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Original research

Inhaled pirfenidone solution (AP01) for IPF: a randomised, open-label, dose–response trial

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/thorax-2022-219391).

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Received 11 July 2022
Accepted 13 January 2023
Published Online First 22 March 2023

To cite: West A, Chaudhuri N, Barczyk A, *et al*. *Thorax* 2023;**78**:882–889.

ABSTRACT

Introduction Oral pirfenidone reduces lung function decline and mortality in patients with idiopathic pulmonary fibrosis (IPF). Systemic exposure can have significant side effects, including nausea, rash, photosensitivity, weight loss and fatigue. Reduced doses may be suboptimal in slowing disease progression.

Methods This phase 1b, randomised, open-label, dose–response trial at 25 sites in six countries (Australian New Zealand Clinical Trials Registry (ANZCTR) registration number ACTRN12618001838202) assessed safety, tolerability and efficacy of inhaled pirfenidone (AP01) in IPF. Patients diagnosed within 5 years, with forced vital capacity (FVC) 40%–90% predicted, and intolerant, unwilling or ineligible for oral pirfenidone or nintedanib were randomly assigned 1:1 to nebulised AP01 50 mg once per day or 100 mg two times per day for up to 72 weeks.

Results We present results for week 24, the primary endpoint and week 48 for comparability with published trials of antifibrotics. Week 72 data will be reported as a separate analysis pooled with the ongoing open-label extension study. Ninety-one patients (50 mg once per day: n=46, 100 mg two times per day: n=45) were enrolled from May 2019 to April 2020. The most common treatment-related adverse events (frequency, % of patients) were all mild or moderate and included cough (14, 15.4%), rash (11, 12.1%), nausea (8, 8.8%), throat irritation (5, 5.5%), fatigue (4, 4.4%) and taste disorder, dizziness and dyspnoea (three each, 3.3%). Changes in FVC % predicted over 24 and 48 weeks, respectively, were −2.5 (95% CI −5.3 to 0.4, −88 mL) and −4.9 (−7.5 to −2.3,−188 mL) in the 50 mg once per day and 0.6 (−2.2 to 3.4, 10 mL) and −0.4 (−3.2 to 2.3, −34 mL) in the 100 mg two times per day group.

Discussion Side effects commonly associated with oral pirfenidone in other clinical trials were less frequent with AP01. Mean FVC % predicted remained stable in the 100 mg two times per day group. Further study of AP01 is warranted.

Trial registration number ACTRN12618001838202 Australian New Zealand Clinical Trials Registry.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Though oral pirfenidone reduces lung function decline and mortality from idiopathic pulmonary fibrosis (IPF), systemic exposure runs the risk of substantial adverse effects.

WHAT THIS STUDY ADDS

⇒ ATLAS phase 1b trial finds inhaled pirfenidone (AP01) has fewer adverse effects than antifibrotics currently used to treat IPF.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The potential for efficacy in slowing progression of fibrosis and ameliorating decline in forced vital capacity warrants further study.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a severe progressive lung disorder, leading to increasing breathlessness and cough with a profound impact on health-related quality of life. Many patients die of respiratory failure or comorbidities within 3–5 years of diagnosis.[1 2] IPF affects up to 200 000 Americans and 135 000 Europeans.[3 4] Worldwide, two oral antifibrotic medications are approved to treat IPF: nintedanib and pirfenidone.[5 6] At the recommended dosing, both can be associated with liver enzyme elevation and gastrointestinal side effects; oral pirfenidone is also associated with photosensitivity and rash.[7 8] In pooled oral pirfenidone trials (pirfenidone: n=1299; placebo: n=624), nausea (38% vs 16%), rash (25% vs 10%), dyspepsia (18% vs 7%), weight loss (16% vs 5%), vomiting (16% vs 6%) and liver enzyme elevation (3% vs 0.9%) were more common with oral pirfenidone than with placebo.[8] In pooled nintedanib trials (nintedanib: n=723; placebo: n=508), diarrhoea (62% v 18%), nausea (24% v 7%), abdominal pain (15% v 6%), liver enzyme elevation (14% v 3%), and vomiting (12% v 3%) were more common with nintedanib than with placebo.[7] These adverse events (AEs) may lead to dose reductions or discontinuations. In US and French studies of patients newly prescribed antifibrotics, more than 20% discontinued oral pirfenidone and 30% nintedanib after 6 months; 12-month discontinuation rates exceeded 40% for both antifibrotics.[9 10] Efficacy of both medications is suboptimal, slowing disease progression by ∼50% but not halting lung function decline.[5 6]

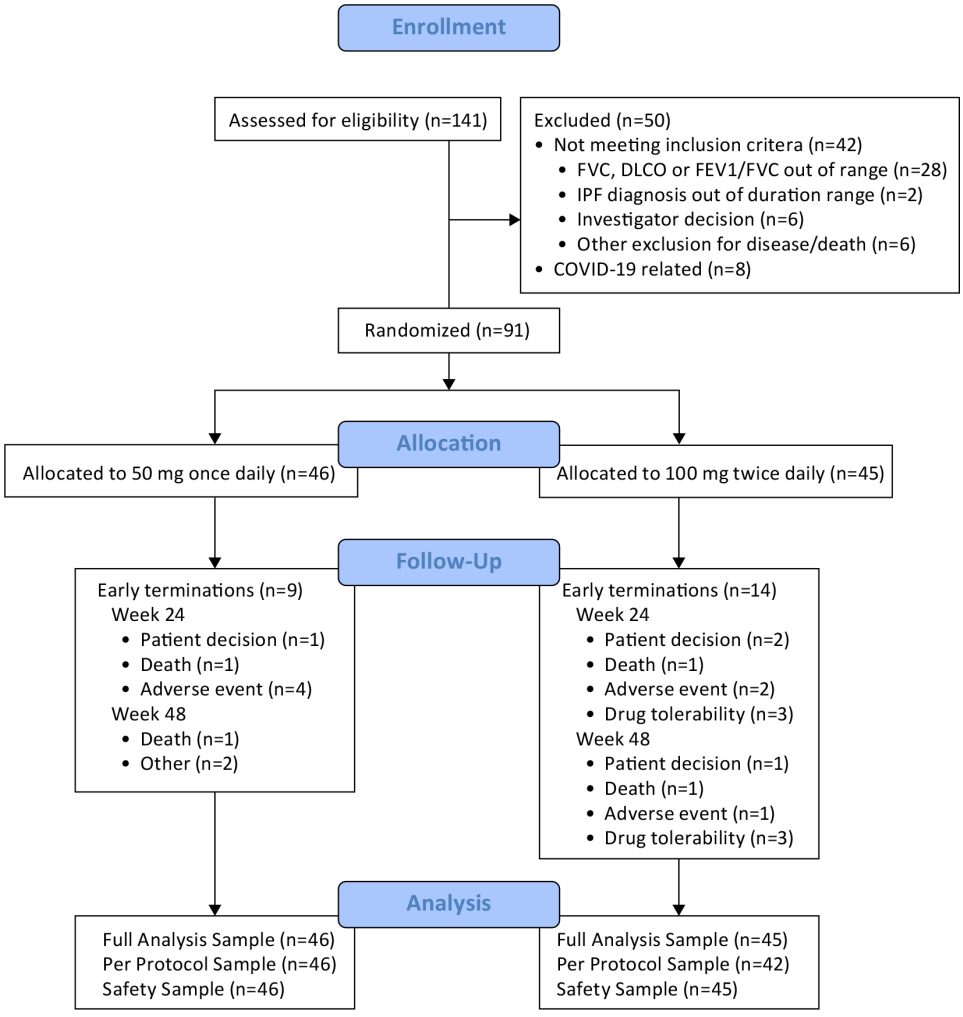

Figure 1 Study population to week 48. Based on a review of safety and efficacy performed after all patients completed 24 weeks, the Data and Safety Monitoring Board recommended all patients transition to the 100 mg twice-daily dose. A total of 31 patients transitioned from 50 mg once per day to 100 mg two times per day: 5 patients transitioned by 48 weeks, 16 more by 72 weeks and an additional 10 after 72 weeks. DLCO, diffusing capacity to carbon monoxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; IPF, idiopathic pulmonary fibrosis.

Aerosol administration improves efficacy and safety of many drugs by increasing delivery to lung tissue and decreasing systemic exposure.[11] AP01 is an inhaled formulation of pirfenidone optimised for lung delivery. In a phase 1, single ascending dose study evaluating safety, tolerability and pharmacokinetics, the PARI investigational eFlow nebuliser delivered >40% of the dose to the lung and enabled alveolar delivery. AP01 was well tolerated by healthy volunteers and patients with IPF. Compared with the approved thrice-daily, 801 mg dose of oral pirfenidone, the highest dose of AP01 tested (100 mg) achieved 35-fold higher peak epithelial lining fluid concentrations with ~1/15 systemic exposure.[12]

The ATLAS study assessed the safety, tolerability and efficacy of two AP01 doses in patients with IPF. We present results for week 24, the primary endpoint and week 48 for comparability to published trials of antifibrotics. The Data and Safety Monitoring Board (DSMB) recommended transitioning all patients to the higher dose midstudy following review of week 24 data in all patients. Only five patients transitioned to the higher dose by the week 48 visit. An additional 16 patients on 50 mg once per day transitioned to 100 mg two times per day by week 72. Therefore, week 72 data will be reported as a separate analysis pooled with the ongoing open-label extension study.

METHODS

Study design

ATLAS is a 24-week (optional extension to 72 weeks) randomised, parallel-group, open-label trial conducted from May 2019 to October 2021 at 25 sites in Australia, New Zealand, Czech Republic, Poland, Netherlands and the UK. The trial was conducted in compliance with the Declaration of Helsinki and the Harmonised Tripartite Guideline for Good Clinical Practice of the International Conference on Harmonisation and approved by local ethics committees. All patients provided written informed consent.

Eligibility

Principal eligibility criteria included being ≥40 years of age, with a confident diagnosis of IPF according to European Respiratory Society/American Thoracic Society (ERS/ATS) criteria within 5 years, forced vital capacity (FVC) ≥40% and ≤90% predicted and forced expiratory volume in 1 s (FEV_1)/FVC ratio ≥0.7, not currently taking oral pirfenidone or nintedanib. Exclusion criteria included acute IPF exacerbation requiring hospitalisation in the previous 3 months, any alternative diagnoses that could lead to pulmonary fibrosis or connective tissue disease,

Table 1 Baseline patient characteristics

	50 mg once per day	100 mg two times per day	Total
Patients, n	46	45*	91
Asia-Pacific region, n (%)	21 (45.7)	21 (46.7)	42 (46.2)
Age at screening (years), mean (SD)	73.4 (7.0)	71.3 (8.1)	72.4 (7.6)
Male, n (%)	32 (69.6)	32 (71.1)	64 (70.3)
Former smoker, n (%)	33 (71.7)	32 (71.1)	65 (71.4)
FVC % predicted at screening, mean (SD)	71.4 (11.7)	72.4 (10.0)	71.9 (10.8)
DLCO % predicted at screening, mean (SD)	48.6 (14.0)	49.1 (10.7)	48.8 (12.4)
IPF duration in months at screening, median (range)	18.1 (2.4–60.4)	27.1 (0.6–58.3)	22.0 (0.6–60.4)
Diagnosis within 1 year, n (%)	10 (21.7)	14 (31.1)	24 (26.4)
CT pattern from scan, n (%)			
Typical UIP pattern	14 (30.4)	24 (53.3)	38 (41.8)
Probable UIP pattern	31 (67.4)	19 (42.2)	50 (54.9)
Indeterminate UIP pattern	1 (2.2)	2 (4.4)	3 (3.3)
QLFCAD mL, mean (SE), n	633.0 (297.1), 44	489.6 (292.3), 42	563.0 (301.8), 86
Prognostic biomarker above median, n (%)			
CXCL13	25 (54.4)	20 (45.5)	45 (50.0)
CCL18	22 (47.8)	23 (52.3)	45 (50.0)
MMP3	27 (58.7)	18 (40.9)	45 (50.0)

*Biomarker data were available for only 44 of the 45 patients in the 100 mg two times per day group.
CT, computed tomography; DLCO, diffusion capacity to carbon monoxide; FVC, forced vital capacity; IPF, idiopathic pulmonary fibrosis; QLFCAD, quantitative lung fibrosis computer-aided diagnosis; SD, standard deviation; SE, standard error; UIP, usual interstitial pneumonia.

asthma, chronic obstructive pulmonary disease or active infection. Full eligibility criteria are provided in online supplemental table 1.

Randomisation and stratification

Patients were randomly assigned using interactive response technology 1:1 to AP01 50 mg once per day or 100 mg two times per day administered with a PARI investigational eFlow nebuliser. Randomisation was stratified by region (Asia-Pacific/Europe) and disease severity (FVC % predicted <50/≥50).

Sample size

The target sample size of 100 patients (50 per dose group) was selected to provide adequate data to assess the safety and tolerability of AP01 given once or two times per day and estimates of changes in outcomes over time. With the target sample size, there was a 92% chance of detecting an AE with a true population rate of 5%.

Study treatment

The first dose was administered at the study site. Patients experiencing cough limiting their ability to complete the first dose were pretreated with one to two puffs of salbutamol for remaining doses, as were patients with a history of asthma, smoking history ≥20 pack-years or ≥15% decrease in FEV_1 % predicted (post–pre dose) unless already receiving a long-acting beta-2 agonist.

Safety assessments

An independent DSMB reviewed safety data after the first 20 patients completed 4 weeks and when all patients completed 24 and 48 weeks. Safety outcome measures included treatment-emergent AEs, change in FEV_1 (post–pre dose) for the initial dose and changes in vital signs and clinical laboratory findings.

Efficacy assessments

Laboratory, in-clinic spirometry and patient-reported outcome (PRO) measures were collected every 4 weeks for 24 weeks, then every 12 weeks through week 72. Spirometry was performed according to ATS/ERS guidelines.[13] PROs included the Leicester Cough Questionnaire (LCQ) and the King's Brief Interstitial Lung Disease (KBILD) questionnaire.[14 15] Laboratory testing was performed using a central laboratory. Leicester Cough Monitor (LCM) 24-hour measurements were collected at baseline and weeks 12 and 24 and centrally scored.[16] High-resolution CT (HRCT) scans were performed at baseline and week 24 and centrally interpreted and quantified, including total lung capacity volume (litres) and quantitative lung fibrosis computer-aided diagnosis (QLFCAD) score (millilitres) for the whole lung.[17]

The primary efficacy endpoint was absolute change from baseline to week 24 in FVC % predicted. Prespecified secondary endpoints included change from baseline in diffusion capacity to carbon monoxide (DLCO), PROs, cough frequency and intensity, and extent of fibrosis and lung volumes measured by quantitative scoring of HRCT.

Analysis

All analyses were consistent with our prespecified Statistical Analysis Plan (provided in online supplemental materials). Safety, disposition and baseline analyses were conducted in patients who received ≥1 dose of AP01, and analyses for all other outcomes were performed on the per-protocol sample specified in the Statistical Analysis Plan. Inferential analyses implicitly handled missing data assuming missingness was explained by observed variables (missing at random). Change in FVC % predicted was analysed using a random coefficients model, with random slopes and intercepts for patients. Baseline FVC % predicted,[18] region, age at screening, sex, baseline DLCO % predicted[19] and baseline

Table 2 Adverse events in 91 treated patients over 48 weeks

	50 mg once per day	100 mg two times per day	Total
Patients, n	46	45	91
AEs reported in ≥10% of patients in either dose group, n (%)*			
Cough	11 (23.9)	14 (31.1)	25 (27.5)
Rash†	6 (13.0)	8 (17.8)	14 (15.4)
Dyspnoea	6 (13.0)	7 (15.6)	13 (14.3)
Nausea	5 (10.9)	5 (11.1)	10 (11.0)
Idiopathic pulmonary fibrosis‡	6 (13.0)	3 (6.7)	9 (9.9)
Fatigue	3 (6.5)	5 (11.1)	8 (8.8)
Lower respiratory tract infection	2 (4.3)	6 (13.3)	8 (8.8)
Upper respiratory tract infection	1 (2.2)	7 (15.6)	8 (8.8)
SAEs, n (%)*			
Idiopathic pulmonary fibrosis‡	1 (2.2)	2 (4.4)	3 (3.3)
Dyspnoea	1 (2.2)	0	1 (1.1)
Haemoptysis	1 (2.2)	0	1 (1.1)
Cardiac failure	1 (2.2)	0	1 (1.1)
Pneumothorax	0	1 (2.2)	1 (1.1)
Pulmonary embolism	0	1 (2.2)	1 (1.1)
Pneumonia	1 (2.2)	1 (2.2)	2 (2.2)
Bacteraemia	1 (2.2)	0	1 (1.1)
Campylobacter infection	1 (2.2)	0	1 (1.1)
Cellulitis	1 (2.2)	0	1 (1.1)
Infectious pleural effusion	0	1 (2.2)	1 (1.1)
Lower respiratory tract infection	0	1 (2.2)	1 (1.1)
Lower respiratory tract infection viral	0	1 (2.2)	1 (1.1)
Parainfluenzae virus infection	0	1 (2.2)	1 (1.1)
Septic embolus	1 (2.2)	0	1 (1.1)
Lung adenocarcinoma stage I	0	1 (2.2)	1 (1.1)
Prostate cancer	0	1 (2.2)	1 (1.1)
Cerebral infarction	1 (2.2)	0	1 (1.1)
Embolic stroke	1 (2.2)	0	1 (1.1)
Chest discomfort	1 (2.2)	0	1 (1.1)
Musculoskeletal chest pain	1 (2.2)	0	1 (1.1)

*n (%) for AEs and SAEs are frequency (percentage) of patients with the event reported.
†Rash includes the following preferred terms: rash, rash macular, rash papular, rash erythematous and rash pruritic.
‡The idiopathic pulmonary fibrosis (IPF) preferred term includes progression, deterioration, exacerbation and worsening of IPF.
AEs, adverse events; SAEs, serious adverse events.

progression-related biomarkers (CXCL13, CCL18, MMP3, each categorised as >or ≤ median level) were included as covariates. The model required all covariates and three follow-up measurements of FVC % predicted for the intercept and slope to be identifiable. The average slope, representing FVC decline, was estimated for each dose group using the random coefficients model and compared between groups. Significance tests comparing dose groups were based on these fixed slope effects and summarised using p values and two-sided 95% CIs for the difference between dose groups. A similar analysis was conducted for FVC in litres. For patients who transitioned to 100 mg two times per day, spirometry data collected after transition were not included when estimating slopes. For visit-based analyses comparing means, a linear mixed-effects model was used with change from baseline in FVC as the dependent variable, patient as a random effect, fixed effect of time and time-varying effects for treatment group (dose group was treated as time-varying to accommodate patients who transitioned) and treatment-by-time interaction and baseline FVC % predicted, region, sex, baseline DLCO % predicted, age at screening, baseline progression-related biomarkers (CXCL13, CCL18, MMP3), each categorised as >median level versus ≤median level as fixed effects. To account for correlated repeated measures within patients, a spatial power variance–covariance matrix was used. These comparisons were summarised using least-squares means and two-sided 95% CIs.

Descriptive analyses were conducted for other endpoints. LCM data analyses include the subgroup with baseline cough frequency during awake hours ≥10 coughs/hour to assess treatment impact in patients for whom coughing is of the greatest concern.[20] The absolute change from baseline in fibrosis in the whole lung obtained from the HRCT scans was calculated and correlated with change from baseline in FVC (millilitres). PROs, including KBILD and LCQ, were summarised as absolute changes from baseline at each visit.

RESULTS

Patient information

Ninety-one patients enrolled (46 at 50 mg once per day, 45 at 100 mg two times per day); 77 (85%) of patients completed 24 weeks and 68 (75%) completed week 48 (figure 1). The target enrolment of 100 was not reached because the COVID-19 pandemic closed most sites from March to April 2020. Ongoing intermittent closures prevented some office visits and spirometry measurements.

All patients had a confident diagnosis of IPF, based on ATS guidelines (online supplemental table 1).[21] Although median IPF duration was shorter in the 50 mg once per day group, the percentage of patients diagnosed within 1 year was lower, and patients had higher quantitative fibrosis on average (table 1). A greater percentage of 50 mg once per day patients had CXCL13 and MMP3 above the median compared with 100 mg two times per day patients.

Safety

Initial doses were well tolerated with no adverse effects on respiratory rate, spirometry or oxygenation during or following administration; median administration times were 5 and 8 min for the 50 mg and 100 mg doses, respectively. Eight patients (9%) had cough associated with nebulisation. Of the eight, one patient received salbutamol before the first dose, four were given salbutamol to continue dosing and three did not require salbutamol. All cough events associated with first-dose nebulisation were mild or moderate in severity and transient.

AEs reported for ≥10% of patients included cough, rash, dyspnoea, nausea, IPF (includes progression, deterioration, exacerbation and worsening), fatigue, lower respiratory tract infection and upper respiratory tract infection (table 2). AEs generally occurred within 3 months, with median time to first AE being 53 (IQR 1–153) days for rash, 32 (9–187) days for nausea and 72 (22–155) days for fatigue. Cough (n=14/91, 15.4%) was the most reported treatment-related AE, with three events related to nebulisation (two in the 50 mg once per day group, one in the 100 mg two times per day group). Except for throat irritation, the incidences of the most common treatment-related AEs were

Table 3 Pulmonary function test results for patients at baseline, 24 and 48 weeks

	50 mg once per day			100 mg two times per day		
		Change from baseline			Change from baseline	
	Baseline	24 weeks	48 weeks	Baseline	24 weeks	48 weeks
FVC % predicted						
Patients, n	46	26	28	42	26	28
Mean (SD)*	71.4 (11.8)	−1.7 (5.4)	−4.6 (5.6)	72.0 (9.6)	0.0 (7.0)	−0.4 (7.9)
Patients, n	–	35	39	–	34	34
Slope (95% CI)†	–	−2.5 (−5.3 to 0.4)	−4.9 (−7.5 to −2.3)	–	0.6 (−2.2 to 3.4)	−0.4 (−3.2 to 2.3)
Patients, n	–	26	28	–	26	28
LS mean (95% CI)‡	–	−1.0 (−3.2 to 1.2)	−3.5 (−5.7 to −1.3)	–	−0.7 (−3.0 to 1.6)	−2.8 (−5.1 to −0.6)
FVC						
Patients, n	46	26	28	42	26	28
Mean (SD)*	2.5 (0.6) L	−66 (191) mL	−191 (191) mL	2.6 (0.6) L	−17 (259) mL	−40 (286) mL
Patients, n	–	35	39	–	34	34
Slope (95% CI)†	–	−88 (−190 to 15)	−188 (−277 to −99)	–	10 (−91 to 110)	−34 (−127 to 60)
DLCO % predicted						
Patients, n	46	26	28	42	25	28
Mean (SD)*	47.7 (12.7)	−0.1 (4.3)	−3.3 (7.8)	49.1 (11.7)	0.2 (5.2)	−2.6 (6.9)
DLCO mL/min/mm Hg						
Patients, n	46	26	28	42	25	28
Mean (SD)*	10.0 (4.2)	−0.1 (0.9)	−0.7 (1.6)	10.4 (3.8)	0.1 (1.1)	−0.5 (1.4)

*Mean (SD) are calculated based on observed data with no imputation.
†Slope (95% CI) for FVC % predicted (or FVC mL) are from a random coefficients model with change from baseline in FVC % predicted (or FVC mL) as the dependent variable, patient and time (continuous) as random effects, fixed effect of time and treatment group and treatment-by-time interaction and baseline FVC % predicted, region, sex, baseline DLCO % predicted, age at screening, baseline progression-related biomarkers (CXCL13, CCL18, MMP3), each categorised as >median level versus ≤median level as fixed effects.
‡LS mean (95% CI) for FVC % predicted are from a random coefficients model with a spatial power variance–covariance matrix, change from baseline in FVC % predicted as the dependent variable, patient as a random effect, fixed effect of time and time-varying effects for treatment group and treatment-by-time interaction and baseline FVC % predicted, region, sex, baseline DLCO % predicted, age at screening, baseline progression-related biomarkers (CXCL13, CCL18, MMP3), each categorised as >median level versu ≤median level as fixed effects.
DLCO, diffusion capacity to carbon monoxide; FVC, forced vital capacity; LS, least squares.

lower for the 50 mg once per day group. One grade 3 event of parainfluenza virus infection was considered serious; all other treatment-related events were mild or moderate.

Thirteen serious AEs were reported in nine patients assigned 50 mg once per day, with 11 serious AEs in 7 patients assigned 100 mg two times per day (table 2). AEs leading to study termination were cough (n=2), progression of IPF (n=2), pneumonia (n=1), rash (as defined in table 2) (n=1) and abnormal CT chest scan (n=1), the latter in a patient suspected of having lung carcinoma who also had abnormal tumour markers at the time.

All liver AEs were mild or moderate and resolved. One patient in the 100 mg two times per day group had elevated hepatic enzymes considered related to treatment, which resolved after dose interruption. The patient was restarted on 50 mg once per day, rechallenged with 100 mg two times per day and hepatic enzyme levels remained within normal limits. All other events were judged unrelated to treatment and included two 50 mg once per day patients with elevated liver function tests, one 50 mg once per day patient with two increased blood bilirubin events, one 100 mg two times per day patient with increased blood potassium and one 100 mg two times per day patient with elevated serum creatinine.

There were four deaths among patients on study. There were two deaths (one in the 50 mg once per day group from embolic stroke/septic embolus and one in the 100 mg two times per day dose from IPF) among patients on study through 24 weeks and two additional deaths (one in the 50 mg once per day group from IPF, one in the 100 mg two times per day group from pulmonary embolism) between 24 and 48 weeks.

Efficacy

Baseline and changes from baseline in pulmonary function testing and sample sizes at landmark timepoints are shown in table 3 and figure 2. Mean changes from baseline in DLCO were comparable between dose groups.

The differences in slopes (100 mg two times a day − 50 mg once per day) were 3.0 (95% CI −0.9 to 7.0; p=0.133) at 24 weeks and 4.5 (95% CI 0.7 to 8.2; p=0.022) at 48 weeks.

The one-sided lower 95% CI limit for the difference with 50 mg once per day and 100 mg two times a day was −0.84 (n=35) and 2.0 (n=35) at 24 weeks and 0.19 (n=39) and 4.2 (n=35) at 48 weeks, respectively.

Change in the QLFCAD score correlated moderately well with change in FVC for 100 mg two times a day but did not correlate for 50 mg once per day (online supplemental figure 1). Three patients assigned 100 mg two times a day had markedly increased FVC at 24 weeks (380, 500 and 850 mL) and 48 weeks (600, 450 and 830 mL) and a corresponding reduction in QLFCAD score in the whole lung at 24 weeks (−237,–151 and −644 mL, respectively).

At baseline, 48% of patients (46% assigned to 50 mg once per day and 50% assigned to 100 mg two times a day) had cough frequency at baseline ≥10/hour while awake (figure 3).

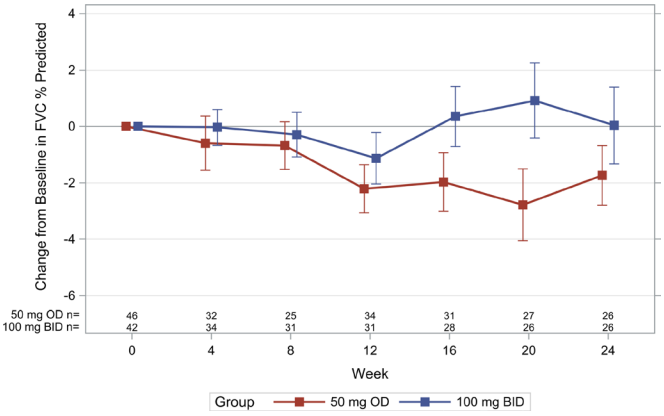

Figure 2 Change from baseline in FVC % predicted. Shown is the observed mean change from baseline in FVC % over 24 weeks. The error bars indicate the SE. BID, two times per day; FVC, forced vital capacity; OD, once per day.

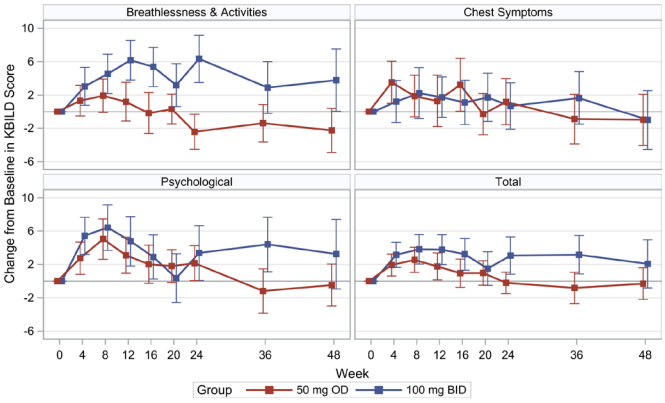

Figure 4 Change from baseline in KBILD scores. Shown is the observed mean change from baseline over 48 weeks in KBILD total and domain scores. The error bars indicate the SE. BID, two times a day; KBILD, King's Brief Interstitial Lung Disease questionnaire; OD, once per day.

Among patients with baseline cough ≥10 /hour, median cough frequency decreased in both dose groups; a more detailed analysis is presented in the online supplemental findings.

Regardless of baseline objective cough frequency, on average, patients reported modest changes in cough-related quality of life (online supplemental table 2). Similarly, small changes were observed in health-related quality of life within dose groups; however, differences in the breathlessness and activities, chest symptoms, psychological and total scores of the KBILD questionnaire were on average higher in the 100 mg two times a day group (online supplemental table 2; figure 4).

Transition to single dosing regimen

Based on a review of safety and efficacy performed after all patients reached the week 24 visit, the DSMB recommended transitioning all patients to 100 mg two times a day for the remainder of the trial because FVC data showed that most patients who received 100 mg two times a day were stabilised. Three patients transitioned to 100 mg two times a day before the week 48 visit and two patients at the week 48 visit. At 72 weeks, all patients had the option of continuing to receive 100 mg two times a day AP01 in an open-label extension trial; 47/54 patients (87%) chose to continue.

