Supplemental Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Campo I, Carey BC, Paracchini E, Kadija Z, De Silvestri A, Rodi G, De Amici M, Torre C, Zorzetto M, Griese M, Meloni F, Corsico AG, Trapnell BC, Mariani F. Inhaled GM-CSF Reduces the Need for Whole Lung Lavage and Improves Gas Exchange in Autoimmune PAP Patients

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TABLE OF CONTENTS **Page**

SECTION 1. ADMINISTRATIVE INFORMATION

Study Title	Inhaled GM-CSF Reduces the Need for Whole Lung Lavage and Improves Gas Exchange in Autoimmune PAP Patients
Trial Registration:	NCT00901511 (ClinicalTrials.gov)
Institutional Registration:	20080037329 (Policlinico San Matteo Institutional Ethics Board)
Sponsor	Agenzia Italiana del Farmaco
Product/Compound	Sargramostim (Leukine®)
Treatment/Procedure	Whole lung lavage (WLL)
Phase of the Study	2
FDA Regulated Study:	No
Principal Investigator	Maurizio Luisetti (Prior to July 2015) Francesca Mariani (After July 2015)
Statistician:	Carmine Tinelli, Annalisa De Silvestri
Clinical Trial Site	I.R.C.C.S. Policlinico San Matteo
Study Period	July 14, 2009 – June 1, 2016

ABBREVIATIONS

A-aDO_2	Alveolar-arterial Difference in oxygen concentration
ABG	Arterial blood gas
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine Aminotransferase
GMAb	Anti-Granulocyte Macrophage Colony Stimulating Factor antibodies
aPAP	Autoimmune Pulmonary Alveolar Proteinosis
AST	Aspartate Aminotransferase
CEA	Carcinoembryonic Antigen
CI	Confidence Interval
CT	Computer Tomography
CTR	Clinical Trial Report
Cyfra 21-1	Cytokeratin 19 Fragment
DLco	Diffusing Capacity of the Lungs for Carbon Monoxide
DSS	Disease Severity Score
EC	Ethics Committee
ECG	Electrocardiogram
FEV1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
HRCT	High resolution computed tomogram
ICU	Intensive care unit
IEC	Independent ethics committee
IMP	Investigational Medicinal Product
ITT	Intention-to-treat
KL-6	Krebs von den Lungen-6
PaO_2	Arterial oxygen tension
$PaCO_2$	Arterial partial pressure of carbon dioxide
PAP	Pulmonary Alveolar Proteinosis
Hct	Hematocrit
PPS	Per Protocol Set
QoL	Quality of Life
rhGM-CSF	Recombinant Human Granulocyte Macrophage Colony Stimulating Factor
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SF-36	Short form (36 question) patient-reported health survey
SOP	Standard Operating Procedure
SP-A	Surfactant Protein A
SP-B	Surfactant Protein B
SpO_2	Oxygen saturation (measured by pulse oximeter)
TLC	Total lung capacity
VC	Vital Capacity
WBC	White blood count
WLL	Whole Lung Lavage

SECTION 2. STATISTICAL ANALYSIS PLAN (SAP)

2.1 Overview

An intention-to-treat (ITT) population will be used as the primary and secondary analysis of efficacy and safety data.

All efficacy and safety variables will be summarized using descriptive methods. Number of cases, mean, standard deviation, median, first quartile, and third quartile will be calculated for the continuous numeric variables. Frequency and percentage will be used to summarize categorical variables. The 95% confidence intervals for all parameters will be calculated.

This SAP is based on the initial protocol and amendments described below (Section 3.2). The full protocol will be made available upon request to appropriate individuals/institutions for non-commercial purposes.

2.2 Protocol Amendments, dates, and rationale for changes

The SAP is based on the initial clinical trial proposal as approved by the Fondazione IRCCS Policlinico San Matteo Ethics Committee (IRCCS PSM EC) and subsequent changes as outlined below.

Document	Date	Summary and rationale
IRCCS PSM EC approval letter	Dec 3, 2008	Initial approval of proposed clinical trial
IRCCS PSM EC letter	Dec 17, 2008	Letter of project activation
IRCCS PSM EC letter	Jun 17, 2009	Approval of revised informed consent and information sheet
IRCCS PSM EC letter	Jul 1, 2015	Communication regarding change of the principal investigator
IRCCS PSM EC letter	Jul 21, 2015	Approval of amendment to change the principal investigator

2.3 Sample size determination

At the time this study was initiated, no outcome measures had been validated in aPAP patients for use in clinical trials or reported as a means to calculate sample size. Therefore, the sample size was based on our experience with WLL in Pavia, Italy; we estimated might result in resolution of the infiltrates (ground glass opacification) on the chest CT scan at a level of 30% in the Control group and 100% in the GM-CSF group. Using a two-tailed Fischer exact test, a sample size of nine patients per group was estimated to provide the trial power of 88% at the 5% significance level to detect a difference of 70% (30% and 100%, respectively) in the GGO score between the GM-CSF group and the Control group. Sample size was calculated with Query Advisor 4.0 software. Furthermore, because our population of aPAP patients receiving medical care in Pavia required an average of 6 WLL procedures per year, we estimated 18 patients with moderate-to-severe aPAP could be enrolled over a three-year period.

