The Effects of a Novel Formulation of Inhaled Cromolyn Sodium (PA101) in Idiopathic Pulmonary Fibrosis and Chronic Cough: A Randomized, Double-blind, Proof-of-concept, Phase 2 Trial

Authors: [1]Professor Surinder S Birring, MD [2]Marlies S Wijsenbeek, MD [3]Sanjay Agrawal, MD [4]Jan WK van den Berg, MD [5]Helen Stone, MD [6]Professor Toby M Maher, MD [7]Ahmet Tutuncu, MD and [8]Professor Alyn H Morice, MD.

Affiliations: [1]Division of Asthma, Allergy and Lung Biology, King's College London, UK; [2]Dept. Respiratory Medicine, Erasmus University Medical Center, Rotterdam, Netherlands; [3]Dept. Respiratory Medicine, Glenfield Hospital, Leicester, UK; [4]Dept. Pulmonology, Isala Hospital, Zwolle, Netherlands; [5]Dept. Respiratory Medicine, Royal Stoke University Hospital, Stoke-on-Trent, UK; [6]Royal Brompton Hospital, London, UK & Fibrosis Research Group, National Heart and Lung Institute, Imperial College, London, UK; [7]Patara Pharma, San Diego, California, USA; [8]Hull York Medical School, Castle Hill Hospital, Hull, UK.

Corresponding Author: Professor Surinder S Birring; King's College London, Division of Asthma, Allergy and Lung Biology, Denmark Hill, London, SE9 5RS. Email address: surinder.birring@nhs.net.

Funding: This study was funded by Patara Pharma, LLC (San Diego, CA, USA).

Key words: Idiopathic pulmonary fibrosis, cough, chronic cough, idiopathic cough, chronic idiopathic cough, cough frequency, cromolyn, cromolyn sodium, disodium cromoglycate, quality of life.

Word count: 4291

ABSTRACT

Background:

Cough can be a debilitating symptom of idiopathic pulmonary fibrosis (IPF) and is difficult to treat. PA101 is a novel formulation of cromolyn sodium delivered via a high efficiency eFlow® nebuliser that achieves significantly higher lung deposition compared to the existing formulations. The efficacy and safety of inhaled PA101 in IPF patients with chronic cough was investigated in a multi-center, randomised, double-blind, placebo-controlled, 2-period, cross-over trial. To explore the mechanism of anti-tussive activity of PA101, a parallel study of similar design was conducted in patients with chronic idiopathic cough (CIC).

Methods:

Twenty-four participants with IPF and chronic cough were randomised to receive PA101 (40 mg) or matching placebo three times a day for 2 weeks, followed by 2 weeks wash out, and then crossed over. The primary outcome measure was objective daytime cough frequency (from 24 hour acoustic recording, Leicester Cough Monitor) and secondary outcomes included subjective cough-specific quality of life (Leicester Cough Questionnaire [LCQ]) assessed at Day 14. The primary efficacy analysis was based on a mixed effects model. In the CIC cohort, twenty-eight participants were randomised in a study with similar design and endpoints. The study was registered with ClinicalTrials.gov (NCT02412020) and the EU Clinical Trials Register (EudraCT Number 2014-004025-40).

Findings:

In IPF patients, PA101 reduced daytime cough frequency by 31.1% at day 14 compared to placebo. ~~ratio of LS Means [95% CI]; 0.67 [0.48, 0.94], p=0.0241.~~ Daytime cough frequency decreased from a mean (SD) of 55 (55) coughs/hour at baseline to 39 (29) coughs/hour at day 14

following treatment with PA101 versus 51 (37) coughs/hour at baseline to 52 (40) cough/hour following treatment with placebo. The reduction in mean daytime cough frequency at Day 14 was significantly greater with PA101 compared to placebo (ratio of LS Means [95% CI]; 0.67 [0.48, 0.94], p=0.0241). ~~There was no significant difference in change in total LCQ scores with PA101 treatment compared to placebo; mean change from baseline to day 14: 1.06 with PA101 compared to 0.01 with placebo (mean treatment difference of 1.1, p=0.091)~~. In contrast, no treatment benefit for PA101 was observed in the CIC cohort; mean reduction of daytime cough frequency at Day 14 for PA101 adjusted for placebo was 6.2%. The ratio of LS Means [95% CI] was 1.27 [0.78, 2.06], p=0.31. PA101 was well tolerated in both cohorts. The incidence of adverse events was comparable between PA101 and placebo treatments, and the majority of AEs were mild in severity.

Interpretation:

This study suggests that the mechanism of cough in IPF may be disease specific. Inhaled PA101 may be a treatment option for chronic cough in patients with IPF and warrants further investigation.

This study was sponsored by Patara Pharma, LLC, San Diego, California, USA.

Panel: Research in context

Evidence before this study

Chronic cough in Idiopathic Pulmonary Fibrosis (IPF) has a significant negative impact on the quality of life of patients, is usually refractory to medical therapy, and is a marker of disease progression. There are currently no approved drugs for the treatment of IPF cough. To date, there has been only one randomized placebo-controlled trial for IPF cough which was a single-centre study conducted in 20 patients with thalidomide. Thalidomide significantly improved cough related quality of life but was poorly tolerated.