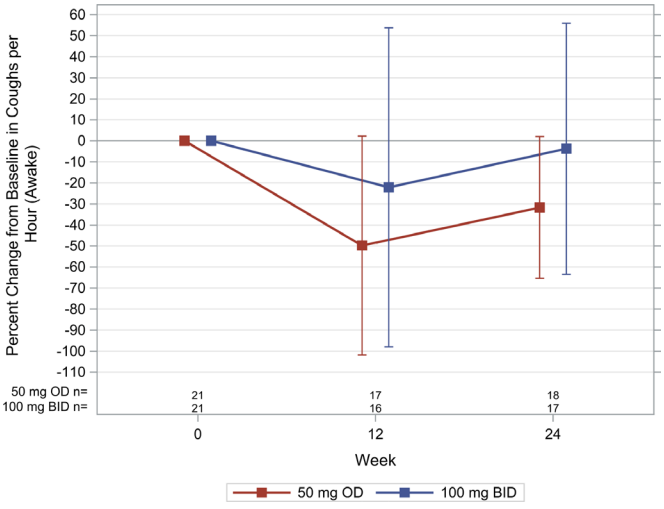

Figure 3 Per cent change from baseline in objective cough frequency measured by Leicester Cough Monitor. Shown is the observed median per cent change from baseline in objective cough frequency over 24 weeks for patients with baseline cough frequency ≥10 per hour. The error bars indicate the IQR. BID, two times a day; OD, once per day.

DISCUSSION

The ATLAS study shows the potential of aerosolised pirfenidone for improved safety and efficacy compared with oral pirfenidone in treating IPF. Both AP01 doses were well tolerated, and the most common dose-limiting AEs associated with oral pirfenidone[22] were reduced or absent. As with oral pirfenidone,[23] AEs generally occurred within 3 months. AEs deemed related to AP01 suggested a possible dose response for cough, rash, nausea, taste disorder, fatigue, dizziness and dyspnoea, with higher incidence in the 100 mg two times a day group than in the 50 mg once per day group.

The incidence of rash for 100 mg two times a day was 17.8%, and all events were mild or moderate. Rash events generally were managed by AP01 dose reduction, topical therapies or sun protection. Because patients intolerant to oral pirfenidone could be enrolled, these rash rates may over-represent expected incidence in the IPF and interstitial lung disease populations. Although patients with prior intolerance may be predisposed to sensitivity, not all patients with a history of rash on oral pirfenidone experienced rash with AP01. Patients with a history of intolerance to oral pirfenidone should not be assumed intolerant to AP01.

Taste disorder was reported in three patients assigned 100 mg two times a day, and dysgeusia was reported in one patient assigned 100 mg two times a day. Although the bitter taste of pirfenidone is masked by adding trace saccharin, sensitivity varied.

The low incidence of systemic AEs observed with AP01 is not surprising because the 100 mg two times a day nebulised dose leads to 1/15 systemic exposure of the approved oral pirfenidone daily dose and, regarding liver toxicity, has no first-pass effect. The elevated hepatic enzymes related to treatment in a single patient returned to normal limits after interruption and rechallenge. Early studies in small populations can miss rare AEs; however, the well-characterised safety profile of oral pirfenidone combined with decreased systemic exposure makes the detection of novel AEs outside the respiratory tract in larger studies unlikely.

Cough is a common IPF symptom. Through 48 weeks, 25 patients were reported to have cough AE with cough AEs in 14 patients judged related to treatment. Most related events (n=12) were considered related to nebulisation and self-resolving or controllable by salbutamol or similar drugs or caused by another trigger such as upper respiratory tract infection and self-resolving. Two related cough events in the 100 mg two times a day group were considered long term. Two patients (one from each dose group) discontinued therapy due to cough. As with oral pirfenidone,[24] both doses of AP01 decreased frequency of cough in IPF patients with high baseline cough frequency, encouraging further study.

The 100 mg two times a day dose group showed significantly less loss of FVC % predicted compared with the 50 mg once per day group at 48 weeks. Changes in quantitative lung fibrosis scores from HRCT correlated well with changes in FVC for the 100 mg two times a day group. PRO results remained stable over 48 weeks in both dose groups with average changes from baseline in LCQ and KBILD domain and total scores less than the minimal clinically important difference.[20 25]

Three patients randomised to 100 mg two times a day had large increases in FVC at 24 and 48 weeks and steady improvement over the course of the study. At screening, their HRCT pattern was typical or probable UIP; time since diagnosis and disease severity varied, and these patients had no ongoing bronchodilator use, no signs of mucus plugging on screening HRCT and no productive cough. Structural changes seen with HRCT are eventually manifested as changes in FVC. FVC increases were consistent with improvements in quantitative fibrosis assessed by HRCT and similar improvements in other secondary efficacy endpoints, including KBILD scores.

The median administration time for the 100 mg two times a day dose was 8 min using the PARI investigational eFlow nebuliser. Duration is important because the pirfenidone half-life in epithelial lining fluid is ~10 min; efficacy due to a peak drug concentration would be decreased with extended nebulisation.[26] Nebulisers with lower delivery efficiency, slower administration or larger particle sizes favouring airway delivery would likely not deliver AP01 with optimal efficacy and safety. Our study design does not lend itself to clearly determining whether the difference seen in efficacy between the 50 and 100 mg doses at 48 weeks resulted from the dose amount or dose frequency.

Findings of this phase 1b study, designed to establish multi-dose safety and tolerability and to obtain variability and effect size estimates, should be interpreted cautiously. Blinding dose groups was not feasible because it would require a diluted formulation to produce equal volumes, leading to reduced C_{max} levels with the 50 mg dose. A placebo-control group without the allowance of background therapies was not included because approved antifibrotic therapies are available for the target IPF population. Spirometry data were missing from some post-baseline visits because of site restrictions due to the COVID-19 pandemic and early terminations. A slope-based analysis was used to leverage all observed data in estimating change over time in FVC % predicted, and a visit-based analysis showed consistent findings at weeks 24 and 48.

In summary, inhaled pirfenidone (AP01) is a promising treatment for IPF. Future studies exploring the potential for fewer adverse effects and improved efficacy compared with current antifibrotics in reducing or slowing progression of fibrosis, ameliorating decline in FVC and meaningfully improving quality of life are warranted.

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Acknowledgements Mark Surber and Stephan Pham developed and supervised the manufacture of the AP01 combination product used in the study. Paul Guttry of Acumen Medical Communications, LLC, assisted in the preparation and submission of the manuscript.

Contributors All authors had the ability to access the data. The authors assume responsibility for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. The initial draft of this manuscript was written by the authors without outside assistance. MLS and ABM have both verified the underlying data, and ABM is acting as guarantor. AW, NC, AB, MLW, TJC, MŠ, AV, EJ, MSW, WP and GR contributed to study conduct, data interpretation and manuscript writing. PH, IG and CG contributed to study design, study conduct, data interpretation,and manuscript writing. DN contributed to data collection, data interpretation and manuscript writing. KO contributed to study design, data analysis, data interpretation and manuscript writing. MLS contributed to data analysis, data interpretation and manuscript writing. LF and ABM contributed to study design, data interpretation and manuscript writing.

Funding Avalyn Pharma sponsored the study and was involved in the study design, the analysis and interpretation of data, the writing of this report and in the decision to submit the paper for publication.

Competing interests NC received a grant from Boehringer Ingelheim and consulting fees from Boehringer Ingelheim, Carrick, Redex, UCB, and Novartis; speaker fees and travel sponsorship from Boehringer Ingelheim and Roche; and payment for participation on data monitoring/advisory board from Boehringer Ingelheim. IG reports consulting fees from Amplia, Ad Alta and Accendatech; speaking fees from Boehringer Ingelheim; and payment for participation in a safety review committee from Accendatech. TJC reports receiving grants/contracts from Boehringer Ingelheim, Roche, Bristol Myers Squibb, Biogen, Galapagos and Avalyn Pharma; speaker's honoraria from Boehringer Ingelheim and Roche; and payment for participation on data safety monitoring/advisory board from Boehringer Ingelheim, Roche, Bristol Myers Squibb and Promedior. EJ received honoraria for lectures from Boehringer Ingelheim, Roche, AstraZeneca, MDS and Chiesi; sponsored travel to meetings from Boehringer Ingelheim and Roche; and payment for participation in advisory boards from Boehringer Ingelheim, Roche, AstraZeneca, Novartis, MDS, Berlin-Chemie and Chiesi. MW reports payments to her institution from Boehringer Ingelheim, Roche, the Netherlands Organisation for Health Research and Development, the Dutch Lung Foundation, the Dutch Pulmonary Fibrosis Patient Association, the Thorax Foundation, ErasmusMC and Sarcoidoisis.nl. Consulting fees were paid to her institution by Boehringer Ingelheim, Roche, Galapagos, Bristol Myers Squibb, Galecto and Respivant; honoraria were paid to her institution by Boehringer Ingelheim, Roche and Novartis. MW received sponsored travel from Boehringer Ingelheim and Roche, and her institution received payment for her participation in data safety monitoring/advisory board from Savara and Galapagos. CG received consulting fees from Avalyn Pharma. GR reports receiving support from Avalyn Pharma for consulting and for chairing the Data Safety Monitoring Board during the conduct of the study; personal and consulting fees from Boehringer Ingelheim, Respivant and Roche; grants from the NIH; and consulting fees from

Bellerophon Therapeutics, Biogen, Bristol Myers Squibb, Fibrogen, Nitto, Promedior, Respivant and Veracyte.

Patient consent for publication Not applicable.

Ethics approval There were over 30 sites involved in this study, in six countries. A table presenting the Ethics Committee/Institutional Board information as well as the approval IDs has been submitted as a separate document titled 'EC:IRB, approval numbers', as supplementary material for editors only. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data are available from Avalyn Pharma on reasonable request.

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Statistical Analysis Plan

Sponsor Name: Avalyn Pharma Inc.

Protocol Number: AP01-002

Protocol Title: A Randomized Open-Label, Phase 1b **S**tudy of the Safety of Pirfenidone So**l**ution for Inhalation (AP01) in P**a**tients with Idiopathic Pulmonary Fibrosi**s** (ATLAS Study)

Protocol Version and Date: Version 2.1 (12-OCT-2020)

Syneos Health Project Code: 1012435

Author: Dean Margeson, Principal Biostatistician

Revision History

Version #	Date (DD-Mmm-YYYY)	Document Owner	Revision Summary
1.0	06-Dec-2019	Dean Margeson	Final Version 1.0
1.1	26-Feb-2020	Dean Margeson	Updated Version to Address Protocol Deviation Classification
2.0	24-Nov-2020	Dean Margeson	Final Version 2.0 – Updated analyses to account for COVID-19 effects on the data.
2.1	02-Jul-2021	Dean Margeson	Updated for changes due to patients in 50 mg once daily group switching to 100 mg twice daily group during Part B.
3.0	15-Jul-2021	Dean Margeson	Final Version 3.0

I confirm that I have reviewed this document and agree with the content.

Approvals

Syneos Health Approval

Dean Margeson, Principal Biostatistician	Dean Margeson	Electronically signed by: Dean Margeson Reason: I am the author Date: Jul 15, 2021 12:27 EDT
Name, Title Lead Biostatistician	Signature	Date (DD-Mmm-YYYY)
Jed Henke, Manager Biostatistics		Electronically signed by: Jed Henke Reason: I am the approver Date: Jul 15, 2021 11:31 CDT
Name, Title Senior Reviewer	Signature	Date (DD-Mmm-YYYY)

Avalyn Pharma, Inc. Approval

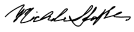
Michele Shaffer, Manager Biostatistics		Electronically signed by: Michele Shaffer Reason: I am the approver Date: Jul 15, 2021 09:37 PDT
Name, Title Sponsor Contact	Signature	Date (DD-Mmm-YYYY)

Table of Contents

1. Glossary of Abbreviations

Abbreviation	Description
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine Aminotransferase
AP01	Pirfenidone Solution for Inhalation
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
BAL	Bronchoalveolar Lavage
BMI	Body Mass index
CI	Confidence Interval
CRF	Case Report Form
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CV	Cardiovascular
DSMB	Data and Safety Monitoring Board
D_{LCO}	Diffusion Capacity for Carbon Monoxide
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELF	Epithelial Lining Fluid
FAS	Full Analysis Set
FEV_1	Forced Expiratory Volume in One Second
FOCBP	Female of Child-Bearing Potential
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GLM	General Linear Model
HRCT	High-Resolution Computed Tomography
ICH	International Conference on Harmonization

Abbreviation	Description
IP	Investigational Product
IPF	Idiopathic Pulmonary Fibrosis
KBILD	The King's Brief Interstitial Lung Disease
LCM	Leicester Cough Monitor
LCQ	Leicester Cough Questionnaire
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
MMRM	Mixed Model Repeated Measures
N/A or NA	Not Applicable
NCI	National Cancer Institute
PE	Physical Exam
PFT	Pulmonary Function Tests
PP	Per-Protocol/Per-Protocol Set
PRO	Patient Reported Outcomes
PT	Preferred Term
QOL	Quality of Life
QTcB	Bazett-Corrected QT Interval
QTcF	Fredericia-Corrected QT Interval
RV	Residual Volume
SAE	Serious Adverse Event
SAF	Safety Set
SaO_2	Saturated Oxygen
SAP	Statistical Analysis Plan
SD	Standard Deviation
SI	Standard International System of Units
SOC	System Organ Class

Abbreviation	Description
SOP	Standard Operating Procedure
TEAE	Treatment Emergent Adverse Event
TFL	Tables, Figures and Listings
TLC	Total Lung Capacity
UIP	Usual Interstitial Pneumonia
VAS	Visual Analogue Scale
VC	Vital Capacity
WHO	World Health Organization

2. Purpose

The purpose of this statistical analysis plan (SAP) is to ensure that the data listings, summary tables and figures which will be produced, and the statistical methodologies that will be used, are complete and appropriate to allow valid conclusions regarding the study objectives.

2.1. Responsibilities

Syneos Health will perform the statistical analyses and is responsible for the production and quality control of all tables, figures and listings.

2.2. Timings of Analyses

An independent Data and Safety Monitoring Board (DSMB) will perform a review of safety data from the first 20 patients after Week 4 to confirm the safety of multiple dose administration. A decision to stop, continue or modify the study will be made based on this review. Throughout the course of the study, all serious adverse events (SAEs) will be sent to the DSMB for review.

An interim analysis will be performed at the completion of Part A. All endpoints will be analyzed and a full set of tables, listings and figures will be generated.

The final analysis of all safety and efficacy endpoints over Part A and Part B is planned after all patients complete the final study visit in Part B or terminate early from the study.

3. Study Objectives

3.1. Primary Objective

The primary objective is to evaluate safety and tolerability of treatment with AP01 given once or twice daily to patients with idiopathic pulmonary fibrosis (IPF) by monitoring adverse events (AEs) and post-dose spirometry.

3.2. Secondary Objectives

The secondary objectives include:

- To estimate the treatment effect of AP01 given 50 mg once daily and 100 mg twice daily on absolute change in % predicted forced vital capacity (FVC) in patients with IPF
- To estimate the treatment effect of AP01 given 50 mg once daily and 100 mg twice daily on absolute change in % predicted Diffusion Capacity for Carbon Monoxide (D_{LCO}) in patients with IPF
- To compare the safety and efficacy of 50 mg once daily vs 100 mg twice daily dosing to provide guidance on dosing regimens for future studies
- To estimate the treatment effect of AP01 given 50 mg once daily and 100 mg twice daily on change in Patient Reported Outcomes (PROs) and cough in patients with IPF
- To estimate the treatment effect of AP01 given 50 mg once daily and 100 mg twice daily on change in the extent of fibrosis and lung volumes as measured by High Resolution Computed Tomography (HRCT) scans in patients with IPF
- To evaluate the following exploratory measurements:
    - Correlation between weekly home spirometry measurements and in-clinic spirometry values
    - Correlation of extent of fibrosis and lung volumes as measured by HRCT at Baseline and Week 24 to other measures of clinical status, e.g. spirometry
    - Change from baseline in serum biomarkers of alveolar epithelial injury (CA19-9 and CA-125).

3.3. Brief Description

This Phase 1b, 2-part, open-label study of AP01 will randomize approximately 100 patients with IPF. Patients will be randomized in a 1:1 ratio to one of two treatment groups: 50 mg once daily or 100 mg twice daily.

Figure 1: Part A Study Schema

PART A – Patients are randomized at Visit 2 to either 50 mg given once daily or 100 mg AP01 given twice daily

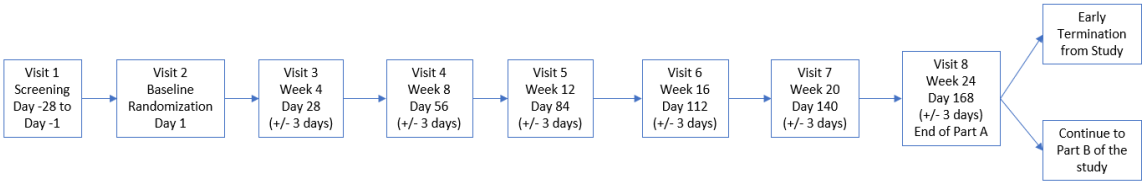


Figure 2: Part B Study Schema

PART B – Patients continue randomized treatment regimen of either 50 mg once daily or 100 mg AP01 twice daily

In Part A, eligible patients at least 40 years of age with a confident clinical and radiographic diagnosis of IPF according to pre-specified criteria, 40 ≤ %FVC ≤ 90, forced expiratory volume in 1 second (FEV_1)/FVC ratio ≥ 70% and 30 ≤ % D_{LCO} ≤ 90 will be randomized with equal probability to receive AP01 50 mg once daily or 100 mg twice daily for 24 weeks. Other than brief periods of medically appropriate treatment for acute IPF exacerbation, patients will not receive any other therapy for the treatment of IPF during Part A. If in the opinion of the investigator, a patient is in need of treatment with oral pirfenidone or nintedanib during Part A, the patient should be discontinued from the study and return to the clinic for their Early Termination visit. Supplemental oxygen will be allowed during the entire study.

The initial dose of drug will be administered in the clinic to confirm airway tolerance. If based on the investigator's opinion, the patient had tolerability issues during the first dose administration, a second dose, at least 4 hours later, will also be observed. Patients who experience cough that limits their ability to complete dosing will be given 1 - 2 puffs (90 - 100 microgram (µg)) of salbutamol in order to complete the in-clinic dose. These patients, as well as patients with a history of asthma or smoking history of 20 pack years or greater, or patients that have a ≥ 15% drop in FEV_1 % predicted in their pre-dose and post-dose readings will be required to use 1 - 2 puffs (90 - 100 µg) of salbutamol prior to

dosing throughout the study unless these patients are currently taking a long-acting beta-2-agonist therapy.

Patients will have a telephone assessment at Week 1 and in-clinic assessments at Weeks 4, 8, 12, 16, 20, and 24. The DSMB will perform a review of safety data from the first 20 patients after Week 4 to confirm the safety and airway tolerability of multiple dose administration. A decision to stop, continue or modify the study will be made based on this review.

Part B will collect long-term safety and efficacy data for an additional 48 weeks. Patients who complete Part A through Week 24 and, in the opinion of the investigator are compliant with treatment, may select to continue the treatment to which they were randomized. If one dosing regimen is determined to be superior either from an efficacy or safety standpoint, Part B may be converted to a single dose regimen and all patients who select to continue or start Part B at that point will be dosed with the selected regimen.

Study duration is up to 80 weeks for patients that complete both Part A and Part B of the study. If patients discontinue study treatment prior to Week 72 for any reason, they should return to the site for an Early Termination visit. All patients who complete the regular study visits through Week 72 in Part B will return for a Follow-up visit 28 days after their End of Study visit.

3.4. Patient Selection

3.4.1. Inclusion Criteria

Inclusion Criteria are described in Section 5.1 of the clinical study protocol.

3.4.2. Exclusion Criteria

Exclusion Criteria are described in Section 5.2 of the clinical study protocol.

3.5. Determination of Sample Size

This is an open-label study to determine safety and tolerability of AP01 50 mg once daily or 100 mg twice daily; both regimens being administered using the eFlow. With 50 patients per group, we have a 92% chance of detecting an AE with a true population rate of 5%, as displayed in Table 1:

Table 1: Probability of observing adverse events

	Actual probability of event		
	1%	**5%**	**10%**
Chance of observing ≥ 1 event	0.395	0.923	0.995

The Phase 3 study of oral pirfenidone in patients with IPF (ASCEND)[1] showed a standard deviation (SD) of 4.5 based on the imputed mean change in % of predicted FVC at Week 26. Assuming a SD of 4.5, with 50 patients per group, a two-sided 95% confidence interval (CI) for mean change from baseline in % of predicted FVC will extend 1.25 from the observed mean. The ASCEND[1] study showed a decrease of 1.5 and 3.9 in % of predicted FVC at Week 26 in the pirfenidone and placebo groups, respectively. Table

2 shows results of the ASCEND[1] active and placebo groups and potential observed differences in the AP01-002 study, assuming a similar SD.

Because we have no prior data on efficacy outcomes with our compound, we are unable to make well-informed predictions of effect size or variability. In lieu of that information, we have included some estimates of confidence intervals based on prior oral pirfenidone studies; however, we cannot be confident that our product will behave similarly and would not base hypothesis tests on this information. This study will give us an idea of which endpoints are most likely to be positively impacted by our product as well as solid estimates of both effect size and variability. In addition, if the effect is due to AUC or multiple peaks/day in the Epithelial Lining Fluid (ELF) rather than peak levels, based on our modeling from the BAL cohort in the AP01-001 study, we have around 75% power to show a difference between groups. With this sample size, we can be confident on selecting dosing regimen(s) for future trials.

Table 2: Observed and potential change in FVC

	ASCEND[1] Placebo (n = 262)	**ASCEND[1] Active (n = 255)**	**AP01-002 Potential Scenarios (n = 50)**		
Observed Change FVC % predicted (mean, SD)	-3.9, 5.2	-1.5, 4.5	-0.75, 4.5	-1.0, 4.5	-1.5, 4.5
95% CI	(-4.53, -3.27)	(-2.05, -0.95)	(-2.0, 0.5)	(-2.25, 0.25)	(-2.75, -0.25)

3.6. Treatment Assignment & Blinding

Patients will be randomized in a 1:1 ratio to receive Pirfenidone Solution for Inhalation (AP01) either 50 mg once daily or 100 mg twice daily; both regimens being administered by the eFlow. The randomization schema will be stratified by region (Asia-Pacific and Europe) and by disease severity (FVC < 50% predicted and FVC ≥ 50% predicted). The first 20 patients randomized in the study must have FVC ≥ 50% predicted at Screening. After the first 20 patients have been randomized, patients with FVC 40% < 50% predicted will be allowed to be randomized in the study but randomization for these patients will be capped at 20.

This is an open-label study; no blinding restrictions are required.

3.7. Administration of Study Medication

Patients will be trained on use of the eFlow and administration of the study drug at the Baseline visit. The first dose of study drug for each patient will be administered and observed at the clinical study site at the Baseline visit to confirm tolerability. All subsequent doses will be administered by the patient at home. All doses must be taken at least 4 hours apart.

Patients who experience cough that limits their ability to complete dosing will be given 1 – 2 puffs (90 -

100 µg) of an inhaled beta 2 agonist, such as salbutamol, in order to complete the in-clinic dose. These patients, as well as patients with a history of asthma or smoking history of 20 pack years or greater, or patients that have a ≥ 15% drop in FEV_1 % predicted in their pre-dose and post-dose readings and are symptomatic will be provided with and required to use 1 - 2 puffs (90 - 100 µg) of salbutamol prior to their second dose and for dosing throughout the study, unless these patients are currently taking a long-acting beta-2-agonist therapy. If symptoms persist with study treatment despite beta-2-agonist therapy, the Investigator will assess and discontinue the patient due to tolerability issues if he/she deems it necessary.

3.8. Study Procedures and Flowchart

3.8.1. Study Schedule

The study schedule can be found in Tables 3 and 4. At-home patient assessments are outlined in Table 5. Detailed information on study assessments is provided in Section 7 of the clinical study protocol.

Table 3: Study Schedule for Part A

	Study Phase – Part A							
	Screening	**Baseline**	**Treatment**					
Visit	1	2	3	4	5	6	7	8
Day	-30 to -1	1	28	56	84	112	140	168
Window (Days)	NA	NA	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3
Informed Consent	X							
Eligibility Assessments	X	X						
Assessment of IPF Diagnosis	X							
Demographics	X							
Medical History	X							
Physical Exam[2]	X	X	X	X	X	X	X	X
Height[3], Weight	X				X			X
Vital Signs	X	X	X	X	X	X	X	X
ECG	X							
Laboratory Tests[4]	X	X	X	X	X	X	X	X
Urinalysis	X	X	X	X	X	X	X	X
Urinary Pregnancy Test[5]	X	X	X	X	X	X	X	X
Spirometry and D_{LCO}	X	X	X	X	X	X	X	X
SaO_2	X		X	X	X	X	X	X
LCM[6] and diary dispensation	X				X			X
Cough VAS, KBILD Questionnaire and LCQ		X	X	X	X	X	X	X

HRCT[7]		X						X
Pre-dose Spirometry		X						
Pre-dose SaO_2		X						
Randomization		X						
Study Material Training		X						
Study Drug Administration First Dose		X						
Post-dose Spirometry (≤ 10 min post-dose)		X						
Post-dose SaO_2		X						
Study Material Dispensation		X	X	X	X	X	X	X[8]
Home Spirometer Training and Dispensation		X						
Concomitant Medications	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X
End of Treatment/Study Form								X

Table 4: Study Schedule for Part B

	Study Phase – Part B				
	Treatment				
Visit	9	10	11	12	Follow-up
Day	252	336	420	504 End of Study	28 days after End of Study
Window (Days)	+/- 7	+/- 7	+/- 7	+/- 7	+/- 7
Cough VAS, KBILD and LCQ	X	X	X	X	X
Physical Exam[2]	X	X	X	X	X
Weight		X		X	
Vital Signs	X	X	X	X	X
Laboratory Tests[4]	X	X	X	X	X
Urinalysis	X	X	X	X	X
Urinary Pregnancy Test[5]	X	X	X	X	
Spirometry and D_{LCO}	X	X	X	X	X

Saturated Oxygen	X	X	X	X	X
Study Material Dispensation	X	X	X		
Concomitant Medications	X	X	X	X	X
Adverse Events	X	X	X	X	X
End of Treatment/Study Form				X	X

Notes for Tables 3 and 4:

1. Follow-up visit will occur 28 days after the End of Study visit in Part B of the study.
2. A complete physical exam will be performed at Screening and Baseline visits. A modified physical exam including the patient's lungs and cardiovascular body systems will be performed at all other visits.
3. Height is collected at the Screening visit only.
4. Blood samples for laboratory tests include: Chemistry (including AST and ALT), Hematology and Biomarkers (Blood samples for biomarkers will not be collected at Screening.)
5. Females of childbearing potential are required to take a urinary pregnancy test at each visit except the Follow-up visit.
6. Patients that pass screening and are scheduled to be randomized will receive a LCM to be worn for 24 hours and returned to the site at the Baseline visit. LCM will be worn for 24 hours at Visits 5 and 8. A paper diary will also be dispensed to the patient to record activities during the 24-hour period.
7. HRCT scans will be performed at Visits 2 and 8. The Baseline HRCT may be skipped if the HRCT for eligibility was performed less than 1 month prior to the Baseline visit.
8. Study material dispensation will occur at Visit 8 for those patients participating in Part B of the study.

Table 5 Patient At-Home Assessments

DAILY: Patients will administer AP01 50 once daily or 100 mg twice daily
WEEKLY: Patients will perform home spirometry readings on the same day each week at approximately the same time of day (+/- 2 hours)

3.8.2. Study Visits

Part A will consist of one Screening visit and 7 study visits, within the 24-week treatment period. Part B will consist of quarterly visits through study termination. A Follow-up visit will occur 28 days after the patient's End of Study visit in Part B.

Details of Study Visits are described in Sections 6.2.1 through 6.2.10 of the clinical study protocol.

4. Endpoints

4.1. Primary Efficacy Endpoint

- Change from baseline in absolute FVC % predicted.

4.2. Secondary Efficacy Endpoints

- Change from baseline in cough frequency as measured by LCM
- Change from baseline in the total KBILD score and in KBILD breathlessness and activity domains
- Change from baseline in the total LCQ score.
- Change from baseline in cough severity as measured by VAS
- Change from baseline in extent of fibrosis and lung volumes as assessed by HRCT
- Change from baseline in D_{LCO}.

4.3. Exploratory Endpoints

- Correlation between weekly home spirometry measurements and in-clinic spirometry values
- Correlation of extent of fibrosis and lung volumes as measured by HRCT at Baseline and Week 24 to other measures of clinical status, e.g. spirometry.
- Change from baseline in serum biomarkers of alveolar epithelial injury (CA19-9 and CA-125).

4.4. Safety Endpoints

- Treatment-emergent adverse events (AEs)
- Change from pre-dose to post-dose FEV_1 after initial dose
- Treatment-emergent deaths
- Treatment-emergent changes in clinical laboratory findings
- Changes in Vital signs.

5. Analysis Sets

5.1. Safety Set

The Safety Set (SAF) will include all randomized patients who were administered at least one dose of study medication. Patients will be analyzed according to treatment received. The SAF will be used for all analyses of safety endpoints.

5.2. Full Analysis Set

The Full Analysis Set (FAS) will include all randomized patients. Patients will be analyzed according to randomized treatment. The FAS will be used for all analyses of patient disposition and baseline analyses and as a supportive sensitivity analyses for all efficacy endpoints. The FAS will also be used for the presentation of patients in all patient listings.

5.3. Per Protocol Set

The Per-Protocol Set (PP) will include all patients who were administered at least one dose of study medication, who have sufficient data to assess the primary efficacy endpoint, and who have no Major Protocol deviations that would impact the ability to appropriately assess the primary endpoint. Patients will be analyzed according to randomized treatment. Criteria for exclusion from the PP includes the following Major Protocol deviations:

- Violation of Inclusion/Exclusion Criteria
- Informed Consent not provided
- Study Drug Deviation
- Concomitant Medicine Restrictions

Summaries of all efficacy endpoints will be created using the PP.

5.4. Protocol Deviations

Protocol deviations will be collected and will be categorized as:

- Informed Consent
- Enrollment Criteria
- Dosing
- Concomitant Medications
- Visits/Procedures Required
- Visit Schedule
- Non-compliance
- Regulatory
- Laboratory
- Other

They will also be graded as Major or Minor.

Major protocol deviations are defined as a subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect the subject's rights, safety or well-being. Major protocol deviations will be included in the Clinical Study Report (CSR). These include: Enrollment Criteria (violation of inclusion/exclusion criteria, Informed Consent (informed consent not provided), Dosing (study drug deviation), Non-compliance, Concomitant Medications (administration of prohibit medication) and Other Good Clinical Practice (GCP) deviations. All protocol deviations will be tracked and corrective live actions implemented.

Protocol deviations will be summarized by grade and category for each treatment group and overall in the following ways:

- Patients with Any Protocol Deviations (Major or Minor)
- Patients with Major Deviations Only
- Patients with Minor Deviations Only (i.e. excluding those patients with Major deviations).
- Protocol Deviations Attributable to COVID-19

All protocol deviations will be listed.

