ORIGINAL ARTICLE

Inhaled GM-CSF for Pulmonary Alveolar Proteinosis

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ABSTRACT

BACKGROUND

Pulmonary alveolar proteinosis is a disease characterized by abnormal accumulation of surfactant in the alveoli. Most cases are autoimmune and are associated with an autoantibody against granulocyte–macrophage colony-stimulating factor (GM-CSF) that prevents clearing of pulmonary surfactant by alveolar macrophages. An open-label, phase 2 study showed some therapeutic efficacy of inhaled recombinant human GM-CSF in patients with severe pulmonary alveolar proteinosis; however, the efficacy in patients with mild-to-moderate disease remains unclear.

METHODS

We conducted a double-blind, placebo-controlled trial of daily inhaled recombinant human GM-CSF (sargramostim), at a dose of 125 μg twice daily for 7 days, every other week for 24 weeks, or placebo in 64 patients with autoimmune pulmonary alveolar proteinosis who had a partial pressure of arterial oxygen (Pao_2) while breathing ambient air of less than 70 mm Hg (or <75 mm Hg in symptomatic patients). Patients with severe pulmonary alveolar proteinosis (Pao_2 <50 mm Hg) were excluded to avoid possible exacerbation of the disease in patients who were assigned to receive placebo. The primary end point was the change in the alveolar–arterial oxygen gradient between baseline and week 25.

RESULTS

The change in the mean (±SD) alveolar–arterial oxygen gradient was significantly better in the GM-CSF group (33 patients) than in the placebo group (30 patients) (mean change from baseline, −4.50±9.03 mm Hg vs. 0.17±10.50 mm Hg; P=0.02). The change between baseline and week 25 in the density of the lung field on computed tomography was also better in the GM-CSF group (between-group difference, −36.08 Hounsfield units; 95% confidence interval, −61.58 to −6.99, calculated with the use of the Mann–Whitney U test and the Hodges–Lehmann estimate of confidence intervals for pseudo-medians). Serious adverse events developed in 6 patients in the GM-CSF group and in 3 patients in the placebo group.

CONCLUSIONS

In this randomized, controlled trial, inhaled recombinant human GM-CSF was associated with a modest salutary effect on the laboratory outcome of arterial oxygen tension, and no clinical benefits were noted. (Funded by the Japan Agency for Medical Research and Development and the Ministry of Health, Labor, and Welfare of Japan; PAGE ClinicalTrials.gov number, NCT02835742; Japan Medical Association Center for Clinical Trials number, JMA-IIA00205.)

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Pulmonary alveolar proteinosis, an uncommon lung disease characterized by the accumulation of pulmonary surfactant within pulmonary alveoli, causes progressive respiratory insufficiency.[1,2] Approximately 90% of cases of pulmonary alveolar proteinosis are autoimmune; autoimmune pulmonary alveolar proteinosis is associated with a high level of autoantibodies against granulocyte–macrophage colony-stimulating factor (GM-CSF).[3] These autoantibodies, which neutralize the biologic activity of GM-CSF,[4] impair the clearance of surfactant and lead to the disease.[5-10]

Pulmonary alveolar proteinosis is commonly treated with whole-lung lavage[11] while the patient is under general anesthesia. In this procedure, each lung is infused with up to 50 liters of saline to physically remove the surfactant sediment. Although this treatment improves lung function in most patients,[12] repeated treatments are usually required because of the reaccumulation of surfactant.[13] Some improvement in pulmonary function has been observed in trials of subcutaneous recombinant human GM-CSF involving patients with pulmonary alveolar proteinosis.[14-16]

In one study involving GM-CSF knockout mice, inhalation (but not extrapulmonary delivery) of GM-CSF corrected pulmonary alveolar proteinosis.[17] By analogy, inhalation of aerosolized exogenous GM-CSF could benefit patients with autoimmune pulmonary alveolar proteinosis. Pilot studies and a prospective multicenter, open-label, phase 2 study of inhaled GM-CSF therapy showed improvements in gas exchange, especially in patients with severe pulmonary alveolar proteinosis, with no serious treatment-related side effects.[18-21] However, these results may be affected by spontaneous remission, which occurs in approximately 20% of patients with pulmonary alveolar proteinosis.[13] Moreover, the efficacy of inhaled GM-CSF in patients with mild-to-moderate disease remains unknown. We conducted the Pulmonary Alveolar Proteinosis GM-CSF Inhalation Efficacy (PAGE) trial, a multicenter, randomized, controlled trial, to test the hypothesis that inhaled GM-CSF would improve oxygenation, findings on lung imaging, and levels of serum markers in patients with mild-to-moderate pulmonary alveolar proteinosis.

