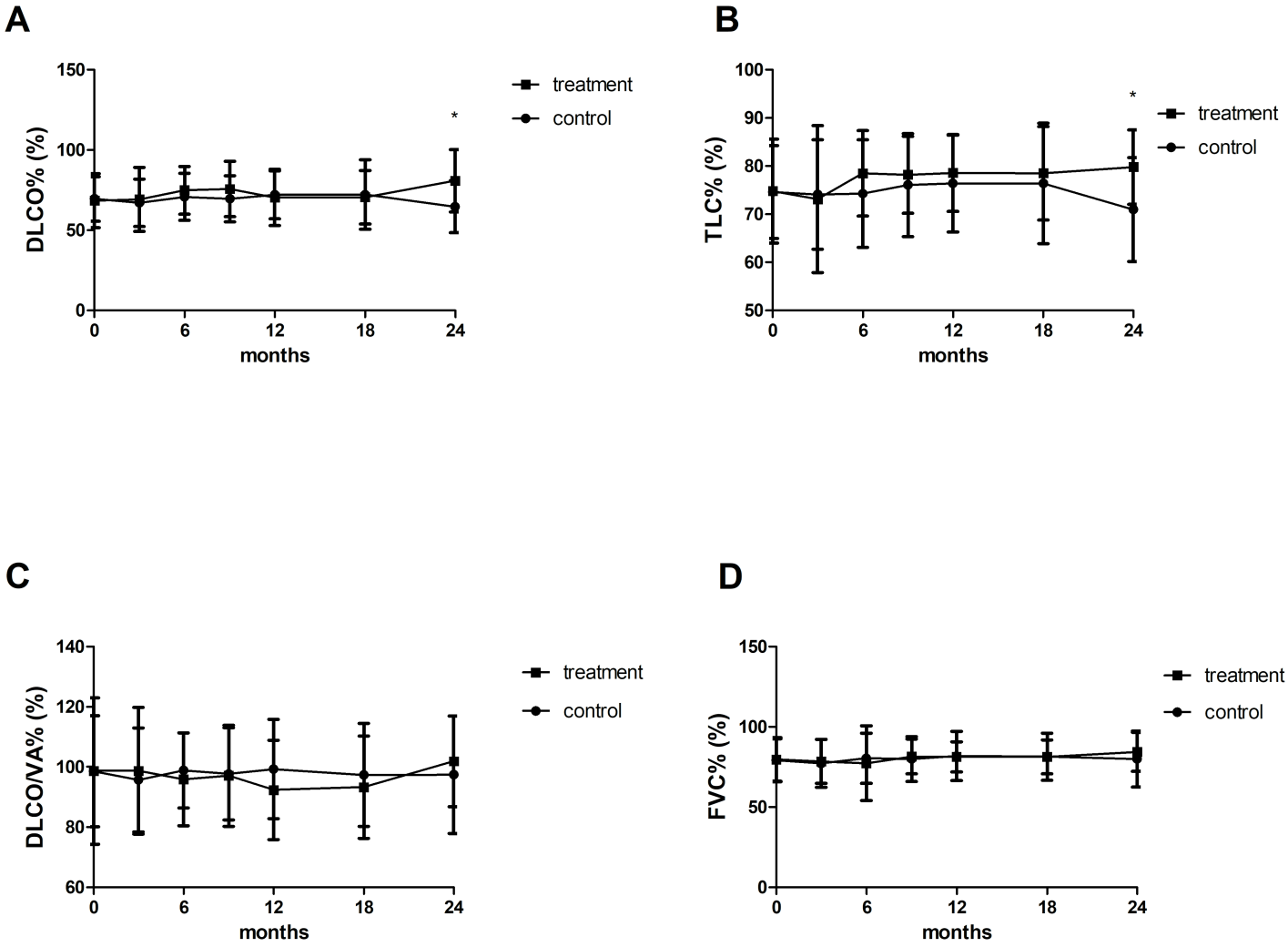

Figure 3

The DLCO% and TLC% showed significant differences between the treatment group compared with the control group at the end of the study ($P<0.05$, respectively, A and B). However, other pulmonary function tests, including FVC and DLCO/VA, did not show any significant differences between the treatment group and the control group, both during the 6-month treatment period and the 18-month follow-up period (C and D). [DLCO% (control group vs. treatment group): 67.12±14.72 vs. 69.19±19.83, p=0.732 (3 months); 70.83±14.62 vs. 74.91±14.80, p=0.465 (6 months); 64.67±16.22 vs. 80.87±19.40, p=0.027 (24 months)]. [TLC% (control group vs. treatment group): 74.09±11.37 vs. 73.12±15.29, p=0.836 (3 months); 74.28±11.18 vs. 78.48±8.88, p=0.269 (6 months); 70.97±10.79 vs. 79.77±7.76, p=0.010 (24 months)].