6. General Aspects for Statistical Analysis

6.1. General Methods

Unless otherwise specified, all data will be summarized by treatment group and overall. A separate summary of data from Part A of will be provided in addition to a full study summary including both Part A and Part B data. Categorical data will be summarized as number and percent of total. Continuous data will be summarized using mean, median, SD and range. Dose response will be evaluated by comparing safety and changes in efficacy variables across the dose regimens.

All relevant patient data will be included in listings. All patients entered into the database will be included in patient data listings.

Pulmonary function tests (PFTs) (including D_{LCO}) are performed at each study visit.

The following PFTs will be performed at all visits (including Screening) using spirometry (and documented on the PFT eCRF form):

- FVC Liters (L)
- FEV_1 Liters (L)
- % Predicated FVC
- % Predicted FEV_1.

Pre-dose and post-dose spirometry will be performed at the Baseline visit.

At every visit, D_{LCO} ($mL/min^{-1}/mmHg^{-1}$) and % Predicted D_{LCO} will be obtained using the site's standard methodology.

FEV_1/FVC ratio will be derived as: 100 x FEV_1/FVC.

For comparisons to baseline, data from Visit 2 prior to the first dose of study drug will be used as baseline, unless otherwise noted. For PFTs, the pre-dose result will be used as baseline. Subgroup analyses will be performed based on baseline groupings for FVC, region, age, sex, D_{LCO} and progression-related biomarkers (CXCL13**,** CCL18, MMP3).

If either regimen is eliminated based on Part A outcomes, patients may be switched to the selected regimen for the remainder of their participation. Data summaries will be created to mimic this switch by using the following treatment grouping for Part B: (1) randomized to 100 mg twice daily; (2) randomized to 50 mg once daily and stayed on 50 mg once daily; (3) randomized to 50 mg once daily and switched to 100 mg twice daily; (4) randomized to 50 mg once daily total [groups 2 and 3 combined]; (5) overall total for Part B data. Descriptive within-patient analyses will be included for those patients who switch from 50 mg once daily to 100 mg twice daily. These analyses will include spider plots of change from baseline showing when the dose switch took place. Data listings will include a variable to denote the regimen switch and flag the time of the switch for affected patients.

No formal hypothesis tests are planned.

6.2. Key Definitions

6.2.1. First Dose Date

First dose date in the study is defined as the first dose date of AP01 in Part A.

6.2.2. Cut-Off Date of Final Analysis

For the purpose of final analysis, the cut-off date of final analysis defined as the date at which all patients in the SAF either discontinue from treatment or 18 months has elapsed from the date of the first day of treatment for the last patient enrolled, whichever occurs first.

6.2.3. Baseline and Change from Baseline

Baseline is defined as the last non-missing measurement prior to or on the first dose of study drug.

Change from baseline = (post-baseline value – baseline value). For the purpose of tabulations, the unscheduled post-baseline values generally will be excluded.

6.2.4. Last Dose Date

The last dose date is defined as the last dose date of AP01 in Part B (or Part A if the patient discontinues treatment prior to Part B).

6.2.5. Study Day

The study day in the study is the days relative to the first dose date in the study. The day of the first dose of study medication in Part A will be defined as study day 1. The day prior to the first dose of study medication is study day -1. There is no study day 0.

6.2.6. First Dose Date of Part B

The first dose date of Part B is defined as first dose of study medication received after the Part A last dose date.

6.2.7. Treatment Duration

The overall treatment duration is defined as the last dose date – first dose date+1.

6.2.8. Dosing Switch Date

The dosing switch date is defined as the start date of the dosing change for those who switch from 50 mg once daily to 100 mg twice daily.

6.3. Missing Data

Missing values will not be imputed for data displayed in summary tables. For imputation of missing data used for the sensitivity analysis of the primary efficacy variable see Section 8.1.

Completely missing or partial dates will be presented in the listings as reported on CRFs.

If an AE has a completely missing onset date, then the AE will be considered a treatment emergent adverse event (TEAE). A medication with a completely missing start date is considered a prior medication. A medication with a completely missing stop date is considered a concomitant medication.

If an adverse event or a medication has a partial missing start or stop date, the following rules will be used to impute the date; then the imputed date will be used to determine whether it is a TEAE for adverse event, or a prior or concomitant medication.

Table 6: Partial Date Derivation

Partial Missing Start or Stop Date	Derived Start Date	Derived Stop Date
Missing month and day, and the year is present	January 1 of that year or first dose date if the year is the same as the year of first dose date	December 31 of that year
Missing day, but year and month are present	First day of that month or first dose date if the year and month are the same as the year and month of first dose date	Last day of that month
Missing month, but year and day are present	Missing month derived as January or same as first dose month if the year is the same as the year of first dose	Missing month derived as December

6.4. Visit Windows

All data collected during study follow-up will be displayed and analyzed according to the actual visit data in the eCRF with the exception of early termination visits. Early termination visits will be mapped to the closest study visit, or analyzed according to the actual visit date, when possible.

The study assessments are summarized in Table 1 and Table 2 of the clinical study protocol. All information required by the protocol must be recorded on the source documents. The study schedule must be followed. However, under special conditions (e.g., holidays, weekends), a window of +/- 3 days is permissible for study procedures as long as the proper order of procedures and assessments is maintained. In Phase 2, a window of +/- 7 days is permissible. These windows are not applicable during the baseline period. Out-of-window data will be retained in the tables, listings, and figures. For the Part A interim analysis, Visit 8 will include data collected up to 36 weeks for HRCT and 28 weeks for all other variables.

6.5. Pooling of Centers

All centers will be pooled for summaries by treatment regimen. No by site analyses will be performed.

6.6. Subgroups

Subgroup analyses will be performed based on baseline groupings for FVC % predicted (FVC % predicted ≤ 65, 65 < FVC % predicted < 80, and FVC % predicted ≥ 80 at Baseline), region (Asia-Pacific and Europe), supplemental oxygen use (Y/N), age at screening categorized as >Median level vs. <=Median level, sex, D_{LCO} % predicted categorized as >Median level vs. <=Median level, and progression-related biomarkers (CXCL13, CCL18, MMP3) each categorized as >Median level vs. <=Median level provided each stratum contains at least 10 patients. Analyses of the primary efficacy endpoint will be performed by each subgroup in addition to by treatment group. In addition, a general

safety summary (overall AE summary, summary of respiratory [SOC respiratory, thoracic and mediastinal disorders] TEAEs by Preferred Term and summary of tolerability including cough due to drug administration and bronchospasm after in-clinic drug administration) will be performed by each subgroup in addition to by treatment group.

7. Demographic, Other Baseline Characteristics and Medication

7.1. Patient Disposition and Withdrawals

The number of patients in each study population will be summarized by treatment group and overall. In addition, for each study part (Part A and Part B), patients' status with regard to study treatment and follow-up will also be summarized by treatment group and overall, along with the reasons for study discontinuations. The timing of the switch to 100 mg twice daily in Part B for 50 mg once daily patients and duration of time on 100 mg twice daily will be summarized.

All patient disposition data will be presented in a listing.

7.2. Demographic and Baseline Characteristics

Demographics and baseline characteristics (age, sex, ethnicity, race and childbearing potential (and reason, if No)) will be summarized by treatment group and overall with descriptive statistics for Part A and Part B.

All demographic and baseline characteristics data will be presented in a listing.

7.3. Medical History

Medical history will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA 21.1 or higher). Medical history will be listed by treatment group, patient number, start date and end date

7.4. Other Baseline Characteristics

Other baseline characteristics will be summarized by treatment group and overall with descriptive statistics for Part A and Part B including:

- IPF Diagnosis
    - Time since diagnosis
    - IPF-related cough (All the time, Most times during the day, Several times during the day, Sometimes during the day, Occasionally through the day, Rarely, Never)
- Smoking history:
    - Smoking Status (Current smoker, former smoker, never smoked)
    - For Current or Former Smokers Only:
        - Years smoked (Current Year – Year Started +1 for Current Smoker; Year Quit – Year Started +1 for Former Smoker)
        - Cigarette pack-years
        - Patients with ≥20 cigarette pack-years vs. patients with <20 cigarette pack-years.
- Region (Asia-Pacific and Europe)

- Disease Severity at Screening (40% predicted ≤ FVC < 50% predicted and FVC ≥ 50% predicted)
- Disease Severity at Baseline (FVC % predicted ≤ 65, 65 < FVC % predicted < 80, and FVC % predicted ≥ 80 at Baseline)
- FVC % predicted at Screening
- FVC (L) at Screening
- Progression related biomarkers at Baseline
    - CXCL13
    - CCL18
    - MMP3

All data for other baseline characteristics will be presented in a listing.

7.5. Drug Exposure

In this study, patients will be dosed in the clinic at Baseline to confirm tolerability for the first (and possibly second dose) and at home by the patient for all post-baseline doses. Drug exposure will be assessed as follows:

Baseline: In the Clinic

- The actual dose (mL) of AP01 for each patient will be the starting volume in the nebulizer minus the remaining volume in the nebulizer (if the full dose was not administered). If a second dose is given due to intolerance at the first dose, the actual dose will be derived in the same manner as the first dose. The number of patients who require a second dose in-clinic will be summarized and the total actual dose for each dose will be summarized by treatment group.
- The number of patients with physician-prescribed salbutamol taken prior to the dose will be summarized for each dose (first dose only if only 1 dose was received or for each dose if 2 doses are received) by treatment group with frequency counts and percentages. For patients who received salbutamol prior to dose, how many of those are because of prior toxicity for each dose and how many were added due to intolerance for the second dose will be summarized by treatment group with descriptive statistics.
- The number of patients with study drug inhalations interrupted will be summarized for each dose (first dose only if only 1 dose was received or for each dose if 2 doses are received) by treatment group with frequency counts and percentages.
- For patients with Inhalation interrupted during the second dose, the number of patients with salbutamol administered to continue dosing will be summarized by treatment group with frequency counts and percentages.
- For each patient, the time of nebulization for each dose will be derived as the study drug administration stop time minus the study drug administration start time and converted to minutes. If the study drug inhalation was interrupted, the time of interruption for each dose will be derived as the Inhalation Re-Start Time minus the Inhalation Interruption Time, converted to minutes and

then subtracted from the time of nebulization. Time of nebulization will then be summarized for each dose by treatment group with descriptive statistics. In addition, the Time of nebulation will be summarized for just those patients who took the full dose with no interruptions for each dose by treatment group with descriptive statistics.

- To assess tolerance, the number of patients with a ≥15% drop from pre-dose FEV_1 % predicted to post-dose FEV_1 % predicted will be summarized for each dose (first dose only if only 1 dose was received or for each dose if 2 doses are received) by treatment group with frequency counts and percentages (see more details in Section 10.6).

Post-Baseline: At Home

- For each patient, the treatment days will be derived cumulatively across all visits as the last dose date minus the first dose date + 1 and will be summarized by treatment group with descriptive statistics.
- For each patient, the actual total dosage (mg) of AP01 will be derived cumulatively across all visits across all patient doses as 50 times the total number of used ampoules returned, and will be summarized by treatment group with descriptive statistics.
- For each patient, the percent compliance will be derived cumulatively across all visits across all patient doses as 100 times (the total number of used ampoules returned divided by the total number of ampoules that would be used if they were fully dosing for the number of days between visits {slightly different from the number of ampoules dispensed}). If a subject discontinues early, the denominator will be the total number of ampoules, which would be used up to, and including the date of discontinuation. Percent compliance will be summarized by treatment group with descriptive statistics. The percent compliance will be categorized (≥80%, 60-<80%, 40-<60%, 20-<40% and <20%) and summarized by treatment group with frequency counts and percentages. The percent compliance will be calculated overall and separately for each dosing regimen for those patients who switch over from 50 mg once daily to 100 mg twice daily in Part B.
- For each patient, the percent compliance with the patient's care plan will be derived cumulatively across all visits across all patient doses as 100 times (the total number of used ampoules returned divided by the total number of ampoules that would be used if they were fully dosing for the number of days between visits minus the number of ampoules that would be missed if following instructions to reduce or interrupt dose). If a subject discontinues early, the denominator will be the total number of ampoules, which would be used up to, and including the date of discontinuation. Percent compliance will be summarized by treatment group with descriptive statistics. The percent compliance will be categorized (≥80%, 60-<80%, 40-<60%, 20-<40% and <20%) and summarized by treatment group with frequency counts and percentages. The percent compliance will be calculated overall and separately for each dosing regimen for those patients who switch over from 50 mg once daily to 100 mg twice daily in Part B.
- The number of patients who had any days where all doses were missed will be summarized cumulatively across all visits by treatment group with frequency counts and percentages. For those patients who had any days where all doses were missing, the maximum number of days between doses will be summarized cumulatively across all visits by treatment group with descriptive statistics.

All recorded information for study medication administration and accountability for AP01 will be listed.

All recorded information for Nebulizer Training, Nebulizer and Nebulizer handset accountability will be listed.

7.6. Prior and Concomitant Medications and Therapies

Medications and therapies that started and stopped prior to the first dose of AP01 are considered as prior medications. Medications and therapies that started prior to the first dose of AP01 and continued into the treatment period are considered as prior and concomitant medications. Medications or therapies with a start date from first dose of AP01 to 30 days after administration of the last dose of AP01 will be considered as concomitant medications.

Prior and concomitant medications will be coded according to World Health Organization (WHO) Drug Dictionary (March 2018 WHO DDE (Enhanced) B2 Format) for Concomitant Medication. The concomitant medications will be summarized for each treatment group and overall by Anatomical Therapeutic Chemical (ATC) level 2 (Therapeutic Main Group) and ATC level 4 (Chemical/Therapeutic Subgroup) using the number and percentage of patients. Medications will be sorted in descending order of frequency of ATC level 2 and ATC level 4 within ATC level 2 in the overall total column. A patient will be counted only once within each level of summation if the patient has taken a medication more than once.

All prior and concomitant medications and therapies will be listed.

8. Efficacy

The following efficacy analyses will be performed:

- Change from baseline in absolute FVC % predicted
- Change from baseline in cough frequency as measured by LCM
- Change from baseline in the total KBILD score and in KBILD breathlessness and activity domains
- Change from baseline in the total LCQ score.
- Change from baseline in cough severity as measured by VAS
- Change from baseline in extent of fibrosis and lung volumes as assessed by HRCT
- Change from baseline in D_{LCO}

The primary endpoint of interest for efficacy is the absolute change from Baseline to Week 24 in FVC % predicted. Linear mixed-effects models will be used to estimate slopes representing the mean change from Baseline to Week 24 in each treatment group, and the difference between treatment groups will be examined. Baseline FVC % predicted, region, age at screening, sex, baseline D_{LCO} % predicted, and baseline progression-related biomarker (CXCL13**,** CCL18, MMP3) values each categorized as >Median level vs. ≤Median level will be included as covariates in the model. Similar modeling strategies will be employed for longer-term data obtained from Part B of the study.

No control group is included in this study; however, there is ample data in the literature showing a consistent rate of decline in IPF patients not receiving anti-fibrotic therapies. Historical data from the placebo groups of the three Phase III clinical trials of oral pirfenidone (ASCEND and CAPACITY trials) showed an average decline in FVC % predicted of ~4% at 6 months. These results will be used as a historical comparator. For each AP01 treatment group, a one sample Wald test will test H_0: mean change from Baseline to Week 24 in FVC % predicted ≤ -4% vs H_1: mean change from Baseline to Week 24 in FVC % predicted > -4%. For each AP01 treatment group, a 95% one-sided confidence interval will be derived for the difference (between A01 and historical reference) in the mean change from Baseline to Week 24 in FVC % predicted. Additionally, we will compare the change from baseline in FVC % predicted at Week 24 for each treatment group to the average decline in FVC % predicted at 24 weeks for patients with no documented antifibrotic treatment calculated from a set of (1) Pulmonary Fibrosis Foundation (PFF) Registry patients and (2) Australian IPF Registry patients, with matching baseline characteristics. For each treatment group and each registry, a two-sided Wald test will test: H_0: 24-week FVC decline$_{\text{AP01 group}}$= 24-week FVC decline$_{\text{registry}}$ vs. H_1: 24-week FVC decline$_{\text{AP01 group}}$ ≠ 24-week FVC decline$_{\text{registry}}$. For each of the 2 AP01 treatment groups and for each of the 2 registries, a 95% two-sided confidence interval will be derived for the difference (between AP01 and registry) in the mean change from Baseline to Week 24 in FVC % predicted.

The primary population used for efficacy analyses will be the PP with the FAS used for specified sensitivity analyses.

8.1. Primary Efficacy Endpoint and Analysis

Spirometry will be conducted at approximately the same time each visit (within 60 minutes of Day 1 spirometry measurement) with the patient in seated position. The test will be performed to American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines[3]. On days of clinic visits (including the screening visit), patients must refrain from strenuous activity at least 12 hours prior to pulmonary function testing (PFT). FVC % predicted will be calculated using the ERS Global Lung Function Initiative 2012 equations[2] as outlined in the clinical study manual.

PFTs will be collected at Screening, Baseline and at each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up) as described in Section 6.1.

The primary efficacy endpoint is the change from baseline in absolute FVC % predicted from Baseline to Week 24. The population used for efficacy analyses of the primary variable will be the PP with the FAS used for the first sensitivity analyses.

A linear mixed-effects model (PROC MIXED) will be used and differences between treatment groups between slopes estimating the change from baseline in absolute FVC % predicted from Baseline to Week 24 will be examined. Baseline FVC % predicted, region, age at screening, sex, baseline D_{LCO} % predicted, and baseline progression-related biomarker (CXCL13**,** CCL18, MMP3) values each categorized as >Median level vs. ≤Median level will be included as covariates in the model. This analysis requires at least three post-baseline FVC % predicted values in addition to baseline be available for a patient to be included in the analysis so that the slope estimated is identifiable. Similar modeling strategies will be employed for longer-term data obtained from Part B of the study. Spirometry data collected after patients switched dosing regimen from 50 mg once daily to 100 mg twice daily will be omitted for this analysis.

For the sensitivity analyses of the primary efficacy endpoint, two different imputation strategies will be used to address missing in-clinic spirometry data used in the calculation of the change in absolute FVC % predicted from Baseline to Week 24. In both imputation strategies, missing data for early terminations other than deaths will be imputed for all visits following termination by assuming the average change at each visit calculated for all patients on the same cohort with FVC % predicted data available at that visit. For patients with missing data due to death, the remaining FVC % predicted values will be imputed as 30%.

For the second imputation strategy, in addition to imputing missing data due to early termination, home spirometry data will be used to replace missing in-clinic data using the following algorithm:

- Flag any biologically implausible home spirometry records for exclusion from analysis defined to be PFT measurements where the corresponding FVC % predicted value is <10 or >136.
- If home spirometry is available within the first two weeks following first dose of study drug, use it to calculate changes from baseline for home spirometry data.
- If home spirometry is not available within the first two weeks following first dose of study drug, use the patient's home spirometry data to fit a linear regression and the estimated intercept as a baseline for home spirometry data.

- Calculate all changes from baseline possible for a patient based on home spirometry data.
- Using any home spirometry data collected within the mapping window for a scheduled visit, create a pseudo office observation as the average of these home spirometry changes from baseline.
- Insert pseudo office observations for missed visits, setting the weeks of the observation to be the target weeks for that visit.

The primary variable will also be summarized by treatment group with descriptive statistics for the following subgroups: FVC (FVC % predicted ≤ 65, 65 < FVC % predicted <80, and FVC % predicted ≥ 80 at Baseline); region (Asia-Pacific and Europe); age at screening (>Median level vs. <=Median level); sex (Male and Female); baseline D_{LCO} % predicted (> Median level vs. <=Median level) and baseline progression-related biomarkers (CXCL13, CCL18, MMP3) each categorized as >Median level vs. <=Median level.

The following statistics for in-clinic FVC and FVC % Predicted will be calculated and summarized by treatment group and displayed in a table:

- Summary statistics of in-clinic FVC and FVC % Predicted at Baseline and at each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up)
- Summary statistics of in-clinic FVC and FVC % Predicted reflecting the absolute change from Baseline to each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up).

The following plot will be produced for in-clinic FVC and FVC % Predicted:

- Mean FVC (or FVC % Predicted) Change from Baseline (±SD) vs. Visit Week (Week 4, Week 8, Week 12, etc.) with lines for each treatment group.

Linear mixed-effects models (PROC MIXED) will be used for the analyses. The model will include the change from baseline in in-clinic FVC (or FVC % Predicted) (measured at any time in the study) as the dependent variable and will include patient and time (continuous) as random effects, fixed effect of time and time-varying effects for treatment group and treatment-by-time interaction to account for those patients who switched dosing regimens from 50 mg once daily to 100 mg twice daily in Part B. Longitudinal plots at selected timepoints displaying the model adjusted, least square means and 95% confidence intervals will be produced.

All in-clinic pulmonary function data will be presented in a listing.

8.2. Secondary Efficacy Endpoint(s) and Analyses

8.2.1. Change from baseline in cough frequency as measured by LCM

The LCM[4] is an audio-based cough detection device that allows sounds to be reconstructed and measured as cough events. Patients that pass all of the screening criteria and will be randomized in the study will be provided with a cough monitor to wear for a 24-hour period prior to coming in for their Baseline visit. This will be used as their baseline reading. The LCM and mailing materials will also be dispensed at Visits 5 and 8. Patients will wear for a 24-hour period, fill out the cough monitor paper diary

and when complete, mail the LCM and diary back using the mailing materials provided. Further details regarding LCM dispensation and return will be outlined in the clinical study manual.

The following statistics for cough frequency (coughs/hour) as measured by LCM will be calculated and summarized separately for 24 hours and for awake time by treatment group and displayed in a table:

- Summary statistics of the cough frequency (coughs/hour) as measured by LCM at Baseline and at Visits 5 and 8 (Week 12 and 24)
- Summary statistics of the cough frequency (coughs/hour) as measured by LCM reflecting the absolute change from Baseline to Visit 5 and 8 (Week 12 and 24).

The following plot will be produced for cough frequency (coughs/hour) as measured by LCM:

- Mean Cough Frequency (coughs/hour) as measured by LCM Change from Baseline (±SD) vs. Visit Week (Week 12, Week 24) with lines for each treatment group.

A linear mixed-effect model (PROC MIXED) will be used and differences between treatment groups between the change from Baseline in coughs/hour as measured by LCM to Weeks 12 and 24 will be examined. Baseline cough frequency (coughs/hour), region, age at screening, sex, baseline D_{LCO} % predicted, and baseline progression-related biomarker (CXCL13**,** CCL18, MMP3) values each categorized as >Median level vs. <=Median level will be included as covariates in the model. The model will include a random effect for patient and fixed effects for treatment, visit (Week 12 or Week 24), and the interaction between treatment and visit. To account for correlated repeated measures within patients a compound symmetric variance-covariance matrix will be used.

In order to assess the impact of treatment on cough in those patients for whom coughing is of greatest concern, additional analyses of cough frequency will be performed on the subset of patients with baseline cough severity VAS ≥ 40 millimeters and in patients with baseline cough frequency ≥10 awake time coughs/hour. When examining these subgroups, the outcome will be % change from baseline.

All data for LCM will be presented in a listing.

8.2.2. Change from baseline in the total KBILD score and in the KBILD breathlessness and activity domain score

The KBILD Questionnaire[8] is a disease specific questionnaire consisting of 15 items that measures three specific domains: breathlessness and activities (questions 1, 4, 11 and 13), psychological (questions 3, 5, 6, 8, 10, 12 and 14) and chest symptoms (questions 2, 7 and 9). All items are scored on a seven-point response scale (1, 2, 3, 4, 5, 6, 7). The KBILD domain and total score ranges are 0–100; 100 represents the best health status A KBILD total score can be derived from the 3 domain scores with the addition of question #15. The algorithm for scoring the KBILD Questionnaire is logit-based and described in Sinha et al. 2019[9].

The KBILD Questionnaire will be completed at Baseline, all subsequent visits and the Follow-up visit. This is a paper-based questionnaire that will be completed by the patient at the relevant visits. The results of the KBILD will be entered into the appropriate eCRF. Appendix Section 14.2 presents the KBILD Questionnaire.

The following statistics for each KBILD questionnaire data domain and total score will be calculated and summarized by treatment group and displayed in a table:

- Number and percentage of patients in each treatment group that completed each survey at Baseline and at each post-Baseline time point.
- Summary statistics of the total KBILD score and each KBILD domain score at Baseline and at each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up)
- Summary statistics of the total KBILD score and each KBILD domain score reflecting the absolute change from Baseline to each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up).

The following plot will be produced for each KBILD domain score and the KBILD total score:

- Mean KBILD domain (or Total) score Change from Baseline (±SD) vs. Visit Week (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up) with lines for each treatment group.

All KBILD Questionnaire data will be presented in a listing.

8.2.3. Change from baseline in the total LCQ score.

The LCQ Questionnaire[10] is a disease specific questionnaire consisting of 19 items that measures three specific domains: physical (questions 1, 2, 3, 9, 10, 11, 14 and 15), psychological (questions 4, 5, 6, 12, 13, 16 and 17) and social (questions 7, 8, 18 and 19). All items are scored on a seven-point response scale (1, 2, 3, 4, 5, 6, 7). Domain scores are calculated as the total score from items in the domain divided by the number of items in the domain (range 1–7). The total score is the addition of all domain scores (range 3–21). For all domains, the higher the score the better the quality of life (QOL). If there are missing items, domain scores can be prorated by averaging the number of items actually answered. When there are missing data, prorating by domain in this way is acceptable as long as at least 50% of the items were answered (e.g., a minimum of 2 of 4 items).

The LCQ will be completed at Baseline, all subsequent visits and the Follow-up visit. This is a paper-based questionnaire that will be completed by the patient at the relevant visits. The results of the LCQ will be entered into the appropriate eCRF. Appendix 14.3 presents the LCQ.

The following statistics for each LCQ questionnaire domain and total score will be calculated and summarized by treatment group and presented in a table:

- Number and percentage of patients in each treatment group that completed each survey at Baseline and at each post-Baseline time point.
- Summary statistics of the total LCQ score and each LCQ domain score at Baseline and at each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up)
- Summary statistics of the total LCQ score and each LCQ domain scores reflecting the absolute change from Baseline to each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up).

The following plot will be produced for each LCQ domain score and the LCQ total score:

- Mean LCQ domain (or total) score Change from Baseline (±SD) vs. Visit Week (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up) with lines for each treatment group.

All LCQ Questionnaire data will be presented in a listing.

8.2.4. Change from baseline in cough severity as measured by VAS

The European Respiratory Society Cough Severity VAS[6] will be completed at Baseline, all subsequent visits and the Follow-up visit. This is a paper-based scale that will be completed by the patient at the relevant visits. The scale will range on a vertical line from 'No Cough' at the bottom to 'Worst Cough Ever' at the top. The patient will put a cross on the line to indicate the severity of their cough in the last 2 weeks. The results of the scale will be measured in millimeters from the bottom of the scale to the marked cross and entered into the appropriate eCRF. Appendix Section 14.1 presents an example of the Cough Severity VAS.

The following statistics for Cough VAS will be calculated and summarized by treatment group and presented in a table:

- Summary statistics of the Cough Severity VAS score at Baseline and at each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up)
- Summary statistics of the Cough Severity VAS score reflecting the absolute change from Baseline to each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up).

The following plot will be produced for the Cough Severity VAS:

- Mean Cough Severity VAS Change from Baseline (±SD) vs. Visit Week (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up) with lines for each treatment group.

All Cough Severity VAS score data will be presented in a listing.

8.2.5. Change from baseline in extent of fibrosis and lung volumes as assessed by HRCT

HRCT scans will be obtained to measure the extent of fibrosis and lung volumes at the Baseline visit and the Week 24 visit, or at an early termination visit if the patient does not complete Part A. These scans will be read and interpreted by a central reader.

The following statistics from the HRCT scans will be calculated and summarized by lung region and treatment group and displayed in a table:

- Summary statistics of the extent of fibrosis and lung volumes as assessed by HRCT at Baseline and the Week 24 post-Baseline time point, or separately for early terminations at the post-Baseline time point available.
- Summary statistics of the extent of fibrosis and lung volumes as assessed by HRCT reflecting the absolute change from Baseline to the Week 24 time point, or separately for early terminations at post-Baseline time point available.

The following plot will be produced for extent of fibrosis and lung volumes as assessment by HCRT:

- Mean Extent of Fibrosis (or Lung Volume) Change from Baseline (±SD) vs. Weeks from Baseline (calculated based on visit date) for each lung region with lines for each treatment group.

A linear model will be used and differences between treatment groups between the change from baseline in whole lung extent of fibrosis and whole lung volume from Baseline to post-Baseline will be examined. Change from baseline will be the dependent variable and baseline whole lung extent of fibrosis (or whole lung volume), region, age at screening, sex, baseline D_{LCO} % predicted and baseline progression-related biomarker (CXCL13**,** CCL18, MMP3) values each categorized as >Median level vs. ≤Median level, and time of HRCT (relative to baseline) will be included as covariates in the model.

All HRCT scan data will be presented in a listing including early termination and unscheduled visits.

8.2.6. Change from baseline in D_{LCO}

D_{LCO} will be collected at each visit. For predicted normal values, different sites may use different prediction formulas, based on the method used to measure D_{LCO}. In any case, the method used must be in compliance with the ATS/ERS guidelines[5] on D_{LCO} measurements and the prediction formula appropriate for that method[7]. Raw data (gas mixture, equation used for prediction of normal, further adjustments made if so) must be traced.

PFTs will be collected at Screening, Baseline (pre-dose and post-dose) and at each Post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up) as described in Section 6.1.

The following statistics for D_{LCO} and D_{LCO} % Predicted will be calculated and summarized by treatment group and displayed in a table:

- Summary statistics of D_{LCO} and D_{LCO} % Predicted at Baseline and at each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up)
- Summary statistics of D_{LCO} and D_{LCO} % Predicted reflecting the absolute change from Baseline to each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up).

The following plot will be produced for D_{LCO} and D_{LCO} % Predicted:

- Mean D_{LCO} (or D_{LCO} % Predicted) Change from Baseline (±SD) vs. Visit Week (Week 4, Week 8, Week 12, etc.) with lines for each treatment group.

A linear mixed-effects model (PROC MIXED) will be used for the analysis. The model will include the change from baseline in D_{LCO} (or D_{LCO} % Predicted) (measured at any time in the study) as the dependent variable and will include patient as a random effect, fixed effect of time and time-varying effects for treatment group and treatment-by-time interaction to account for those patients who switched dosing regimens from 50 mg once daily to 100 mg twice daily in Part B. To account for correlated repeated measures within patients a spatial power matrix will be used. Longitudinal plots at selected timepoints displaying the model adjusted, least square means and 95% confidence intervals will be produced.