Added value of this study

This is a randomised placebo-controlled multi-centre trial of a novel formulation of cromolyn sodium (inhaled PA101) for the treatment of IPF cough. A parallel study of similar design was also conducted in patients with chronic idiopathic cough (CIC) to explore the mechanism of cough and the potential of PA101. Treatments were delivered via a high efficiency nebuliser (eFlow) over 14 days. Treatment with PA101 in IPF was associated with a significant reduction in both objective daytime and 24-hour cough frequency compared to placebo. PA101 was well tolerated, with adverse events comparable to placebo. In contrast, in patients with chronic idiopathic cough, no treatment benefit was observed for PA101.

Implications of all available evidence

The mechanism of cough in IPF appears to be disease specific. Inhaled PA101 may offer a treatment option for chronic cough in IPF. Future studies need to evaluate dose response, treatment duration, and any potential effect on disease progression.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a progressive life-threatening disease with a median survival of 3-5 years.[1] The prevalence of IPF in the United States has been estimated to be 42.7 per 100,000 people.[2] Cough in IPF is often a debilitating symptom and is usually refractory to medical therapy.[3-5] The prevalence of cough in IPF has been reported to be 80%, and it is an independent predictor of disease severity, time to death, or need for lung transplantation.[3,6]

Mast cells are thought to be important in the pathogenesis of both cough and IPF.[7,8] Cromolyn sodium is used for maintenance treatment of allergic asthma and indolent systemic mastocytosis. The mechanism of action of cromolyn sodium is not known but it is believed to have pleotropic effects.[9,10] Historically, cromolyn's activity has been attributed to inhibition of mast cell degranulation and the consequential inhibition of mast-cell mediated immune activation.[9,11] Recently, cromolyn has been reported to reduce c-fibre sensory nerve activity via an orphan G-protein coupled receptor, GPR35.[12] Furthermore, GPR35 has been described as a target for cromolyn and is known to be expressed on immune cells and small diameter sensory neurons.[13-15] Previously approved formulations of inhaled cromolyn sodium are thought to achieve low lung deposition due to their low concentration and inefficient delivery devices.[16] PA101 is a novel high concentration formulation of cromolyn sodium with osmolality and pH adjusted to a physiologically tolerable range and delivered via a high efficiency electronic nebulizer, eFlow® (PARI, Germany).

This pilot, proof of concept study investigated the safety and efficacy of inhaled PA101 on objective cough frequency and cough specific quality of life in a randomised, double-blind, placebo-controlled trial in IPF patients with chronic cough. To explore the mechanism of chronic

cough and the anti-tussive activity of PA101, a parallel study of similar design was conducted in patients with chronic idiopathic cough (CIC).

METHODS

Study Design and Participants

This multi-center, randomised, double-blind, placebo-controlled, 2-cohort, 2-period, cross-over trial was performed in participants with IPF across 7 centers in the United Kingdom and the Netherlands. The study was conducted between 13 February 2015 and 02 February 2016. The diagnosis of IPF was based on the consensus of a multidisciplinary team based on the presence of definitive or possible usual interstitial pneumonia (UIP) pattern on high-resolution computed tomography (HRCT) and after excluding alternative diagnoses such as lung diseases associated with environmental and occupational exposure, connective tissue diseases and drug therapy. Chronic cough was defined as duration greater than 8 weeks and cough that was not responsive to targeted therapies for possible underlying triggers (gastro-esophageal reflux, asthma, and rhinitis). Participant inclusion criteria were adults aged 40 to 79 years old, daytime cough severity ≥ 40 mm on a visual analogue scale (VAS), mean daytime objective cough frequency ≥15 coughs per hour as measured with the Leicester Cough Monitor (LCM), TLCOc > 25% of predicted value within 12 months and FVC > 50% of the predicted value within 1 month.

Participant exclusion criteria were presence of significant coronary artery disease (myocardial infarction within 6 months or unstable angina within 1 month), upper or lower respiratory tract infection within 4 weeks, productive cough, acute exacerbation of IPF within 3 months, long-term daily oxygen therapy (>10 hours/day) and pulmonary arterial hypertension with limitation of activity. Participants who had taken the following medications within 2 weeks of the study

were also excluded: prednisone, narcotic antitussives, gabapentin, inhaled corticosteroids, dextromethorphan, carbetapentane, H1 antihistamines, and cromolyn sodium. Anti-fibrotic therapy (i.e., pirfenidone and nintedanib) was allowed during the course of the study provided that participants had taken a stable dose for at least 3 months prior to study start and remained on stable doses during the study. All eligible participants provided written informed consent.

A second cohort of participants with chronic idiopathic cough (CIC) was investigated across 4 centers using the same study design and assessments. A total of 28 participants with CIC that were unresponsive to targeted treatment for identified underlying triggers (i.e., post-nasal drip, asthmatic/non-asthmatic eosinophilic bronchitis, and gastro-esophageal reflux disease) were enrolled across 4 centers. Participant inclusion criteria were adults aged 18 to 75 years old, daytime cough severity ≥ 40 mm using VAS, daytime objective cough frequency ≥15 coughs per hour, and not using other antitussive medications within 2 weeks of the study. Exclusion criteria were similar to the IPF cohort.