Methods

Trial Design and Oversight

The trial was designed by the investigators of the PAGE Trial Study Group at the Niigata University Medical and Dental Hospital and at 11 other hospitals in Japan. The trial was approved by the institutional review board of each participating hospital and conducted by the clinical trial coordinating committee. Data were collected from case-report forms in an electronic data-capture system and submitted to the Clinical and Translational Research Center (CTRC) at the Niigata University Medical and Dental Hospital. The CTRC members (listed in the Supplementary Appendix, available with the full text of this article at NEJM.org) analyzed the data, and the authors who are members of the CTRC vouch for the accuracy and completeness of the data and analyses and for adherence of the trial to the protocol, available at NEJM.org. All the authors participated in writing the manuscript and made the decision to submit the manuscript for publication.

Patient Recruitment

Patients with pulmonary alveolar proteinosis were identified from questionnaires completed by chief pulmonologists at major hospitals throughout Japan. These surveys were based on the laboratory records of patients who had undergone testing to detect autoantibodies against GM-CSF.

Patients were eligible to participate in the trial if they were between 16 and 80 years of age; had received a diagnosis of autoimmune pulmonary alveolar proteinosis on the basis of findings on high-resolution computed tomography (CT) and biopsy, cytologic findings on bronchoalveolar lavage, or both; had a positive serum GM-CSF antibody level (>1.0 μg per milliliter)[22]; and had a partial pressure of arterial oxygen (Pao_2) of less than 70 mm Hg after 5 minutes in the supine position while breathing ambient air, or less than 75 mm Hg and at least one of the following symptoms: cough, sputum production, or exertional dyspnea.

The exclusion criteria included lung-lavage therapy within the previous 6 months, previous GM-CSF or other cytokine therapy, or current or planned pregnancy. Patients with a Pao_2 of less than 50 mm Hg while breathing ambient air were excluded. All the patients provided written informed consent with documents approved by the institutional review board of each participating hospital. Further details on the inclusion and exclusion criteria are provided in Section 2 in the Supplementary Appendix.

RANDOMIZATION

Patients who met the eligibility criteria were randomly assigned by computer, in a 1:1 ratio, to receive either inhaled GM-CSF (at a dose of 125 μg twice daily on days 1 through 7 and none on days 8 through 14 for 12 2-week cycles) or matched placebo. All the patients and physicians in charge were unaware of the trial-group assignments during the 24-week intervention period. Details regarding randomization are provided in Section 3 in the Supplementary Appendix.

TRIAL AGENTS AND PROCEDURE

The recombinant human GM-CSF (sargramostim, lyophilized formulation) and placebo were provided and delivered to Japan free of charge by Sanofi Genzyme, which had no role in the design or execution of the trial or in the analyses or reporting of the data. Both GM-CSF and placebo were inhaled, as described previously.[19-21,23] Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events.[24] Laboratory tests to assess patient safety included hematologic, serum, and urinary tests.

TRIAL END POINTS

The primary end point was the change in the alveolar–arterial oxygen gradient between baseline and week 25, as described previously.[19,20] This end point was compared between the GM-CSF group and the placebo group. Detailed methods of arterial blood gas analyses and calculations of the alveolar–arterial oxygen gradient used in this trial are provided in Section 3 in the Supplementary Appendix.