All D_{LCO} data will be presented in a listing.

9. Exploratory

9.1. Correlation between weekly home spirometry measurements and in-clinic spirometry

Patients will obtain spirometry readings at home on the same day and at approximately the same time each week (+/- 2 hours). Home spirometers will be dispensed at the Baseline visit; patients will be trained on the use of home spirometers prior to their departure from the Baseline visit. Home spirometer training and accountability data will be presented in a listing.

The home spirometry measurements (FVC, FVC % predicted) will be summarized by treatment group and visit including change from the baseline in-clinic pre-dose spirometry value using descriptive statistics. For the tabular summary and graphical summary, biologically implausible values and outliers will be removed as described previously in Section 8.1. The remaining spirometry measurements (FVC, FVC % predicted) within the mapping window for a scheduled visit will be averaged. All home spirometry data will be included in a listing.

Bland-Altman plots will be produced to describe the agreement between the home and in-clinic spirometry measurements at each visit. In these plots, the average of home spirometry measurements within the mapping window for a scheduled visit will be compared with the in-clinic spirometry at each visit.

A linear mixed-effects model (PROC MIXED) approach[12] will be used to determine the repeated-measures correlation coefficient estimates for in-clinic FVC measurements with averages of home FVC measurements across visits for each treatment group. A normal approximation will be used to determine the estimate for the correlation coefficient (ρ_{hat}), the 95% confidence intervals for the correlation coefficient estimate and p-value. This analysis for Part B will only use measurements in the 50 mg once daily group taken prior to the switch to the 100 mg twice daily group if the patient switched dosing regimens.

9.2. Change in absolute FVC % predicted from home spirometry Baseline visit to Week 24 and Week 72

A linear mixed-effects model (PROC MIXED) will be used for analysis. The model will include FVC % predicted (measured at home) as the dependent variable and will include patient and time of spirometry reading (study day) as random effects, and fixed effects of treatment group, time of spirometry reading (study day), along with a treatment-by-time interaction. Longitudinal plots at selected timepoints displaying the model adjusted, least square means and 95% confidence intervals will be produced. Spirometry data collected after patients switched dosing regimens from 50 mg once daily to 100 mg twice daily will be omitted for this analysis.

9.3. Correlation of extent of fibrosis and lung volume as measured by HRCT at Baseline and Week 24 to other measures of clinical status

The following HRCT measurements will be correlated with the following In-Clinic Spirometry Measurements (FVC) at Baseline and Week 24 or early termination:

- Extent of Fibrosis as Measured by HRCT with FVC

- Lung Volume as Measured by HRCT with FVC.
- Change in Extent of Fibrosis as Measured by HRCT with Change in FVC
- Change in Lung Volume as Measured by HRCT with Change in FVC

A linear mixed-effects model (PROC MIXED) will be used for the analyses of the absolute measures. The models will include the Extent of Fibrosis (or Lung Volume) (measured at Baseline and Week 24 or early termination) as the dependent variable and will include patient as a random effect, and fixed effects of treatment group, FVC (Baseline, Week 24 or early termination), visit, timing of HRCT in weeks from Baseline, and a treatment-by-visit interaction. To account for correlated repeated measures within patients a compound symmetric variance-covariance matrix will be used. Longitudinal plots at selected timepoints displaying the model adjusted, least square means and 95% confidence intervals will be produced. A linear model will be used for the change in Extent of Fibrosis (or Lung Volume). The model will include the change from baseline in Extent of Fibrosis (or Lung Volume) (measured at Week 24 or early termination) as the dependent variable and will include fixed effects of change from baseline in FVC, treatment group, baseline Extent of Fibrosis (or Lung Volume), and timing of HRCT in weeks from Baseline. The model adjusted, least squares mean and 95% confidence interval at Week 24 will be produced.

A linear mixed-effects model will be used to determine the repeated-measures correlation coefficient estimate for Extent of Fibrosis (or Lung Volume) with FVC across visits (Baseline, Week 24) for each treatment group. A normal approximation will be used to determine the estimate for the correlation coefficient (ρ_{hat}), the 95% confidence intervals for the correlation coefficient estimate and p-value. A partial correlation coefficient will be used to determine the correlation coefficient estimates for change in Extent of Fibrosis (or Lung Volume) with change in FVC for each treatment group. The corresponding 95% confidence intervals and p-values for the correlation coefficient estimates also will be reported.

9.4. Change from baseline in serum biomarkers of alveolar epithelial injury (CA19-9 and CA-125)

The serum biomarkers of alveolar epithelial injury (CA19-9 and CA-125)[11] will be collected at Baseline, all subsequent visits and the Follow-up visit. The following statistics for CA19-9 and CA-125 will be calculated and summarized by treatment group and displayed in a table:

- Summary statistics of CA19-9 and CA-125 at Baseline and at each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up)
- Summary statistics of CA19-9 and CA-125 reflecting the absolute change from Baseline to each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up).

The following plot will be produced for CA19-9 and CA-125:

- Mean CA19-9 (or CA-125) Change from Baseline (±SD) vs. Visit Week (Week 4, Week 8, Week 12, etc.) with lines for each treatment group.

Change from baseline in CA19-9 and CA-125 will be correlated with change from baseline in FVC at Weeks 4, 8, 12, 16, 20, 24, 36, 48, 60, and 72. Linear mixed-effects models (PROC MIXED) will be used for the analyses. The model will include the change from baseline in CA19-9 (or CA-125) (measured at Weeks 4, 8, 12, 16, 20, 24, 36, 48, 60, and 72) as the dependent variable and will include patient as a

random effect, fixed effects of time, change from baseline in FVC (measured at Weeks 4, 8, 12, 16, 20, 24, 36, 48, 60 and 72), baseline CA19-9 (or CA-125), visit and time-varying effects for treatment group and treatment-by-time interaction to account for those patients who switched dosing regiments from 50 mg once daily to 100 mg twice daily in Part B. To account for correlated repeated measures within patients a spatial power variance-covariance matrix will be used. Longitudinal plots at selected timepoints displaying the model adjusted, least square means and 95% confidence intervals will be produced.

A linear-mixed effects model (PROC MIXED) approach will be used to determine the repeated-measures correlation coefficient estimates for change from baseline in CA-19 (or CA-125) with change from baseline in FVC for each treatment group using change from baseline at Weeks 4, 8, 12, 16, 20, 24, 36, 48, 60 and 72. A normal approximation will be used to determine the estimate for the correlation coefficient (ρ_{hat}), the 95% confidence intervals for the correlation coefficient estimate and p-value. Spirometry data collected after patients switched dosing regimens from 50 mg once daily to 100 mg twice daily will be omitted from this analysis.

10. Safety

The population used for safety analyses will be the SAF. Safety will be assessed on the basis of AE or adverse drug reactions (ADR) reports, clinical laboratory data, ECG parameters, physical examinations, vital signs and change from pre-dose to post-dose FEV_1 after initial dose.

Treatment-emergent AEs (any AEs recorded during or following the first study drug administration) will be summarized by treatment group and categorized by severity and relationship to the study procedures and to the investigational product (IP). If a patient has more than 1 occurrence of the same AE, he/she will be counted only once within that preferred term in the summary tables. The most severe occurrence of an AE, as well as the most extreme relationship of the AE to the study procedures and IP, will be indicated in cases of multiple occurrences of the same AE. SAEs will be summarized separately.

All AE data will be presented in a listing. Additionally, listings of SAEs and AEs leading to discontinuation will be generated. All SAEs will be evaluated to determine whether they are SUSARs or Unexpected Adverse Device Effects.

10.1. Adverse Events / Adverse Drug Reactions

An adverse event (AE) is any untoward medical condition that occurs in a patient while participating in this clinical study. Adverse events will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA 21.1 or higher) terminology and the severity of the toxicities will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), v5.0, where applicable. An adverse drug reaction (ADR) means any AE caused by a drug or biologic.

Treatment-Emergent Adverse Events (TEAEs) are those adverse events/adverse drug reactions that are recorded during or following the initiation of AP01 medication administration, and do not necessarily have a causal relationship to the use of the study medication. Treatment-Emergent Adverse Events (simply referred to as adverse events in summary tables) will be summarized.

The following adverse event summary tables will be summarized by treatment group and combined over both treatment groups:

1) An overall summary with the number and percentage of patients reporting AEs, serious AEs, grade 3 or higher AEs, treatment-related AEs, AEs leading to study treatment (AP01) discontinuation and AEs with outcome of deaths. In addition, this overall summary will be performed for each subgroup listed in Section 6.6.

2) AEs overall and by system organ class and preferred term. In this summary, a patient is counted once at the system organ class and once at each preferred term within the system organ class. In addition, this summary will be performed for each subgroup listed in Section 6.6.

3) A separate summary of respiratory [SOC respiratory, thoracic and mediastinal disorders] TEAEs by Preferred Term and summary of tolerability including cough due to drug administration and bronchospasm after in-clinic drug administration) will be performed by each subgroup listed in Section 6.6.

4) AEs overall and by system organ class, preferred term and highest CTCAE grade.

In this summary, a patient is counted once at the highest grade for which the event occurred in the system organ class and the highest grade for each unique preferred term within that system organ class. Therefore, patients may only contribute once to each preferred term and once to each system organ class. The missing severity grade will be reported in a separate category.

5) Study-treatment-related AEs overall and by system organ class and preferred term.

All those AEs with relationship to AP01 marked as “Possibly Related” or “Probably Related” or missing will be reported in the table.

6) Study-treatment related AEs by system organ class, preferred term and Highest CTCAE grade

7) AEs leading to study treatment termination by system organ class and preferred term

8) AEs leading to study treatment termination by system organ class, preferred term and highest CTCAE grade

9) CTCAE Grade 3 or higher AEs, overall and by system organ class and preferred term

A Serious Adverse Event (SAE) is an AE that falls into one or more of the following categories:

a. Results in death
b. Is life-threatening
c. Requires inpatient hospitalization or prolongation of existing hospitalization
d. Results in persistent or significant disability or incapacity
e. Is a congenital anomaly/birth defect
f. Is any other important medical event

Serious adverse events will be summarized by treatment group and combined over both treatment groups.

1) SAEs overall and by system organ class and preferred term.

In this summary, a patient is counted once at the system organ class and once at each preferred term within the system organ class.

2) SAEs overall and by system organ class, preferred term and highest CTCAE grade.

10.2. Laboratory Evaluations

At each visit, clinical laboratory tests will be conducted at the study’s central laboratory. Clinical laboratory tests will include:

- Hematology: complete blood count with differential, platelet count

- Serum Biochemistry: bilirubin, alkaline phosphatase, sodium, potassium, chloride, bicarbonate, urea, creatinine, total protein, albumin, glucose, ALT, AST
- Urinalysis: pH, specific gravity, presence/absence of protein, glucose, blood
- Biomarkers: MMP3, CCL18, CXCL13, CA19-9, CA-125

Clinical laboratory results will be summarized using Standard International System of Units (SI) units. Laboratory measurements will be summarized for all protocol scheduled time points. Descriptive statistics (n, mean, SD, median, min and max) will be presented for all clinical chemistry, hematology, urinalysis (continuous) and biomarker (CA19-9 and CA125) laboratory parameters at each scheduled visit. Change from baseline at each scheduled post-Baseline visit will also be presented.

Patient counts and percentages of patient for each category of each urinalysis (categorical) parameters will be summarized at each scheduled visit.

Shift tables (i.e., low-normal-high at Baseline versus low-normal-high at each post-Baseline visit in a 3-by-3 contingency table) will be provided for hematology, clinical chemistry, urinalysis and biomarker to assess changes from baseline in laboratory values by visit.

A listing for all laboratory results for each laboratory category (hematology, serum biochemistry, urinalysis and biomarkers) and a listing of treatment-emergent abnormal values will be presented.

10.3. Vital Signs

Vital sign measurements including systolic blood pressure, diastolic blood pressure, pulse rate, respiration rate, and body temperature at each scheduled time and changes from baseline in vital sign measurements will be summarized with descriptive statistics at each scheduled time point by treatment group. Temperature will be displayed in Celsius.

Body height will be measured at Screening only and will be summarized by treatment group. Weight will be measured at Screening and Week 12, 24, 48 and 72 visits and, along with changes from baseline, will be summarized with descriptive statistics at each scheduled time point by treatment group. Weight will be displayed in kilograms; height will be displayed in centimeters.

Body mass index (BMI) will be derived from the weight (at Week 12, 24, 48 and 72) and height (at Screening) along with changes from baseline, will be summarized with descriptive statistics at each schedule timepoint by treatment group. BMI will be displayed in kilograms per meter squared.

All vital signs data will be presented in a listing.

10.4. Physical Examination

A complete PE will be performed at the Screening and Baseline visits. This will include physical examination of the following body areas and systems: examination of general appearance, skin, head, neck (including thyroid), eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system.

A modified PE of the lungs and CV body systems will be performed at each subsequent visit.

A complete or modified physical exam is optional at unscheduled visits, per the investigator's discretion.

The physical examination data will be presented in a listing.

10.5. Saturated Oxygen (SaO_2) Assessment

Saturated Oxygen (SaO_2) assessments will be performed at Screening, Baseline (Pre-Dose and Post-Dose) and at all post-Baseline Visits (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up). The SaO_2 % result will be captured and an indication will be noted if a patient received supplemental oxygen and, if so, the number of liters per minute. For Pre-Dose at Baseline, patients with SaO_2 < 90% will be administered supplemental oxygen until the SaO_2 reaches 93% prior to dosing.

The following statistics for SaO_2 will be calculated and summarized by treatment group and displayed in a table:

- Number and percentage of patients in each treatment group that had a SaO_2 result at screening, Baseline (pre-dose) and at each post-Baseline time point.
- Summary statistics of SaO_2 at screening, Baseline (pre-dose) and at each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up)
- Summary statistics of SaO_2 reflecting the absolute change from Baseline (pre-dose) to each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up).

The following plot will be produced for SaO_2:

- Mean SaO_2 Change from pre-dose Baseline (±SD) vs. Visit Week (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up) with lines for each treatment group.

All SaO_2 data will be presented in a listing.

10.6. Pre and Post-Dose Spirometry at Baseline

At the Baseline visit, the first dose of study drug will be administered at the clinic; post-dose Spirometry will be performed and the post-dose spirometry FEV_1 results will be compared with the pre-dose FEV_1 results. Patients that have a ≥ 15% drop in FEV_1 % predicted in their pre-dose to post-dose readings and who are symptomatic will be required to use 1 - 2 puffs (90 - 100 µg) of salbutamol prior to dosing throughout the study unless these patients are currently taking a long-acting beta-2-agonist therapy.

If based on the investigator's opinion, the patient had tolerability issues during the first dose administration, a second dose, at least 4 hours later, will also be observed at the Baseline visit. For the second dose, a second pre-dose spirometry and post-dose spirometry will be performed. In addition, pre- and post-dose SaO_2 will also be recorded for the second dose.

For the first dose, the following statistics for pre- and post-dose FEV_1 results will be calculated and summarized by treatment group and displayed in a table:

- Number and percentage of patients in each treatment group that had an FEV_1 result at Baseline (pre-dose) and post-dose at Baseline
- Summary statistics of FEV_1 at Baseline (pre-dose) and at Baseline (post-Baseline)
- Summary statistics of FEV_1 reflecting the absolute change from Baseline (pre-dose) to Baseline (post-dose).

- Number and percent of patients who had a ≥ 15% drop in FEV_1 % predicted in their pre-dose and post-dose reading at Baseline.
    - For those with this drop, the number and percent that were symptomatic:
        - For those who were symptomatic, the number and percent that were discontinued due to intolerance or were given salbutamol and re-dosed at clinic,
            - For those who were re-dosed, summary statistics of their FEV_1 % predicted drop after that second dose and the number and percent who discontinued study.

All Baseline Pre- and Post-dose (first dose and second dose, if needed) spirometry data will be presented in a listing.

10.7. FEV_1/FVC Ratio

The following statistics for FEV_1/FVC ratio (as described in Section 6.1) will be calculated and summarized by treatment group and displayed in a table:

- Number and percentage of patients in each treatment group that had a FEV_1/FVC ratio result at Baseline and at each post-Baseline time point.
- Summary statistics of FEV_1/FVC ratio at Baseline and at each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up)
- Summary statistics of FEV_1/FVC ratio reflecting the absolute change from Baseline to each post-Baseline time point (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up).

The following plot will be produced for FEV_1/FVC ratio:

- Mean FEV_1/FVC ratio Change from Baseline (±SD) vs. Visit Week (Week 4, 8, 12, 16, 20, 24, 36, 48, 60, 72 and Follow-Up) with lines for each treatment group.

10.8. Urine Pregnancy Test

At all visits except the Follow-up visit, a urinary pregnancy test will be performed for all FOCBP and the results from these tests will be presented in a listing.

10.9. 12-Lead ECG

A 12-lead ECG will be performed at the Screening visit. The following results will be collected:

- Pulse rate (bpm)
- QRS duration (msec)
- QT interval (msec)
- QTcF interval (msec)
- QTcB interval (msec)
- ECG evaluation (Normal; Abnormal, Not Clinically Significant; Abnormal, Clinically Significant).

The FAS will be used. An ECG is optional at unscheduled visits, per the investigator's discretion. All ECG data will be presented in a listing.

11. Interim Analyses

The DSMB will perform a review of safety data from the first 20 patients after Week 4 to confirm the safety of multiple dose administration. A decision to stop, continue or modify the study will be made based on this review. Throughout the course of the study, all SAEs will be sent to the DSMB for review.

At the completion of Part, A, all endpoints will be analyzed and a full set of tables, listings and figures will be generated. At the end of Part B, all analyses will be produced including data from both Parts A and B of the study.

12. Changes from Analysis Planned in Protocol

The FAS will be added and will be used for disposition and baseline analyses and as supporting sensitivity analyses for efficacy analyses. The FAS population is considered a standard analysis population used in most studies.

13. Reference List

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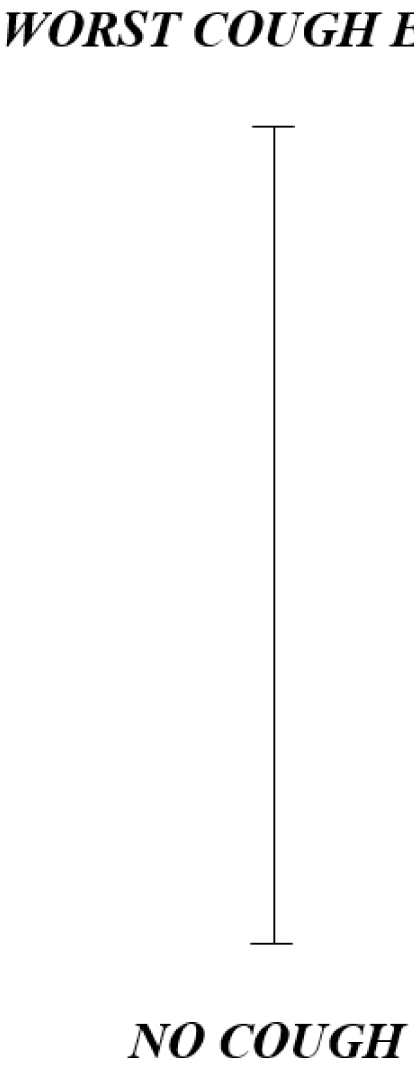
14. Appendices

14.1. Cough Visual Analogue Scale (Example)

Please put a cross on the line to indicate the severity of your cough in the last 2 weeks.



14.2. The King's Brief Interstitial Lung Disease Questionnaire (K-BILD)

The King's Brief Interstitial Lung Disease Questionnaire (K-BILD)©2011

This questionnaire is designed to assess the impact of your lung disease on various aspects of your life. Please circle the response that best applies to you for each question

1. In the last 2 weeks, I have been breathless climbing stairs or walking up an incline or hill.						
1. Every time	2. Most times	3. Several Times	4. Some times	5. Occasionally	6. Rarely	7. Never
2. In the last 2 weeks, because of my lung condition, my chest has felt tight.						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
3. In the last 2 weeks have you worried about the seriousness of your lung complaint?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
4. In the last 2 weeks have you avoided doing things that make you breathless?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
5. In the last 2 weeks have you felt in control of your lung condition?						
1. None of the time	2. Hardly any of the time	3. A little of the time	4. Some of the time	5. A good bit of the time	6. Most of the time	7. All of the time
6. In the last 2 weeks, has your lung complaint made you feel fed up or down in the dumps?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
7. In the last 2 weeks, I have felt the urge to breathe, also known as 'air hunger'.						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
8. In the last 2 weeks, my lung condition has made me feel anxious.						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
9. In the last 2 weeks, how often have you experienced 'wheeze' or whistling sounds from your chest?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
10. In the last 2 weeks, how much of the time have you felt your lung disease is getting worse?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
11. In the last 2 weeks has your lung condition interfered with your job or other daily tasks?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
12. In the last 2 weeks have you expected your lung complaint to get worse?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
13. In the last 2 weeks, how much has your lung condition limited you carrying things, for example, groceries?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
14. In the last 2 weeks, has your lung condition made you think more about the end of your life?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
15. Are you financially worse off because of your lung condition?						
1. A significant amount	2. A large amount	3. A considerable amount	4.A reasonable amount	5. A small amount	6. Hardly at all	7. Not at all

14.3. Leicester Cough Questionnaire

This questionnaire is designed to assess the impact of cough on various aspects of your life. Read each question carefully and answer by CIRCLING the response that best applies to you. Please answer ALL questions, as honestly as you can.

1. In the last 2 weeks, have you had chest or stomach pains as a result of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

2. In the last 2 weeks, have you been bothered by sputum (phlegm) production when you cough?

1	2	3	4	5	6	7
Every time	Most times	Several times	Some times	Occasionally	Rarely	Never

3. In the last 2 weeks, have you been tired because of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

4. In the last 2 weeks, have you felt in control of your cough?

1	2	3	4	5	6	7
None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

5. How often during the last 2 weeks have you felt embarrassed by your coughing?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

6. In the last 2 weeks, my cough has made me feel anxious

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

7. In the last 2 weeks, my cough has interfered with my job, or other daily tasks

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

8. In the last 2 weeks, I felt that my cough interfered with the overall enjoyment of my life

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

9. In the last 2 weeks, exposure to paints or fumes has made me cough

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

10. In the last 2 weeks, has your cough disturbed your sleep?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

11. In the last 2 weeks, how many times a day have you had coughing bouts?

1	2	3	4	5	6	7
All of the time (continuously)	Most times during the day	Several times during the day	Some times during the day	Occasionally through the day	Rarely	None

12. In the last 2 weeks, my cough has made me feel frustrated

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

13. In the last 2 weeks, my cough has made me feel fed up

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

14. In the last 2 weeks, have you suffered from a hoarse voice as a result of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

15. In the last 2 weeks, have you had a lot of energy?

1	2	3	4	5	6	7
None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

16. In the last 2 weeks, have you worried that your cough may indicate serious illness?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

17. In the last 2 weeks, have you been concerned that other people think something is wrong with you, because of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

18. In the last 2 weeks, my cough has interrupted conversation or telephone calls

1	2	3	4	5	6	7
Every time	Most times	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

19. In the last 2 weeks, I feel that my cough has annoyed my partner, family or friends

1	2	3	4	5	6	7
Every time I cough	Most times when I cough	Several times when I cough	Some times when I cough	Occasionally when I cough	Rarely	Never

Thank you for completing this questionnaire.

Supplementary Table 1: Key inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
≥40 years of age	Acute idiopathic pulmonary fibrosis (IPF) exacerbation requiring hospitalisation in the previous 3 months
Forced vital capacity (FVC) ≥40% and ≤90% predicted *	Alternative diagnoses of interstitial lung disease or history of significant environmental exposure that could cause pulmonary fibrosis
Forced expiratory volume in 1 second (FEV_1)/FVC ratio ≥0.7	Connective tissue disease, asthma, chronic obstructive pulmonary disease, or active infection
Not taking oral pirfenidone or nintedanib due to national formulary restrictions, or were intolerant to/unwilling to, if previously offered	High likelihood of death or requiring significant surgery within 6 months
A confident diagnosis of IPF according to American Thoracic Society/European Respiratory Society criteria ** within the previous 5 years	End-stage renal disease requiring dialysis; end-stage liver disease; alanine aminotransferase or aspartate aminotransferase >5 times the upper limit of normal
If IPF diagnosis ≥1 year Diagnosis was based on high-resolution computed tomography (HRCT) and/or surgical lung biopsy findings consistent with usual interstitial pneumonia (UIP). If honeycombing was not present on the HRCT, then evidence of disease progression by HRCT and/or an absolute loss of FVC % predicted ≥5% over the previous 12 months was required. IPF diagnosis <1 year Typical or probable UIP pattern by HRCT within the previous 12 months was required. If surgical lung biopsy was available, a definite, probable, or possible UIP pattern was required.	Participated in a clinical study within the previous 30 days or 5 half-lives of the previous investigational product
Clinical symptoms consistent with IPF ≥1 year duration	Pregnant or breastfeeding
A diffusion capacity to carbon monoxide ≥30% and ≤90% predicted	
Fibrotic changes on HRCT scan greater than the extent of emphysema	
No evidence of improvement in IPF disease severity over the preceding year	
<10% relative change in FVC between screening and first dose	

* Quanjer PH, Stanojevic S, Cole TJ, *et al*. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. ERS Global Lung Function Initiative. *Eur Respir J* 2012;40:1324–1343.
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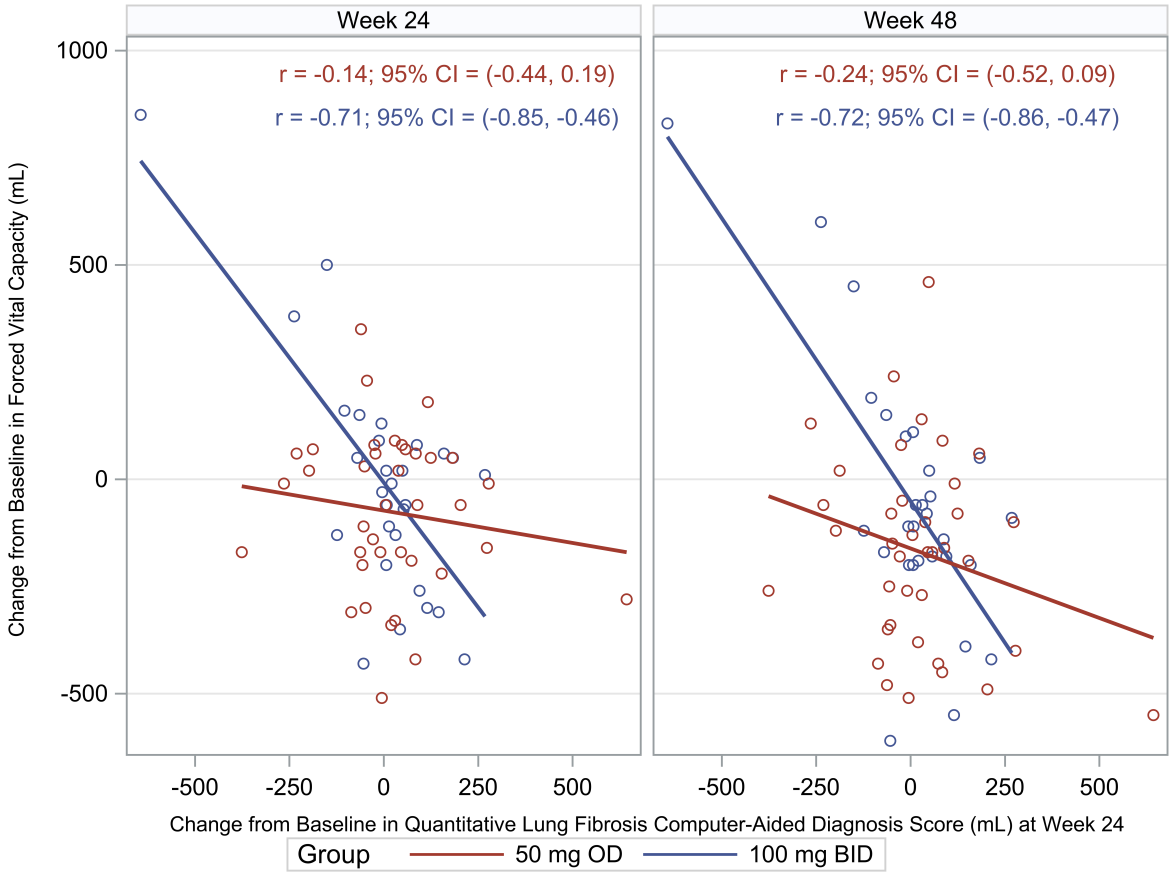

Supplementary Figure 1: **Correlation of change from baseline in quantitative lung fibrosis computer-aided diagnosis score in whole lung at week 24 with change from baseline in forced vital capacity at weeks 24 and 48**
BID=twice daily. CI=confidence interval. OD=once daily. r=Pearson correlation coefficient.

Supplementary Findings

At baseline, 48% of patients (46% assigned to 50 mg QD and 50% assigned to 100 mg BID) had cough frequency at baseline ≥10/hour while awake. This subgroup's median (IQR) cough frequency at baseline was greater for 50 mg QD [25.6 (20.8–36.1)] than for 100 mg BID [18.5 (14.5−33.7)]. There was a reduction in objective cough rates for these patients at week 24 (−31.7% versus −3.8%) for 50 mg QD and 100 mg BID, respectively. Sixteen patients (nine patients: 50 mg QD, seven patients: 100 mg BID) experienced a >30% decrease in objective cough at week 24. Eleven patients (five patients: 50 mg QD, six patients: 100 mg BID) experienced a >30% increase in objective cough at week 12 or week 24. Five (one patient: 50 mg QD, four patients: 100 mg BID) of the 11 patients had an accompanying respiratory AE or infection, including thrush, respiratory tract infection, dyspnoea, and worsening of IPF, and one patient had a cough AE.

