A Phase II Clinical Trial of Low-Dose Inhaled Carbon Monoxide in Idiopathic Pulmonary Fibrosis

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BACKGROUND: Preclinical studies have demonstrated that low-dose carbon monoxide (CO) can abrogate experimental lung fibrosis. To test the therapeutic role of inhaled CO, we designed a clinical study in patients with idiopathic pulmonary fibrosis (IPF).

METHODS: We conducted a multicenter, phase IIa, double-blinded, sham-controlled, clinical trial. Patients with IPF were randomized to treatment with inhaled CO at 100 to 200 parts per million or to inhaled 21% oxygen for 2 h daily, twice weekly, for 12 weeks. The primary study end point was the difference in change in matrix metalloproteinase-7 (MMP7) serum concentration after 12 weeks of treatment. Secondary end points included pulmonary function test measures, 6-min walk distance, rates of adverse events, acute exacerbation, hospitalization and death, and quality of life measures.

RESULTS: Fifty-eight subjects were randomized to treatment with inhaled CO (n = 29) or placebo (n = 29). Despite modest increases in CO blood levels, the change in MMP7 concentrations after 12 weeks of treatment did not significantly differ between the study arms (MMP7 difference at week 12, -0.90 ng/mL; 95% CI, -4.18 to 2.38 ng/mL). No differences were observed in physiologic measures, incidence of acute exacerbations, hospitalization, death, or patient-reported outcomes. Importantly, no differences in distribution of adverse events were noted between the treatment arms.

CONCLUSIONS: Inhaled CO is well tolerated and can be safely administered to patients with IPF in the ambulatory setting; however, inhaled CO did not result in significant changes in study end points. Our findings support testing the efficacy of inhaled therapies in future IPF clinical trials.

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ABBREVIATIONS: CO = carbon monoxide; DLCO = diffusing capacity for carbon monoxide; HO-1 = heme oxygenase-1; IPF = idiopathic pulmonary fibrosis; MMP7 = matrix metalloproteinase-7; ppm = parts per million

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Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, fibrosing parenchymal lung disease with increasing prevalence and rising mortality.¹ Until recently, lung transplantation was the only intervention known to improve survival²; recent clinical trials testing the efficacy of nintedanib³ and pirfenidone⁴ have demonstrated that antifibrotic agents reduce the rate of decline in lung function, may decrease the risk of acute exacerbations, and potentially improve survival in patients with IPF. Although these groundbreaking trials signal a new era for the treatment of progressive fibrotic lung diseases, the modest improvement in lung function, the frequency of significant side effects leading to discontinuation of therapy, and the lack of improvement in quality of life highlight the pressing need for the development of new therapies.⁵

Carbon monoxide (CO) is an endogenously produced diatomic gas that exerts diverse biologic functions, including protection against oxidative injury and cell death, inhibition of cell proliferation, suppression of matrix production and inflammation, and increased fibrinolysis, all of which are important in the pathogenesis of pulmonary fibrosis.^{6,7} CO is made in the body by heme oxygenase-1 (HO-1), one of the few inducible molecules that can protect the lungs from an increased oxidant burden under circumstances of stress.⁸ HO-1 is ubiquitously expressed, and is responsible for degradation of heme to biliverdin, free iron, and CO. Although all three products of HO-1 activity have been shown to possess cytoprotective properties, CO has been most extensively studied with respect to its effects on lung disease. Tsuburai et al⁹ reported that adenoviral transfer of HO-1 protected mice from bleomycin-induced fibrosis. Extending these findings to CO, we have shown that low concentrations (250 parts per million [ppm]) of inhaled CO, even when administered for as little as 3 h/d after bleomycin treatment, can attenuate the development of fibrosis.¹⁰ Mice treated with CO had significantly lower fibrotic/reparative histology scores

than animals receiving bleomycin alone. Over the last two decades, numerous studies have shown that CO exerts cytoprotective effects in preclinical models of organ injury developed in rodents, pigs, and nonhuman primates.¹¹ In humans, administration of low-dose inhaled CO to healthy volunteers and patients with COPD is well tolerated.^{12,13}