2.4 Hypotheses

The null hypothesis was that there would be no difference in time between baseline WLL and first use of rescue WLL between the treatment groups. An additional null hypothesis (regarding secondary endpoints) was that physiologic measures gas exchange (PaO_2, A-aDO_2, DLCO%), pulmonary function tests, biomarkers of PAP, and/or SF-36 Health Survey component scores would not be different the GM-

CSF group and the Control group.

The alternative hypothesis is that there would be an increase in the time between baseline WLL and first use of rescue WLL in the GM-CSF group compared to the Control group. An additional alternative hypothesis is that there would be a further improvement in physiologic measures gas exchange (P_AO_2, A-aDO_2, DLCO%), pulmonary function tests, biomarkers of PAP, or SF-36 Health Survey component scores in the GM-CSF group compared to the Control group.

2.5 Randomization and statistical analysis methods

2.5.1 Randomization

Study participants were screened and recruited from the population of patients receiving medical care for treatment of aPAP at the Pneumology Unit, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia, Italy. After providing written informed consent, each participant was randomly assigned, in a 1 to 1 ratio, to either the GM-CSF (Treatment) group or the WLL (Control) group with the use of a randomization list. The study statistician, Carmine Tinelli (IRCCS), created the randomization list using software located at the internet-based website 'Random.org' before the trial started. Notifications of each participant's assignment was provided in writing by the statistician to study investigators only after the assignment was made. The randomization list was maintained by the statistician and not available to the other study investigators at any time. Study clinicians performing whole lung lavage treatment of study participants were informed of each new participant's treatment assignment only after they had been screened, consented, enrolled, and assigned to a study group. Following assignment, participants, clinicians, and study investigators were unblinded about participants' group assignments and interventions except that radiologists providing assessments of participants' chest computed scans were blinded as to the treatment group before and during provision of their evaluations.

2.5.2 Statistical analysis methods

The analyses were performed according to the intention-to-treat (ITT) principle. The primary end point was the median difference in time to rescue WLL between the GM-CSF and Control groups compared using the Mann-Whitney test. Primary analysis of key secondary outcomes was performed by determining between-group differences using repeated measures analysis of variance (RM-ANOVA) after adjustment for baseline values, gender, age, and the number of patients at risk at each time point. Secondary analysis was performed by determining the between-group difference at each visit, after imputation of missing data using the last-observation carried forward (LOCF) method. Comparisons of the corresponding group means (parametric data), medians (non-parametric data), or numbers (categorical data) were made using Student's t-test, Mann-Whitney test, or Fisher's exact test, as appropriate. All reported p values are two-sided and have not been adjusted for multiple testing. P values of less than 0.05 were considered to indicate statistical significance. Analysis by RM-ANOVA was performed using Stata software version 14.2 and other analyses were performed using Prism for Mac OS software, version 9.51.

3.6 Analysis Set

The ITT population is defined as all individuals who were randomized to participate in the trial. The ITT population was used for the primary analysis, secondary analysis, and the safety analysis.

3.7 Definitions

A-aDO_2	Alveolar-arterial oxygen difference is a measure of the difference between the alveolar concentration (A) of oxygen and the arterial (a) concentration of oxygen
AE	An adverse event is defined as any untoward medical occurrence, including an exacerbation of a pre-existing condition, in a subject in a clinical investigation who signed informed consent. The event does not necessarily have to have a causal relationship with trial procedures.
Baseline	Baseline refers to the time immediately following administration of the initial, planned bilateral WLL, which occurred in the week prior to Study Month 0.
DLCco%	Diffusing capacity for carbon monoxide is the extent to which oxygen passes from the air sacs of the lungs into the blood, expressed as a percentage of the predicted value.
FEV1%	Forced expiratory volume is the amount of air that can forcibly be blown out in one second, after full inspiration, expressed as a percentage of the predicted value.
FVC%	Forced vital capacity is the maximum amount of air a person can expel from the lungs after a maximal inhalation, expressed as a percentage of the predicted value.
Relative day	The relative day of an event is derived as follows: Relative day = (Start date) - (Date of the second planned baseline WLL) + 1. For days before the start date, calculate as Relative day = (Start date) - (Date of the second planned baseline WLL). In this way, there will be no day = 0. Day 1 is the day after the second planned baseline WLL.
SpO_2	Indirect measurement of oxygen saturation using a finger probe, ear sensor or similar device.
SAE	A serious adverse event is defined as any adverse event which results in death, is immediately life-threatening, results in persistent or significant disability / incapacity, requires or prolongs subject hospitalization, is a congenital anomaly / birth defect, or is to be deemed serious for any other reason if it is an important medical event when based upon appropriate medical judgement which may jeopardize the subject and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions.
UAE	An unexpected adverse event is one, the nature or severity of which is not consistent with information in the relevant source document(s).
VC%	The vital capacity is the maximum amount of air a person can slowly expel from the lungs after a maximum slow inhalation, expressed as a percentage of the predicted value.