Supplementary Table 2: Patient-reported outcome (PRO) results [mean (standard deviation)]

	50 mg once daily			100 mg twice daily		
		Change from Baseline			Change from Baseline	
	Baseline	**24 weeks**	**48 weeks**	**Baseline**	**24 weeks**	**48 weeks**
Patients, n	46	40	36	42	35	29
Leicester Cough Questionnaire						
Total	15.74 (4.11)	0.22 (3.61)	−1.28 (3.51)	16.67 (3.44)	−0.44 (3.11)	−0.53 (2.84)
Physical	5.07 (1.20)	0.15 (1.06)	−0.28 (1.08)	5.32 (1.03)	0.02 (0.94)	−0.05 (0.91)
Psychological	5.23 (1.54)	0.06 (1.34)	−0.46 (1.22)	5.63 (1.31)	−0.22 (1.27)	−0.20 (0.97)
Social	5.43 (1.50)	0.01 (1.36)	−0.54 (1.38)	5.72 (1.26)	−0.24 (1.11)	−0.29 (1.15)
King's Brief ILD Questionnaire						
Total	53.5 (14.0)	−0.2 (8.1)	−0.3 (11.4)	57.2 (10.9)	3.1 (13.2)	2.1 (15.5)
Breathlessness and activities	40.0 (18.1)	−2.4 (13.3)	−2.3 (15.9)	45.0 (17.7)	6.3 (16.7)	3.8 (20.1)
Chest symptoms	64.8 (23.6)	1.2 (17.6)	−1.0 (18.4)	70.1 (19.5)	0.7 (16.5)	−1.0 (19.1)
Psychological	53.7 (20.0)	2.2 (13.3)	−0.5 (15.1)	58.1 (15.3)	3.4 (19.5)	3.2 (22.5)

ILD: interstitial lung disease.

Avalyn Pharma, Inc. Protocol v2.1
AP01-002 12 OCT 2020

CLINICAL STUDY PROTOCOL

Title: **A** Randomized Open-Label, Phase 1b S**t**udy of the Safety of Pirfenidone So**l**ution for Inhalation (AP01) in P**a**tients with Idiopathic Pulmonary Fibrosi**s** (ATLAS Study)

Protocol Number:	AP01-002
Version:	Version 2.1 (12 OCT 2020)
Investigational Drug:	Pirfenidone Solution for Inhalation (AP01)

INVESTIGATOR'S SIGNATURE

By signing this Protocol, the Investigator(s) acknowledges and agrees:

The Investigator will conduct this study as detailed herein, in compliance with current Good Clinical Practice (GCP) and the applicable regulatory requirements and will make every reasonable effort to complete the study within the time designated.

The Protocol and all relevant information on the Investigational Product relating to nonclinical and prior clinical experience, which was furnished by Avalyn Pharma, will be made available to all physicians, nurses and other personnel who participate in the conduct of this study. The Investigator will discuss this material with them to assure that they are fully informed regarding the investigational product(s) and the conduct of the study.

This document contains information that is privileged or confidential. As such, it may not be disclosed unless specific prior permission is granted in writing by Avalyn Pharma or such disclosure is required by federal or other laws or regulations. Persons to whom any of this information is to be disclosed must first be informed that the information is confidential. These restrictions on disclosure will apply equally to all future information supplied, which is indicated as privileged or confidential.

Avalyn Pharma will have access to any source documents from which Case Report Form (CRF) information may have been generated. The CRFs and other data pertinent to this study are the sole property of Avalyn Pharma, which may utilize the data in various ways, such as for submission to government regulatory authorities, or in publication of the results of the study.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

Except where the Principal Investigator's signature is specifically required, it is understood that the term 'Investigator' as used in this Protocol and on the CRFs refers to the Principal Investigator or an appropriately qualified member of the staff that the Principal Investigator designates to perform specified duties of the Protocol. The Principal Investigator is ultimately responsible for the conduct of all aspects of the study.

Avalyn Pharma, Inc.
AP01-002

Protocol v2.1
12 OCT 2020

SPONSOR'S SIGNATURE

Approved by:

Bruce Montgomery, MD (Print Name)

Chief Executive Officer

Signature

12 Oct 2020

Date

West A, *et al. Thorax* 2023;0:1–8. doi: 10.1136/thorax-2022-219391

SYNOPSIS

Protocol Title

A Randomized Open-Label, Phase 1b Study of the Safety of Pirfenidone Solution for Inhalation (AP01) in Patients with Idiopathic Pulmonary Fibrosis (ATLAS Study)

Protocol Number

AP01-002

Clinical Phase

Phase 1b

Product Sponsor

Avalyn Pharma, Inc.

Indication

Idiopathic Pulmonary Fibrosis (IPF)

Investigational Drug

Pirfenidone Solution for Inhalation (AP01)

Control

None (Open-label)

Objectives

To evaluate the safety and tolerability of AP01 given once or twice daily to patients with IPF.

To estimate the treatment effect of AP01 given 50 milligrams (mg) once daily and 100 mg twice daily on the absolute change in percent of predicted forced vital capacity (% FVC) in patients with IPF.

Safety Outcome Measures

- Treatment-emergent adverse events (AEs)
- Change from pre-dose to post-dose FEV_1 after initial dose
- Treatment-emergent deaths
- Treatment-emergent changes in clinical laboratory findings
- Changes in vital signs

Efficacy Outcome Measures

- Change from Baseline in % FVC
- Change from Baseline in diffusing capacity of the lungs for carbon monoxide (D_{LCO})
- Change from Baseline in Patient Reported Outcomes (PROs) scores
- Change from Baseline in cough frequency and intensity
- Change from Baseline in the extent of fibrosis and lung volumes as measured by High Resolution Computed Tomography (HRCT)

Exploratory Endpoints

- Correlation between weekly home spirometry measurements and in-clinic spirometry values
- Correlation of extent of fibrosis and lung volumes as measured by HRCT at Baseline and Week 24 to other measures of clinical status, e.g. spirometry
- Change from Baseline in serum biomarkers of alveolar epithelial injury (CA19-9 and CA-125)

Investigational Products

Investigational Drug Dosage

50 mg AP01, 100 mg AP01

Number of Patients

Approximately 100 patients will be treated; 50 each with 50 mg once daily or 100 mg twice daily

Study Duration

This study has two parts. In Part A, each patient will be screened and those enrolled will then receive 24 weeks treatment with AP01. Patients that complete Part A and in the opinion of the investigator are compliant with study procedures will be allowed to continue to Part B of the study for 48 weeks additional treatment.

If patients discontinue study treatment prior to Week 72 for any reason, they should return to the site for an Early Termination visit. All patients who complete the regular study visits through Part B will return for a Follow-up visit 28 days after their End of Study visit (EOS/Visit 12) unless the patient has consented to enroll in the Rollover study, AP01-005.

The end of study for patients that plan to participate in the Rollover study (AP01-005) will be their last treatment visit (EOS/Visit 12) or an safety monitoring visit after Visit 12. The end of the study for patients not enrolling in AP01-005 will be the day of their last Follow-up or Early Termination visit.

Study Population

- Male and female patients at least 40 years of age

- 40% ≤ FVC ≤ 90% predicted; The first 20 patients randomized must have FVC ≥ 50% predicted. After the first 20 patients have been randomized, patients with FVC 40% < 50% predicted will be allowed to be randomized in the study but randomization for these patients will be capped at 20.
- FEV_1/FVC ratio ≥ 70%
- Not eligible for oral pirfenidone and nintedanib due to national formulary restrictions OR intolerant to or unwilling to start oral pirfenidone and nintedanib, if previously offered (Nintedanib use is allowed in Part B of the study)
- Confident diagnosis of IPF based on clinical, radiologic and pathologic data without evidence or suspicion of an alternative diagnosis that may contribute to their interstitial lung disease
- 30 ≤ % D_{LCO} ≤ 90%

Study Overview/Design

This is a randomized, open-label study of Pirfenidone Solution for Inhalation (AP01) 50 mg once daily or 100 mg twice daily. This study has 2 parts.

Part A (24 weeks): Patients will be randomized in a 1:1 ratio to one of two treatment arms: 50 mg once daily or 100 mg twice daily. On Day 1, the initial dose of the drug will be administered in the clinic to confirm airway tolerance. If in the investigator's opinion, the patient had tolerability issues during the first dose administration, a second dose, at least 4 hours later, will also be observed. The remainder of the doses will be administered by the patient outside of the clinic. Patients will have a telephone assessment at Week 1 and an in-clinic assessment at Weeks 4, 8, 12, 16, 20, and 24. Patients who do not continue to Part B or who are withdrawn from the study prior to completion should return for an Early Termination visit. Week 4 safety data from the first 20 patients will be reviewed by a Data and Safety Monitoring Board (DSMB), who may suggest changes to design or stopping of the study based on safety concerns.

Part B (48 weeks): Patients who, in the opinion of the investigator, are compliant with study treatment dosing and study procedures will be permitted to enter Part B. All patients continuing to Part B prior to the implementation of this amendment will continue to receive the treatment regimen (50 mg once daily or 100 mg twice daily) to which they were randomized in Part A. If one dosing regimen is determined to be superior either from an efficacy or safety standpoint, Part B may be converted to a single dose regimen.

The DSMB for this study met on 12 OCT 2020 to review preliminary dose comparison data. It was determined that the 100 mg twice daily dose showed a trend for amelioration of FVC loss and recommended all patients in Part B be treated with the 100 mg twice daily dose. Therefore, all patients who participate in Part B after the implementation of this amendment will be treated with the 100 mg twice daily dose. Any patients already participating in Part B on the 50 mg once daily dose will be converted to the 100 mg twice daily dose regimen. Patients will have monthly telephone assessments and quarterly in-clinic assessments. All patients that complete the study visits through Part B will have the option to continue receiving AP01 by enrolling or planning to enroll in the AP01-005 study or to stop dosing after the EOS/Visit 12 and return for a Follow-up visit, 28 days after their End of Study visit.

Statistics

Power and Sample Size

This is an open-label study; no formal hypothesis testing is planned. 50 patients per arm will be adequate to assess the safety and tolerability of AP01 given once or twice daily, as well as provide estimation of changes in response endpoints over time.

Primary Analysis

Safety and tolerability will be assessed by treatment emergent AE rates, post-first dose spirometry, deaths, clinically significant laboratory findings and vital signs.

Secondary Analyses

Change from baseline in FVC % predicted, $D_{LCO,}$ PRO domain scores, cough frequency, extent of fibrosis and lung volumes over 24 and 72 weeks will be analyzed by treatment arm.

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LIST OF ABBREVIATIONS

The following abbreviations are used in this study protocol.

6MWT	6-Minute Walk Test
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine Aminotransferase
ADL	Activities of Daily Living
AP01	Pirfenidone Solution for Inhalation
AST	Aspartate Aminotransferase
ATS	American Thoracic Society
AUC	Area Under the Concentration-Time Curve
$AUC_{(0-x)}$	Area Under the Concentration-Time Curve from Time 0 to *x* Hours Post Dose
BAL	Bronchoalveolar Lavage
BFS	Blow-Fill-Seal
C_{max}	Maximum Concentration
CE	Conformité Européene
CFR	Code of Federal Regulations
CI	Confidence Interval
CRF	Case Report Form
CRO	Contract Research Organization
CV	Cardiovascular
CYP	Cytochome P450 Family
CYP1A2	Cytochome P450 Family Subfamily A Member 2
DSMB	Data and Safety Monitoring Board
D_{LCO}	Diffusion Capacity for Carbon Monoxide
EC	Ethics Committee
EC_{50}	Half Maximal Effective Concentration
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eFlow	eFlow® Nebulizer System
ELF	Epithelial Lining Fluid
EOS	End of Study
ERS	European Respiratory Society
FEV_1	Forced Expiratory Volume in One Second
FOCBP	Female of Child-Bearing Potential
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GSD	Geometric Standard Deviation
HRCT	High-Resolution Computed Tomography
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IP	Investigational Product
IPF	Idiopathic Pulmonary Fibrosis
IRB	Institutional Review Board
KBILD	The King's Brief Interstitial Lung Disease
Kg	Kilogram
L	Liters

LCM	Leicester Cough Monitor
LCQ	Leicester Cough Questionnaire
LDPE	Low Density Polyethylene
MedDRA	Medical Dictionary for Regulatory Activities
µg	Micrograms
µg/mL	Micrograms per Milliliter
µg·h/mL	Micrograms per Hour per Milliliter
µm	Micrometer
mg	Milligram
mg/d	Milligrams per Day
mg/mL	Milligrams per Milliliter
mL	Milliliter
mM	Millimolar
MMAD	Mass Median Aerodynamic Diameter
NaCl	Sodium Chloride
NCI-CTCAE	National Cancer Institute's Common Terminology Criteria for Adverse Events
NOAEL	No Observed Adverse Effect Level
PE	Physical Exam
PP	Per-Protocol
PRO	Patient Reported Outcome
q.s.	quantum sufficit ("as much as is sufficient")
QSR	Quality Systems Regulations
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SaO_2	Saturated Oxygen
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Suspected Adverse Reaction
$T_{(0)}$	Time Zero
$T_{1/2}$	Half-life (lives)
UIP	Usual Interstitial Pneumonia
VAS	Visual Analogue Scale
VC	Vital Capacity
VS	Versus

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1. INTRODUCTION

1.1 Idiopathic Pulmonary Fibrosis (IPF) and Pirfenidone

Idiopathic pulmonary fibrosis is a fatal lung disease caused by both genetic and environmental factors resulting in progressive lung scarring and death due to respiratory failure and/or co-morbidities. Characterized by a dry cough, shortness of breath and decreased exercise capacity, this disease exhibits a post-diagnosis survival period of approximately 2-5 years and affects up to 200,000 Americans and 135,000 Europeans [1]. The true prevalence of IPF in Australia is unknown, however there are nearly 700 patients in the Australian IPF Registry[2].

Oral pirfenidone is marketed in the United States, Europe, Australia and Canada (Roche/Genentech Esbriet®) and Japan (Shionogi Pirespa®). Although Esbriet® has proven to slow IPF disease progression, it is a low potency drug that requires a very large oral dose to achieve efficacious lung levels. While the Esbriet® dose has been established near the upper safety threshold (3 X 267 milligram (mg) capsules/dose or 801 mg three times daily; 2403 mg/day (mg/d)), the distribution of drug is uniform resulting in lung levels that are below that required for optimal effect and systemic levels that are often poorly tolerated. Moreover, because oral-delivered blood levels exist at the upper-safety threshold, oral-dose escalation for additional efficacy is not practical. Complicating matters, dose-absorbing food, first-pass metabolism, and safety-driven dose-reduction and stoppage protocols further reduce pirfenidone lung dose and interrupt required maintenance therapy.[3-5]

Aerosol administration of multiple classes of drugs, including bronchodilators, corticosteroids, and antibiotics have been proven to improve both efficacy and safety by increasing delivery to lung tissue and decreasing systemic exposure[6]. Oral pirfenidone has been shown to provide amelioration of forced vital capacity (FVC) decline, as well as a positive impact on mortality in IPF patients, the latter by meta-analysis of Phase 3 studies.[3][7] However, systemic side effects and likely under-treatment of the alveoli suggest that an aerosolized formulation of pirfenidone has the potential to provide improved tolerability and efficacy profiles.

1.2 Summary of Nonclinical and Clinical Data

1.2.1 Nonclinical Summary

The systemic toxicities and target organs associated with the approved pirfenidone dosage forms have been well established. Nonclinical data revealed no special hazard for patients with IPF based on conventional studies of safety pharmacology, repeated dose toxicity, genotoxicity and carcinogenic potential. These studies are detailed in the Investigator's Brochure (IB).

The nonclinical studies conducted for the eFlow® Nebulizer System (eFlow) evaluated and identified potential localized pulmonary and systemic toxicities associated with the inhalation delivery.

To date, five Good Laboratory Practices (GLP) repeat dose inhalation toxicology studies have been conducted in rats and dogs using pirfenidone solution for inhalation. These inhalation studies included toxicokinetic determinations at the beginning and end of each study. In addition, a GLP cardiovascular safety pharmacology study was conducted in telemetered Beagle dogs and a respiratory safety pharmacology study was performed in association with the 28-day repeat dose inhalation toxicology study in rats. The studies completed to date are listed in Table 1. A 6-month repeat dose inhalation toxicology study in rats with a 3-month interim sacrifice showed no drug-related histological findings at any dose level at either the 3-month interim sacrifice or the 6-month terminal sacrifice.

Table 1: Completed GLP Nonclinical Inhalation Studies in support of Pirfenidone Solution for Inhalation (AP01)

Study Number	Study Title
CRL 7300392 GP-TX-001	Pirfenidone: A Maximum Tolerated Dose and 7-Day Repeat Dose Toxicity Study with Nebulized Aerosol Formulation in Rats
CRL 7300394 GP-TX-004	A 28 Day Study of Pirfenidone by Inhalation with a Nebulized Aerosol Formulation in Rats with a 28 Day Recovery Period
CRL 7300393 GP-TX-002	Pirfenidone: A Maximum Tolerated Dose and 7-Day Repeat Dose Toxicity Study with Nebulized Aerosol Formulation in Dogs
CRL 7300395 GP-TX-003	A 28 Day Study of Pirfenidone by Inhalation with a Nebulized Aerosol Formulation in Dogs with a 28 Day Recovery Period
CRL 6900865 GP-SP-001	A Pharmacological Assessment of the Effects of Pirfenidone on the Cardiovascular System of the Beagle Dog Using Telemetry
CRL 7300652 GP-TX-005	A 26 Week (with 13-Week Interim) Study of Pirfenidone by Inhalation with a Nebulized Aerosol Formulation in Rats with a 28 Day Recovery Period

The maximum tolerated dose level for each species tested was determined to be the maximum deliverable dose level using a clinically relevant formulation and the maximum duration of exposure allowed by the Contract Research Organization (CRO). Each study had 5 dose groups, including vehicle and air only control treatment groups and 3 pirfenidone treatment groups. In the 7-day rat study, the average achieved pulmonary dose for combined sexes over the 7-day treatment period were 2.78, 6.85 and 10.7 mg/kilogram (kg)/day. No pirfenidone treatment-related adverse effects were observed for any measured parameter. The No Observed Adverse Effect Level (NOAEL) was considered 10.7 mg/kg/day.

Administration of pirfenidone by inhalation for 28 days was well tolerated in rats. The average achieved pulmonary dose for combined sexes over the 28-day exposure period was 1.91, 3.94 and 6.05 mg/kg/day for the low, mid and high dose groups respectively. The determined mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) for pirfenidone ranged from 4.2 micrometers (μm) ± 1.6 to 4.4 μm ± 1.6. All animals survived until scheduled termination. There were no clinical signs or effects on body weights observed. Ocular and respiratory functions as well as blood parameters were unaffected by treatment. Minimal to mild, reversible, centrilobular hepatocellular hypertrophy was observed in males and females administered ≥ 3.94 mg/kg/day with associated increased liver weights in males at all dose levels and females at ≥ 3.94 mg/kg/day. No adverse effects were observed in a subset of rats from the high dose group allowed a 28-day treatment free recovery period. Based on the low severity and reversibility of the centrilobular hypertrophy observed in the liver, 6.05 mg/kg/day was considered the NOAEL, correlating to an average maximum concentration (C_{max}) values in males of 5.56 micrograms per milliliter (μg/mL) and in females of 6.89 μg/mL, and an average Area Under the Concentration-Time Curve (AUC) values in males of 21.0 micrograms per hour per milliliter (μg·h/mL) and in females of 26.5 μg·h/mL.

Results from the 7-Day dog study indicate that at the high pulmonary dose of 6.73 mg/kg/day in male and female Beagle dogs there were no adverse effects observed in clinical observations, food consumption, clinical pathology, necropsy, organ weights or histopathology that were considered related to treatment with pirfenidone.

Administration of pirfenidone by inhalation for 28 days was well tolerated in dogs. The average achieved pulmonary dose for combined sexes over the 28-day exposure period was 2.40, 4.90 and 10.2 mg/kg/day for the low, mid and high dose groups respectively. The determined MMAD ± GSD for pirfenidone ranged from 1.8 micrometers (μm) ± 1.6 to 1.9 μm ± 1.6. All animals survived until scheduled termination. There were no pirfenidone related changes in clinical signs, body weight, food consumption, ophthalmology, electrocardiogram (ECG), clinical pathology, necropsy, organ weights or histopathology following the 28-day treatment period. The NOAEL was considered 10.2 mg/kg/day. The average C_{max} and AUC values at day 28 were 10.3 (male) and 7.3 μg/mL (females) and 27.7 (males) and 19.3 μg·h/mL (females), respectively.

A Cardiovascular Safety Pharmacology Study was performed in telemetered Beagle dogs. Air and vehicle control and three pirfenidone dose levels were administered in an ascending manner to a group of 4 telemetered dogs with a minimum washout period of 4 days between each treatment. The highest achieved pulmonary dose given was 7.07 mg/kg. Measured cardiovascular parameters included mean arterial, systolic and diastolic blood pressure, pulse pressure, heart rate, body temperature, electrocardiographic waveform analysis including PR, QRS, QT and QT_c duration and intervals. No treatment-related abnormalities were observed in any measured parameter.

A Respiratory Safety Pharmacology study was conducted in association with the 28-day repeat inhalation dose study in rats. Baseline respiratory function measurements were conducted prior to treatment initiation, then in 5 animals/sex/group on Day 3 or 4 of treatment. Using plethysmography, respiratory parameters were measured including tidal volume, respiratory rate and derived minute volume. No treatment-related adverse effects were observed in any of the measured or derived parameters at the high dose level.

The average C_{max} and AUC values for the 28-day inhalation studies and for the anticipated clinical dose for the Phase 1b study (AP01-002) are listed in Table 2. Results from the nonclinical studies indicate that a margin of safety should range from 3.2 to 6.8 depending upon the comparison of exposure made. For comparison purposes, the C_{max} and AUC values for the approve oral dose is provided. The approved oral dose is 801 mg administered three times daily. Comparison between human routes of exposure indicates that the systemic exposure for inhalation is 4.6 (C_{max}) to 14.8 (AUC) fold lower than the oral dose.

Table 2: Safety Margins based on System Exposure (C_{max}, AUC) Comparisons to the Phase 1 Clinical Study (AP01-001)

Species	Achieved Pulmonary Dose (mg/kg/day)	C_{max} (µg/mL)	AUC (µg·h/mL)
Rat	6.05	5.56 to 6.89	21.0 to 26.5
Beagle dog	10.2	7.3 to 10.3	19.3 to 27.2
Human	100 mg*	1.7**	4.0
Human oral dose	801 mg	7.9	59.3
Margin of Safety	Not applicable	3.2 to 6.1	4.8 to 6.8

*The human dose is the total dose delivered (100 mg) but does not represent the expected pulmonary dose. The anticipated pulmonary exposure in humans is 40% of the total dose or 40 mg.**Data based on the average C_{max} of 6 patients from the Phase 1 study (AP01-001) back extrapolated to time zero ($T_{(0)}$) or immediate post dosing.

Based on the liver weight and liver histology findings in the rat, a 6-month repeat dose inhalation study with toxicokinetics in rats was performed. The treatment groups included an air only and vehicle control groups and 3 pirfenidone treatment groups at target pulmonary dose levels of 1, 2 and 4 mg/kg/day. The purpose of this study was to evaluate and chronic effects upon the respiratory system, as well as potential systemic effects following inhalation of pirfenidone solution. This study fulfilled the requirements of a single species chronic study, as indicated in the Food and Drug Administration guidance, "Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route", Guidance for Industry and Review Staff, (October 2015) Pharmacology/Toxicology. This study included a 90-day interim sacrifice. Plasma samples were taken on Day 1, at the 90-day time interval and at termination of the 6-month treatment period for toxicokinetics. In addition, a 28-day treatment-free recovery period for a subset of rats was included at the 90-day interval, as well as the end of the in-life phase of the 6-month study to evaluate any potential reversibility of adverse effects. The average achieved pulmonary dose levels for combined sexes determined over the 26-week treatment period was 1.09, 2.01 and 3.70 mg/kg/day for the low, mid and high dose levels of pirfenidone. No pirfenidone related adverse effects were observed in clinical observations, body weights, organ weights or ratios, clinical pathology investigations or histopathology at any dose level tested. Adverse effects observed included squamous metaplasia of the larynx in all treatment groups including controls that was reversible upon a 28 day treatment-free recovery period. These findings are commonly found in rat studies due in part to the oropharyngeal-laryngeal anatomy of this species. It is considered an adaptive response to a local irritant and has no human correlate[8],[9]. The NOAEL was considered the high dose level of 3.70 mg/kg/day corresponding to a sex averaged C_{max} of 2.975 µg/mL and an $AUC_{(0-t)}$ of 15.9 µg·h/mL as determined from plasma samples taken on Day 182.

In conclusion, the inhalation studies conducted to date demonstrate minimal, reversible adverse effects in the rat only and generally provide a better safety profile than similar studies conducted in support of the oral indication. The high dose levels in the inhalation studies were close to the maximum deliverable dose allowed by the CRO for reasons of animal welfare. While the systemic exposure of pirfenidone in rats and dogs provided a somewhat narrow safety margin compared to human inhalation exposure, the overall exposure for patients using this route of administration is significantly less that the oral indication.

1.2.2 Clinical Summary

Oral Pirfenidone

As of 2017, more than 3500 IPF patients have received oral pirfenidone in clinical studies and named patient programs at doses ranging from 801 mg/d to 4805 mg/d. The following clinical studies defined the safety profile, limits for gastrointestinal tolerability and dose-responsive human efficacy.[3, 5, 10-12]

- InterMune-sponsored PIPF-005 Phase 1 study (single- and multi-dose 801 to 4005 mg/d) – Demonstrated both pirfenidone and its primary metabolite, 5-carboxy-pirfenidone (inactive), have rapid elimination half-lives ($T_{1/2}$) of ~2.4 and ~2.0 hours, respectively. Single-dose plasma pharmacokinetic characteristics following an 801 mg dose in fed normal patients were C_{max} = 7.9 µg/mL and $AUC_{Time\ 0\text{-}infinity\ (0\text{-}\infty)}$ = 59.3 µg·h/mL. Significant multi-dose plasma accumulation was not observed. Initial dose-escalation over two weeks and food were necessary to reach a tolerated maintenance dose (up to 801 mg three times a day).

- InterMune-sponsored PIPF-010 Phase 1 study (drug-drug interaction study) – Demonstrated that pirfenidone clearance is reduced with co-administration of fluvoxamine, which inhibits Cytochrome P450 Family 1 Subfamily A Member 2 (CYP1A2) and several other Cytochrome P450 (CYP) isoforms. Further, pirfenidone clearance was significantly higher in cigarette smokers than non-smokers, presumably due to higher CYP1A2 enzyme activity in smokers. The human lung contains very low levels of CYP1A2.

- Shionogi-sponsored SP3 Phase 3 study – Data at 52 weeks demonstrated a significant benefit of oral pirfenidone compared with placebo where a reduced change in vital capacity (VC) from baseline was observed (-90 milliliters (mL) versus (vs.) -160 mL; a 44% relative difference; p = 0.042). As a secondary outcome, this study showed pirfenidone reduced disease progression by 55% relative to placebo (hazard ratio 0.45; 95% Confidence Interval (CI) 0.11-0.79; p = 0.028).

- InterMune-sponsored PIPF-004 (CAPACITY 2) Phase 3 study (2403 and 1197 mg/d vs. placebo) – Data at 72 weeks demonstrated a significant benefit of oral pirfenidone compared with placebo where a marked reduction in decline of percent predicted FVC from baseline was observed (2403 mg/d: mean decline from baseline of -8.0% vs. -12.4% for placebo, a 35% relative difference; p = 0.001). This study also demonstrated a dose response with 1197 mg/d showing a mean decline from baseline of -10% vs. -12.4% for placebo, a 19.4% relative difference (p-value not reported).

- InterMune-sponsored PIPF-006 (CAPACITY 1) Phase 3 study (2403 mg/d vs. placebo) – Data at 48 weeks demonstrated a significant benefit of oral pirfenidone compared with placebo where a marked reduction in decline of percent predicted FVC from baseline was observed (2403 mg/d: mean decline from baseline of -5.0% vs. -6.9% for placebo, a 27% relative difference; p = 0.005). However, at 72 weeks this effect was insignificant (p = 0.501). At 72 weeks, this study did not show that pirfenidone significantly reduced disease progression (hazard ratio 0.84; 95% CI 0.58-0.1.22; p = 0.355). Pirfenidone did show a significant reduced mean decline from baseline in 6MWT compared to placebo (-45.1 vs. -76.9 m; relative difference 41%; p < 0.001).

- Pooled analysis of InterMune-sponsored CAPACITY 2 and CAPACITY 1 (2403 mg/d vs. placebo) – At 72 weeks, categorical change in FVC ≥ 10%, progression-free survival and mean change in 6MWT were significant (p = 0.003, 0.025 and 0.0009, respectively).

- Meta-analysis of InterMune CAPACITY 2, CAPACITY 1, and Shionogi SP3 (high dose only) – Demonstrated a reduced disease progression with a hazard ratio of 0.70; 95% CI 0.56-0.88; p = 0.002 and clearly excluded no-effect from serial lung capacity treatment measurements.

- Adverse events (AEs) of InterMune CAPACITY 2 and CAPACITY 1 – The most commonly reported (≥ 10%) and important AEs during clinical study experience at a dose of 2403 mg/d compared to placebo, respectively, were nausea (32.8% vs. 13.3%), rash (28.7% vs. 8.6%), fatigue (22.3% vs. 13.3%), diarrhea (21.7% vs. 13.5%), dyspepsia (16.8% vs. 5.5%), photosensitivity reaction (12.2% vs. 1.7%) and elevated liver enzymes (>3X upper limit of normal aspartate aminotransferase/alanine aminotransferase (AST/ALT): 4.1% vs. 0.6%). Serious adverse events (SAEs) were recorded at similar frequencies between active and placebo groups. In the InterMune open-label Phase 3 extension AE and SAE observations out to 3 years treatment were similar in event and frequency.

- InterMune-sponsored ASCEND (2403 mg/d vs. placebo) – At 52 weeks, categorical change in FVC ≥ 10%, progression-free survival and categorical change in 6MWT were significant (p = 0.000001, 0.0001 and 0.036, respectively).

The recommended daily dose of Esbriet® for patients with IPF is three 267 mg capsules three times a day with food for a total of 2403 mg/d. Doses above 2403 mg/d are not recommended for any patient. Upon initiating treatment, it is recommended that the dose be titrated over a 14-day period.

During the InterMune CAPACITY studies[10], 48% of patients were dose-reduced or intermittently stopped due to AEs. Dose reductions were permitted to 50% before discontinuation. 6.2% more patients in the 2403 mg/d pirfenidone group discontinued treatment due to an AE than those on placebo (14.8% vs. 8.6%, respectively).