A major obstacle to the efficient design and execution of clinical trials in IPF is the reliance on lung physiology measurements (FVC), functional outcomes (6-min walk distance), or hospitalization and survival as primary outcome measures.¹⁴ These measurements appear poorly responsive to therapy in IPF (likely because of their slow rates of change), and their use as primary end points requires the design of clinical trials with relatively large populations and of long duration. Molecular markers that predict disease progression and/or survival are attractive candidates for cohort enrichment (ie, identifying subjects at increased risk of disease progression) and may represent alternative molecular outcomes that could demonstrate therapeutic response more efficiently. Matrix metalloproteinase-7 (MMP7) is one of the most extensively studied biomarkers in IPF. MMP7 levels are increased in the lung and peripheral blood compartments of patients with IPF.¹⁵ Several studies have independently demonstrated that increases in peripheral blood MMP7 levels are associated with increased disease severity and reduced survival.¹⁶ Moreover, MMP7 plasma levels have been previously measured in a phase I IPF clinical trial.¹⁷

Here we report the results of a phase IIa, randomized, sham-controlled, multicenter study designed to test the safety, tolerability, and efficacy of inhaled CO in patients with IPF. We hypothesized that low-dose inhaled CO could be safely administered in the ambulatory setting, would reduce MMP7 serum levels, and improve clinical indicators of disease progression.

Methods

Subjects were randomized in a 1:1 fashion to treatment with inhaled CO at 100 to 200 ppm, or to placebo administration of inhaled 21% oxygen. CO or placebo was administered under close supervision twice weekly in the clinic setting, for 2 h per session, for a total of 12 weeks, at eight participating clinical centers. Participants were followed for up to 48 weeks after randomization. Study staff was trained to monitor subjects for CO toxicity and to monitor ambient CO levels during drug administration (subsequently

Drs Rosas and Goldberg contributed equally to this manuscript.

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discussed). All participants provided informed consent. Data safety monitoring (Data Safety Monitoring Board) and site-specific institutional review board approval at each of the participating sites were obtained prior to study initiation and subject enrollment. All participants provided informed consent.

Inclusion and Exclusion Criteria

Adults 18 to 85 years of age were considered eligible if their diagnoses of IPF were made in accordance with published guidelines.¹⁸ Patients with mild to moderate lung disease as defined by an FVC of $\geq 50\%$ predicted, measured in accordance with guidelines published by the American Thoracic Society,¹⁹ and the absence of supplemental oxygen requirement at rest, were eligible for study participation. Subjects were excluded if they had evidence of active infection within the month prior to screening, significant obstructive respiratory defect (postbronchodilator ratio of FEV₁/FVC $< 70\%$ predicted), supplemental oxygen requirement at rest (to maintain an oxygen saturation $> 88\%$), history of myocardial infarction within 1 year prior to screening, heart failure within 3 years prior to screening, or cardiac arrhythmia requiring drug therapy. Additionally, subjects were excluded if they were pregnant or breastfeeding, participating in other IPF clinical trials, or actively smoking within 4 weeks of screening (according to self-report).

Randomization

Randomization occurred via random assignment. The trial statistician generated randomization codes using the permuted block method; a block size of 12 was chosen. SAS 9.3 (SAS Institute) PROC PLAN statement was used to generate the randomization schema. Opaque randomization envelopes were prepared, sequentially numbered with study identification, and used under the supervision of a designated investigator at the Brigham and Women's Hospital.

CO Dosing and Administration

Certified medical grade CO gas (single-use cylinder units with predetermined CO gas concentration of 100 or 200 ppm) or oxygen (21%) was delivered through a CPAP facemask at 15 L/min (Fig 1). Noninvasive co-oximetry measurements were performed with a Massimo device,²⁰ correlated with measurement of CO levels via arterial blood gas during the screening visit and performed at multiple time points during subsequent dosing sessions (every 15 min during the 2-hour treatment session and 15 and 30 min postdosing). Unblinded study staff monitored co-oximetry at multiple time points and after each drug administration. CO dosage was adjusted to maintain co-oximetry levels $\leq 8\%$ (see study

protocol in e-Appendix 1). Ambient air CO levels were measured in real time by an industrial infrared CO analyzer.