% predicted Calculation of % predicted of spirometry and DLCO parameters collected during the study will be made using the same equation for all determinations.

2.8 Summary statistics

Numeric data will be summarized by means of summary statistics. For continuous variables, the following summary statistics will be presented: number of observations, mean value, standard deviation, minimum, first quartile, median and third quartile. Categorical data will be presented as counts and percentages. The data will be presented by visit. Summary statistics will be presented by treatment group and assessment time and/or visit, as applicable.

2.9 Demographic and baseline clinical characteristics of the participants

A subject disposition will be produced, including number of subjects screened, randomized, withdrawn from trial (with reason for withdrawal), number of subjects withdrawing from treatment but remaining in trial, number of subjects completing the study, number of subjects included in the safety analysis, full analysis and per protocol sets. Demographics, standard baseline characteristics and aPAP history data will be presented using summary statistics.

2.10 Outcome Measures

2.10.1 Primary Outcome Measure

The primary outcome measure is the time, in months, between the scheduled baseline WLL and first administration of unscheduled 'rescue' WLL, which is referred to as 'time to rescue WLL'.

2.10.2 Secondary Outcome Measures

Key secondary outcome measures include change from baseline in:

- The number of patients requiring unscheduled, rescue WLL in the first 30 months following the baseline scheduled WLL.
- Peripheral arterial oxygen concentration (PaO_2).
- Alveolar-arterial difference in oxygen concentration (A-aDO_2).
- Diffusing capacity of the lungs for carbon monoxide (DLCO%).
- Ground glass opacification (GGO) of the lungs measured by visual scoring of chest computed tomography (CT) scan.
- Vital Capacity (VC%).
- Serum biomarkers of PAP (carcinoembryonic (CEA) antigen, Krebs von den Lungen-6 (KL-6) antigen, and cytokeratin fragment (Cyfra-21.1).

Other secondary outcome measures include change from baseline in:

- Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) component scores
- Serum GM-CSF autoantibody concentration.
- Peripheral blood white blood cell count.

- Peripheral blood platelet counts.

2.11 Primary Analyses

2.11.1 Efficacy of the Baseline WLL

The effects of the scheduled, WLL performed at baseline was analyzed as the change from the Pre-WLL (month -1) value to the Post-WLL (month 0) in the following outcome measures: PAP disease severity score, SF-36 component score (General health, Health change, Physical function, Energy/Fatigue, Pain, Social function, Emotional well-being, role limitations due to physical health, role limitations due to emotional problems), Spirometry (FEV1%, FVC%, FEV1%/FVC%, TLC%, VC%), pulmonary gas exchange (PaO_2, A-aDO_2, DLco%, $PaCO_2$), radiologically measured surfactant burden (change in GGO score), peripheral blood WBC and platelet count, GM-CSF autoantibody levels, and serum biomarkers of aPAP (serum Cyfra21.1, CEA, and KL-6 antigens).

2.11.2 Primary End Point

The primary end point will be analyzed as the between group difference in median time between the baseline WLL and the first administration of rescue WLL for patients in the GM-CSF group and the Control group. The between-group comparison will be done using the Mann Whitney test.

2.11.3 Secondary End Points

The occurrence and timing (i.e., study month) of rescue WLL administration in each group will be evaluated using Kaplan-Meyer analysis. The between-group difference in the number of patients requiring rescue WLL during the 30 months after the baseline WLL will be evaluated using Fisher's Exact test.

Primary analysis of key secondary end points will be evaluated using repeated measures analysis of variance (RM-ANOVA) after adjustment for baseline values, gender, age, and the number of patients at risk at each time point.

2.12 Secondary Analyses

Secondary analysis of secondary outcome measures were performed by determining between-group difference at each visit, after imputation of missing data using the last observation carried forward (LOCF) method for the following outcome measures: PaO_2, A-aDO_2, DLco%, VC, GGO, serum biomarkers of aPAP (serum Cyfra21.1, CEA, and KL-6 antigens), GM-CSF autoantibody levels, peripheral blood white blood cell count and platelet count, and SF-36 general health score.

The A-aDO_2 was measured consistently at our hospital in Pavia, Italy, and calculated using the following equation:

$$A\text{-}aDO_2 - (FiO_2 (P_{atm} - P_{H2O}) - PaCO_2/0.8) - PaO_2$$

where FiO_2 is fraction of inspired oxygen,

P_{atm} is ambient atmospheric pressure,
P_{H2O} is saturated vapour pressure of water at body temperature (set to 47 mmHg / 6.266 kPa),
$PaCO_2$ is arterial partial pressure of carbon dioxide, and
PaO_2 is arterial partial pressure of oxygen.