Randomisation, Treatments and Protocol

Participants were randomly allocated PA101 40mg or identical matching placebo (manufactured by Holopack GmbH, Sulzbach, Germany) three times daily (TID) via oral inhalation for 14 days (±1 day) in a 1:1 ratio to one of two sequences (PA101 40 mg in treatment period 1, identical matching placebo in treatment period 2; placebo in treatment period 1, PA101 40 mg in treatment period 2) using a computer-generated randomisation schedule. The randomisation was stratified by cohort (IPF, CIC) and a block size of 4 was used in each of the two strata. There was a washout period of 14 days (±2 days) between the two treatment periods (figure 1). Study participants, investigators and study staff, and the sponsor remained blinded to the randomisation

scheme until the blind was formally broken after all subjects completed the study. Both the active and the placebo treatments were administered using the eFlow high efficiency nebuliser. The eFlow is a single-patient, multi-use, portable, silent, battery-operated, electronic nebuliser that uses a vibrating mesh membrane to generate smaller particle aerosol that allows for more targeted and homogenous deposition of drug to the lung compared to a general-purpose nebuliser.[17,18]

Each participant attended a screening visit within 14 days of the start of the study to undergo eligibility assessments that included medical history, physical examination, vital signs, electrocardiograph (ECG), blood and urinary laboratory assessments. A cough monitor (LCM) was attached in the morning for 24 hours during pre-treatment (day -1), day 7 and day 14 visits for assessment of efficacy. A further safety follow-up call was arranged within 7 days following the last study treatment.

Outcome measures and safety assessments

The primary efficacy outcome daytime cough frequency was assessed from 24 hours cough recording using the Leicester Cough Monitor (LCM), a validated, objective, semi-automated and ambulatory cough monitoring device.[19-21] The patient-reported secondary efficacy outcomes were cough specific quality of life as measured by the Leicester Cough Questionnaire (LCQ)[22] and cough severity as measured by a visual analogue scale (VAS, 0-100 mm). The LCQ is a validated 19-item cough-specific health-related quality of life questionnaire. Overall scores range from three to twenty-one with a higher score indicating a better quality of life. The minimal clinically important difference for LCQ is 1.3.[23]

Exploratory efficacy variables included the ILD-specific quality of life measured by King's Brief Interstitial Lung Disease Questionnaire (K-BILD),[24] pulmonary function tests (PFTs), and fraction of exhaled nitric oxide (FeNO) as measured by Niox Vero. PFTs were conducted in accordance with the American Thoracic Society/European Respiratory Society (ATS/ERS) 2005 guidelines.[25]

Ethics and trial registration

Liverpool Central/United Kingdom, reference no. 14/NW/1405, and METC Isala Zwolle and METC Erasmus Medical Center/Netherlands. The study was registered at ClinicalTrials.gov (NCT02412020) and the EU Clinical Trials Register (EudraCT Number 2014-004025-40).

Statistical Analysis

Primary efficacy analysis for both IPF and CIC cohorts was based on the intent-to-treat population (efficacy analysis set, EAS), which included all participants who received at least one dose of study drug and who had at least one post-baseline efficacy measurement. An additional analysis was also performed using a per-protocol population (PP), which included all participants who completed both treatment periods and who did not have major protocol violations. The endpoints for IPF and CIC cohorts were similar except the additional secondary endpoint of IPF specific quality of life assessed with the K-BILD for the IPF cohort. Safety analysis was based on all participants who took at least one dose of study drug. The primary efficacy endpoint for both IPF and CIC cohorts was the change from baseline in daytime cough counts per hour. The 24-hour and night-time cough frequencies were also assessed. Daytime was defined by the time the participant reported they were awake in the morning and the time they went to bed. Prior to analysis of the daytime, nighttime, and 24-hour average cough counts at day 7 and day 14, a log

transformation was applied at each visit. A mixed effects model was used for the change from baseline at each visit with sequence, treatment, and period as fixed effects, the baseline value as a covariate, and patient nested within sequence as a random effect. The percent change in cough frequency at Day 14 was obtained from data from both treatment periods and no statistical comparisons were made. No imputation for dropouts or missing data for assessments not completed at individual visits were performed. For the analyses, if there were any missing data, then the F-tests from PROC MIXED were based on Kenward-Roger's adjusted degrees of freedom. Responders were defined as a greater than 30% percentage decrease in day-time cough frequency from baseline to day 14. A sample size of 24 subjects would have approximately 80% power to detect 30% change in daytime cough frequency at the 5% statistical significance level with a 2-sided α error level of 5% in a 2-treatment crossover study for each study cohort.[26] Treatment-emergent adverse events (TEAE) were coded according to the MedDRA dictionary version 18.0. All statistical analyses were performed using SAS software version 9.2.

Role of funding source

This study was sponsored by Patara Pharma, LLC, San Diego, California, USA. The sponsor participated in the study design, study oversight, medical monitoring, data management, analysis, and reporting of the data. SSB and AT had access to the data, which was analysed by a statistician (Chuck Davis, PhD), SBB and AT. SSB, AT and AHM made the decision to submit for publication. All authors contributed to the writing and edited this manuscript. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

RESULTS

IPF COHORT

Participants

A total of 33 participants with IPF were screened and 24 eligible patients (mean age 67 years, male 63%, mean forced vital capacity [FVC] 73% predicted, FEV_1/FVC 83%, and median cough duration 5.6 years) were randomised. All but two patients completed both treatment periods; two patients were withdrawn from the study following completion of period 1 and before period 2 (figure 2). The baseline demographics and clinical characteristics of randomised patients are summarized in table 1. No participant had a diagnosis of asthma and all had blood eosinophil levels at baseline within normal limits ($<0.5x10^9$/L). No patient was taking angiotensin converting enzyme inhibitor medication. The proportion of patients on IPF treatments (pirfenidone or nintedanib), proton pump inhibitor, or H2 antagonist therapy were 54% (13/24), 67% (16/24) and 0% (0/24), respectively. The baseline characteristics were comparable for each treatment period between PA101 and placebo groups.