Study End Points

The primary study end point was the difference in change in MMP7 serum concentration (ng/mL) after 12 weeks of inhaled CO therapy or placebo. Peripheral blood samples were obtained and processed at designated research visits at participating sites. Serum sample aliquots were stored locally at -80°F and subsequently shipped in dry ice to a Brigham and Women's Hospital central laboratory for processing. Serum MMP7 levels were measured using an enzyme-linked immunosorbent assay kit (R&D Systems). Briefly, serum samples were added in triplicate to 96-well plates coated with MMP7 antibody and then incubated at room temperature for 2 h. Conjugated secondary antibody was added, and the plate was incubated for another 2 h. Plates were then incubated with the substrate solution for 1 h, and reaction was terminated with stop solution. Concentration of MMP7 was calculated from a standard curve using optical density (490 nm) measurements.

Secondary end points included the difference in change in MMP7 concentration after completion of the 12-month study and the change in pulmonary function measurements, 6-min walk distance, quality of life as assessed by the St. George's Respiratory Questionnaire,²¹ rates of acute exacerbations as defined by published criteria²² and designated by site principle investigators, and rates of adverse events, serious adverse events, hospitalization, and survival at the completion of 12 weeks of treatment and at the completion of a total of 48 weeks of follow-up. Analysis of end points was conducted when all subjects completed the 12-week dosing period and at the completion of 48-week follow-up.

Sample Size Calculation

The study was powered to detect a 2.4-ng/mL difference in mean serum concentrations with a common SD of 2.2 ng/mL (effect size, 1.1) in the MMP7 level between treatment groups with two-sided α of 0.05 and $> 80\%$ power. The calculation accounted for a loss to follow-up rate of up to 15.5%.

Statistical Analysis

All analyses were performed according to the intention-to-treat principle. All subjects were included in the analysis and assigned to the randomly allocated treatment arm. Repeated-measure analysis of variance was used to assess (1) mean change in serum MMP7 levels over time, (2) group difference between placebo

Figure 1 – Administration of CO or placebo: certified medical grade CO gas (single-use cylinder units with predetermined CO gas concentrations of 100 or 200 ppm) or oxygen (21%) was delivered through a tight-fitting CPAP facemask at 15 L/min. The mask was connected to tubing with a one-way expiratory valve to prevent accumulation and rebreathing of exhaled gas. ASCO = ASCO Power Technologies; CO = carbon monoxide; ppm = parts per million.

and CO treatment, and (3) interaction between time and group effect. The continuous secondary outcomes obtained at different time points were analyzed using repeated-measure analysis of variance. Tukey-Kramer adjustment was made to all post hoc pairwise comparisons. The categorical secondary outcomes were

analyzed using either χ^2 or Fisher exact tests. Time to event analyses were performed for mortality, acute exacerbations, and hospitalization using Kaplan-Meier method and Cox proportional hazards regression. All statistical analyses were conducted using SAS 9.3.

Results

Study Enrollment and Patient Characteristics

Sixty-five subjects were screened in interstitial lung disease clinics at eight academic pulmonary fibrosis programs in the United States between December 1, 2011, and March 12, 2014. Fifty-eight subjects were equally randomized to the CO treatment ($n = 29$) or placebo ($n = 29$) groups. Of these, 51 subjects (88%) completed the 12-week dosing period, 45 (77%) completed both the dosing and 48-week follow-up periods, and 13 (22%) were prematurely

terminated (Fig 2). One subject randomized to the CO group was administered 21% oxygen in the context of a protocol deviation (Fig 2). This subject was included in the CO group for the purposes of study analysis, as per our intention-to-treat analysis plan. A post hoc analysis with subject assignment per protocol did not yield alternative results. Baseline demographics, imaging, biopsy findings, and pulmonary function testing of randomized subjects are outlined in Table 1. No meaningful differences in baseline characteristics were noted between subjects randomized to the two study arms.

Figure 2 – Flowchart of study enrollment: a total of 65 subjects were screened and 58 subjects were randomized in 1:1 fashion to receive inhaled CO or placebo. See Figure 1 legend for expansion of abbreviation.