Assessments reported in kPa were converted to mmHg before the calculations were done.

The GGO was graded as the average of assessments made by three independent radiologists who evaluated CT scans visually and calculated the PAP-related pulmonary surfactant burden according to the following scale:

- Grade 1 = 1 segment affected
- Grade 2 = 2 – 5 segments affected
- Grade 3 = 6 – 9 segments affected
- Grade 4 = 10 – 14 segments affected
- Grade 5 = > 14 affected segments

2.13 Safety Analyses

Safety analyses were performed on the ITT population.

The primary safety analyses included enumeration of adverse events, serious adverse events, adverse drug reactions, and adverse events leading to discontinuation of the intervention, including clinically significant changes in laboratory tests, assessed at each study visit (1-9, Supplemental Table 1) and during periodic phone calls (at month 12 and 24) from enrollment through the 30 months following the scheduled baseline WLL (month 0).

The potential for bronchospasm was assessed by pulmonary function testing 20 minutes after the first administration of inhaled sargramostim.

2.14 Interventions

2.14.1 GM-CSF Group

- Scheduled Baseline WLL: All participants in this group will receive scheduled WLL at baseline (month 0).

- GM-CSF induction treatment: All participants in this group will receive inhaled GM-CSF (250 mcg daily, 7 consecutive days every other week for 12 weeks beginning 1 week after the scheduled baseline WLL).

- Washout period: All participants in this group will not receive inhaled GM-CSF treatment for 4 weeks immediately following GM-CSF induction treatment.

- GM-CSF maintenance treatment: All participants in this group will receive inhaled recombinant GM-CSF (sargramostim (Leukine®), 250 mcg daily on days 1 and 3 of every consecutive 14-day period for 6 months beginning 17 weeks after the scheduled baseline WLL at month 0).

- Unscheduled Rescue WLL: No participants in this group will receive any scheduled rescue WLL during the 30 months after the baseline WLL but all participants will be eligible to receive an unscheduled rescue WLL during this period if and only if they experience progression of aPAP resulting in respiratory failure (strictly defined as PaO_2 <60 mm Hg at rest OR PaO_2 > 60 mmHg at rest AND desaturation <90% at rest OR decline in SpO_2 of 5% or more during exercise testing). Any participant receiving a rescue WLL will be judged to have failed this intervention group and the time (study month) at which the rescue WLL was first required will be noted.

2.14.2 Control Group

- Scheduled Baseline WLL: All participants in this group will receive scheduled WLL at baseline (month 0).

- Unscheduled Rescue WLL: No participants in this group will receive any scheduled rescue WLL during the 30 months after the baseline WLL but all participants will be eligible to receive an unscheduled rescue WLL during this period if and only if they experience progression of aPAP resulting in respiratory failure (strictly defined as PaO_2 <60 mm Hg at rest OR PaO_2 > 60 mmHg at rest AND desaturation <90% at rest OR decline in SpO_2 of 5% or more during exercise testing). Any participant receiving a rescue WLL will be judged to have failed this intervention group and the time (study month) at which the rescue WLL was first required will be noted.

2.15 Concomitant medications

A history of all prescription and nonprescription medications will be collected. Concomitant medications lists will be updated at each visit to include prescription and nonprescription medication taken since the previous visit. The information to be collected will include name of drug (using the drug trade name if known), total daily dose and units, indication or clinical condition for which the medication was used.

2.16 Adverse events and serious adverse events

Absolute number of AEs, SAEs, ADRs, severe AEs and AEs leading to treatment discontinuation, including clinically significant changes in laboratory tests during the 30 months immediately following administration of the initial WLL at Study Month 0.

Adverse events have been evaluated on the basis of

- Worsening of the underlying disease or other pre-existing conditions, if they are judged clinically relevant by the investigator,
- Changes in vital signs, physical examination and laboratory test results, if they are judged clinically relevant by the investigator,
- Changes in clinical endpoints (e.g. DLco%, spirometry, HRCT), if they are judged clinically relevant by the investigator.

The intensity of the adverse event will be judged based on the following:

- Mild: Awareness of sign(s) or symptom(s) which is/are easily tolerated
- Moderate: Enough discomfort to cause interference with usual activity

- Severe: Incapacitating or causing inability to work or to perform usual activities

Medical judgment will be used to determine the causal relationship of the adverse event to any trial-related procedure, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge. The causal relationship of the adverse event will be judged as

- Definitely not related
- Probably not related
- Possibly related
- Probably related
- Definitely related

2.17 Adjustment for covariates

The absolute between-group mean difference for continuous outcome variables during follow-up were analyzed using repeated measures analysis of variance after adjustment for baseline values, gender, age, and the number of patients at risk at each time point.