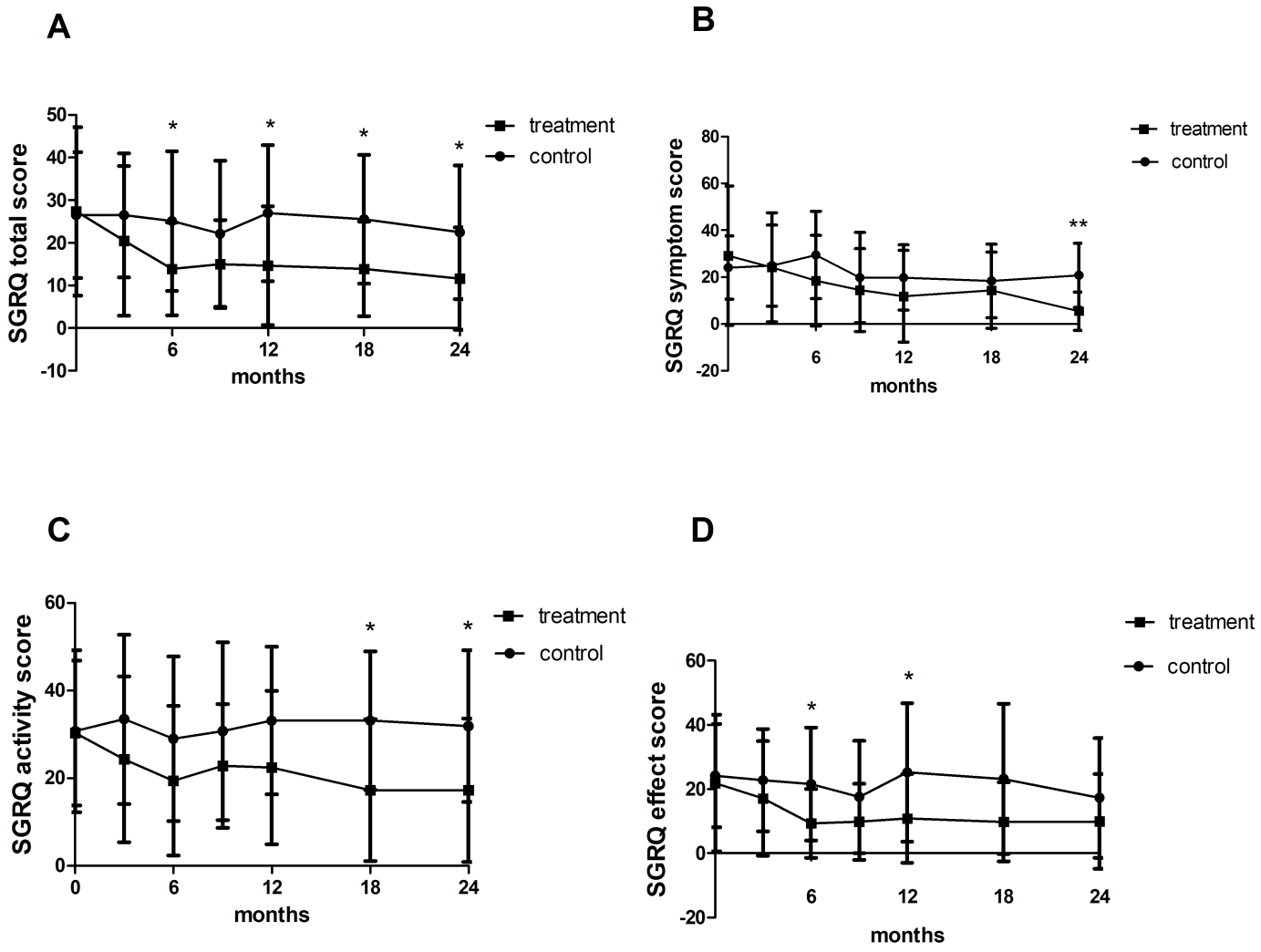

Figure 4

The SGRQ score during the 24-month study period. A, total SGRQ score; B, SGRQ symptom score; C, SGRQ activity score; D, SGRQ effect score. *:P <0.05, **: P <0.01.