Inhaled Pirfenidone

Avalyn Pharma has sponsored a study (AP01-001) to investigate the safety/tolerability and pharmacokinetics of a single administration pirfenidone solution for inhalation (AP01) delivered by a high efficiency vibrating plate nebulizer in volunteers and patients with IPF. Forty-four adults in 6 cohorts were consented to receive single doses of AP01 to assess tolerability, effect on lung function, and pharmacokinetics. Cohorts 1, 2, and 3 (normal healthy volunteers) (n = 2 placebo; n = 6 active in each cohort) breathed a single ascending dose, of 25, 50, and 100 mg dose of AP01. Cohort 4 (normal healthy volunteers (n = 6 active, no placebo) were administered 100 mg of AP01 and underwent bronchoalveolar lavage (BAL) to measure epithelial lining pirfenidone concentrations. Cohort 5 (prior or current smokers) (n = 2 placebo; n = 6 active), and Cohort 6 (IPF patients) (n = 6 active, no placebo), were administered 100 mg of AP01. All treatments were administered with an eFlow; PARI Pharma GMBH, Starnberg, Germany. Serial measures of urine and plasma pirfenidone were collected during the 24-hour post administration in all subjects. The same formulation of AP01 used in the AP01-001 study will be used in this study (AP01-002). See Table 8 for drug product composition.

Table 3: AP01-001 Adverse Events

	Cohort 1 NHV	Cohort 2 NHV	Cohort 3 NHV	Cohort 4 BAL in NHV	Cohort 5 Smokers	Cohort 6 IPF
Status	Completed	Completed	Completed	Completed	Completed	Completed
Active Placebo	6 2	6 2	6 2	6 0	6 2	6 0
Dose	25 mg 2 mL	50 mg 4 mL	100 mg 8 mL	100 mg 8 mL	100 mg 8 mL	100 mg 8 mL
Treatment Time Range	3 – 4 min	4 – 8 min	11 – 15 min	12– 15 min	9 – 14 min	11 - 15 min
Subject with AEs	4	3	4	2	3	5
<u>Possibly/Probably Related</u>						
Cough	1 mild	1 mild	3 mild	1 mild	1 mild	1 mild
↑ upper airway secretion	2 mild	0	0	0	0	0
Dizziness	0	1 mild	0	0	0	1 mild
Headache	0	1 mild	0	0	0	1 moderate
Dysphonia	0	0	0	0	0	1 mild
<u>Not/Unlikely Related</u>						
Nasal Congestion	0	0	1 mild*	0	0	0
Anxiety	1 mild	0	0	0	0	0
Headache	1 moderate	0	0	1 moderate	2 mild 1 moderate	0
Toothache	0	1 moderate*	0	0	0	0
Nausea	0	0	0	1 mild	0	0
Abdominal distension	0	0	0	0	0	1 mild
Oropharyngeal pain	0	0	0	0	0	1 mild
Fatigue	0	0	0	0	0	1 mild
Soft tissue injury	0	0	0	0	0	1 moderate

*Placebo patients

Following the 100 mg dose, mean peak concentrations extrapolated back to time zero (T_0) in the six volunteers that underwent BAL in plasma were 1.7 µg/mL, and in epithelial lining fluid (ELF) concentrations, 135.9 µg/mL. To place context on the aerosol pirfenidone pharmacokinetics, the approved dose of oral pirfenidone is 801 mg[4] administered 3 times daily. Following the 801 mg oral dose, the peak plasma mean concentration is 7.9 µg/mL and the $AUC_{0\text{-}18hr}$ is 60.9 µg·hr/mL. Following a 100 mg inhalation, the $AUC_{0\text{-}18hr}$ is 4 µg·hr/mL. Therefore, the 100 mg nebulizer dose leads to on average 1/15th the systemic exposure of the oral dose. (Data on file, Avalyn Pharma.)

The CAPACITY 004 phase 3 study of oral pirfenidone established that 400 mg three times daily compared to 801 mg three times daily had a better AE profile, but had about half the efficacy, as measured by amelioration of the rate of FVC decline[10]. This study demonstrated that AEs were

dose related, not idiosyncratic, suggesting that a much lower systemic dose achieved with the 100 mg AP01 nebulized dose may have a superior safety profile. The dose response seen in the CAPACITY 004 study suggests that higher local concentrations after aerosol administration may also result in improved efficacy.

1.2.3 Known and Potential Risks and Benefits

Pirfenidone has well established animal and human toxicity profiles after oral administration which are dose dependent, including gastrointestinal side effects, photosensitivity, and hepatic dysfunction.

Total systemic absorption anticipated after inhaled administration will be on average 15-fold less than standard 801 mg oral dose, therefore, the known systemic side effects are not expected to present in this study. Liver function tests (AST/ALT) will be monitored throughout the study as an extra precaution, however liver toxicity is not expected due to the low systemic exposure. In the case of an AST or ALT value greater than 5 times the upper limit of normal, an alert will be sent to the site and to the medical monitor informing them of the value and providing further instructions regarding eligibility and assessment for potential adverse events. An AST or ALT value of greater than 5 times the upper limit of normal is exclusionary for enrollment.

In the AP01-001 single ascending dose study, the drug was well tolerated in both normal volunteers and IPF patients. The most commonly reported event was cough, which was noted in 7/38 volunteers and 1/6 IPF patients in the Phase 1 study. Cough was mild, self-limited, and did not prevent the full administration of aerosol dose in any subject. Refer to Table 3 for the full summary of AEs observed in the single aerosol dose AP01-001 Phase 1 study.

Forced expiratory volume in one second (FEV_1) values were obtained 10 minutes post-dosing to detect whether the inhaled dose would lead to bronchospasm. One volunteer had a 17% asymptomatic reduction of FEV_1 after a 100 mg nebulizer dose, with a post dose FEV_1 of 3.9 liters (L). No other subject had a decline greater than 15%.

1.2.4 Rationale for Study Design

Oral pirfenidone has been shown to provide improvements in FVC as well as a positive impact on mortality in IPF patients. However, systemic side effects and likely under-treatment of the airways indicate an inhaled formulation has the potential to provide improved tolerability and efficacy profiles. AP01-001, demonstrated that a single dose up to 100 mg/mL was safe and well-tolerated while achieving greatly improved pirfenidone ELF concentrations and reduced systemic levels. AP01-002 is designed to demonstrate AP01 multiple dose safety and tolerability in IPF patients, as well as provide estimates of the variability and effect of AP01 on important efficacy outcomes. The results of AP01-002 will assist in the design of future studies. The preliminary 6 month results from AP01-002 allowed the DSMB to select the optimal dosing regimen of 100 mg twice daily for future AP01 studies.

2. OBJECTIVES

- To evaluate the safety and tolerability of treatment with AP01 when given either once or twice daily to patients with IPF
- To estimate the treatment effect of AP01 given 50 mg once daily and 100 mg twice daily on absolute change in percent predicted FVC in patients with IPF
- To estimate the treatment effect of AP01 given 50 mg once daily and 100 mg twice daily on absolute change in percent predicted Diffusion Capacity for Carbon Monoxide (D_{LCO}) in patients with IPF
- To compare the safety and efficacy of 50 mg once daily vs 100 mg twice daily dosing to provide guidance on dosing regimens for future studies
- To estimate the treatment effect of AP01 given 50 mg once daily and 100 mg twice daily on change in Patient Reported Outcomes (PROs) and cough in patients with IPF
- To estimate the treatment effect of AP01 given 50 mg once daily and 100 mg twice daily on change in the extent of fibrosis and lung volumes as measured by High Resolution Computed Tomography (HRCT) scans in patients with IPF
- To evaluate the following exploratory measurements:
    - Correlation between weekly home spirometry measurements and in-clinic spirometry values
    - Correlation of extent of fibrosis and lung volumes as measured by HRCT at Baseline and Week 24 to other measures of clinical status, e.g. spirometry
    - Change from Baseline in serum biomarkers of alveolar epithelial injury (CA19-9 and CA-125)

3. STUDY ENDPOINTS

3.1. Safety Outcome Measures

- Treatment-emergent AEs
- Change from pre-dose to post-dose FEV_1 after initial dose
- Treatment-emergent deaths
- Treatment-emergent changes in clinical laboratory findings
- Changes in vital signs

3.2. Efficacy Outcome Measures

- Change from Baseline in FVC % predicted
- Change from Baseline in D_{LCO}
- Change from Baseline in Patient Reported Outcomes (PRO)
- Change from Baseline in cough frequency and intensity
- Change from Baseline in extent of fibrosis and lung volumes

3.3. Exploratory Outcome Measures

- Correlation between weekly home spirometry measurements and in-clinic spirometry values
- Correlation of extent of fibrosis and lung volumes as measured by HRCT at Baseline and Week 24 to other measures of clinical status, e.g. spirometry
- Change from Baseline in serum biomarkers of alveolar epithelial injury (CA19-9 and CA-125)

4. INVESTIGATIONAL PLAN

4.1. Study Design

This Phase 1b, 2-part, open-label study of AP01 will randomize approximately 100 patients with IPF. Patients will be randomized in a 1:1 ratio to one of two treatment arms: 50 mg day once daily or 100 mg twice daily. The primary objective is to evaluate safety and tolerability of treatment with AP01 by monitoring AEs and post-dose spirometry. This study will also evaluate the effect of AP01 on various efficacy measures as detailed in Section 2.

Figure 1: Part A Study Schema

PART A – Patients are randomized at Visit 2 to either 50 mg given once daily or 100 mg AP01 given twice daily

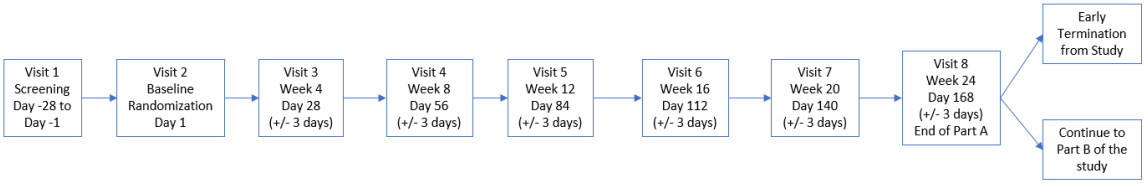
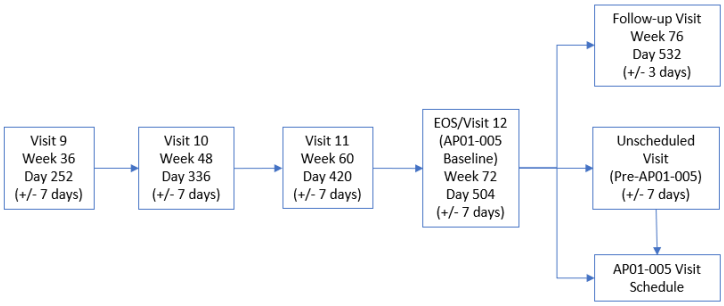


Figure 2: Part B Study Schema

PART B – Patients continue randomized treatment regimen of either 50 mg once daily or 100 mg AP01 twice daily



In Part A, eligible patients at least 40 years of age with a confident clinical and radiographic diagnosis of IPF according to pre-specified criteria, $40 \leq$ %FVC ≤ 90, FEV_1/FVC ratio $\geq 70\%$ and $30 \leq \% D_{LCO} \leq 90$ will be randomized with equal probability to receive AP01 50 mg once daily or 100 mg twice daily for 24 weeks. Other than brief periods of medically appropriate treatment for acute IPF exacerbation, patients will not receive any other therapy for the treatment of IPF during Part A. If in the opinion of the investigator, a patient is in need of treatment with oral pirfenidone or nintedanib during Part A, the patient should be discontinued from the study and return to the clinic for their Early Termination visit. Supplemental oxygen will be allowed during the entire study.

The initial dose of drug will be administered in the clinic to confirm airway tolerance. If based on the investigator's opinion, the patient had tolerability issues during the first dose administration, a second dose, at least 4 hours later, will also be observed. Patients who experience cough that limits their ability to complete dosing will be given 1 - 2 puffs (90 - 100 micrograms (µg)) of salbutamol in order to complete the in-clinic dose. These patients, as well as patients with a history of asthma or smoking history of 20 pack years or greater, or patients that have a ≥ 15% drop in FEV_1 percent predicted in their pre-dose and post-dose readings will be required to use 1 - 2 puffs (90 - 100 µg) of salbutamol prior to dosing throughout the study unless these patients are currently taking a long-acting beta-2-agonist therapy.

Patients will have a telephone assessment at Week 1 and in-clinic assessments at Weeks 4, 8, 12, 16, 20, and 24. The Data and Safety Monitoring Board (DSMB) will perform a review of safety data from the first 20 patients after Week 4 to confirm the safety and airway tolerability of multiple dose administration. A decision to stop, continue or modify the study will be made based on this review.

Part B will collect long term safety and efficacy data for an additional 48 weeks. Patients who complete Part A through Week 24 and, in the opinion of the investigator are compliant with treatment, may select to continue the treatment to which they were randomized. The DSMB for this study met on 12 OCT 2020 to review preliminary dose comparison data. It was determined that the 100 mg twice daily dose showed a trend for amelioration of FVC loss and recommended all patients in Part B be treated with the 100 mg twice daily dose. Therefore, all patients who participate in Part B after the implementation of this amendment will be treated with the 100 mg twice daily dose. Patients participating in the AP01-005 study will continue treatment that they are receiving at Visit 12 on this study and then transition to the Rollover study upon approval of AP01-005 at their site.

Study duration is up to 80 weeks for patients that complete both Part A and Part B of the study and up to 92 weeks for patients that plan to continue AP01 on the AP01-005 Rollover study. If patients discontinue study treatment prior to Week 72 for any reason, they should return to the site for an Early Termination visit. All patients who complete the regular study visits through Week 72 in Part B will return for a Follow-up visit 28 days after their End of Study visit unless the patient plans to participate in AP01-005. Patients planning to enroll in AP01-005 will continue administering AP01 using EOS/Visit 12 as their Baseline visit for AP01-005. If Regulatory approval for AP01-005 has not been achieved by the time a patient is ready to enroll in AP01-005, the patient will continue treatment that they are receiving at Visit 12 on this study and then transition to the Rollover study upon approval of AP01-005 at their site. Patients will have a visit to assess safety and compliance at least every 3 months while awaiting approval of AP01-005 at their site. The end of the study for all patients will be the day of their last Follow-up or Early Termination visit or their last treatment visit for those enrolling in AP01-005.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1. Patient Inclusion Criteria

A patient must meet all of the following inclusion criteria to be eligible to be randomized in the clinical study:

Population

1. Male and female patients, at least 40 years of age at Screening
2. Not eligible for oral pirfenidone and nintedanib due to national formulary restrictions OR intolerant to or unwilling to start oral pirfenidone and nintedanib, if previously offered

Diagnosis of IPF

3. Clinical symptoms consistent with IPF of ≥ 12 months duration (with or without IPF diagnosis)
4. Diagnosis of IPF, defined as the first instance in which a patient was informed of having IPF, no more than 60 months before randomization.
    - Patients that have had an IPF diagnosis ≥ 1 year, the following criteria must be met:
        - HRCT and/or Surgical Lung Biopsy findings consistent with UIP. If honeycombing is not present on the HRCT, then one or both of the following criteria must be present:
            - Disease progression since diagnosis by HRCT and/or
            - An absolute loss of FVC ≥ 5% percent predicted over the past 12 months
    - Patients that have had IPF diagnosis within the last year, the following criteria must be met:
        - Diagnosis of Usual Interstitial Pneumonia (UIP) or IPF by HRCT (HRCT must be performed within 12 months prior to Screening) and/or Surgical Lung Biopsy as outlined in Table 4:

Table 4: Diagnosis of UIP or IPF by HRCT and Surgical Lung Biopsy

CT pattern as determined by Fleischner Society White Paper[13]	Surgical Lung Biopsy Not Available	Pathology Panel: Definite UIP	Pathology Panel: Probable UIP	Pathology Panel: Possible UIP	Pathology Panel: Inconsistent with UIP or Not Classifiable
Typical UIP Pattern	Eligible	Eligible	Eligible	Eligible	NOT Eligible
Probable UIP Pattern	Eligible	Eligible	Eligible	Eligible	NOT Eligible
Indeterminate or most consistent with non-IPF diagnosis	NOT Eligible	NOT Eligible	NOT Eligible	NOT Eligible	NOT Eligible

5. Extent of fibrotic changes (honeycombing, reticular changes) greater than the extent of emphysema on HRCT scan
6. No features supporting an alternative diagnosis on transbronchial biopsy, BAL, or surgical lung biopsy, if performed

IPF Disease Severity and Progression

7. 40% ≤ FVC ≤ 90 % predicted at Screening based on Global Lung Initiative[14] equations. The first 20 patients randomized must have FVC ≥ 50% predicted. After the first 20 patients have randomized, patients with FVC 40% < 50% predicted will be allowed to be randomized in the study but randomization for these patients will be capped at 20
8. Change in FVC (measured in liters) between Screening and Day 1 (pre-dose measurement) must be a < 10% relative difference, calculated as:

$$\frac{\text{Screening FVC (L)} - \text{Day 1 FVC (L)}}{\text{Screening FVC (L)}} \times 100\%$$

9. $30 \leq \%\ D_{LCO} \leq 90\%$ at Screening
10. In the investigator's opinion, no evidence of improvement in measure of IPF disease severity over the preceding year
11. $FEV_1/FVC \geq 70\%$

Informed Consent and Protocol Adherence

12. Able to understand and sign a written informed consent form
13. Able to understand the importance of adherence to study treatment and the study protocol and willing to follow all study requirements, including the concomitant medication restrictions, throughout the study

5.2. Patient Exclusion Criteria

The presence of any of the following exclusion criteria excludes a patient from study enrollment:

Disease-Related Exclusions

1. Significant clinical worsening of IPF between Screening and Day 1, in the opinion of the investigator
2. Not a suitable candidate for enrollment or unlikely to comply with the requirements of this study, in the opinion of the investigator
3. History of acute IPF exacerbation requiring hospitalization in the last 3 months
4. History of clinically significant environmental exposure known to cause pulmonary fibrosis, including but not limited to drugs (such as amiodarone), asbestos, beryllium, radiation, and domestic birds
5. Known explanation for interstitial lung disease, including but not limited to radiation, drug toxicity, sarcoidosis, hypersensitivity pneumonitis, bronchiolitis obliterans organizing pneumonia, human immunodeficiency virus, viral hepatitis, and cancer
6. Clinical diagnosis of any connective tissue disease, including but not limited to scleroderma, polymyositis/dermatomyositis, systemic lupus erythematosus, and rheumatoid arthritis
7. Current diagnosis of asthma or chronic obstructive pulmonary disease
8. Clinical evidence of active infection, including but not limited to bronchitis, pneumonia, sinusitis, urinary tract infection, or cellulitis

Medical Exclusions

9. Females with a positive pregnancy test at Screening or are currently breastfeeding
10. Any history of malignancy likely to result in significant disability or likely to require significant medical or surgical intervention within the next 6 months. This does not include minor surgical procedures for localized cancer (e.g., basal cell carcinoma)
11. Any condition other than IPF that, in the opinion of the investigator, is likely to result in the death of the patient within the next 6 months
12. History of severe hepatic impairment or end-stage liver disease or ALT or AST greater than 5 times the upper limit of normal at Screening
13. History of end-stage renal disease requiring dialysis
14. Participation in a clinical study with administration of an investigational drug product within the previous 30 days, or five half-lives of the previously administered investigational product

5.3. Study Restrictions

Patients are not to receive pirfenidone or nintedanib within 3 days prior to randomization to the study and during participation in Part A of the study. Nintedanib, but not oral pirfenidone, use is allowed if deemed necessary by the investigator during Part B of the study. In the event of an acute exacerbation, any treatment deemed necessary by the investigator is acceptable until the resolution of the event. Other than brief periods of medically appropriate treatment for acute IPF exacerbation, patients will not receive any other therapy for the treatment of IPF during Part A. If in the opinion of the investigator, a patient is in need of treatment with oral pirfenidone or nintedanib during Part A, the patient should be discontinued from the study and return to the clinic for their Early Termination visit. Oxygen use is allowed during the study.

5.4. Screening Rules and Rescreening

Patients who do not meet all the inclusion criteria or who meet at least one of the exclusion criteria will be considered screen failures. Patients may be rescreened after failing screening in the event of changes in responses to the eligibility criteria.

5.5. Randomization Criteria

Randomization will occur at Baseline/Study Visit 2. Patients must continue to meet all of the inclusion and none of the exclusion criteria to be randomized.

5.6. Patient Withdrawal Criteria

Patient participation in the study is purely voluntary; patients can withdraw consent at any time for any reason without effect on subsequent care. This is an open-label study in which the investigator is aware of the patient's treatment assignment. The investigator, in consultation with the Medical Monitor, or the Medical Monitor may exercise his or her medical judgment to terminate a patient's participation in the study due to clinically significant changes in any clinical or laboratory parameter. If in the opinion of the investigator, the patient is failing the study drug for lack of efficacy, as evidenced by an excessive decline of FVC, during Part A of the study, the patient can be withdrawn and placed on alternative therapies. During Part B of the study, the investigator may choose to add nintedanib, but not oral pirfenidone, to the patient's treatment regimen.

Any randomized patient desiring to discontinue prior to study completion should be encouraged to discuss his or her reasons and concerns with the investigator. If, after discussion, the patient still chooses to discontinue participation in the study, the patient should be encouraged to attend an Early Termination visit. (Patients that discontinue during the screening process will be considered screen failures.) Patients who are discontinued during a scheduled visit will be encouraged to complete all unique assessments for both that study visit and the End of Treatment visit at the time of discontinuation. If a patient is still participating in Part A and has had a HRCT scan within the past month, the HRCT from the End of Treatment visit will not be performed.

An End of Study case report form (CRF) page will be completed for every patient who receives study medication whether or not the patient completes the study. The reason for any early discontinuation from the study will be indicated on this form. The primary reason for any early termination will be selected from the following standard categories:

- Adverse Event: Clinical or laboratory events occurred that in the medical judgment of the investigator, in consultation with the Medical Monitor and Avalyn Pharma, represents an unacceptable risk to the patient if he/she continues in the study. This includes serious and non-serious AEs regardless of relationship to study medication. The investigator must follow the patient until the AE resolves or satisfactorily stabilizes
- Withdrawal of Consent: The patient desires to withdraw from further participation in the study in the absence of a medical need to withdraw determined by the investigator. If the patient gives a reason for this desire, this will be recorded on the CRF
- Pregnancy
- Lack of tolerability to drug or device
- Lost to Follow-up: The patient fails to complete the study and site personnel are unable to contact the patient within 30 days post-dose
- Death

5.7. Stopping Rules

This study may be discontinued at any time if, in the opinion of the DSMB, investigator, or Avalyn Pharma, continuation of the study represents a significant medical risk to participating patients. The DSMB will perform a safety data review after 20 patients have completed Week 4 and will make a recommendation at that time as to whether the study should continue or be modified. Avalyn Pharma also reserves the right to terminate the study at any time for any reason.

6. STUDY SCHEDULE AND PROCEDURES

6.1. Study Schedule

The study schedule can be found in Tables 5 and 6. At-home patient assessments are outlined in Table 7. Detailed information on study assessments is provided in Section 7.

6.2. Study Visits

Part A will consist of one Screening visit and 7 study visits, within the 24-week treatment period. Part B will consist of quarterly visits through study termination. A Follow-up visit will occur 28 days after the patient's End of Study visit in Part B.

6.2.1. Visit 1 – Screening (Day -30 to Day -1)

- Informed Consent
- Demography
- Medical history and concomitant medications including past 3 months
- Complete physical exam (PE) and vital signs, including height and weight
- Standard 12-lead ECG
- Saturated Oxygen (SaO_2)
- Spirometry and D_{LCO}
- Blood samples for laboratory tests (Chemistry including AST and ALT, Hematology)
- Urinalysis
- Urinary pregnancy test for females of childbearing potential (FOCBP) only
- Confirmation of IPF diagnosis as described in Section 5
- Inclusion/Exclusion Review

Leicester Cough Monitor (LCM) training. Patients that pass all of the screening criteria and are deemed eligible for the study will be provided with a LCM between the Screening and Baseline visit to wear for 24 hours. Patients will bring the LCM with them to the site at the Baseline visit. Optionally, patients may return to the clinic one day prior to the Baseline visit to receive their LCM and perform the 24-hour Baseline LCM reading; returning the LCM to the clinic the following day.

6.2.2. Visit 2 - Baseline (Day 1)

The following procedures will be conducted on Day 1 in chronological order:

- Cough Visual Analogue Scale (VAS), King's Brief Interstitial Lung Disease (KBILD) Questionnaire and Leicester Cough Questionnaire (LCQ)
- Concomitant medications since previous visit
- Record any AEs after signing informed consent
- Complete PE and vital signs

- HRCT (If not performed at Screening or Screening HRCT was performed more than 1 month prior to Baseline. For scheduling purposes, the Baseline HRCT may be performed up to 1 month prior to or 1 week after the Baseline visit.)
- Pre-dose Spirometry and D_{LCO}
- Pre-dose SaO_2; Patients with $SaO_2 < 90\%$ will be administered supplemental oxygen until the SaO_2 reaches 93% prior to dosing
- Blood samples for laboratory tests (Chemistry including AST and ALT, Hematology, Biomarkers (CXCL13**,** CCL18, MMP3, CA19-9 and CA-125)
- Urinalysis
- Urinary pregnancy test (FOCBP only)
- Confirm Inclusion/Exclusion criteria
- Randomization
- Study material training and first study drug administration; patients experiencing excessive cough or bronchospasm will be administered 1 – 2 puffs (90 - 100 µg) of salbutamol prior to subsequent doses or during initial dosing
- Post-dose Spirometry to be performed ≤ 10 minutes after completion of AP01 dose (Patients with a ≥ 15% drop from pre-dose spirometry who are symptomatic will be treated as described in section 8.6.)
- Post-dose SaO_2
- Study material dispensation, including salbutamol and instructions for use of salbutamol (if needed as described in Sections 8.6 and 8.7.)
- Home spirometry training and dispensation

6.2.3. Weekly Home Spirometry

Patients will perform home spirometry weekly during both Part A and Part B of the study. Home spirometry readings will be transferred electronically to the vendor.

6.2.4. Visit 2A

One week after the Baseline visit, the site will conduct a follow-up call to the patient to assess tolerability, study drug compliance, AEs, concomitant medications, status of the LCM return and use of the home spirometer.

6.2.5. Visits 3, 4, 5, 6, and 7 (Day 28, 56, 84, 112, 140 +/- 3 days, respectively)

The following procedures will be conducted at the listed visits in chronological order:

- Cough VAS, KBILD Questionnaire and LCQ
- AEs and concomitant medications since previous visit
- Modified PE (Lungs and Cardiovascular (CV) body systems only)
- Weight (Visit 5 only)

- Vital signs
- SaO_2
- Spirometry and D_{LCO}
- Blood samples for laboratory tests (Chemistry including AST and ALT, Hematology, Biomarkers (CA19-9 and CA-125 only))
- Urinalysis
- Urinary pregnancy test (FOCBP only)
- LCM dispensation (Visit 5 only)
- Study material dispensation

6.2.6. Visit 8 - End of Part A (Day 168 +/- 3 days)

The following procedures will be conducted at the listed visits in chronological order:

- Cough VAS, KBILD Questionnaire and LCQ
- AEs and concomitant medications since previous visit
- Modified PE (Lungs and CV body systems only)
- Weight
- Vital signs
- SaO_2
- Spirometry and D_{LCO}
- Blood samples for laboratory tests (Chemistry including AST and ALT, Hematology, Biomarkers (CA19-9 and CA-125 only))
- Urinalysis
- Urinary pregnancy test (FOCBP only)
- HRCT
- LCM dispensation
- End of Treatment form for Part A completion
- Study material dispensation (3-month supply for patients participating in Part B only)

6.2.7. Monthly Calls to Patient

During Part B, the site will conduct a monthly call to the patient between visits to assess tolerability, study drug compliance, AEs, concomitant medications, status of the LCM return (when applicable) and use of the home spirometer.

6.2.8. Visits 9, 10, and 11 – Part B (Day 252, 336, and 420 +/- 7 days, respectively)

The following procedures will be conducted at the listed visits in chronological order:

- Cough VAS, KBILD Questionnaire and LCQ

- AEs and concomitant medications since previous visit
- Modified PE (Lungs and CV body systems only)
- Weight (Visit 10 only)
- Vital signs
- SaO_2
- Spirometry and D_{LCO}
- Blood samples for laboratory tests (Chemistry including AST and ALT, Hematology, Biomarkers (CA19-9 and CA-125 only))
- Urinalysis
- Urinary pregnancy test (FOCBP only)
- Study material dispensation (3-month supply)

6.2.9. Visit 12 - End of Study (Day 504 +/- 7 days)

The following procedures will be conducted at the listed visits in chronological order:

- Cough VAS, KBILD Questionnaire and LCQ
- AEs and concomitant medications since previous visit
- Modified PE (Lungs and CV body systems only)
- Weight
- Vital signs
- SaO_2
- Spirometry and D_{LCO}
- Blood samples for laboratory tests (Chemistry including AST and ALT, Hematology, Biomarkers (CA19-9 and CA-125 only))
- Urinalysis
- Urinary pregnancy test (FOCBP only)
- End of Treatment for Part B form completion
- Informed Consent for continued treatment until enrollment in the AP01-005 Rollover study (Patients waiting approval of AP01-005 at their site only.)

6.2.10 Safety Monitoring Visits for Patients Awaiting Site Approval to Enroll in AP01-005 Only (Day 588 +/- 7 days)

- Spirometry
- AST and ALT
- Saturated Oxygen (SaO_2)
- Urinary pregnancy test for females of childbearing potential (FOCBP) only

- Abnormalities on chest and skin exam
- Vitals
- AEs and concomitant medications since previous visit

6.2.11 Follow-up Visit for Patients NOT Enrolling in AP01-005 (28 days +/- 7 days after the Visit 12 - End of Study visit)

Follow-up visits will occur 28 days after the End of Study visit for Part B. The following procedures will be conducted in chronological order:

- Cough VAS, KBILD Questionnaire and LCQ
- AEs and concomitant medications since previous visit
- Modified PE (Lungs and CV body systems only)
- Vital signs
- SaO_2
- Spirometry and D_{LCO}
- Blood samples for laboratory tests (Chemistry including AST and ALT, Hematology, Biomarkers (CA19-9 and CA-125 only))
- Urinalysis
- End of Study form completion

Patients may experience AEs that necessitate an unscheduled visit. There may also be situations in which the investigator asks a patient to report for an unscheduled visit following the report of an AE or a significant decrease in FVC during home spirometry readings. Additional examinations may be conducted at these visits as necessary to ensure the safety and well-being of patients during the study. Electronic case report forms (eCRFs) should be completed for each unscheduled visit.