2.18 Handling of Dropouts and Missing Data

An intention-to-treat analysis without imputation of missing data was used to determine the absolute between-group mean difference in outcome variables during follow-up using repeated measures analysis of variance after adjustment for baseline values, gender, age, and the number of patients at risk at each time point.

SECTION 3. SUPPLEMENTAL TABLE AND FIGURES

Table S1. Schedule of Events and Procedures [a]

Event	Visit type	Screening	Pre-WLL	Baseline	Treatment				Follow-Up	
	Visit number	1	2	3	4	5	6	7	8	9
	Study month	-3	-1	0	1	3	6	10	18	30
Informed consent		+								
Eligibility [b]		+								
Medical history		+								
PFT (full)		+	+	+	+	+	+	+	+	+
Exercise testing [c]		+	+	+	+	+	+	+	+	+
ABG analysis (PaO_2, A-aDO_2) [d]		+	+	+	+	+	+	+	+	+
Chest radiograph		+		+	+		+			
Chest CT scan [e]			+	+		+		+	+	+
SF36 health survey questionnaire			+	+	+	+	+	+	+	+
Serum anti-GM-CSF antibody test [f]			+	+	+	+	+	+		
Biomarker tests [g]										
Safety assessment [h]			+	+	+	+	+	+	+	+
Bronchial hyper-responsiveness [i]					+	+	+	+	+	+
Scheduled baseline WLL [j]				+						

Definition of abbreviations: ABG = arterial blood gas, A-aDO_2 = alveolar arterial difference in oxygen concentration, CT = computed tomogram, GM-CSF = granulocyte/macrophage-colony stimulating factor PaO_2 = peripheral arterial oxygen concentration, PFT = pulmonary function testing, SF-36 = short form 36 question health surface, WLL = whole lung lavage.

[a] Study procedures and events are indicated by +.

[b] Includes evaluation of both inclusion and exclusion criteria as listed in the manuscript.

[c] Performed using a treadmill with the modified Bruce protocol as described in the methods.

[d] Performed with the subject breathing room air in the supine position for at least ten minutes.

[e] Visual assessment of the degree of ground glass opacification performed as reported (Tazawa, Am J Resp Crit Care Med. 2010; 181(12): 1345-1354).

[f] Performed at the Translational Pulmonary Science Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA as reported (Uchida, J Immun. Meth. 2014; 402: 57-70).

[g] Includes serum carcinoembryonic (CEA), Krebs von den Lungen-6 (KL-6), and Cyfra-21-1.

[h] Includes solicitation and recording of adverse events related to history, physical exam, vital signs, laboratory tests (complete blood count, cell differential; urinalysis), electrocardiogram.

[i] Evaluated by measurement of spirometry 20 minutes after the first administration of inhaled sargramostim.

[j] Scheduled baseline bilateral whole lung lavage (WLL) performed according to an institutional standardized protocol as reported (Beccaria, Eur. Resp. J. 2004; 23 (4): 526-531). One week after the baseline WLL, participants were evaluated as indicated (exercise testing, ABG analysis, Chest radiography, CT scan, SF-36 health survey questionnaire, serum anti-GM-CSF antibody test and safety assessment).

Figure S1. Schematic of the clinical trial design. The study comprised a 3-month observation period, a 10-month randomized treatment period, and a 20-month follow up period during which no further therapy was scheduled to be administered. All enrolled participants entered the observation period and underwent a planned baseline bilateral whole lung lavage (WLL) at month 0 and were randomized to receive inhaled GM-CSF or no further therapy (GM-CSF and Control groups, respectively). Inhaled GM-CSF (Leukine®) was administered using high efficiency vibrating mesh nebulizer in two phases: a GM-CSF-induction therapy phase (250 μg daily, 7 consecutive days every other week for 12 weeks beginning 1 week after the baseline WLL (indicated by thick black bars)) followed by a 4-week washout period without intervention administration and then a GM-CSF-maintenance therapy phase (250 μg daily on days 1 and 3 of every consecutive 14-day period for 6 months beginning 17 weeks after the baseline WLL (indicated by thin black bars)). All patients were eligible to receive unscheduled WLL 'rescue' therapy at any time during the trial as necessary for aPAP disease progression, which was strictly defined by a resting PaO_2 of <60 mm Hg or PaO_2 >60 mm Hg and SpO_2 <90% at rest or a decline of 5% during exercise testing. All patients were followed for at least 30 months after the baseline WLL.