TABLE 1] Baseline Characteristics by Study Cohort

Characteristic	CO Group (n = 29)	Placebo Group (n = 29)	P Value
Age, y	66.6 ± 6.1	68.6 ± 8.4	.3234
Male sex	25 (86.2)	22 (75.9)	.5045
Ethnicity			> .99
Hispanic or Latino	1 (3.5)	2 (6.9)	
Other	28 (96.5)	27 (93.1)	
Race			.5125
White	25 (86.2)	27 (93.1)	
Black	1 (3.4)	2 (6.9)	
Asian	2 (7.0)	0 (0.0)	
Other	1 (3.4)	0 (0.0)	
HRCT scan			.2790
UIP pattern	16 (55.2)	20 (69.0)	
Possible UIP pattern	13 (44.8)	9 (31.0)	
Biopsy available ^a			> .99
Definite UIP	16 (88.9)	17 (94.4)	
Probable UIP	2 (11.1)	1 (5.6)	
Predose carboxyhemoglobin, %	1.03 ± 1.59	1.32 ± 1.52	> .99
FVC, L	2.97 ± 0.63	2.72 ± 0.87	.9622
FVC % predicted	72.37 ± 17.14	69.87 ± 14.01	.9999
TLC % predicted	67.72 ± 13.05	65.36 ± 13.61	.9996
Dlco % predicted	40.82 ± 12.28	41.46 ± 13.95	> .99

Values are No. (%), mean ± SD, or as otherwise indicated. CO = carbon monoxide; Dlco = diffusing capacity for carbon monoxide; HRCT = high-resolution CT; TLC = total lung capacity; UIP = usual diffusing capacity for carbon monoxide interstitial pneumonitis.

^aCO group: n = 18; placebo group: n = 18.

Carboxyhemoglobin Monitoring

The maximum carboxyhemoglobin level measured during each treatment ranged from 2.24% ± 2.2% to 3.82% ± 2.79% in the CO group and 1.75% ± 1.78% to 2.62% ± 2.70% in the placebo group. There was evidence that the maximum mean change in carboxyhemoglobin levels was significantly higher in the treatment group than the placebo group ($P = .02$); however, no significant differences in co-oximetry levels were observed at any individual time point between the two study arms (Fig 3). Twenty-eight out of a total of 29 subjects randomized to treatment with CO received at least one dose of 200 ppm. During the treatment visits, mean maximum carboxyhemoglobin levels for subjects treated with 200 ppm ranged from 2.41 ± 2.01 to 3.67 ± 2.76, which was similar to that reported for all doses administered in the treatment group.

Analysis of the Primary End Point

There was no significant difference in the change of serum MMP7 concentrations between the CO-treated group and placebo after the 12-week dosing period ($P = .207$). Least

squares mean for change from baseline to week 12 was −0.15 ng/mL (95% CI, −1.31 to 1.01) for the CO group and 0.88 ng/mL (95% CI, −0.25 to 2.02) for the

Figure 3 – Max COHB during drug administration by treatment group: when all doses were averaged, the Max mean change in COHB levels was significantly higher in the treatment group than placebo ($P = .02$). No significant differences in co-oximetry levels were observed at any individual time point between the two study arms. COHB = carboxyhemoglobin; Max = maximum.

placebo group. Although over the course of the study there was a significant change in MMP7 levels when combining both groups ($P = .006$), no significant differences in MMP7 levels were observed between groups after the 48-week follow-up period ($P = .815$) (Fig 4).

Analysis of Secondary End Points

Although there was evidence of decline in FVC ($P = .002$), total lung capacity ($P = .0001$), and diffusing capacity of the lungs for carbon monoxide (DLCO) ($P = .0006$) over the course of the study when combining both groups, there was no evidence that the change in measurement of FVC percent predicted was influenced by treatment at either completion of treatment ($P = .574$) or study completion ($P = .262$). Least squares mean for change from baseline to week 12 was -1.57% (95% CI, -3.38% to 0.23%) in the CO group and -0.84% (95% CI, -2.65% to 0.96%) in the placebo group (Fig 5). Least squares mean for change from end of treatment to study completion was -2.53% (95% CI, -5.83 to 0.78) in the CO group and -4.45 (95% CI, -7.67 to -1.22) in the placebo group. No differences were observed when absolute FVC values were used for the analysis over the course of the study. We also did not observe a treatment effect over time in TLC percent predicted values at completion of treatment ($P = .59$) or at study completion ($P = .641$). Least squares mean for change from baseline to week 12 was -2.29% (95% CI, -4.53% to -0.05%) in the CO group and -1.44% (95% CI, -3.63% to 0.76%) in the