Primary Efficacy Variable –Daytime Cough Frequency

The efficacy results for the efficacy analysis set (EAS) population and the per-protocol (PP) population were comparable for all variables, therefore only the results for the EAS population are presented. At baseline, there was a significant correlation between daytime cough frequency and cough severity (VAS) and cough quality of life (LCQ total score), correlation coefficients r= 0.683 (p=0.0003) and r= -0.682 (p=0.00002), respectively. There was no period effect (p=0.28), baseline value effect (p=0.18) or treatment sequence effect (p=0.91). Data from combined periods 1 and 2 are presented.

At baseline, mean daytime cough counts for PA101 and placebo treatments were comparable (55 vs 51 coughs/hour, respectively) as were mean 24-hour cough counts (mean of 40 vs 38 coughs/hour, respectively, figure ~~3~~4). Following 14 days of treatment with PA101, there was a mean reduction in daytime and 24-hour cough frequency at day 14 of 31.1% and 29.1% respectively with PA101, when adjusted for placebo. The reduction in daytime mean cough frequency with PA101 was statistically significant at day 14 compared to placebo (ratio of LS Means [95% CI]; 0.67 [0.48, 0.94], p=0.0241) (table 2a). Similarly, the reduction in 24-hour mean cough frequency with PA101 was statistically significant at day 14 compared to placebo (ratio of LS Means [95% CI]; 0.68 [0.49, 0.95], p=0.0245) (figure ~~4~~5). Similar differences were seen at day 7 for daytime and 24-hour cough frequency in favor of PA101 compared to placebo (p=0.0329, table 2a, and p=0.0163, respectively). Nocturnal cough was also significantly reduced with PA101 at day 7 (ratio of LS Means [95% CI]; 0.53 [0.33, 0.85], p=0.0122), but not at Day 14 (ratio of LS Means [95% CI]; 0.64 [0.26, 1.57], p=0.30).

Responder Analysis

A clinically meaningful response was defined as a reduction in daytime cough frequency greater than 30% at Day 14 compared to baseline. There were no significant differences in patient characteristics between those who responded to therapy with PA101 versus non-responders (age (p=0.53), gender (p=0.66), duration of cough (p=0.58), IPF duration (p=0.28) and proportion of patients taking anti-fibrotic therapy (p=0.37)). The percentage of patients with at least a 30% reduction from baseline in daytime cough frequency following PA101 treatment was nearly twice that with placebo; 8/23 patients (35%) with PA101 treatment, and 4/21 patients (19%) with placebo (figure ~~5~~6). In patients who responded to PA101, the mean reduction in daytime cough frequency was 59%, reduction in cough severity VAS was 56%, and improvement in cough

quality of life was LCQ 2.3 points. No difference in responder rate was observed in patients on PA101 treatment with respect to anti-fibrotic IPF treatments (4 of 8 responders were on an IPF treatment).

Secondary Outcome Measures

There was no statistically significant change in cough specific quality of life (LCQ total score) with PA101 at Day 14 compared to placebo (p=0.09, table 3a). There was no statistically significant change in cough severity (VAS score) with PA101 at Day 14 compared to placebo (p=0.13, table 4a). The study sample size was powered to detect differences in objective cough frequency and was insufficient to detect significant differences in subjective cough measures. There was however a significant correlation coefficient between change in cough severity (VAS score) and change in daytime cough frequency with PA101 at Day 14, r= 0.415 (p=0.048), suggesting that a reduction in cough frequency may lead to an improvement in subjective measures. There were statistically significant improvements in favour of PA101 treatment at Day 14 compared to placebo in two domains of the K-BILD, chest symptoms and psychological (p=0.027 and p=0.032, respectively) (table 5). There were no significant changes from baseline to Day 14 in FEV_1, FVC and FeNO measurements with PA101 or placebo or between groups.

CHRONIC IDIOPATHIC COUGH COHORT

Twenty-eight participants with CIC were enrolled and 27 received study treatments (mean age 62 years, female 78%, Caucasian 93%, and median cough duration 9.9 years). One patient withdrew consent prior to receiving any study treatment (~~online supplementary appendix,~~ Figure ~~1~~3). The baseline demographics and clinical characteristics are summarised in table 1. The mean reduction in daytime cough frequency was 25.9% with PA101 and 19.7% with placebo (6.2%

greater mean reduction for PA101 when adjusted for placebo). The difference between PA101 and placebo treatments was not statistically significant using the ratio of LS means for the change from baseline in the log-transformed daytime average cough count at Day 14 (ratio of LS Means [95% CI]; 1.27 [0.78, 2.06], p=0.31) (table 2b). Similarly, the difference between PA101 and placebo treatments was not statistically significant using the ratio of LS means for the change from baseline in the log-transformed 24-hour average cough count at Day 14 (Figure 7). There were no statistically significant differences between PA101 and placebo treatments at Day 14 for the changes in patient-reported subjective endpoints (LCQ: LS Means difference [95% CI] of 0.5 [-0.80, 1.80], p=0.42, and VAS: LS Means difference [95% CI] of -0.4 [-9.37, 8.58], p=0.92) (tables 3b and 4b).