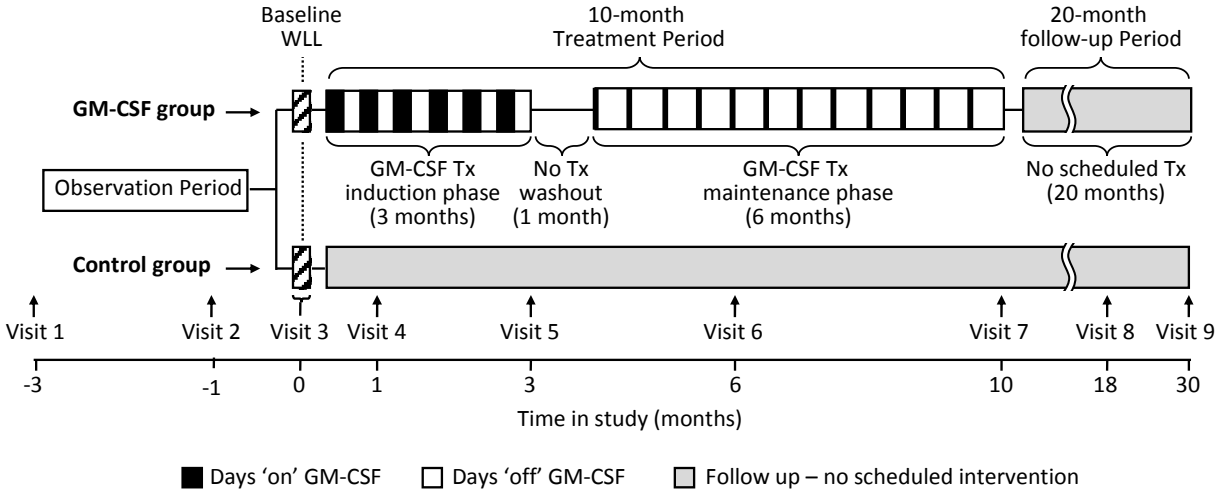


Figure S2. Administration of interventions to the patients. All patients received a scheduled, baseline WLL (black arrow before month 0) and were randomized to receive either no further scheduled treatment or inhaled GM-CSF as described in the methods (Control and GM-CSF groups, respectively). All patients were eligible to receive rescue WLL (black boxes) as necessary for progression of aPAP lung. Patients with progression of aPAP requiring rescue WLL, defined by strict adherence to prespecified measures, were judged to have failed their assigned intervention. Individuals that withdrew for personal reasons and the time they withdrew are indicated (X).

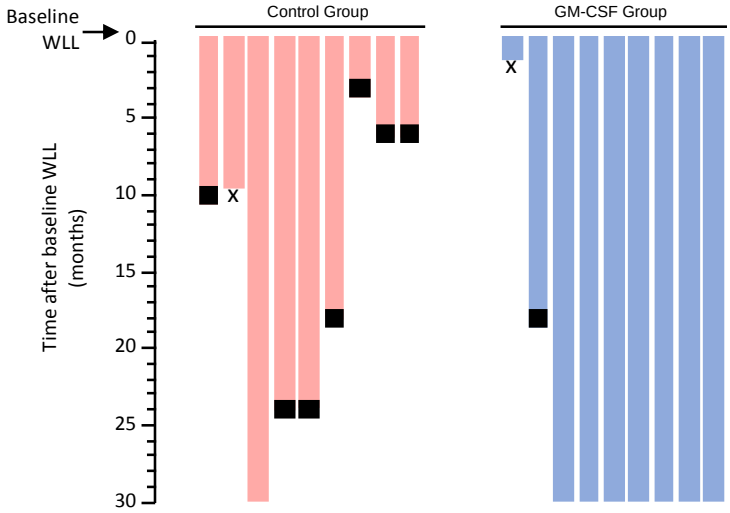


Figure S3. Effects of WLL and inhaled GM-CSF on pulmonary surfactant burden. Patients in the GM-CSF group (filled symbols) or Control group (open symbols) received a chest CT scan with visual analysis to quantify ground glass opacification (GGO) at the indicated times (symbols). Data are median (+/- interquartile range) GGO after imputation of missing data by the last observation carried forward method. The timing of administration of inhaled GM-CSF as induction-therapy (thick vertical black lines) or maintenance-therapy (thin vertical black lines) to participants in the GM-CSF group as well as periods with no scheduled intervention (open boxes) are shown.

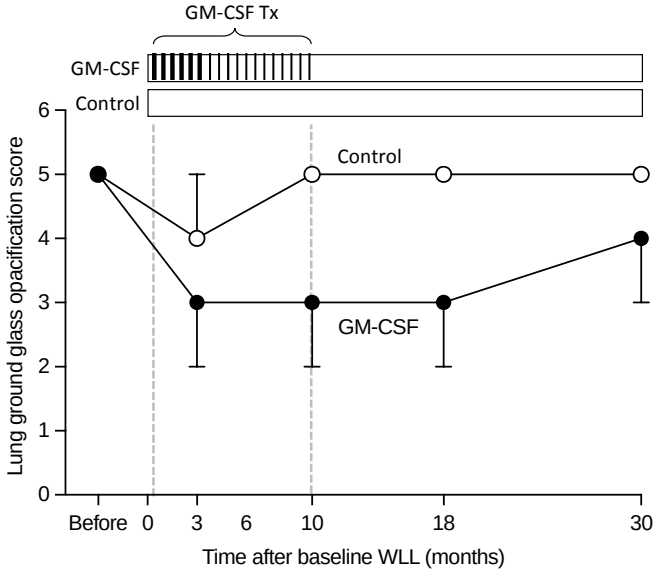


Figure S4. Effects of WLL and inhaled GM-CSF on serum biomarkers of PAP. Data are mean (+/- standard deviation) serum biomarkers for patients in the GM-CSF group (filled symbols) or the Control group (open symbols) after imputation of missing data by the last observation carried forward method. The timing of administration of inhaled GM-CSF as induction-therapy (thick vertical black lines) or maintenance-therapy (thin vertical black lines) to participants in the GM-CSF group as well as periods with no scheduled intervention (open boxes) are shown.