placebo group. Least squares mean for change from end of treatment to study completion was -3.06 (95% CI, -5.91 to -0.21) in the CO group and -4.46 (95% CI, -7.20 to -1.72) in the placebo group. Similarly, the change in DLCO percent predicted values was not different between groups at the completion of treatment ($P = .740$) or at study completion ($P = .904$). Least squares mean for change from baseline to week 12 was 1.10% (95% CI, -1.72% to 3.93%) in the CO group and 0.46% (95% CI, -2.18% to 3.10%) in the placebo group. Least squares mean for change from end of treatment to study completion was -4.90% (95% CI, -8.63 to -1.17) in the CO group and -5.93 (95% CI, -9.47 to -2.40) in the placebo group. Finally, we did observe a significant change over time in the 6-min walk distance in both groups at both treatment completion ($P = .010$) and study completion ($P = .010$). Least squares mean for change from baseline to week 12 was 35.54 m (95% CI, -61.24 to -9.85 m) in the CO group and 12.92 m (95% CI, -12.78 to 38.61 m) in the placebo group (Fig 5). Least squares mean for change from end of treatment to study completion was 20.24 m (95% CI, -10.94 to 51.43 m) in the CO group and -55.56 m (95% CI, -86.46 to -24.67 m) in the placebo group.

Patient-Reported Outcomes

There was no evidence that change in St. George's Respiratory Questionnaire scores was influenced by treatment at either treatment completion ($P = .812$) or study completion ($P = .126$). Least squares mean for change from baseline to week 12 was -2.12 (95% CI, -5.53 to 1.28) in the CO group and -1.55 (95% CI, -4.99 to 1.89) in the placebo group (Fig 6). Least squares mean for change from end of treatment to study completion was 4.32 (95% CI, -0.09 to 8.73) in the CO group and 7.92 (95% CI, 3.45 to 12.40) in the placebo group.

No differences were observed in the incidence of acute exacerbation, hospitalization, death, or patient-reported outcomes between study groups. Six subjects withdrew from participation during the course of the study because of worsening pulmonary symptoms or disease progression, two in the placebo group and four in the treatment group. One acute exacerbation of IPF was reported during the course of the study, occurring in a patient receiving CO, but no significant differences in the distribution of acute exacerbations were observed. Three of the subjects who withdrew ultimately died, two in the placebo group and one in the treatment group.

Figure 4 – Primary end point, MMP-7 serum concentration, during the study period by treatment group: although MMP-7 levels overall significantly increased over time combining the two groups ($P = .006$), there was no significant difference observed in the primary study end point of reduction in serum MMP-7 levels between the carbon monoxide-treated group and placebo after the 12-wk dosing period. MMP-7 = matrix metalloproteinase-7.

Figure 5 – A-D, Secondary end points, (A) FVC, (B) TLC, (C) DLCO, and (D) 6-min walk distance: no significant differences were observed in measures of pulmonary function or 6-min walk distance between the two treatment arms after the 12-wk dosing period or after 12-mo of follow-up. DLCO = diffusing capacity for carbon monoxide; TLC = total lung capacity.

Subjects that withdrew had a statistically significant ($P = .0258$) reduced DLCO (24.75 ± 1.71) compared with the remaining participants in the treatment arm (43.39 ± 11.20). No other differences in baseline clinical characteristics were observed.

Adverse Events

A total of 254 nonserious adverse events from 51 subjects were reported during the course of the study. The most common reports were respiratory adverse events; study subjects reported 37% and 38% adverse events in the CO and placebo treatment arms, respectively (Table 2). A total of 17 serious adverse events from 10 subjects were reported during the study period. These included five reports of IPF disease progression, two reports of pneumonia, and one report each of worsening dyspnea, acute pancreatitis, acute

Figure 6 – Self-reported outcomes: no significant differences were observed in scores on the St. George's Respiratory Questionnaire between the two treatment arms after the 12-wk dosing period or after 12-mo of follow-up.