ADVERSE EVENTS

Treatment with PA101 was well tolerated by the participants in both cohorts. The incidence of all AEs were similar for both treatments in both cohorts (table 6, figure 2). The majority of AEs were mild in severity with both treatments, and there were no severe AEs or SAEs reported. No disturbance of taste was reported by any subject. In the IPF cohort, two patients withdrew from the study due to AEs: One patient during placebo treatment (increased cough and shortness of breath), and one patient during PA101 treatment (common cold). In the CIC cohort, four patients discontinued the study due to AEs: two patients during placebo treatment (headache, cough and oropharyngeal pain; dizziness), and two patients during PA101 treatment (angioedema and sinus tachycardia; pharyngeal hypoesthesia, cough and dyspnea).

DISCUSSION

This is the first study of the efficacy and safety of inhaled PA101 in IPF patients with chronic cough. There was a significant 31% reduction in daytime and 24-hour objective cough frequency following treatment with PA101 compared to placebo. PA101 was also associated with an improvement in chest symptom and psychological domains of ILD specific quality of life. PA101 was well tolerated; the incidence of adverse events were comparable to placebo, and most were mild in severity. In contrast, no treatment benefit for PA101 was observed in patients with chronic idiopathic cough.

We assessed efficacy with objective cough frequency. This has a number of advantages over other objective and subjective cough measures; it is not susceptible to the patient's perception of their symptom, and they can demonstrate an improvement in cough earlier than subjective measures.[27,28] There was a significant reduction in cough frequency with PA101 following seven days of therapy, and cough frequency continued to reduce at Day 14. The responder analysis was also consistent with the key finding of a reduction in cough frequency. A greater proportion of patients had a meaningful reduction in cough frequency with PA101 compared to placebo. An analysis with differing thresholds for the definition of response was also consistent with an improvement in cough favouring PA101 compared to placebo. A 30% reduction in cough frequency is likely to be clinically meaningful to patients. The minimal clinical important difference for objective cough frequency has not been studied, but our finding of a 30% reduction in cough frequency adjusted for placebo is comparable to that reported recently for other anti-tussive therapies for chronic idiopathic cough, such as MK7264 (formerly AF-219), a P2X3 receptor antagonist, and physiotherapy and speech and language therapy intervention.[27,29] There was an improvement in subjective measures of ILD-specific quality of life, favouring PA101 over placebo. The improvement in cough-specific quality of life with PA101 approached

the LCQ MCID of 1.3 units.[23] There were no issues reported by patients relating to the use of eFlow nebuliser device. This may reflect the compact size and portability of using the eFlow in and outside the patient's home.

The mechanism of cough in IPF and other chronic cough conditions is poorly understood.[30,31] Cough in IPF may be caused by several mechanisms such as inflammation, mechanical stimulation from distorted lung, or gastro-esophageal reflux, which may lead to cough reflex hypersensitivity.[32-34] Airway inflammation has been reported in IPF, for example, increased numbers of mast cells, neutrophils and eosinophils and their mediators in bronchoalveolar lavage and sputum have been reported.[8,35-39] Mast cells are thought to be important in the pathogenesis of cough. Degranulated mast cells release mediators such as Substance P, histamine, serotonin, and proteases that activate sensory nerve C-fibers.[33,34,40] Airway inflammatory mediators and increased cough reflex sensitivity to capsaicin and substance P have been reported in IPF.[34] Mast cells and their mediators are thought to be pro-fibrotic as well as inflammatory.[8,41,42] Mast cells are found in close proximity to fibroblastic foci in IPF and they have been reported to stimulate, migrate, proliferate and activate fibroblasts.[41-43] A recent study reported that mast cells in IPF can activate a phenotypic change in lung fibroblasts to myofibroblasts that contribute to fibrosis.[43]

Pirfenidone and nintedanib have been recently approved for the treatment of IPF. However, there are currently no drugs approved for treatment of cough in IPF. In phase 3 trials, no antitussive effect of pirfenidone was reported.[44] However, in a post-hoc analysis, stabilisation of cough was reported in a subset of patients when treated with low dose pirfenidone.[45] A recent study of pirfenidone in IPF cough reported a reduction in cough frequency but this study did not include a control group for comparison.[46] The only randomized, double-blind, placebo-controlled trial to date in IPF cough has

been with thalidomide.[47] Thalidomide is known to be anti-inflammatory and may also interact with sensory nerves.[48,49] In a study of 20 IPF patients receiving treatment with thalidomide for 24 weeks, there was a significant improvement in cough specific quality of life compared to placebo; however a significant proportion of patients reported troublesome side-effects with thalidomide.[47] An open label study of oral corticosteroids in six IPF patients reported a significant reduction in subjective cough scores,[34] however a number of immunosuppressive drug combinations with oral corticosteroids have failed in treating IPF and not well tolerated by patients.[50] In our study, subjects had been evaluated for common causes of cough that included asthma prior to entering the study. In an open label study of six IPF patients receiving anti-fibrotic drug interferon-gamma, there was an improvement in cough specific quality of life.[51] There have been no further reports of this medication in IPF cough. In an open label trial of a high dose proton pump inhibitor plus ranitidine for 2 months in IPF, there was no improvement in objective cough frequency.[52]