Table 5: Study Schedule for Part A

	Study Phase – Part A							
	Screening	Baseline	Treatment					
Visit	1	2	3	4	5	6	7	8
Day	-30 to -1	1	28	56	84	112	140	168
Window (Days)	NA	NA	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3	+/- 3
Informed Consent	X							
Eligibility Assessments	X	X						
Assessment of IPF Diagnosis	X							
Demographics	X							
Medical History	X							
Physical Exam[2]	X	X	X	X	X	X	X	X
Height[3], Weight	X				X			X
Vital Signs	X	X	X	X	X	X	X	X
ECG	X							
Laboratory Tests[4]	X	X	X	X	X	X	X	X
Urinalysis	X	X	X	X	X	X	X	X
Urinary Pregnancy Test[5]	X	X	X	X	X	X	X	X
Spirometry and D_{LCO}	X	X	X	X	X	X	X	X
SaO_2	X		X	X	X	X	X	X
LCM[6] and diary dispensation	X				X			X
Cough VAS, KBILD Questionnaire and LCQ		X	X	X	X	X	X	X
HRCT[7]		X						X
Pre-dose Spirometry		X						
Pre-dose SaO_2		X						
Randomization		X						
Study Material Training		X						
Study Drug Administration First Dose		X						
Post-dose Spirometry (≤ 10 min post-dose)		X						
Post-dose SaO_2		X						
Study Material Dispensation		X	X	X	X	X	X	X[8]
Home Spirometer Training and Dispensation		X						
Concomitant Medications	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X
End of Treatment/Study Form								X

Table 6: Study Schedule for Part B

	Study Phase – Part B					
	Treatment					
Visit	9	10	11	12[9]	Follow-up[10]	Safety Visit[11]
Day	252	336	420	504 End of Study	28 days after End of Study	588
Window (Days)	+/- 7	+/- 7	+/- 7	+/- 7	+/- 7	+/- 7
Cough VAS, KBILD and LCQ	X	X	X	X	X	
Physical Exam[2]	X	X	X	X	X	X
Weight		X		X		
Vital Signs	X	X	X	X	X	X
Laboratory Tests[4]	X	X	X	X	X	X
Urinalysis	X	X	X	X	X	
Urinary Pregnancy Test[5]	X	X	X	X		X
Spirometry and D_{LCO}	X	X	X	X	X	X[12]
Saturated Oxygen	X	X	X	X	X	X
Study Material Dispensation	X	X	X	X[13]		X
Concomitant Medications	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X
End of Treatment/Study Form				X	X	
Informed Consent to continue AP01 until enrollment in AP01-005[11]				X		

Notes for Tables 5 and 6:

1. Follow-up visit will occur 28 days after the End of Study visit in Part B of the study.
2. A complete physical exam will be performed at Screening and Baseline visits. A modified physical exam including the patient's lungs and cardiovascular body systems will be performed at all other visits. For Safety Visits, only chest and skin exam are required.
3. Height is collected at the Screening visit only.
4. Blood samples for laboratory tests include: Chemistry (including AST and ALT), Hematology and Biomarkers (Blood samples for biomarkers will not be collected at Screening.) For Safety Visits, only AST and ALT are required.
5. Females of childbearing potential are required to take a urinary pregnancy test at each visit except the Follow-up visit.
6. Patients that pass screening and are scheduled to be randomized will receive a LCM to be worn for 24 hours and returned to the site at the Baseline visit. LCM will be worn for 24 hours at Visits 5 and 8. A paper diary will also be dispensed to the patient to record activities during the 24-hour period.
7. HRCT scans will be performed at Visits 2 and 8. The Baseline HRCT may be skipped if the HRCT for eligibility was performed less than 1 month prior to the Baseline visit.
8. Study material dispensation will occur at Visit 8 for those patients participating in Part B of the study.
9. Patients will be assessed for eligibility to participate in AP01-005 at EOS/Visit 12. Those enrolling in AP01-005 will use EOS/Visit 12 as their AP01-005 Baseline visit.
10. Patients enrolling in AP01-005 will not participate in the Follow-up visit.
11. Patients awaiting site approval of AP01-005 only.

12. For Safety Visits, D_{LCO} not required.
13. Study Material Dispensation only for patients continuing AP01 at Visit 12.

Table 7: Patient At-Home Assessments

DAILY: Patients will administer AP01 50 once daily or 100 mg twice daily
WEEKLY: Patients will perform home spirometry readings on the same day each week at approximately the same time of day (+/- 2 hours) during Part A and Part B of the study

7. ASSESSMENTS

7.1. Background Assessments

7.1.1. Demographic/Medical History

Information relating to the patient's sex, age, race, height, and weight will be recorded at Screening on the appropriate eCRF page. Medical history of each patient will be collected at Screening and recorded on the appropriate eCRF page.

7.2. Efficacy Assessments

7.2.1. FVC

Pre and post-dose spirometry will be conducted the day of first dose. The post-dose spirometry reading should be performed ≤ 10 minutes after completion of the AP01 dose. Spirometry will be conducted at approximately the same time each visit (within 60 minutes of Day 1 pre-dose spirometry measurement) with the patient in seated position. The test will be performed to American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines[15]. On days of clinic visits (including the screening visit), patients must refrain from strenuous activity at least 12 hours prior to pulmonary function testing. FVC % predicted will be calculated using the ERS Global Lung Function Initiative 2012 equations[14] as outlined in the clinical study manual.

7.2.2. Leicester Cough Monitor[16]

The LCM is an audio-based cough detection device that allows sounds to be reconstructed and measured as cough events. Patients that pass all of the screening criteria and will be randomized in the study will be provided with a cough monitor to wear for a 24-hour period prior to coming in for their Baseline visit. This will be used as their baseline reading. The LCM and mailing materials will also be dispensed at Visits 5 and 8. Patients will wear for a 24-hour period, fill out the cough monitor paper diary and when complete, mail the LCM and diary back using the mailing materials provided. Further details regarding LCM dispensation and return will be outlined in the clinical study manual.

7.2.3. D_{LCO}

D_{LCO} will be collected at each visit. For predicted normal values, different sites may use different prediction formulas, based on the method used to measure D_{LCO}. In any case, the method used must be in compliance with the ATS/ERS guidelines[17] on D_{LCO} measurements and the prediction formula appropriate for that method[18]. Raw data (gas mixture, equation used for prediction of normal, further adjustments made if so) must be traced.

7.2.4. Cough Visual Analogue Scale (VAS)[19]

The Cough VAS will be completed at Baseline, all subsequent visits and the Follow-up visit. This is a paper-based scale that will be completed by the patient at the relevant visits. The results of the scale will be measured in millimeters and entered into the appropriate eCRF. Appendix 17.1 presents an example of the Cough VAS.

7.2.5. The King's Brief Interstitial Lung Disease (KBILD) [20]

The KBILD Questionnaire will be completed at Baseline, all subsequent visits and the Follow-up visit. This is a paper-based questionnaire that will be completed by the patient at the relevant visits. The results of the KBILD will be entered into the appropriate eCRF. Appendix Section 17.2 presents the KBILD Questionnaire.

7.2.6. Leicester Cough Questionnaire (LCQ)[21]

The LCQ will be completed at Baseline, all subsequent visits and the Follow-up visit. This is a paper-based questionnaire that will be completed by the patient at the relevant visits. The results of the LCQ will be entered into the appropriate eCRF. Appendix 17.3 presents the LCQ.

7.3. Additional Endpoint(s) Assessments

7.3.1. Weekly home spirometry measurements

Patients will obtain spirometry readings at home on the same day and at approximately the same time each week (+/- 2 hours). Home spirometers will be dispensed at the Baseline visit; patients will be trained on the use of home spirometers prior to their departure from the Baseline visit.

7.3.2. Extent of fibrosis and lung volumes as assessed by HRCT

HRCT scans will be obtained to measure the extent of fibrosis and lung volumes at Baseline and the Week 24 visits. These scans will be read and interpreted by a central reader.

7.4. Safety Assessments

All patients who enter the study will be assessed for safety. Safety will be monitored by observation of and direct inquiry regarding AEs at each visit after the first dose of study drug.

7.4.1. Adverse Events

All AEs will be collected from informed consent through the Follow-up visit. Details regarding AE definitions, collection, recording, and reporting are found in Section 10.1.

7.4.2. Physical Examination

A complete PE will be performed at the Screening and Baseline visits. This will include physical examination of the following body areas and systems: examination of general appearance, skin, head, neck (including thyroid), eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system.

A modified PE of the lungs and CV body systems will be performed at each subsequent visit.

A complete or modified physical exam is optional at unscheduled visits, per the investigator's discretion.

Body height will be measured at Screening only. Weight will also be measured at the Screening and Week 24 and 48 visits.

7.4.3. Vital Signs

Blood pressure and pulse will be assessed at each visit. Patients should be comfortably seated for 5 minutes prior to blood pressure readings. Study staff will take care to select the appropriate cuff size for each patient. Respiratory rate and temperature will be measured at each visit.

7.4.4. Concomitant Medications/Therapies

All concomitant medications/therapies collected throughout the study must be recorded on the Concomitant Medications and Therapies eCRF. The prohibited and allowed concomitant medications/therapies for the study are discussed in Section 8.9.1.

7.4.5. Electrocardiography (ECG)

A 12-lead ECG will be recorded at Screening by the investigator or other designated, qualified individual from the study research team. ECGs will be assessed for clinical relevance including rhythm and repolarization.

7.4.6. Laboratory Assessments for Hematology, Serum Chemistry, Biomarkers and Urinalysis

At each visit, clinical laboratory tests will be conducted at the study's central laboratory with the exception of biomarkers, which may be sent to a specialty lab. Clinical laboratory tests will include:

- Hematology: complete blood count with differential, platelet count
- Serum Biochemistry: bilirubin, alkaline phosphatase, sodium, potassium, chloride, bicarbonate, urea, creatinine, total protein, albumin, glucose, ALT, AST
- Urinalysis: pH, specific gravity, presence/absence of protein, glucose, blood
- Biomarkers[22, 23]: CXCL13**,** CCL18, MMP3 (progression-related) will be tested at Baseline only. CA19-9 and CA-125 (predictive of alveolar epithelial damage) will be collected at all visits except for the Screening visit.)

7.4.7. Pregnancy Testing

At all visits except the Follow-up visit, a urinary pregnancy test will be performed for all FOCBP.

Contraceptive Requirements

Patients must be willing to comply with the contraceptive requirements of the study.

To prevent pregnancy in a FOCBP, FOCBP patients must agree to use one of the following contraceptive methods during the study and for 30 days after the last dose of IP:

- Abstinence from heterosexual intercourse OR
- Use a highly-effective form of contraception (e.g. hormonal contraception, or

  an intrauterine device) AND

  use of a barrier method by the female or her sexual partner.

Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

7.5. Pharmacokinetic Assessments

This study will have no pharmacokinetic parameters assessed.

8. INVESTIGATIONAL DRUG INFORMATION AND MANAGEMENT

8.1. Investigational Drug Dose Regimen

Two dose levels/regimen combinations will be tested in this study:

- Pirfenidone 50 mg in 4 mL aqueous solution delivered once daily
- Pirfenidone 100 mg in 8 mL aqueous solution delivered twice daily

8.2. Dose Rationale

In addition to demonstrating safety and tolerability of AP01 multiple dosing in IPF patients, a key goal of the Phase 1b study is to select an optimal dosing regimen for further development of AP01. The initial dosing regimen was selected by formulation considerations, time and frequency of administration and local lung pharmacokinetics. Results from this study will help to elucidate whether therapeutic effect is dependent on peak dose, frequency of administration after a peak dose, or AUC in the lung.

Aqueous solubility limits the stability of the AP01 pirfenidone formulation concentration to 12.5 mg/mL. Time and frequency of administration are also important considerations; improved compliance is expected with fewer dose administrations per day and shorter administration times.

The 801 mg oral pirfenidone plasma pharmacokinetics have been well described. From this, one can estimate drug levels in the ELF and lung tissue from plasma concentrations. Assuming 50% of the drug is tightly protein-bound with the rest freely capable of local diffusion and with a known plasma $T_{1/2}$ (2.4 hours), we may estimate AUC in both the ELF and lung tissue. Following an 801 mg oral dose, a measured plasma C_{max} is 7.9 μg/mL[11], with the estimated ELF and lung tissue C_{max} of 3.9 μg/mL. The $AUC_{0-\infty}$ is 59.3 μg·hr/mL in plasma and estimated as 29.7 μg·hr/mL in ELF and lung tissue.

One can also estimate inhaled pirfenidone ELF pharmacokinetics from a single collected BAL fluid sample from healthy volunteers. Due to the time to administer anesthesia and perform the procedure, BAL collection is typically 30 - 50 minutes post-drug administration, which is several $T_{1/2}$ from the true C_{max} seen at the end of inhaled administration. Therefore, because the ELF clearance $T_{1/2}$ is unknown, one can only estimate the ELF pirfenidone C_{max} and local lung AUC. To strengthen this estimation, a large animal sheep pharmacokinetic study was performed. Sheep were administered inhaled pirfenidone and both serial BAL and plasma samples collected. Analysis of this data provided a pirfenidone plasma/ELF pharmacokinetic relationship curve, which was used to extrapolate the single human ELF data point back to C_{max} and estimate AUC. In normal human volunteers, the 100 mg dose provided an average ELF C_{max} of 135.9 μg/mL and an $AUC_{0\text{-}last}$ of 54.5 μg·hr/mL. With these data, one can also then confirm adequate pirfenidone ELF delivery in the IPF population in the absence of BAL fluid sampling by correlating obtained plasma levels and 24-hour urine collections between normal volunteers and IPF patients.

The 24-hour urine collection in the normal human volunteers BAL cohort compared to the IPF cohort was similar on average but with less variability (40.2 ± 20.5 mg vs. 42.2 ± 14 mg). Since the IPF cohort has less lung surface area but the same absorption, this suggests that the IPF cohort had higher C_{max} ELF levels. The C_{max} plasma levels in the IPF cohort were lower than the volunteer cohorts, but absorption was prolonged, also suggesting the ELF AUCs were similar or superior to the BAL cohort.

There are other preclinical and clinical observations that provide considerations for potential dose regimens. The first is that the inhaled C_{max} with the 100 mg dose exceeds the pirfenidone half maximal inhibitory concentration (~25 µg/mL) from in vitro models and nears the observed half maximal effective concentration (EC_{50}) (~100 µg/mL) in animal models of bleomycin injury[24]. As noted before, the C_{max} in the ELF of the 801 mg oral dose is 3.9 µg/mL and does not approach the required concentrations from in vitro and in vivo models of lung injury. These observations suggest that a higher C_{max} may improve efficacy. Second, as noted above, preclinical models of IPF using bleomycin suggest that efficacy is correlated with C_{max} rather than AUC, this finding would be ideal for an aerosol dose with high ELF concentrations and fast clearance. Although these same animal models suggest that once a day dosing can be effective, the duration of action after a peak dose in IPF patients is not known.

The high dose chosen for the Phase 1b study is 100 mg administered twice daily. The 100 mg dose based on the modeling noted above would provide an average C_{max} in the ELF of 135.9 µg/mL and an $AUC_{0\text{-}18\ hr}$ of 54.5 µg·hr/mL. The C_{max} is approximately 35-fold higher than what is achieved with the oral 801 mg dose, with the total $AUC_{0\text{-}18\ hr}$ 2-fold higher. IPF's pathogenesis is postulated to pivotally depend upon alveolar epithelial cell damage. This dose would provide dramatically higher ELF C_{max} and a daily cumulative AUC with the twice daily aerosol dose 1.5-fold higher compared to a three times daily 801 mg oral dose. The 100 mg aerosol dose requires a 9 - 15 minutes administration time.

The second dose chosen for the Phase 1b study is 50 mg administered once daily. In the Phase 1 dose escalation study, the plasma pharmacokinetics were proportional to the dose. Assuming proportionality, the 50 mg once daily dose would have a C_{max} 17-fold higher than the oral 801 mg dose, and a total 24-hour AUC in the ELF that would be 2/3 of the 801 oral three times daily regimen. The administration time of the 50 mg dose is expected to be 5 - 7 minutes and a shorter regimen would likely lead to more compliance, better patient acceptance and perhaps fewer adverse respiratory effects.

Both of these doses will have substantially less systemic pirfenidone exposure compared to the 801 mg oral dose. Following an 801 mg oral dose, the peak plasma mean concentration is 7.9 µg/mL and the $AUC_{0\text{-}18hr}$ is 60.9 µg·hr/mL.[11] Following a 100 mg inhalation, the peak plasma mean concentration is 1.7 µg/mL and the AUC $_{0\text{-}18hr}$ is 4 µg·hr/mL. Therefore, the 100 mg nebulizer dose leads to less than 1/15, and the 50 mg dose 1/30, the systemic exposure of the oral dose.

No clinical study has examined fewer than three oral pirfenidone doses per day. Since oral pirfenidone is taken at meals, the time between dinner and breakfast is usually twelve hours, suggesting the duration of action is at least that long. Like inhalation, the three times daily oral regimen delivers a short-duration peak with very low trough levels. Testing a 100 mg twice daily regimen would mimic the duration between evening and morning dose and would also increase the AUC in the ELF over a 24-hour period by 1.5-fold compared to the 801 mg three times daily oral dose. If efficacy is driven by C_{max} rather than AUC, the 100 mg twice daily and 50 mg once daily doses would provide a 34-fold and 17-fold higher peak concentration, respectively, than is achieved from an 801 mg oral dose. The 50 mg once daily regimen would provide a 17-fold higher peak concentration than is achieved from an 801 mg oral dose. The 50 mg once daily regimen was chosen to test the hypothesis that only once daily dosing is adequate and efficacy is driven by C_{max} in the ELF. The 100 mg twice daily regimen was chosen to test whether efficacy is also dependent on total ELF AUC or multiple daily peaks.

8.3. Investigational Drug Manufacturing, Packaging and Labeling

Investigational drug used in this study will be prepared, packaged, and labeled in accordance with the standard operating procedures (SOPs), Good Manufacturing Practice (GMP) guidelines, International

Conference on Harmonization (ICH) guidelines for Good Clinical Practice (GCP), guidelines for Quality System Regulations (QSR), and applicable regulations.

8.3.1. Drug Substance

The manufacturer of cGMP pirfenidone drug substance for this study is Procos S.p.A, Italy.

8.3.2. Drug Product

AP01 is a sterile, aqueous solution formulation consisting of pirfenidone (active ingredient) dissolved in a 5 mM citrate buffer containing sodium chloride (for osmolality and tolerability improvement) and sodium saccharin (to improve taste and tolerability). AP01 is formulated with an osmolality ~380 milliosmoles/kg with a final pH ~6.0. All excipients have good history of use in pharmaceutical products and have been used in United States Food and Drug Administration (US FDA), European and Australian-approved inhalation solutions. AP01 IP has been tested in a Phase 1 study with the composition presented in Table 8. All excipients have good history of use in pharmaceutical products and have been used in US FDA, European and Australian-approved inhalation solutions.

Table 8: Drug Product Composition

Ingredient	Function	Concentration
Pirfenidone	Active pharmaceutical ingredient	12.5 mg/mL
Sodium citrate dihydrate	Buffering agent	4.5 mM
Citric acid monohydrate	Buffering agent	0.5 mM
NaCl	Osmolality adjustment/Tolerability improvement	150 mM
Sodium saccharin dihydrate	Taste/Tolerability improvement	0.75 mM
Sterile Water for Injection	Vehicle	q.s.

8.3.3. Manufacture of Drug Product

AP01 IP is manufactured by Holopack GmbH in Sulzbach-Laufen, Germany. The manufacturing process is comprised of formulation compounding, aseptic filling into low density polyethylene (LDPE) ampoules by the blow-fill-seal (BFS) operation, and secondary packaging filled ampoules in foil laminate overwrap. Release and stability testing is conducted at Pharmaceutical Product Development, Inc., located in Middleton, Wisconsin, United States of America.

8.3.4. Nebulization Device

The nebulizer is a re-usable device intended for single patient use. AP01 will be delivered to the lung by oral inhalation using the PARI (Germany) eFlow® Nebulizer System. The eFlow is a high-efficiency, 510(k)-cleared, Conformité Européene (CE)-marked device that is marketed with other products for other disease indications.

With the AP01 formulation, the eFlow nebulizer (model with large holding chamber and with 35 membrane head) produces an aerosol with a narrow particle size distribution with a mass median diameter of 2.9 – 3.9 μm and a GSD < 1.8, which is well suited to lower airway and alveolar drug deposition.

8.3.5. Packaging and Labeling of AP01 and Nebulizer Kits

AP01 IP Supply

Overwrapped AP01 IP ampoules will be packaged and labelled as required by regional regulation by PPD Development, L.P. in Athlone, Ireland. Each IP carton will contain 18 AP01 overwrapped pouches, each pouch containing two ampoules. Each ampoule will contain 4 mL AP01 12.5 mg/mL formulation, which is a 50 mg dose. The 50 mg once daily inhaled dose cohort will use one IP carton every 4 weeks (1/2 pouch per day; 1 ampoule per once daily dose). The 100 mg twice daily inhaled dose cohort will use four IP cartons every 4 weeks (2 pouches per day; 2 ampoules per twice daily dose). Details of IP distribution will be outlined in the clinical study manual.

Nebulizer Starter Kits and Replacement Handsets

Randomized patients will receive an eFlow nebulizer starter kit with their initial AP01 IP cartons at the Baseline visit. At subsequent visits, patients will receive an eFlow replacement handset to exchange with their used handset every 4 weeks or as needed. Nebulizer starter kits will be packaged separately from IP cartons. Nebulizer starter kits will comprise of a single box with a study-specific label as required by regional regulation. Replacement handsets will be packaged separately from eFlow starter kits and IP cartons. Each eFlow replacement handset will be packaged with a study-specific label as required by regional regulation. Details of device distribution will be outlined in the clinical study manual.

8.4. Investigational Drug and Device Storage

Manufactured IP and nebulizer supplies must be kept in a secure, limited access storage area maintained under room temperature (15° - 25° Celsius).

8.5. Investigational Drug Preparation

The IP is to be kept in the aluminum foil pouch until the point of use.

The patient should be instructed not to use this IP with any other nebulizers, and not to use this eFlow with any other drug products. Details regarding drug preparation will be outlined in the clinical study manual.

8.6. Investigational Drug Administration

Patients will be trained on use of the eFlow and administration of the study drug at the Baseline visit. The first dose of study drug for each patient will be administered and observed at the clinical study site at the Baseline visit to confirm tolerability. All subsequent doses will be administered by the patient at home. All doses must be taken at least 4 hours apart.

Patients who experience cough that limits their ability to complete dosing will be given 1 – 2 puffs (90 - 100 µg) of an inhaled beta 2 agonist, such as salbutamol, in order to complete the in-clinic dose. These patients, as well as patients with a history of asthma or smoking history of 20 pack years or greater, or patients that have a ≥ 15% drop in FEV_1 percent predicted in their pre-dose and post-dose readings and are symptomatic will be provided with and required to use 1 - 2 puffs (90 - 100 µg) of salbutamol prior to their second dose and for dosing throughout the study, unless these patients are currently taking a long-

acting beta-2-agonist therapy. If symptoms persist with study treatment despite beta-2-agonist therapy, the Investigator will assess and discontinue the patient due to tolerability issues if he/she deems it necessary.

8.7. Missed Doses and Dose Modifications

Doses that are missed can be taken in a 24-hour period, as long as doses are 4 hours apart.

In the event of excessive coughing while drug administration at home, the patient will contact the site to determine how to proceed. Salbutamol may be prescribed to be administered prior to dosing, as described in section 8.6, if deemed necessary by the investigator.

If AP01 is stopped during treatment due to intolerance, restart dosing when the patient has improved. The initial dose after restarting should be observed for tolerance.

It is recommended to reduce the 100 mg twice daily dose to 50 mg twice daily if the patient cannot tolerate the 100 mg dose. If the 50 mg twice daily dose cannot be tolerated, the patient may have their dose reduced to 50 mg once daily.

8.8. Investigational Drug Accountability, Handling and Disposal

8.8.1. Investigational Drug Handling and Disposal

Administration of the first dose of IP will be observed by the investigator or designee; time of administration and confirmation of full dose being administered will be noted on the eCRFs.

The investigator or designee is responsible for IP and accountability, reconciliation and record maintenance. In accordance with all applicable regulatory requirements, designated research or pharmacy staff at each study site must maintain IP accountability records for study drug throughout the course of the study.

Patients will be instructed to bring back the box(es) of unused ampoules at each study visit for drug accountability purposes. Patients will be instructed to dispose of all used ampoules. Details regarding drug accountability procedures will be captured in the clinical study manual.

8.8.2. Salbutamol Handling and Disposal

Patients requiring salbutamol as described in Sections 8.6 and 8.7 will be provided with salbutamol at the study visits or as needed. Patients will dispose of the salbutamol at the end of the study.

8.8.3. Device Handling, Cleaning and Disposal

The eFlow instructions for use provide detailed instructions for the patient to follow regarding cleaning and maintenance of the handsets for this study.

In the event of a nebulizer (base unit, handset or both) malfunction, the patient will contact the site immediately for a replacement unit. The patient will return the malfunctioning nebulizer to the site. The site will return the malfunctioning nebulizer to the manufacturer (via the clinical packager) for a root cause analysis. Further details regarding the handling of malfunctioning devices will be outline in the clinical study manual.

In accordance with all applicable regulatory requirements, designated research or pharmacy staff at each study site must maintain accountability records for the nebulizer system (base units and handsets) throughout the course of the study. Details regarding device accountability procedures will be captured in the clinical study manual.

8.9. Treatment of Patients

8.9.1. Concomitant Medications and/or Treatments

Patients are prohibited from receiving pirfenidone or nintedanib within 3 days prior to randomization to the study and during participation in Part A of the study. Other than brief periods of medically appropriate treatment for acute IPF exacerbation, patients will not receive any other therapy for the treatment of IPF in Part A of the study. If in the opinion of the investigator, a patient is in need of treatment with oral pirfenidone or nintedanib during Part A, the patient should be discontinued from the study and return to the clinic for their Early Termination visit. Nintedanib, but not oral pirfenidone, use is allowed if deemed necessary by the investigator during Part B of the study. If AP01 is stopped during treatment of an exacerbation due to a patient's intolerance, it should be restarted when the patient has improved, however the first dose should be observed for tolerance. Oxygen use is allowed during the study.

Due to the low systemic levels expected with delivery by inhalation, drug-drug interactions are unlikely and unexpected.

Patients who are experience excessive coughing during study drug administration will be required to inhale 1 - 2 puffs (90 - 100 µg) of salbutamol within 10 minutes prior to subsequent study drug dosing. Pre-treatment with salbutamol will be documented on the Concomitant Medications eCRF page.

8.9.2. Treatment Compliance

Administration of the first dose of IP will be observed by the investigator or designee; time of administration and confirmation of full dose being administered will be noted in the eCRF. Patients will bring all unused ampoules to each visit for drug accountability and compliance assessment. At the discretion of the investigator, patients that are not in compliance with study dosing and procedures will be discontinued from the study.

9 RANDOMIZATION AND BLINDING PROCEDURES

9.1 Randomization

Patients will be randomized in a 1:1 ratio to receive AP01 either 50 mg once daily or 100 mg twice daily; both regimens being administered by the eFlow. The randomization schema will be stratified by region (Asia-Pacific and Europe) and by disease severity (FVC < 50% predicted and FVC ≥ 50% predicted). The first 20 patients randomized in the study must have FVC ≥ 50% predicted at Screening. After the first 20 patients have been randomized, patients with FVC 40% < 50% predicted will be allowed to be randomized in the study but randomization for these patients will be capped at 20.

9.2 Blinding

This is an open-label study; no blinding restrictions are required.

10 ADVERSE EVENTS

10.1 Adverse and Serious Adverse Events

Adverse Events (AEs) will be recorded after the patient signs informed consent and throughout the study. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

The investigator is responsible for the detection and documentation of AEs regardless of treatment group or suspected causal relationship to the IP. For all AEs, the investigator must pursue and obtain information adequate to determine the outcome of the AE and to assess whether the AE meets the criteria for classification as an SAE requiring immediate notification to Avalyn Pharma or its designated representative.

10.1.1 Definitions of AEs

10.1.1.1 Adverse Event

An AE is defined as any untoward or unfavorable medical occurrence associated with the patient's participation in the research, whether or not considered related to the patient's participation in the research (ICH E6 Guidelines for GCP). Any medical condition that is present at the time that the patient is screened will be considered as medical history and not recorded as an AE; however, if the condition worsens at any time during the study, it will be recorded and reported as an AE.

AEs are defined as any untoward medical occurrence in a patient administered a pharmaceutical product and which do not necessarily have to have a causal relationship with this treatment.

Clinical Abnormalities

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding, or other abnormal assessment is clinically significant, and thus constitutes an AE.

10.1.1.2 Serious Adverse Event

An AE is considered "serious" if, in the view of either the investigator or Avalyn Pharma, it results in any of the following outcomes (21 Code of Federal Regulations (nacl) 312.32(a)):

- Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up period must be reported to Avalyn Pharma whether it is considered treatment related or not.
- A life-threatening event: An AE or suspected adverse reaction (SAR) is considered "life-threatening" if, in the view of either the investigator or Avalyn Pharma, its occurrence places the participant at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood

dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of IP dependency or abuse.
- Congenital anomaly or birth defect.

If an event meets any of the above definitions, regardless of the severity or relationship of the event to the study product, the event must be reported to Avalyn Pharma as described in Section 10.1.5.

AEs reported from clinical studies associated with hospitalization or prolongation of hospitalization are considered serious. Any hospitalization except observational admissions of less than 24 hours meets these criteria. This category also includes transfer within the hospital to an acute/intensive care unit (e.g., from a standard of care unit to an acute/intensive care unit).

Hospitalization does not include the following:

- Rehabilitation facilities, hospice facilities or respite care (e.g. caregiver relief)
- Nursing homes or skilled nursing facilities
- Emergency room visits
- Same day surgeries (as outpatient/same day/ambulatory procedures)
- < 24-hour admissions for observation or evaluation

Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical AE is not in itself an SAE. Examples include:

- Admission for treatment of a preexisting condition that did not worsen
- Protocol-specified admission (e.g. for a procedure required by the study protocol)
- Hospitalizations for cosmetic elective surgery, social, and/or convenience admissions
- Diagnostic and therapeutic procedures, such as surgery, should not be reported as AEs; however, the medical condition for which the procedure was performed should be reported if it occurs during the reporting period and meets the definition of an AE. For example, an acute appendicitis that begins during the AE reporting period should be reported as an AE, and the resulting appendectomy should be recorded as treatment of the AE.

10.1.1.3 Adverse Drug Reaction and Suspected Adverse Reaction

An adverse drug reaction (ADR) means any AE caused by a drug or biologic.

Suspected adverse reaction (SAR) means any AE for which there is a reasonable possibility that the drug or biologic caused the AE. For the purposes of safety reporting, “reasonable possibility” means there is evidence to suggest a causal relationship between the drug or biologic and the AE. An SAR implies a lesser degree of certainty about causality than an ADR (21 CFR 312.32(a)).