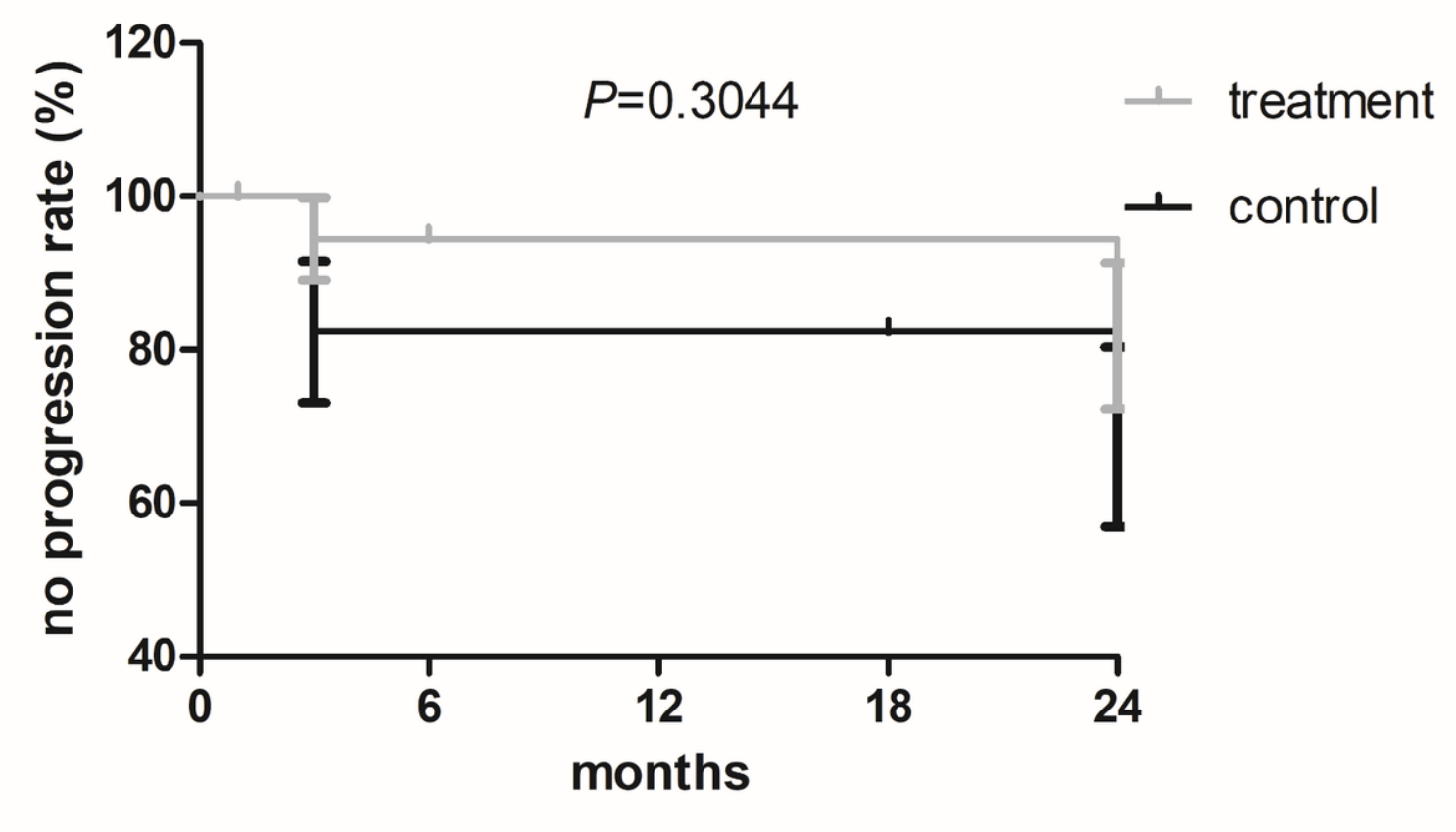

Figure 5

No disease progression rate (measured by time to rescue therapy) during the 24 months study has no significant difference between the treatment and control groups. P >0.05.

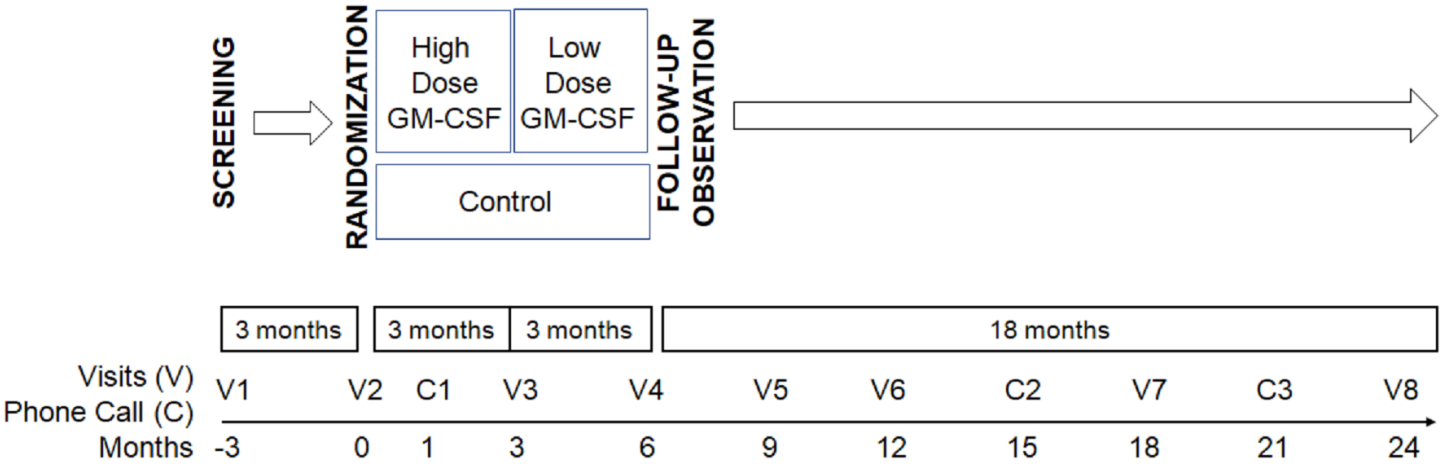

Figure 6

The clinical trial comprising three sequential periods: high-dose therapy for 3 months (150 μg twice daily every other week), low-dose therapy for 3 months (150 μg once daily every other week) and observation for 18 months. Study visits during treatment were designed at 0, 1, 3 and 6 months. Thereafter, patients were followed up by visits at 9, 12, 15, 18, 21 and 24 months.

Supplementary Files

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