Cromolyn was the first non-steroidal anti-inflammatory drug approved for asthma in 1973. Its use declined following the introduction of inhaled corticosteroids. Previous formulations of cromolyn were limited by the poor efficiency of drug delivery devices, such as dried powder inhaler, metered dose inhaler or nebulisation via jet nebulizer,[16] which possibly led to sub-optimal lung delivery and deposition. PA101 is a high-concentration formulation of cromolyn, coupled with a high efficiency nebuliser to optimise lung deposition. The combination of PA101 and eFlow nebuliser delivers an approximately 5-10-fold higher lung concentration when using systemic levels of cromolyn as a surrogate marker.[53] The mechanism of the antitussive effect of PA101 is not known. PA101 has the potential to modulate a wide range of inflammatory cells and their mediators known to play an important role in cough, for example, mast cells, eosinophils, neutrophils and their derivatives.[54-56] PA101 may also modulate airway sensory

nerve function. Cromolyn has been reported to reduce c-fibre sensory nerve activity.[57] A recent study reported that the effect of cromolyn on sensory nerves is mediated via an orphan G-protein coupled receptor, GPR35.[12] We did not study the possibility that PA101 may modulate the underlying disease biology of IPF and reduce disease progression; this warrants further investigation with higher doses and longer duration of therapy. Cromolyn has the potential to reduce disease progression due to its anti-fibrotic activity. Anti-fibrotic activity of cromolyn has been reported in lungs, heart, liver, kidney, and skin.[58-63] The efficacy of cromolyn in an MDI formulation has been investigated in a small, single center study in patients with advanced lung cancer with cough. There was a reduction in cough severity compared to placebo following 2 weeks of treatment.[64]

The differential results obtained with PA101 in patients with IPF compared to chronic idiopathic cough cohorts in this study suggest that cromolyn may suppress cough selectively in patients with underlying respiratory disease. To our knowledge, this is the first clinical study suggesting that the mechanism of chronic cough may be disease specific. In CIC, there is no underlying respiratory disease and the potential drivers of cough are not clearly defined. In IPF-related cough, increased numbers of resident mast cells in fibrotic lungs is a well-established observation, and an interaction between mast cells and the cough reflex may provide a mechanistic basis.[8,34,43,65,66] The sensory nerve innervation of the distal airways is thought to differ to proximal airways and this may be another factor why the efficacy of an antitussive therapy may differ between IPF and laryngeal/upper airway disorders causing cough.[31]

There are some limitations with our study. We investigated a small number of patients, and the study was under-powered to assess subjective measures. Our findings need confirmation in a larger population with a parallel group design. A larger study may also determine the

characteristics of patients that are associated with a response to therapy. The duration of therapy was brief, and it is possible that improvements in cough frequency and quality of life may have been greater if the therapy had been administered for longer. As expected, there was gender difference between the cohorts, with more males in the IPF cohort and more females in the CIC cohort. Cough frequency monitors have been utilised largely in patients with chronic idiopathic cough and in a limited number of studies in IPF. A strong correlation between objective cough frequency and subjective measures of cough such as the LCQ has however been reported in IPF.[4] The increase in LCQ scores with PA101 did not meet statistical significance in contrast to cough frequency. The potential reasons for this are the study sample size was underpowered to detect changes in subjective endpoints and the study duration was too brief to demonstrate an improvement in quality of life. The assessment of cough frequency on a daily basis would have been useful to establish the onset of efficacy. However, wearing a cough monitor continuously for seven days would be burdensome for participants; consideration should be given to a daily cough severity diary in future studies. We did not assess bronchial hyper-responsiveness at baseline but a diagnosis of asthma is unlikely in our participants since none had a diagnosis of asthma and none had raised blood eosinophil counts at baseline.

In conclusion, treatment with inhaled PA101 was associated with a significant reduction in objective cough frequency in patients with IPF associated chronic cough, but not in patients with chronic idiopathic cough. These results suggest that the mechanism of cough in IPF appears to be disease specific. PA101 was well tolerated, and there were no severe or serious adverse events. Future studies, in particular dose-response studies and longer duration of treatment, are planned to assess the potential for inhaled PA101 to improve cough in IPF.

Contributors

SSB, MSW, AT, and AHM were involved in designing the study. SSB, MSW, SA, JWKB, HS, TMM, and AHM recruited, screened, monitored patients, collected and interpreted data. SBB and AT had access to the data, which was analysed by a statistician (Chuck Davis), SBB and AT. SBB, AT and AHM made the decision to submit for publication. All authors wrote and edited this manuscript.

Declaration of interests

SSB was supported by London National Institute for Health Research (NIHR) *I* Wellcome Trust King's Clinical Research Facility and the NIHR Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health, UK. SSB has received fees for consulting and King's College Hospital for cough monitor analyses from Patara Pharma, LLC. AT is an employee of Patara Pharma, LLC. Patara Pharma, LLC paid research grant for this study to each participating institution (the NHS Foundation Trust, university or hospital). The other authors declared no conflicts of interest.

Acknowledgements

Patara Pharma, LLC sponsored this study. Statistical expertise was provided by Chuck Davis, PhD (CSD Biostatistics, Oro Valley, Arizona, USA). We thank all patients who participated in this study.