Efficacy was also evaluated with respect to the following secondary end-point measures: symptoms (cough, sputum production, and exertional dyspnea); the score on the modified Medical Research Council Dyspnea Scale (scores range from 0 to 4, with higher scores indicating worse respiratory status); vital capacity; diffusion capacity of the lung for carbon monoxide (DL_{CO}); Pa_{O_2} while the patient was breathing ambient air; distance walked in a 6-minute walk test; findings on chest high-resolution CT; serum levels of mucinlike glycoprotein KL-6, carcinoembryonic antigen, surfactant protein D, surfactant protein A, high-sensitivity C-reactive protein, monocyte chemotactic protein 1 (MCP-1), and autoantibodies against GM-CSF; and scores on the chronic obstructive pulmonary disease (COPD) assessment test questionnaire (scores range from 0 to 40, with higher scores indicating a more severe effect on a patient's quality of life). Trial visits occurred at screening, baseline, and at weeks 7, 13, 19, 25, 31, 37, and 43.

Chest high-resolution CT scans were evaluated with CT densitometry techniques[25] that were modified for the Synapse Vincent volume analyzer (Fujifilm); this analyzer was used instead of a visual scoring system.[21] The analyzer calculated a density value of the lung field on CT from density signals (Hounsfield units) and pixel numbers for each image slice, and the mean CT density values were calculated for each patient.

Primary and secondary end points were measured at baseline and during every visit, as described in the trial calendar in Section 4 in the Supplementary Appendix. Furthermore, we conducted univariate and multivariate analyses of the relationship between the clinical characteristics at baseline and the change in the alveolar–arterial oxygen gradient as a prespecified subgroup analysis.

STATISTICAL ANALYSIS

The sample size was calculated with the use of a two-tailed t-test. For the primary analysis, we determined that a sample size of 30 patients in each group (for a total of 60 patients) would provide the trial with a power of 80% to detect an effect size of 1.31 (95% confidence interval [CI], 0.76 to 1.81) at the 5% significance level in the change from baseline to week 25 in the alveolar–arterial oxygen gradient between the GM-CSF group and the placebo group. This calculation was based on Cohen's d formula (the difference of means divided by the pooled standard deviation).

Numerical results are presented as means (±SD) or medians with interquartile ranges. Analyses were performed according to the intention-to-treat principle. The Mann–Whitney U test was used to assess the difference between the two groups, and the sign test was used to assess the differences from baseline to week 25 within each group. A linear mixed-effects model and a generalized linear model were used to evaluate differences between and within groups. The model included the time elapsed since enrollment, treatment assignment, and interaction between time and treatment. The model procedure using statistics with the Kenward–Roger

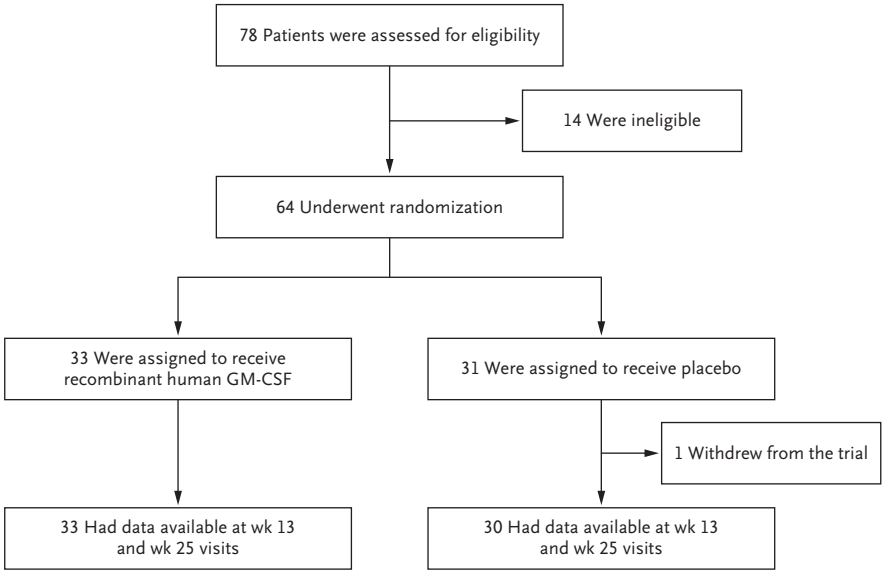

Figure 1. Screening, Randomization, and Follow-up.