TABLE 2] Nonserious Adverse Events by Cohort

Organ System	CO Group (119 events)	Placebo Group (135 events)
Respiratory/thoracic/mediastinal	44 (36.97)	51 (37.78)
Nervous system	11 (9.24)	13 (9.63)
Gastrointestinal	11 (9.24)	11 (8.15)
Musculoskeletal/connective tissue	10 (8.40)	7 (5.19)
Cardiac	3 (2.52)	11 (8.15)
Infection	7 (5.88)	7 (5.19)
Skin/subcutaneous	9 (7.56)	3 (2.22)
General/administration site	2 (1.68)	7 (5.19)
Injury/procedural	4 (3.36)	3 (2.22)
Renal/urinary	4 (3.36)	3 (2.22)
Metabolism/nutrition	3 (2.52)	3 (2.22)
Eye	2 (1.68)	3 (2.22)
Vascular	1 (0.84)	3 (2.22)
Reproductive/breast	0 (0)	2 (1.48)
Neoplasm	1 (0.84)	0 (0)
Blood/lymphatic	1 (0.84)	0 (0)
Ear/labyrinth	0 (0)	1 (0.74)
Investigations	4 (3.36)	3 (2.22)
Surgical and medical procedures	2 (1.68)	4 (2.96)

Values are No. of events (% of events). See [Table 1](#) legend for expansion of abbreviation.

coronary syndrome, worsening cirrhosis, colonic obstruction, and hyponatremia. Site investigators determined that these adverse events were not related to the study drug. No statistically significant differences in the distribution of adverse events or serious adverse events were noted between treatment arms.

Discussion

Despite encouraging preclinical studies examining the efficacy of CO therapy in fibrotic lung disease, in our randomized, multicenter, sham-controlled study, we observed no significant improvement in prespecified primary or secondary study end points after a 12-week treatment regimen with inhaled CO. Specifically, no statistically significant differences were observed in pulmonary function testing, functional assessments, or patient-reported outcomes between subjects receiving CO and those receiving ambient oxygen.

A large number of molecular biomarkers have been associated with a diagnosis of IPF; however, only a limited number of peripheral blood proteins have been associated with a decline in lung function and reduced survival in patients with IPF. These prognostic biomarkers include several alveolar epithelial (MMP7

and surfactant protein D [SP-D]) and macrophage-derived proteins (C-C Motif Chemokine Ligand 18 [CCL-18]). Of note MMP7 has been implicated in the pathogenesis of IPF²³; furthermore, we have shown that MMP7 levels are increased in lung tissue, BAL fluid, and the peripheral blood compartment of patients with IPF when compared with control subjects.¹⁵ Additionally, several groups have shown that increased MMP7 levels are associated with reduced survival,¹⁶ and a recent study suggests that metalloproteinase degradation products are both increased and correlate with increased mortality in patients with IPF.²⁴ Taken together, these findings suggest that select biomarkers may have value as surrogate molecular end points in clinical trials. In this trial, we choose MMP7 above other molecular markers because it may identify patients at risk for disease progression and its predictive attributes have been independently validated, suggesting a potential role as a therapeutic biomarker. However, in the present study, there was no difference in change in MMP7 concentration between study groups after the 12-week dosing period, or after 48 weeks of follow-up.

Although an overall treatment effect on the walk distance was not observed over the course of this study, a significant interaction effect between time and

treatment was observed after both the 12-week dosing period and the completion of the study. These findings could in part be accounted for by a longitudinal decline in walk distance over the 12-week course of treatment in the treated group, which was observed to recover from week 12 to study conclusion, or by the observed decline in the 6-min walk distance from the end of the treatment period to study conclusion in the placebo group, which was not observed in the treated group (Fig 5).

A number of observational and interventional studies suggest that inhaled CO can be safely administered. Stewart et al¹² performed 25 exposures to known CO concentrations in healthy volunteers; an 8-h exposure to inhaled CO at 100 ppm resulted in carboxyhemoglobin levels ranging from 11% to 13%. Similarly, Ren et al²⁵ exposed 11 normal volunteers to a CO regimen aiming to maintain a carboxyhemoglobin level of 10% for 8 h; carboxyhemoglobin levels ranged from 9.1% to 10.5% (mean, 9.7%). Finally, Zevin et al²⁶ exposed healthy volunteers to CO inhalations at 1,200 to 1,500 ppm once every minute for 10 min, and repeated inhalations once every 45 min for 16 h. The authors reported a mean carboxyhemoglobin of $5\% \pm 1\%$.