10.1.1.4 Unexpected Adverse Reaction

Avalyn Pharma is responsible for assessing AEs for expectedness. With regards to reporting to the Health Authority, an AE is considered “unexpected” when its nature (specificity), severity, or rate of occurrence is not consistent with applicable product information as described in the safety information provided in the protocol or IB for AP01. "Unexpected," as used in this definition, also refers to AEs or SARs that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular

drug under investigation (21 CFR 312.32(a)). In addition, clinical events that commonly occur in IPF patients, such as pulmonary exacerbations, may be unexpected in a specific patient, but are likely to occur during the course of the study. These events should be classified as 'expected' rather than 'unexpected' as they are part of the natural history of the disease.

10.1.2 Severity of AEs/SAEs

The study site will grade the severity of AEs experienced by study participants according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version 5.0.* This document (referred to herein as the "NCI-CTCAE manual") provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of AEs. Please refer to the NCI-CTCAE manual for the desired event and specific grading for that event.

If the event is not listed in the NCI-CTCAE manual, please refer to the following standards in the NCI-CTCAE manual:

- Grade 1 = mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 = moderate minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL) e.g. preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
- Grade 3 = severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
- Grade 4 = life-threatening consequences; urgent intervention indicated.
- Grade 5 = death related to AE.

For additional information and a printable version of the NCI-CTCAE manual, go to
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Note: The terms serious and severe are not synonymous. Serious criteria as defined in Section 10.1.1.2 above serve as a guide for defining regulatory reporting obligations. The term severe is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe headache); the event itself, however, may be of relatively minor medical significance. This is not the same as serious, which is based on patient/adverse outcome. Therefore, an AE of severe headache might not be considered serious, but a moderate infection for which a patient is hospitalized should be reported as an SAE.

10.1.3 Relationship to Investigational Drug Treatment

An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the IP caused or contributed to an AE and must be provided for all AEs (serious and non-serious).

Avalyn Pharma's determination of attribution will be used for reporting to the appropriate health authorities. The relation of an AE to study participation will be determined using the descriptors and definitions provided in Table 9:

Table 9: Relationship of the AEs to the Study Drug

Relationship	Description
Not Related	The event is related to an etiology other than the investigational study drug (the alternative etiology will be documented in the study patient's medical record OR, the time of occurrence of AE is not reasonably related to administration of the study medication.
Unlikely Related	The event is unlikely to be related to the investigational study drug and likely to be related to other factors.
Possibly Related	There is an association between the event and the administration of study drug and there is a plausible mechanism for the even to be related to study drug, but there may also be alternative etiology, such as characteristics of the patient' clinical state or underlying disease.
Probably Related	There is an association between the event and the administration of study drug, a plausible mechanism for the event to be related and causes other than the study drug have been ruled out, and/or the event reappeared on re-exposure to the study drug.

The investigator is obligated to assess the relationship between IP and the occurrence of each AE. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the administration of IP will be considered and investigated. The investigator will also consult the IB and/or product information in the determination of his/her assessment.

The causal relationship of the AE to the IP, study devices or procedures should be assessed by the investigator (or medically qualified delegate) using the classifications provided in Table 9.

10.1.4 Collecting and Recording AEs

10.1.4.1 Period of Collection

All AEs will be collected from the time of informed consent through the final study visit. All AEs and SAEs should be treated as medically appropriate and followed until event resolution.

10.1.4.2 Methods of Collection

AEs may be collected as follows:

- Observing the patient
- Questioning the patient in an unbiased and non-leading manner
- Receiving an unsolicited complaint from the patient

An abnormal value or result from a clinical evaluation, laboratory value, etc. can also indicate an AE if it is determined by the investigator to be clinically significant. If this is the case, it must be recorded in the source document and as an AE on the appropriate AE/SAE form(s). The evaluation that produced the value or result should be repeated until that value or result returns to normal or is stabilized and the patient’s safety is not at risk.

10.1.4.3 Recording Method

10.1.4.3.1 AEs

All AEs occurring during this clinical study after informed consent is signed will be recorded by the investigator on the appropriate eCRF in precise medical terms, along with the date of onset and the date of resolution. To avoid vague, ambiguous, or colloquial expressions, the AE should be recorded in standard medical terminology rather than the patient's own words. Whenever possible, the investigator should combine signs and symptoms into a single term that constitutes a single diagnosis. Each AE is to be evaluated for duration, severity, seriousness, and relatedness to study drug, therefore if the AE occurs on the date of first dose, whether it occurred prior to the dose or after the dose will be also be recorded.

The severity of the AE and its relationship to the study drug will be assessed by the investigator. The investigator will treat participants experiencing AEs appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes. If any medication is administered in response to the AE, this medication should be noted on the Concomitant Medications and Therapies eCRF as a concomitant medication administered. The action taken and the outcome must also be recorded. The investigator will follow a non-serious AE until resolution, stabilization of the event up to the Follow-up visit. The investigator will follow an SAE (regardless of relationship to study drug until the event resolves, stabilizes, or becomes non-serious. The terms of AE resolution (i.e., recovered/resolved, not recovered/not resolved, recovered/resolved with sequelae, recovering/resolving, fatal, unknown) should also be recorded.

10.1.4.3.2 SAEs

SAEs will be recorded both on the AE eCRF and on the SAE form. Health authorities will be notified as outlined in Section 10.1.5.2.

10.1.5 Reporting AEs

10.1.5.1 Reporting SAEs to Avalyn Pharma

The following process for reporting an SAE ensures compliance with 21 CFR 312 and ICH guidelines. After learning that a participant has experienced an SAE, the investigator or designee is responsible for reporting the SAE, regardless of relationship or expectedness, within 24 hours of becoming aware of the event. The initial SAE report should include as much information as possible, but at a minimum must include the following:

- Reporter
- Patient Identifier
- Study product or intervention
- Serious AE term
- Relationship to study medication(s)
- Reason why the event is serious

Supplemental eCRF pages should be current at the time of SAE reporting: medical history, concomitant medications, demographics, study drug administration, and death as applicable.

Unavailable details of the event should not delay submission of the known information. As additional details become available, the SAE report form should be updated and re-submitted.

For additional information regarding SAE reporting, please refer to the clinical study manual. Safety monitoring will be handled by Syneos Health.

10.1.5.2 Reporting SAEs to Health Authorities

Avalyn Pharma or designee is responsible for reporting SAEs to the health authorities in accordance with the regulations for each country.

After the SAE has been reported by the site investigator and assessed by Avalyn Pharma, there are 2 options to report an event to the appropriate health authorities:

Standard reporting is required. This option applies if the AE is classified as one of the following:

- Serious, SAR per the definitions section (Section 10.1.1)
- Serious and not an SAR per the definitions section (Section 10.1.1)

Expedited reporting is required. This option applies if the AE/safety finding is classified as one of the following:

1. Serious and unexpected suspected adverse reaction (SUSAR) per the definitions section (Section 10.1.1)

    - Avalyn Pharma must report any SAR that is both serious and unexpected. Avalyn Pharma must report AE as an SAR only if there is evidence to suggest a causal relationship between the study product and the AE, such as:
    - A single occurrence of an event that is uncommon and known to be strongly associated with the product treatment (e.g. relevant associated events, e.g. angioedema, hepatic injury, or Stevens-Johnson Syndrome);
    - One or more occurrences of an event that is not commonly associated with product treatment but is otherwise uncommon in the population exposed to the product (e.g. relevant associated events, e.g. tendon rupture);
    - An aggregate analysis of specific events observed in a clinical study (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of IP therapy) that indicates those events occur more frequently in the treatment group than in a concurrent or historical control group.

2. Any safety findings from other studies: Avalyn Pharma must report any findings from other epidemiological studies, pooled analysis of multiple studies, or clinical or nonclinical studies that suggest a significant risk in humans exposed to the IP that would result in a safety-related change in the protocol, informed consent, IB, or other aspects of the overall conduct of the study.

These events must be reported by Avalyn Pharma to the appropriate health authorities within 15 calendar days; fatal or life-threatening events must be reported within 7 calendar days.

10.1.5.3 Reporting SAEs to IRBs or ECs

It is the responsibility of the investigators to promptly notify their respective Institutional Review Board(s) (IRBs) or Ethics Committees (ECs) of safety reports or other matters involving risk to patients as mandated by the IRBs/ECs.

10.1.5.4 Reporting SAEs to the Data Safety Monitoring Board

Avalyn Pharma will provide the DSMB with data of all SAEs on an ongoing basis.

10.1.5.5 Reporting Pregnancy

During the study, all patients should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period). If pregnancy is confirmed, the patient will be withdrawn from the study and followed until the pregnancy comes to term.

The investigator is responsible for reporting all available pregnancy information on the Pregnancy form within 24 hours of becoming aware of the event, although pregnancy itself if not considered an AE. The investigator should counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. The patient should be referred to an obstetrician/gynecologist experienced in reproductive toxicity for further evaluation and counseling. Monitoring of the participant should continue until the conclusion of the pregnancy. Follow-up information detailing the outcome of the pregnancy and the health of the newborn should be reported as it becomes available.

Any pregnancy complication or premature terminations including miscarriage, spontaneous abortion or elective termination of a pregnancy for medical reasons will be reported as an SAE, as described in Section 10.1.5. Should the pregnancy result in a congenital abnormality or birth defect, a separate SAE report must be submitted. Furthermore, all neonatal deaths that occur within 30 days of the birth should be reported as SAEs, without regard to causality. In addition, any infant death that occurs after the 30-day reporting period that the investigator suspects is related to the in-utero exposure to the study treatment should also be reported.

11 STATISTICS

11.1 Power and Sample Size Determination

This is an open-label study to determine safety and tolerability of AP01 50 mg once daily or 100 mg twice daily; both regimens being administered using the eFlow. With 50 patients per arm, we have a 92% chance of detecting an AE with a true population rate of 5%, as displayed in Table 10:

Table 10: Probability of observing adverse events

	Actual probability of event		
	1%	**5%**	**10%**
Chance of observing ≥ 1 event	0.395	0.923	0.995

The Phase 3 study of oral pirfenidone in patients with IPF (ASCEND)[12] showed a standard deviation (SD) of 4.5 based on the imputed mean change in % of predicted FVC at Week 26. Assuming a SD of 4.5, with 50 patients per arm, a two-sided 95% CI for mean change from baseline in % of predicted FVC will extend 1.25 from the observed mean. The ASCEND[12] study showed a decrease of 1.5 and 3.9 in % of predicted FVC at Week 26 in the pirfenidone and placebo arms, respectively. Table 11 shows results of the ASCEND[12] active and placebo arms and potential observed differences in the AP01-002 study, assuming a similar SD.

Because we have no prior data on efficacy outcomes with our compound, we are unable to make well informed predictions of effect size or variability. In lieu of that information, we have included some estimates of confidence intervals based on prior oral pirfenidone studies; however, we cannot be confident that our product will behave similarly and would not base hypothesis tests on this information. This study will give us an idea of which endpoints are most likely to be positively impacted by our product as well as solid estimates of both effect size and variability. In addition, if the effect is due to AUC or multiple peaks/day in the ELF rather than peak levels, based on our modeling from the BAL cohort in the AP01-001 study, we have around 75% power to show a difference between arms. With this sample size we can be confident on selecting dosing regimen(s) for future trials.

Table 11: Observed and potential change in FVC

	ASCEND[12] Placebo (n = 262)	**ASCEND[12] Active (n = 255)**	**AP01-002 Potential Scenarios (n = 50)**		
Observed Change FVC % predicted (mean, SD)	-3.9, 5.2	-1.5, 4.5	-0.75, 4.5	-1.0, 4.5	-1.5, 4.5
95% CI	(-4.53, -3.27)	(-2.05, -0.95)	(-2.0, 0.5)	(-2.25, 0.25)	(-2.75, -0.25)

11.2 General Considerations

All data will be summarized by treatment group and overall. A separate summary of data from Part A of will be provided in addition to a full study summary including both Part A and Part B data. Categorical data will be summarized as number and percent of total. Continuous data will be summarized using

mean, median, SD and range. Dose response will be evaluated by comparing safety, and changes in efficacy variables across the dose regimens.

All individual patient data will be displayed in listings. For comparisons to baseline, data from Visit 2 prior to the first dose of study drug will be used as baseline. Subgroup analyses will be performed based on baseline groupings for FVC, region and progression-related biomarkers (CXCL13**,** CCL18, MMP3).

If either regimen is eliminated based on Part A outcomes, patients may be switched to the selected regimen for the remainder of their participation. Data summaries will be created to mimic this switch. Data listings will include a variable to denote the regimen switch and flag the time of the switch for affected patients.

Patients will be analyzed according to randomized treatment.

Details of data handling and summaries will be further defined in the Statistical Analysis Plan (SAP).

11.2.1 Significance Levels

No formal hypothesis tests are planned.

11.2.2 Multiple Comparisons

No formal hypothesis tests are planned.

11.2.3 Missing Data

Due to the COVID-19 pandemic, which occurred while the study was actively enrolling, additional strategies are included for handling missing data. Missing values will not be imputed for data displayed in summary tables. For patients with missing data due to death, remaining FVC values will be imputed as a sensitivity analysis using a lower value of FVC than the patient's last observed value. Because home spirometry data collection continues when COVID-19 prevented in-clinic visits, home spirometry FVC will be used to inform missing office spirometry data handling for reasons other than early terminations. Full details of imputation methods will be included in the SAP.

11.2.4 Visit Windows

All data collected during study follow-up will be displayed according to the actual visit in the eCRF. Assessments taken outside of windows described in the protocol will be displayed and analyzed as outlined in the SAP.

11.3 Analysis Populations

The analysis populations are defined as follows:

- The safety population is defined as all randomized patients who receive the study drug.
- The per-protocol (PP) population will include all patients who have sufficient data to assess the primary efficacy endpoint, and who have no major protocol deviations that would impact the ability to appropriately assess the primary endpoint. The details of major protocol deviations that exclude patients from the PP population will be defined in the SAP.

Safety analyses will be performed on the safety population.

Summaries of efficacy endpoints will be created using the PP population.

11.4 Background and Demographic Characteristics

Demographic and baseline characteristics will be summarized by treatment arm and overall.

11.5 Efficacy and Safety Analyses

11.5.1 Efficacy Analyses

The following analyses will be performed:

- Change from Baseline in absolute FVC % predicted
- Change from Baseline in cough frequency as measured by LCM
- Change from Baseline in the total KBILD score and in KBILD breathlessness and activity domains
- Change from Baseline in the total LCQ score.
- Change from Baseline in cough severity as measured by VAS
- Change from Baseline in extent of fibrosis and lung volumes as assessed by HRCT
- Change from Baseline in D_{LCO}

The primary endpoint of interest for efficacy is the absolute change from Baseline to Week 24 in FVC % predicted. Linear mixed-effects modeling will be used and differences between treatment arms will be examined. Baseline FVC, region and progression-related biomarker (CXCL13**,** CCL18, MMP3) values will be considered as covariates in the model. Further details on the assumptions and planned analyses will be provided in the SAP. Similar modeling strategies will be employed for longer term data obtained from Part B of the study. No control group is included in this study, however exploratory analyses will be conducted using registry and historical data as comparators. Details of all analyses will be provided in the SAP.

In order to assess the impact of treatment on cough in those patients for whom coughing is of greatest concern, additional analyses of cough frequency will be performed on the subset of patients with baseline cough severity VAS > 40 millimeters and in patients with baseline cough frequency ≥ 10 coughs/hour during awake hours.

11.5.2 Safety Analyses

Treatment-emergent AEs (any AEs recorded during or following the first study drug administration) will be summarized by treatment group and categorized by severity and relationship to the study procedures and to the IP. If a patient has more than 1 occurrence of the same AE, he/she will be counted only once within that preferred term in the summary tables. The most severe occurrence of an AE, as well as the most extreme relationship of the AE to the study procedures and IP, will be indicated in cases of multiple occurrences of the same AE. SAEs will be summarized separately.

All AEs will be presented in a listing. Additionally, listings of SAEs and AEs leading to discontinuation will be generated. All SAEs will be evaluated to determine whether they are SUSARs or Unexpected Adverse Device Effects.

11.6 Pharmacokinetic Analyses

This study will have no pharmacokinetic parameters assessed.

11.7 Interim Analyses

The DSMB will perform a review of safety data from the first 20 patients after Week 4 to confirm the safety of multiple dose administration. A decision to stop, continue or modify the study will be made based on this review. Throughout the course of the study, all SAEs will be sent to the DSMB for review.

At the completion of Part A, all endpoints will be analyzed and a full set of tables, listings and figures will be generated. At the end of Part B, all analyses will be produced including data from both Parts A and B of the study.

12 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

12.1 Study Monitoring

According to ICH GCP guidelines, Avalyn Pharma is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of data recorded on the eCRFs. The CRO is responsible for assigning the study monitor(s) to this study. The study monitor's duties are to aid the investigator in the maintenance of complete, accurate, legible, well-organized, and easily retrievable data. The study monitor will advise the investigator of the regulatory necessity for study-related monitoring, audits, IRB(s) or EC(s) review, and inspection by providing direct access to the source data/documents. In addition, the study monitor will explain to and interpret for the investigator all regulations applicable to the clinical evaluation of an investigational drug and nebulizer, as documented in ICH guidelines.

It is the study monitor's responsibility to inspect the eCRFs and source documentation throughout the study to protect the rights of the patients; to verify adherence to the protocol; to verify completeness, accuracy, and consistency of the data; and to confirm adherence of study conduct to any local regulations. Details will be outlined in the clinical monitoring plan.

12.2 Source Documents

Avalyn Pharma requires that the investigator prepare and maintain adequate and accurate records for each patient treated with the investigational drug. Source documents such as any hospital, clinic, or office charts and the signed informed consent forms are to be included in the investigator's files with the patient's study records.

Study data will be captured electronically; study site personnel will record eCRF data from source documents. Patients will record selected study assessments directly into the eCRF. If any data are first recorded onto documents such as laboratory reports, these documents will be considered source.

12.3 Data Collection and Management

This study will be conducted in compliance with the ICH document "E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1)," dated March 2018. This study will also be conducted in accordance with the Declaration of Helsinki (2013).

This study will use electronic data collection techniques to collect data directly from the investigational site using eCRFs. The data will be stored centrally in a fully validated clinical database. The investigator is responsible for ensuring that all sections of each eCRF are completed promptly and correctly and that entries can be verified against any source data.

Study monitors will perform source document verification to ensure there are no inconsistencies between the eCRFs and source documents. Discrepancies will be resolved in accordance with the principles of GCP. Detailed study monitoring procedures are provided in the clinical monitoring plan.

Data management will be coordinated by the CRO in accordance with their SOPs and a formal study data management plan.

AEs will be coded with MedDRA. Concomitant medications will be coded using World Health Organization – Drug Reference List.

Data from outside sources (e.g. HRCT results, home spirometry results, LCM results, lab results, etc.) will be integrated into the clinical database.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Quality assurance includes all the planned and systematic actions that are established to ensure that the clinical study is performed and the data are generated, documented (recorded), and reported according to ICH GCP and local/regional regulatory standards.

A quality assurance representative from Avalyn Pharma, the CRO or its designee, who is independent of and separated from routine monitoring, may periodically arrange inspections/audits of the clinical study by reviewing the data obtained and procedural aspects. These inspections may include on-site inspections/audits and source data checks. Direct access to source documents is required for the purpose of these periodic inspections/audits.

14 ETHICS

14.1 Ethics Review

The investigator will not start this study, nor will devices be shipped to the investigator's site, before providing Avalyn Pharma and/or the CRO with evidence of IRB(s) or EC(s) approval. The investigator will promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to patients. The investigator will not make any changes in the research without IRB(s) or EC(s) approval, except where necessary to eliminate apparent immediate hazards to the patients. The investigator will provide progress reports to the IRB(s) or EC(s) as required by the IRB(s) or EC(s). The investigator will provide a final report to the IRB(s) or EC(s) after completion of participation in the study.

14.2 Ethical Conduct of the Study

The investigator should conduct the study in accordance with this protocol, the Declaration of Helsinki, and ICH GCP guidelines. The investigator and Avalyn Pharma and/or the CRO will sign the protocol and study contract to confirm agreement. The investigator will not implement any amendment (deviation or changes of the protocol) without agreement by Avalyn Pharma and/or the CRO and the IRB(s) or EC(s) approval/information, except where necessary to eliminate immediate hazards to study patients or when changes involve only logistical or administrative aspects of the study.

14.3 Written Informed Consent

14.3.1 Patient Information and Informed Consent

The informed consent document will be approved by the IRB(s) or EC(s) that is appropriate for each study site. The investigator is responsible for ensuring that the patient fully understands the nature and purpose of the study. Information should be given in both oral and written form whenever possible. No patient should be obliged to participate in the study. Patients, their relatives, guardians, or (if applicable) legal representatives must be given ample opportunity to inquire about details of the study. The information must make clear that refusal to participate in the study or withdrawal from the study at any stage is without any prejudice to the patient's subsequent care. Patients must be allowed sufficient time to decide whether they wish to participate. Patients will provide consent after they have read the Informed Consent Form and the investigator or designee has answered any questions they may have about the study.

The patient must be made aware of and give consent to direct access to his/her source medical records by study monitors, auditors, the IRB(s) or EC(s), and regulatory authorities. The patient should be informed that such access will not violate patient confidentiality or any applicable regulations. The patient should also be informed that he/she is authorizing such access by signing the informed consent form.

Each patient will be given a signed copy of the informed consent form to keep for his/her records.

Patient's choosing to participate in the Rollover study (AP01-005) may be asked to provide consent to continue the treatment that they are receiving at Visit 12 on this study and then transition to the Rollover study upon approval of AP01-005 at their site.

14.3.2 Provision of New and Important Information Influencing Patient's Consent

When any new and important information that may be relevant to the patient's consent is obtained, the investigator and Avalyn Pharma, the CRO, and/or their designee(s) will consult with each other on how to deal with the information. When Avalyn Pharma, the CRO, and/or their designee(s), and a responsible investigator judge it necessary, the investigator must immediately provide the patients with such information, revise the written information and other explanatory documents based on the new information, and obtain approval from the IRB(s) or EC(s). In this instance, the investigator should also immediately inform patients currently participating in the clinical study of such information, confirm their intention to continue participation, re-explain the study to them using the revised written information and other explanatory documents, and obtain written consent to continue participation based on their voluntary decision.

14.4 Patient Confidentiality

Individual patient medical information obtained as a result of this study is considered confidential, and disclosure to third parties is prohibited. Information will be accessible to authorized parties or personnel only. Medical information may be given to the patient's physician or to other appropriate medical personnel responsible for the patient's well-being. Each patient will be asked to complete a form allowing the investigator to notify the patient's primary health care provider of his/her participation in this study.

15 ADMINISTRATIVE PROCEDURES

15.1 Publications of the Clinical Study

The clinical study plan and the results of the study will be published on the World Health Organization's International Clinical Trials Registry Platform. The results of and data from this study belong to Avalyn Pharma Inc.

15.2 Protocol Amendments and Deviations

No change or amendment to this protocol may be made by the investigator, Avalyn Pharma and/or the CRO after the protocol has been agreed to and signed by all parties unless such change(s) or amendment(s) has (have) been agreed upon by the investigator, Avalyn Pharma and/or the CRO. Any change agreed upon will be recorded in writing, and the written amendment will be signed by the investigator and Avalyn Pharma and/or the CRO. IRB or EC approval is required prior to the implementation of an amendment, unless overriding safety reasons warrant immediate action, in which case the IRB(s) or EC(s) will be promptly notified.

No waivers to inclusion/exclusion criteria will be granted; patients need to meet all criteria, exactly as specified, to be enrolled. No deviation from the protocol or investigational plan will be made except to protect the life or physical well-being of a patient in an emergency. Additionally, prospective deviations from the protocol or investigational plan are not permitted except to protect the life or physical well-being of a patient in an emergency. Deviations that occur unintentionally or are the result of action by the patient must be documented and reported to Avalyn Pharma and to the IRB(s) or EC(s), if applicable, according to regulations. Further details about the documentation, evaluation, and follow-up of protocol deviations are detailed in this study's clinical monitoring plan.

15.3 Data and Safety Monitoring Board

An independent DSMB will review accumulated individual safety data for the first 20 patients after their first four weeks of treatment. The data to be reviewed will be unblinded since this is an open-label study. Based on this review, the DSMB will make a recommendation to continue, modify or stop the study. Enrollment will continue during the DSMB review unless 5 or more of the first 20 patients are discontinued due to intolerance.

At any time during the study, should any untoward safety issue be observed, the DSMB Chair may schedule an immediate meeting to review the relevant safety data.

16 DATA HANDLING AND RECORD KEEPING

16.1 Inspection of Records

Avalyn Pharma and/or the CRO, their designee(s), the IRB(s) or EC(s), or regulatory authorities will be allowed to conduct site visits to the investigational facilities for the purpose of monitoring or inspecting any aspect of the study. The investigator agrees to allow Avalyn Pharma and/or the CRO, their designee(s), the IRB(s) or EC(s), or regulatory authorities to inspect the investigational drug and device storage area, investigational drug and device stocks, investigational drug and device records, patient charts and study source documents, and other records relative to study conduct.

16.2 Retention of Records

The principal investigator must retain all documentation relating to the study for a period of at least 2 years after the last marketing application approval or, if not approved, 2 years following the discontinuance of the test article for investigation. If this requirement differs from any local regulations, the local regulations will take precedence unless the local retention policy is less than 2 years.

16.3 Sample Retention

Samples may be used for purposes related to this research. The samples may be stored until the study team has determined that specimens are no longer needed and the decision has been made that there are no samples to be re-assayed. In addition, identifiable samples can be destroyed at any time at the request of the patient.

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17 APPENDICES

17.1 ERS Cough Visual Analogue Scale for Cough (Example)

17.2 The King's Brief Interstitial Lung Disease (K-BILD) Questionnaire

17.3 Leicester Cough Questionnaire

17.4 Protocol Amendments

Appendix 17.1 Cough Visual Analogue Scale (Example)

Please put a cross on the line to indicate the severity of your cough in the last 2 weeks.

WORST COUGH EVER

NO COUGH

17.2 The King's Brief Interstitial Lung Disease Questionnaire (K-BILD)

The King's Brief Interstitial Lung Disease Questionnaire (K-BILD)©2011

This questionnaire is designed to assess the impact of your lung disease on various aspects of your life. Please circle the response that best applies to you for each question

1. In the last 2 weeks, I have been breathless climbing stairs or walking up an incline or hill.						
1. Every time	2. Most times	3. Several Times	4. Some times	5. Occasionally	6. Rarely	7. Never
2. In the last 2 weeks, because of my lung condition, my chest has felt tight.						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
3. In the last 2 weeks have you worried about the seriousness of your lung complaint?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
4. In the last 2 weeks have you avoided doing things that make you breathless?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
5. In the last 2 weeks have you felt in control of your lung condition?						
1. None of the time	2. Hardly any of the time	3. A little of the time	4. Some of the time	5. A good bit of the time	6. Most of the time	7. All of the time
6. In the last 2 weeks, has your lung complaint made you feel fed up or down in the dumps?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
7. In the last 2 weeks, I have felt the urge to breathe, also known as 'air hunger'.						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
8. In the last 2 weeks, my lung condition has made me feel anxious.						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
9. In the last 2 weeks, how often have you experienced 'wheeze' or whistling sounds from your chest?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
10. In the last 2 weeks, how much of the time have you felt your lung disease is getting worse?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
11. In the last 2 weeks has your lung condition interfered with your job or other daily tasks?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
12. In the last 2 weeks have you expected your lung complaint to get worse?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
13. In the last 2 weeks, how much has your lung condition limited you carrying things, for example, groceries?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
14. In the last 2 weeks, has your lung condition made you think more about the end of your life?						
1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. Hardly any of the time	7. None of the time
15. Are you financially worse off because of your lung condition?						
1. A significant amount	2. A large amount	3. A considerable amount	4.A reasonable amount	5. A small amount	6. Hardly at all	7. Not at all

17.3 Leicester Cough Questionnaire

This questionnaire is designed to assess the impact of cough on various aspects of your life. Read each question carefully and answer by CIRCLING the response that best applies to you. Please answer ALL questions, as honestly as you can.

1. In the last 2 weeks, have you had chest or stomach pains as a result of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

2. In the last 2 weeks, have you been bothered by sputum (phlegm) production when you cough?

1	2	3	4	5	6	7
Every time	Most times	Several times	Some times	Occasionally	Rarely	Never

3. In the last 2 weeks, have you been tired because of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

4. In the last 2 weeks, have you felt in control of your cough?

1	2	3	4	5	6	7
None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

5. How often during the last 2 weeks have you felt embarrassed by your coughing?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

6. In the last 2 weeks, my cough has made me feel anxious

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

7. In the last 2 weeks, my cough has interfered with my job, or other daily tasks

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

8. In the last 2 weeks, I felt that my cough interfered with the overall enjoyment of my life

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

9. In the last 2 weeks, exposure to paints or fumes has made me cough

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

10. In the last 2 weeks, has your cough disturbed your sleep?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

11. In the last 2 weeks, how many times a day have you had coughing bouts?

1	2	3	4	5	6	7
All of the time (continuously)	Most times during the day	Several times during the day	Some times during the day	Occasionally through the day	Rarely	None

12. In the last 2 weeks, my cough has made me feel frustrated

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

13. In the last 2 weeks, my cough has made me feel fed up

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

14. In the last 2 weeks, have you suffered from a hoarse voice as a result of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

15. In the last 2 weeks, have you had a lot of energy?

1	2	3	4	5	6	7
None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

16. In the last 2 weeks, have you worried that your cough may indicate serious illness?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

17. In the last 2 weeks, have you been concerned that other people think something is wrong with you, because of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

18. In the last 2 weeks, my cough has interrupted conversation or telephone calls

1	2	3	4	5	6	7
Every time	Most times	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

19. In the last 2 weeks, I feel that my cough has annoyed my partner, family or friends

1	2	3	4	5	6	7
Every time I cough	Most times when I cough	Several times when I cough	Some times when I cough	Occasionally when I cough	Rarely	Never

Thank you for completing this questionnaire.

17.4 Protocol Amendments

Version 2.1 dated 12 OCT 2020

Date	Section	Change from	Change to	Justification
12OCT2020	Synopsis, Sections 1..2.4, 4.1, 8.2	Previous verbiage	Various revisions and additions of verbiage to document the DSMB change in dose for 50 mg daily dose during Part B of the study	DSMB meeting on 12 OCT 2020 advised for all patients in Part B to receive the 100 mg twice daily dose
12OCT2020	Synopsis	Unscheduled treatment visit	Monitoring safety visit	Clarification of visit name