During the 24-week randomized, double-blind, placebo-controlled trial, all the patients remained unaware of their trial-group assignments. An analysis of arterial blood gas was performed every 12 weeks. Of the 78 patients with pulmonary alveolar proteinosis who underwent screening, 64 were enrolled and 63 completed the trial. One patient in the placebo group withdrew from the trial because of anxiety caused by rapidly progressing autoimmune pulmonary alveolar proteinosis. GM-CSF denotes granulocyte–macrophage colony-stimulating factor.

adjustment method was used to fit the model without imputation of missing data. For categorical end points, the data were compared with the use of either the chi-square test or Fisher's exact test. For continuous variables, the medians were compared with a Mann–Whitney U test. The P value was two-sided and used for the primary end point only. A P value of less than 0.05 was considered to indicate statistical significance.

We used the linear mixed-effects model to evaluate the interaction of treatment with smoking history, with the alveolar–arterial oxygen gradient as the dependent variable. The model included the time elapsed since enrollment (in months), the treatment assignment (GM-CSF or placebo), smoking history (in persons who had never smoked vs. current smokers or former smokers), and the interaction among time, treatment, and smoking history. Estimates of the effects obtained from the full model used in the analysis are shown in Table S4 in the Supplementary Appendix. All analyses were performed with the use of SAS software, version 9.4 (SAS Institute).

RESULTS

PATIENTS

From September 2016 through December 2016, a total of 78 patients were assessed for eligibility (Fig. 1). Of these patients, 64 patients with mild-to-moderate autoimmune pulmonary alveolar proteinosis were deemed to be eligible to participate in the trial and were randomly assigned to either the GM-CSF group (33 patients) or the placebo group (31 patients) (Table 1, and Table S1 in the Supplementary Appendix). One patient assigned to the placebo group withdrew from the trial, and a total of 63 patients completed the 24-week double-blind intervention period.

PRIMARY END POINT

The change in the alveolar–arterial oxygen gradient was significantly greater in the GM-CSF

Table 1. Clinical Characteristics of the Patients at Baseline.*

Variable	GM-CSF Group (N=33)	Placebo Group (N=31)
Age — yr	56.5±12.4	57.2±12.9
Female sex — no. (%)	14 (42)	13 (42)
Tobacco use — no. (%)		
Current smoker	3 (9)	1 (3)
Former smoker	12 (36)	18 (58)
Never smoked	18 (55)	12 (39)
Dust exposure — no. (%)	13 (39)	14 (45)
Symptom score on modified Medical Research Council Dyspnea Scale†	1.55±0.94	1.42±0.96
Total COPD assessment test score‡	13.5±8.52	14.5±7.95
Density of lung field on CT		
No. of patients with data	31	28
Value — Hounsfield units	−664.2±95.07	−676.9±88.02
Results of pulmonary-function tests		
Percentage of predicted vital capacity	77.2±17.6	82.3±14.9
DL_{CO}		
No. of patients with data	32	30
Percentage of predicted value	64.7±22.1	64.1±19.5
Pa_{O_2} — mm Hg§	66.4±8.66	68.8±8.96
Alveolar–arterial oxygen gradient — mm Hg¶	37.5±9.99	35.2±11.4
Serum markers		
Mucinlike glycoprotein KL-6 — U/ml	5264±3102	8104±10345
Carcinoembryonic antigen — ng/ml	7.95±6.36	8.34±7.28
Surfactant protein D — ng/ml	271.8±187.7	344.1±236.1
Surfactant protein A — ng/ml	107.2±53.8	128.4±112.1
High-sensitivity C-reactive protein — ng/ml	600.9±697.0	1087±2515
MCP-1		
No. of patients with data	33	30
Value — pg/ml	410.5±137.6	415.2±149.5
Autoantibodies against GM-CSF — μg/ml	66.8±71.7	61.8±53.5

* Plus–minus values are means ±SD. COPD denotes chronic obstructive pulmonary disease, CT computed tomography, DL_{CO} diffusion capacity of the lung for carbon monoxide, GM-CSF granulocyte–macrophage colony-stimulating factor, and MCP-1 monocyte chemotactic protein 1.

† Scores on the Medical Research Council Dyspnea Scale range from 0 to 4, with higher scores indicating worse respiratory status.

‡ Total COPD assessment test scores range from 0 to 40, with higher scores indicating a more severe effect on a patient's quality of life.