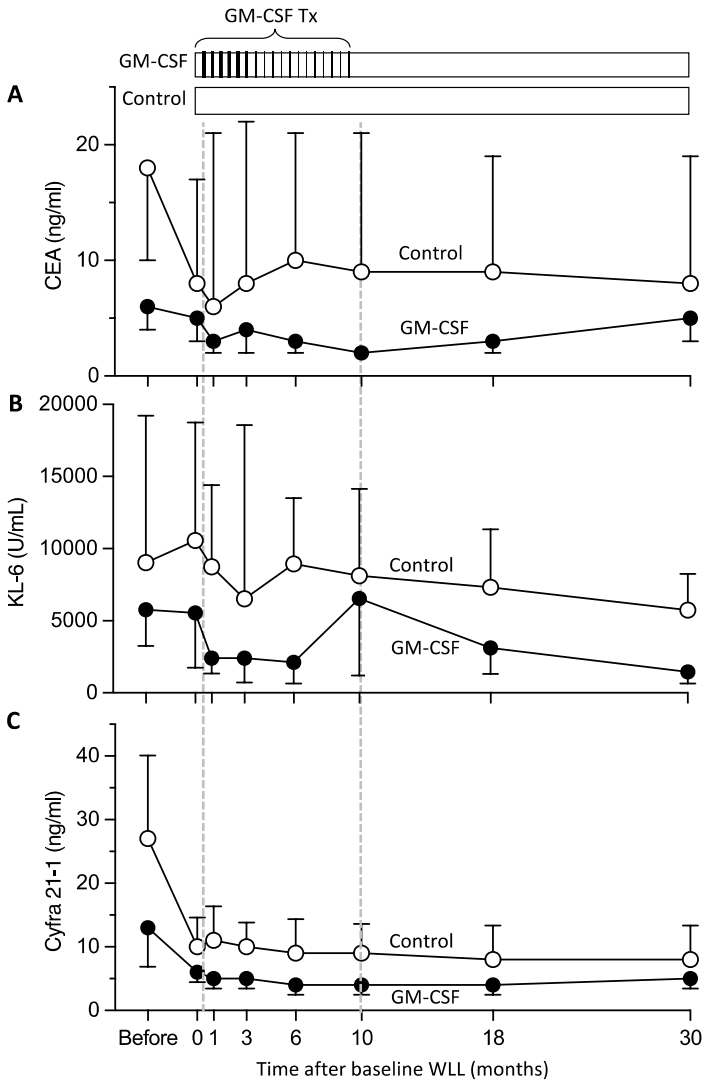


Figure S5. Effects of WLL and inhaled GM-CSF on serum GM-CSF autoantibody. Data are mean (+/- standard deviation) serum GM-CSF autoantibody levels for patients in the GM-CSF group (filled symbols) or the Control group (open symbols) after imputation of missing data by the last observation carried forward method. The timing of administration of inhaled GM-CSF as induction-therapy (thick vertical black lines) or maintenance-therapy (thin vertical black lines) to participants in the GM-CSF group as well as periods with no scheduled intervention (open boxes) are shown.