Ours is the first study to demonstrate the feasibility and safety of administration of inhaled CO in patients with IPF. Our study cohort appeared to be representative of patients with IPF in general, with a significant decline in lung function parameters such as FVC, total lung capacity, and DLCO during the study period. In the setting of a rigorous dosing and monitoring schedule in this cohort, 88% of subjects completed the study treatment regimen and 77% completed the 48-week follow-up period. Administration of CO was well tolerated and not associated with increased adverse events. In general, development of CO therapeutics is limited by data on neurologic and cardiovascular toxicities associated with accidental exposure to high ambient CO levels.²⁷ Importantly, we did not observe an increase in neurologic or cardiovascular events in the CO-treated arm when compared with the placebo group. Moreover, we did not find increased rates of hospitalization from any cause or an increase in all-cause mortality associated with CO treatment. Taken together with safety findings reported in prior phase I studies, our data suggest that inhaled therapies can be safely administered to patients with IPF in the ambulatory setting under appropriate monitoring conditions.

A number of preclinical studies have outlined the homeostatic properties of endogenous CO and therapeutic benefits of CO in diverse pathologic conditions.^{7,11} In a lung fibrosis model, mice treated with intratracheal bleomycin and exposed to low-concentration inhaled CO had significantly lower hydroxyproline accumulation than control mice exposed to ambient air.¹⁰ This *in vivo* study demonstrated that CO modulates key pathologic profibrotic processes, including synthesis and deposition of extracellular matrix, mesenchymal cell proliferation, and cytoprotection. The well-documented beneficial effects of CO have led to the design of several phase I and II clinical trials in COPD,¹³ acute lung injury (No. NCT02425579), pulmonary arterial hypertension (No. NCT01523548), and kidney transplantation (No. NCT00531856). However, these findings did not translate to significant improvement in either biomarker or clinical end points in the present study.

Several aspects of the study design should be considered when interpreting the lack of therapeutic efficacy. First, absence of safety data in the IPF population and a potential narrow therapeutic index prompted us to implement a conservative dosing algorithm to achieve CO blood levels $\leq 8\%$ (see Methods). The average maximum carboxyhemoglobin level in the treatment arm was $3.82\% \pm 2.79\%$ and differed by only 1.2% compared with placebo. In a recent clinical trial of patients with stable COPD, subjects were treated with inhaled CO at 100 to 125 ppm for 2 h/d on 4 consecutive days. This produced a mean maximal individual carboxyhemoglobin level of 4.5%.¹³ Because baseline carboxyhemoglobin levels of 3% have been reported in some urban areas,²⁸ and levels as high as 10% to 15% may be observed in asymptomatic chronic smokers,²⁹ it is possible that in this trial subjects may have been underdosed. Additional dose titration studies are required to determine the concentration of inhaled CO required to achieve mean carboxyhemoglobin levels of 6 to 8. Second, study subjects were treated twice a week for a total of 12 weeks. This limited dosing period was chosen to test tolerance and improve compliance with study visits. It is plausible that more frequent dosing (ie, daily) and longer treatment duration (ie, 6-12 months) would be more likely to improve study outcomes. Third, we hypothesized that a molecular surrogate end point (ie, MMP7) could be more sensitive than traditional study end points (ie, FVC). Although overall we observed an increase in MMP7 levels over the 48-week study period, it remains unclear if MMP7 is a

potential CO therapeutic target. Fourth, findings from a recent phase II clinical trial in IPF suggest that a large study population is required to elicit an efficacy signal when using traditional primary end points such as FVC.³⁰ These findings also suggest that our phase IIa study may have been underpowered to detect differences in traditional clinical end points.

In summary, we have shown that low-dose inhaled CO is well tolerated and can be safely administered to patients with IPF in the ambulatory setting. Further studies are needed to determine the appropriate dosing and administration schedules to achieve therapeutic local and/or systemic concentrations of inhaled therapies, and to fully assess their effectiveness in IPF.

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Additional information: The e-Appendix can be found in the Supplemental Materials section of the online article.

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