§ The partial pressure of alveolar oxygen (Pa_{O_2}) is measured while the patient is in a supine position and breathing ambient air.

¶ The alveolar–arterial oxygen gradient is calculated with the use of the following equation: alveolar–arterial oxygen gradient $= (PB - P_{H20}) \times F_{IO_2} - Pa_{CO_2}/R + \{Pa_{CO_2} \times F_{IO_2} \times (1-R)/R\} - Pa_{O_2}$, where F_{IO_2} indicates fraction of inspired oxygen, Pa_{CO_2} partial pressure of arterial carbon dioxide, PB barometric pressure measured by validated barometers, P_{H20} partial pressure of water vapor in inspired air (assumed to be 47 mm Hg), and R the respiratory exchange ratio (assumed to be 0.8).

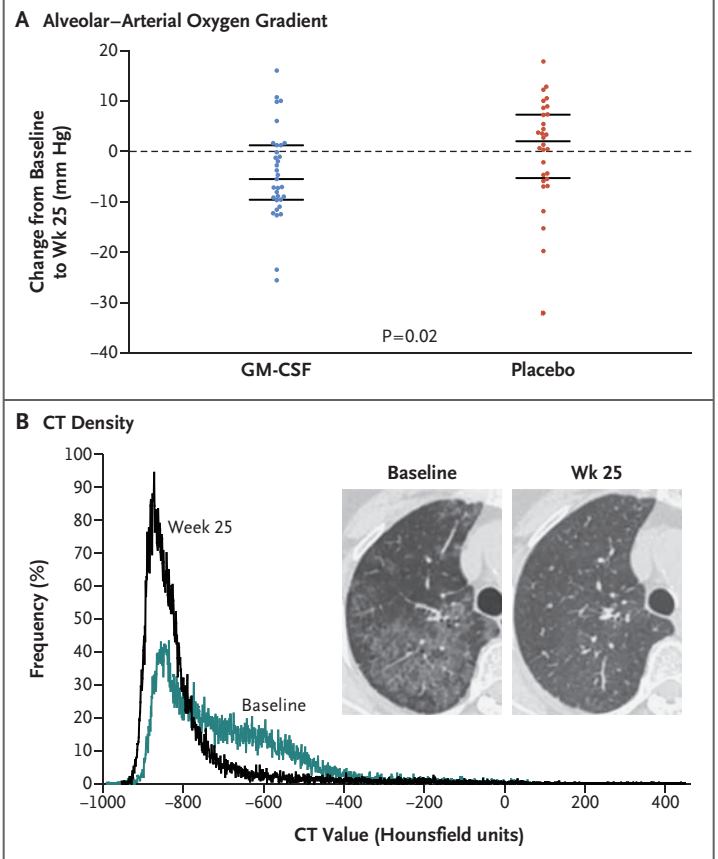

Figure 2. Changes in the Alveolar–Arterial Oxygen Gradient.

Panel A shows changes in the alveolar–arterial oxygen gradient from baseline to week 25 in the GM-CSF group (33 patients) and placebo group (30 patients) during the 24-week randomized intervention period. The middle solid horizontal lines show the median values, the upper solid horizontal lines show the 75th percentiles, and the lower solid horizontal lines show the 25th percentiles. Each circle represents an individual patient. Panel B shows high-resolution computed tomographic (CT) images of the chest in a representative patient from the GM-CSF group at baseline and at week 25, with a reduction in ground-glass opacity during treatment. The distribution of numbers of pixels with various CT density values for the same patient is shown.

group than in the placebo group (mean [±SD] change from baseline, −4.50±9.03 mm Hg vs. 0.17±10.50 mm Hg; P=0.02) (Fig. 2A and Table 2). The greater decrease in the alveolar–arterial oxygen gradient in the GM-CSF group was driven by the higher Pao_2 in that group than in the placebo group (4.77±9.43 mm Hg vs. −0.05±9.48 mm Hg; difference vs. placebo, 5.40; 95% CI, 1.00 to 9.90).