REFERENCES

1. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011; **183**:788-824.

2. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2016; **174**:810-816.

3. Ryerson CJ, Abbritti M, Ley B, Elicker BM, Jones KD, Collard HR. Cough predicts prognosis in idiopathic pulmonary fibrosis. *Respirology* 2011; **16**:969-975.

4. Key AL, Holt K, Hamilton A, Smith JA, Earis JE. Objective cough frequency in Idiopathic Pulmonary Fibrosis. *Cough* 2010; **6**:4.

5. Swigris JJ, Stewart AL, Gould MK, Wilson SR. Patients' perspectives on how idiopathic pulmonary fibrosis affects the quality of their lives. *Health and Quality of Life Outcomes* 2005; **3**:61.

6. Faruqi S, Murdoch RD, Allum F, Morice AH. On the definition of chronic cough and current treatment pathways: an international qualitative study. *Cough* 2014; **10**:5-13.

7. McGarvey LP, Forsythe P, Heaney LG, MacMahon J, Ennis M. Bronchoalveolar lavage findings in patients with chronic nonproductive cough. *Eur Respir J* 1999; **13**: 59-65.

8. Overed-Sayer C, Rapley L, Mustelin T, Clarke DL. Are mast cells instrumental for fibrotic diseases? *Front Pharmacol* 2013; **4**:174.

9. Cox JS, Beach JE, Blair AM, et al. (1970). Disodium cromoglycate (Intal®). *Advances in Drug Research* 1970; **5**:115-196.

10. Storms W, Kaliner MA. Cromolyn sodium: fitting an old friend into current asthma treatment. *J Asthma* 2005; **42**: 79-89.

11. Bernstein IL. Cromolyn sodium. *Chest* 1985; **87** (1 Suppl): 68S-73S.

12. Maher SA, Birrell MA, Baker KE, et al. Cromoglycate: breathing life into an old asthma drug. European Respiratory Society International Congress, Amsterdam 2015. DOI: 10.1183/13993003.congress-2015.PA1017 (abstr).

13. Yang Y, Lu JYL, Wu X, et al. G-protein-coupled receptor 35 is a target of the asthma drugs cromolyn disodium and Nedocromil sodium. *Pharmacology* 2010; **86**:1-5.

14. Ohshiro H, Tonai-Kachi H, Ichikawa K. GPR35 is a functional receptor in rat dorsal root ganglion neurons. *Biochem Biophys Res Commun* 2008; **365**:344-348.

15. Vieria dos Santos R, Magerl M, Martus M, et al. Topical sodium Cromoglycate relieves allergen- and histamine-induced dermal pruritus. *Br J Dermatol* 2010; **162**:674-676.3

16. Keller M, Schierholz J. Have inadequate delivery systems hampered the clinical success of inhaled disodium cromoglycate? Time for reconsideration. *Expert Opin Drug Deliv* 2011; **8**:1-17.

17. Leaker BR, Barnes PJ, Jones CR, Tutuncu A, Singh D. Efficacy and safety of nebulized glycopyrrolate for administration using a high efficiency nebulizer in patients with chronic obstructive pulmonary disease. *Br J Clin Pharmacol* 2015; **79**:492-500.

18. Knoch M, Keller M. The customized electronic nebulizer: a new category of liquid aerosol drug delivery systems. *Expert Opin Drug Deliv* 2005; **2**:377-390.

19. Birring SS, Fleming T, Matos S, Rai AA, Evans DH, Pavord ID. The Leicester Cough Monitor: preliminary validation of an automated cough detection system in chronic cough. *Eur Respir J* 2008; **31**:1013–1018.

20. Ryan NM, Birring SS, Gibson PG. Gabapentin for refractory chronic cough: a randomized, double blind, placebo-controlled trial. *Lancet* 2012; **380**:1583-1589.

21. Boulet LP, Coeytaux RR, McCroy DC, et al. Tools for assessing outcomes in studies of chronic cough. Chest Guideline and Expert Panel Report. *Chest* 2015; **147**:804-814.

22. Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MDL, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). *Thorax* 2003; **58**:339–343.

23. Raj AA, Pavord DI, Birring SS. Clinical cough IV: What is the minimal important difference for the Leicester Cough Questionnaire? *Handb Exp Pharmacol* 2009; **187**:311–320.

24. Patel AS, Siegert RJ, Keir GJ, et al. The minimal important difference of the King's Brief Interstitial Lung Disease Questionnaire (K-BILD) and forced vital capacity in interstitial lung disease. *Respir Med* 2013; **107**:1438–1443.

25. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of Spirometry. ATS/ERS Task Force: Standardisation of Lung Function Testing. *Eur Respir J* 2005; **26**:319–338.

26. Kelsall A, Houghton LA, Jones H, Decalmer S, McGuinneess K, Smith JA. A novel approach to studying the relationship between subjective and objective measures of cough. *Chest* 2011; **139**:569-575.

27. Smith J, Kitt M, Sher M, Butera P, Ford A. Tackling the burden of chronic cough: A dose escalation study of AF-219. European Respiratory Society International Congress, London 2016. 48: OA1976; DOI: 10.1183/13993003.congress-2016.OA1976 (abstr)

28. Raj AA, Birring SS. Clinical assessment of chronic cough severity. *Pulm Pharmacol Ther* 2007; **20**:334-337.

29. Charmberlain-Mitchel SA, Garrod R, Clark L, et al. Physiotherapy, and speech and language therapy intervention for patients with refractory chronic cough: a multicentre randomised control trial. *Thorax* 2017; **72**:129-136.