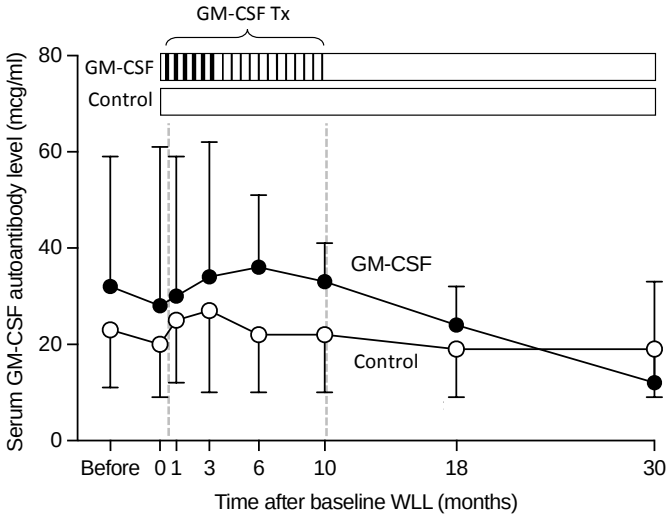
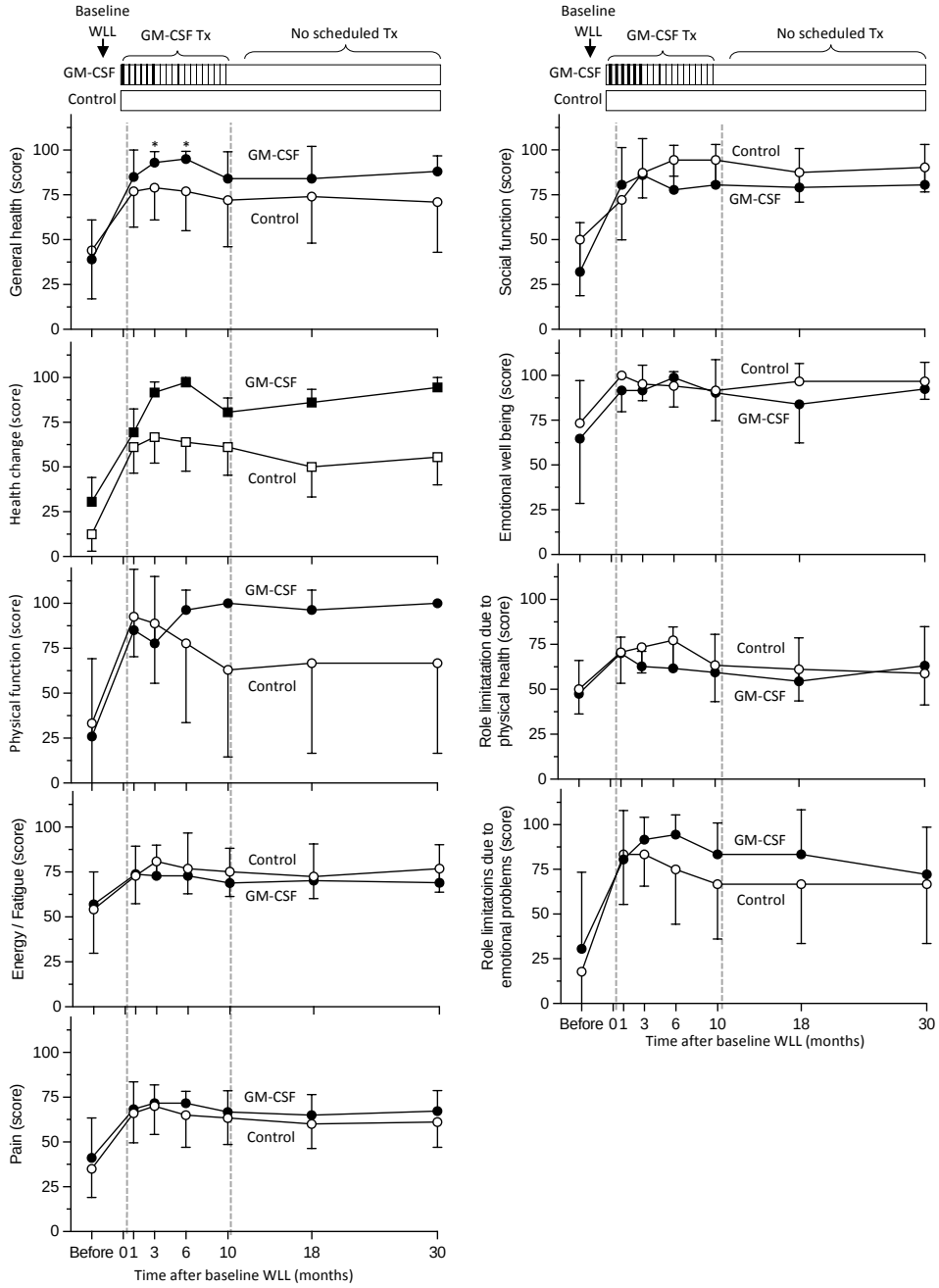


Figure S6. Effects of WLL and inhaled GM-CSF on health status. Data are mean (+/- standard deviation) SF-36 component scores for patients in the GM-CSF group (filled symbols) or the Control group (open symbols) after imputation of missing data by the last observation carried forward method. The timing of administration of inhaled GM-CSF as induction-therapy (thick vertical black lines) or maintenance-therapy (thin vertical black lines) to participants in the GM-CSF group as well as periods with no



scheduled intervention (open boxes) are shown.

Figure S7. Effects of WLL and inhaled GM-CSF on WBC and platelet count. Data are mean (+/- standard deviation) WBC count and platelet count for patients in the GM-CSF group (filled symbols) or the Control group (open symbols) after imputation of missing data by the last observation carried forward method. The timing of administration of inhaled GM-CSF as induction-therapy (thick vertical black lines) or maintenance-therapy (thin vertical black lines) to participants in the GM-CSF group as well as periods with no scheduled intervention (open boxes) are shown.