SECONDARY AND EXPLORATORY END POINTS

Scores on the COPD assessment test did not differ substantially between the groups (Table 2); the change in the score on the modified MRC Dyspnea Scale differed between the groups (interaction between time and treatment per month, 0.07; 95% CI, 0.01 to 0.13, as assessed by means of a linear mixed-effects model).

CT values for radiographic density were available for 31 of 33 patients in the GM-CSF group and 28 of 30 patients in the placebo group, and these values were used in statistical analyses. The change in CT density values between baseline and week 25 was −22.4 in the GM-CSF group and −2.5 in the placebo group, for a pseudo-median between-group difference of −36.08 (95% CI, −61.58 to −6.99, calculated with the use of the Mann–Whitney U test and the Hodges–Lehmann estimate of confidence intervals for pseudo-medians) (Table 2 and Fig. 2B). Similarly, the slope of the change in CT density between baseline and week 25 in the GM-CSF group was steeper than that in the placebo group (interaction between time and treatment per month, 3.27; 95% CI, 1.80 to 4.73, as assessed by means of a linear mixed-effects model).

We conducted seven statistical tests of hypotheses to compare the trial groups with respect to changes in serum levels of biomarkers, including KL-6, MCP-1, carcinoembryonic antigen, surfactant protein D, surfactant protein A, high-sensitivity C-reactive protein, and autoantibodies against GM-CSF. The changes in the levels of KL-6 between baseline and week 25 were −1199±3098 U per milliliter in the GM-CSF group and 4.70±9154 U per milliliter in the placebo group (Table 2). This finding suggests that GM-CSF may have been effective (estimated difference, −0.13; 95% CI, −0.25 to −0.01, as assessed by means of a generalized linear model); this finding was consistent with the results of a previous study.[21] In addition, the change from baseline in serum levels of MCP-1, a small cytokine that recruits monocytes, was greater in patients in the GM-CSF group than in patients in the placebo group (−36.1±91.5 pg per milliliter vs. 17.0±102.4 pg per milliliter; pseudo-median between-group difference, −58.45 pg per milliliter [95% CI, −97.49 to −17.51], as assessed by means of a Mann–Whitney U test) (Table 2).

Vital capacity did not significantly improve in either group between baseline and week 25, with a mean change of 1.89±6.24% in the GM-CSF group and −0.74±7.42% in the placebo group, for a pseudo-median between-group difference of 3.31 percentage points (95% CI, −0.59 to 6.35).

Table 2. Change from Baseline in Primary and Selected Secondary End-Point Variables.*

Variable	Value at Wk 25		Change from Baseline		Estimated Difference, GM-CSF vs. Placebo (95% CI)†
	GM-CSF (N=33)	Placebo (N=30)	GM-CSF (N=33)	Placebo (N=30)	
Alveolar–arterial oxygen gradient (mm Hg)	33.0±12.9	35.5±14.1	−4.50±9.03	0.17±10.50	−5.70 (−10.50 to −1.40)‡
Symptom score on modified Medical Research Council Dyspnea Scale§	1.09±1.04	1.37±0.85	−0.45±0.79	−0.03±0.09	0.07 (0.01 to 0.13)¶
Total score on COPD assessment test‖	13.03±8.16	10.87±7.24	0.48±6.86	−3.43±7.16	2.00 (−1.00 to 5.00)‡
Density of lung field on CT (Hounsfield units)**	−686.6±112.9	−681.1±106.8	−22.42±65.23	−2.47±55.9	−36.08 (−61.58 to −6.99)‡
Pulmonary function					
Percentage of predicted vital capacity	78.30±19.78	82.67±15.56	1.89±6.24	−0.74±7.42	3.31 (−0.59 to 6.35)‡
Percentage of predicted DL_{CO}	68.75±21.56	64.53±22.34	4.70±15.57	0.37±14.46	6.87 (0.62 to 13.05)‡
Distance on 6-min walk test (m)††	429.8±140.6	367.5±188.5	19.19±71.80	5.63±178.57	1.52 (−42.00 to 50.00)‡
Serum marker					
KL-6 (U/ml)	4065±3832	8175±11806	−1199±3098	4.70±9154	−0.13 (−0.25 to −0.01)‡‡
Carcinoembryonic antigen (ng/ml)	6.59±6.34	8.07±12.33	−1.36±3.20	−0.37±6.76	−0.40 (−1.90 to 1.10)‡
Surfactant protein D (ng/ml)	215.9±166.0	321.4±287.9	−55.90±121.2	−15.9±104.0	−21.00 (−63.00 to 19.00)‡
Surfactant protein A (ng/ml)	85.41±47.09	97.55±84.39	−21.82±24.80	−30.71±47.30	0.44 (−12.00 to 14.30)‡
High-sensitivity C-reactive protein (ng/ml)	1145±2437	1569±3643	544±2536	457±4505	77 (−113 to 329)‡
MCP-1 (pg/ml)	374.4±124.3	434.4±152.3	−36.1±91.5	17.0±102.4	−58.45 (−97.49 to −17.51)‡
Autoantibodies against GM-CSF (μg/ml)	75.35±68.01	51.95±47.90	8.58±24.94	−4.88±10.36	10.75 (1.70 to 17.05)‡

* Plus–minus values are means ±SD. CI denotes confidence interval.
† Between-group differences are expressed in one of the following ways: as a pseudo-median difference calculated with the use of the Hodges–Lehmann estimate based on the Mann–Whitney U test; as a coefficient of the interaction between time and trial agent per month calculated with the use of the linear mixed-effects model; or as a coefficient of trial agent calculated with the use of the generalized linear model. Because of the use of the Hodges–Lehmann estimator, the estimated difference is not the crude difference between the medians. These intervals have not been adjusted for multiple comparisons and hence cannot be used to infer the significance of the individual findings.
‡ This confidence interval was calculated with the use of the Mann–Whitney U test.
§ Scores on the modified Medical Research Council Dyspnea Scale range from 0 to 4, with higher scores indicating worse respiratory status.
¶ This confidence interval was calculated with the use of the linear mixed-effects model according to months since baseline.
‖ Scores on the COPD assessment test questionnaire range from 0 to 40, with higher scores indicating a more severe effect on a patient's quality of life.
** At week 25, data were not available for two patients in the GM-CSF group and one patient in the placebo group.
†† At baseline, data were not available for two patients in the GM-CSF group and three patients in the placebo group.
‡‡ This confidence interval was calculated with the use of the generalized linear model.

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The change in the percentage of predicted DLco from baseline to week 25 was 4.70±15.57 in the GM-CSF group and 0.37±14.46 in the placebo group, for a pseudo-median between-group difference of 6.87 (95% CI, 0.62 to 13.05, as assessed by means of a Mann–Whitney U test) (Table 2).

The mean distance on the 6-minute walk test in both groups at baseline was greater than 360 m; this indicates that most patients were able to engage in mild exercise. There was no substantial difference in the change in walking distance between baseline and week 25 in the GM-CSF group and the placebo group (19.19±71.80 m vs. 5.63±178.57 m, for a pseudo-median between-group difference of 1.52 m [95% CI, −42.00 to 50.00]) (Table 2).

EFFICACY IN CURRENT AND FORMER SMOKERS

We created an expanded linear mixed-effects model with all the variables including time, treatment, smoking history, and the two-way and three-way interactions of time, treatment, and smoking history, as well as trial site, symptom, disease severity, sex, age, dust exposure, and previous whole-lung lavage. This model was used to assess the interactions of background variables with the change in the alveolar–arterial oxygen gradient from baseline to week 25 (Table S4 in the Supplementary Appendix). The results of this analysis indicated that there was a minimal effect of inhaled GM-CSF in patients who were smokers.

AUTOANTIBODIES AGAINST GM-CSF

It is notable that the change in the level of autoantibodies against GM-CSF was greater in the GM-CSF group than in the placebo group (8.58±24.94 μg per milliliter vs. −4.88±10.36 μg per milliliter, for a pseudo-median difference of 10.75 [95% CI, 1.70 to 17.05]). This finding suggests that inhaled GM-CSF accelerated production of these autoantibodies (Fig. S2A in the Supplementary Appendix). The neutralizing capacity of the GM-CSF antibody did not change significantly in the GM-CSF group during the trial (Fig. S2B in the Supplementary Appendix).

ADVERSE EVENTS

No deaths occurred during the trial. Adverse events that occurred during the trial did not differ significantly between the two groups (Table S2 in the Supplementary Appendix). Serious adverse events occurred in 6 of the 33 patients who received GM-CSF. These events were ileus, congestive heart failure, worsening of autoimmune pulmonary alveolar proteinosis, pneumothorax, influenza type A infection, lacunar infarction, and breast cancer; 1 patient had both influenza type A infection and worsening of autoimmune pulmonary alveolar proteinosis. Three of the 31 patients who received placebo also reported serious adverse events during the trial; these events were cataract, worsening of autoimmune pulmonary alveolar proteinosis, and peripheral sensory neuropathy.

DISCUSSION

In this randomized, controlled trial involving patients with mild-to-moderate autoimmune pulmonary alveolar proteinosis, the change in the alveolar–arterial oxygen gradient was better with inhaled GM-CSF than with placebo. The effect of spontaneous remission, which occurs in approximately 20% of patients, and the placebo effect may have played a role in the earlier open-label phase 2 study. The effects of spontaneous remission on the reduction in symptoms of pulmonary alveolar proteinosis were obviated by the present randomized trial design.

Effects were observed on laboratory rather than clinical measures. This outcome may be a result of the exclusion of patients with the most severe cases to prevent exacerbation of the condition if they received placebo for 24 weeks. In the previous phase 2 study,[21] patients with severe autoimmune pulmonary alveolar proteinosis had a response to inhaled GM-CSF, and the exclusion of these patients from this trial may explain the rather modest effects on the alveolar–arterial oxygen gradient in the GM-CSF group and the modest between-group difference in symptoms as measured by the score on the COPD assessment test or 6-minute walk test. Since the primary and secondary end-point measures in the GM-CSF group changed together in parallel (although some changes were small), the data across multiple end-point measures indicated, at best, a modest therapeutic benefit.

The difference between current or former smokers and patients who had never smoked with respect to the increase in the alveolar–arterial oxygen gradient suggests that cigarette smoking

may affect the pathway of antibody production and thus stimulate GM-CSF neutralization. We speculate that long-term cigarette smoking may cause minimal remodeling of small airways, leading to changes in mucus production that affect the distribution of inhaled GM-CSF in the lung. In addition, alveolar macrophages in smokers may be functionally impaired beyond the defects caused by GM-CSF autoantibodies; this impairment may be responsible for the poor response to inhaled GM-CSF in smokers with autoimmune pulmonary alveolar proteinosis.

During the trial, we observed an increase in serum levels of autoantibodies against GM-CSF in the GM-CSF group but not in the placebo group. However, the neutralizing capacity did not differ between the groups, probably because of the duration of inhalation. Administration of subcutaneous, long-term, intermittent recombinant human GM-CSF can cause a transient induction of autoantibodies against GM-CSF in patients with metastatic colon cancer[26] and, in some patients, the antibody can be neutralizing.[27] Infusion of yeast-derived GM-CSF has also induced GM-CSF–reactive autoantibodies in patients with cancer.[28] Because of the lack of a placebo group, the previous phase 2 study could not show an increase in levels of autoantibodies against GM-CSF in patients who received GM-CSF; this finding may have been associated with the production of autoantibodies against the extrinsic, inhaled GM-CSF.

This trial has some limitations. First, only one dose of GM-CSF was tested. The effect of higher doses is not known but could be a basis for further research. Second, only one type of nebulizer was used for inhalation therapy. Although jet nebulizers are inexpensive and commonly used, nebulizers with newer vibrating mesh or membrane components, which are more efficient and facilitate shorter treatment times, could be evaluated. Third, the lyophilized formulation of recombinant human GM-CSF that was used in this trial required patients to dissolve the agent in saline before inhalation. Face-to-face instruction and a brochure and video instructions for home reference are needed. It is possible that a liquid formulation of GM-CSF would make administration simpler.

The results of our randomized, controlled trial showed that inhaled GM-CSF had a significant but very modest effect on the alveolar–arterial oxygen gradient in patients with pulmonary alveolar proteinosis. At the dose used, there were changes in some laboratory measures, but no clinically important changes in outcomes were noted.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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APPENDIX

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