30. Canning BJ, Chang AB, Bolser D, et al. Anatomy and neurophysiology of cough. Chest guideline and expert panel report. *Chest* 2014; **146**:1633-1648.

31. Mazzone SB, Undem BJ. Vagal afferent innervation of the airways in health and disease. *Physiol Rev* 2016; **96**:975-1024.

32. Raghu G, Freudenberger TD, Yang S, et al. High prevalence of abnormal acid gastroesophageal reflux in idiopathic pulmonary fibrosis. *Eur Respir J* 2006; **27**:136-142.

33. Van Manen MJG, Birring SS,Vancheri C, et al. Cough in idiopathic pulmonary fibrosis. *Eur Respir Rev* 2016; **25**:278-286.

34. Hope-Gill BD, Hilldrup S, Davies C, Newton RP, Harrison NK. A study of the cough reflex in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2003; **168**:995–1002.

35. Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE Jr. Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. *Chest* 2008; **133**:226-232.

36. Birring SS, Parker D, McKenna S, et al. Sputum eosinophilia in idiopathic pulmonary fibrosis. *Inflamm Res* 2005; **54**:51-56.

37. Madison JM, Irwin RS. Chronic cough in adults with interstitial lung disease. *Curr Opin Pulm Med* 2005; **11**:412-416.

38. Andersson CK, Andersson-Sjoland A, Mori M, et al. Activated MCTC mast cells infiltrate diseased lung areas in cystic fibrosis and idiopathic pulmonary fibrosis. *Respir Res* 2011; **12**:139.

39. Kawanami O, Ferrans VJ, Fulmer JD, Crystal RG. Ultrastructure of pulmonary mast cells in patients with fibrotic lung disorders. *Lab Invest* 1979; **40**:717-734.

40. Lavinka PC, Dong X. Molecular signaling and targets from itch: lessons for cough. *Cough* 2013; **9**:8.

41. Cha S-I, Chang CS, Kim EK, et al. Lung mast cell density defines a subpopulation of patients with idiopathic pulmonary fibrosis. *Histopathology* 2012; **61**:98-106.

42. Inoue Y, King TE, Tinkle SS, Dockstader K, Newman LS. Human mast cell basic fibroblast growth factor in pulmonary fibrotic disorders. *Am J Pathology* 1996; **149**:2037-2054.

43. Wygrecka M, Dahal BK, Kosanovic D, et al. Mast cells and fibroblasts work in concert to aggregate pulmonary fibrosis: role of transmembrane SCF and the PAR-2/PKC-α/Raf-1/p44/42 signaling pathway. *Am J Pathology* 2013; **182**:2094-2108.

44. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomized trials. *Lancet* 2011; **377**:1760-1769.

45. Azuma A, Taguchi Y, Ogura T, et al. Exploratory analysis of a phase III trial of pirfenidone identifies a subpopulation of patients with idiopathic pulmonary fibrosis as benefiting from treatment. *Respiratory Research* 2011; **12**:143.

46. Van Manen, Birring SS, Vancheri C, et al. Effect of pirfenidone on cough in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2016; **193**:A2696.

47. Horton MR, Santiopietro V, Mathew L, et al. Thalidomide for the treatment of cough in idiopathic pulmonary fibrosis. *Ann Intern Med* 2012; **157**:398-406.

48. Ye Q, Chen B, Tong Z, et al. Thalidomide reduces IL-18, IL-8 and TNF-α release from alveolar macrophages in interstitial lung disease. *Eur Respir J* 2006: **28**:824-831.

49. Cata JP, Weng HR, Dougherty PM. The effects of thalidomide and minocycline on taxol-induced hyperalgesia in rats. *Brain Res* 2008; **1229**:100-110.

50. Rochwerg B, Neupane B, Zhang Y, et al. Treatment of idiopathic pulmonary fibrosis: a network meta analysis. *BMC Medicine* 2016; **14**:18.

51. Lutherer LO, Nugent KM, Schoettle BW, et al. Low-dose oral interferon α possibly retards the progression of idiopathic pulmonary fibrosis and alleviates associated cough in some patients. *Thorax* 2011; **66**:446-447.

52. Kilduff CE, Counter MJ, Thomas GA, Harrison NK, Hope-Gill BD. Effect of acid suppression therapy on gastroesophageal reflux and cough in idiopathic pulmonary fibrosis: an intervention study. *Cough* 2014; **10**:4.

53. Mensinga T, Diamant Z, Qude-Elberink JNG, Tutuncu A. Improved systemic bioavailability of cromolyn sodium using inhaled PA101 via eFlow nebulizer. *Am J Respir Crit Care Med* 2017; 195:A3476 (abstr).

54. Moqbel R, Walsh GM, Macdonald AJ, Kay B. Effect of disodium cromoglycate on activation of human eosinophils and neutrophils following reversed (anti-IgE) anaphylaxis. *Clinical Allergy* 1986; **16**:73-83.

55. Shapiro, GG, König P. Cromolyn sodium: a review. *Pharmacotherapy* 1973; **5**:156–70.

56. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nature Medicine* 2013; **18**:693-704.

57. Dixon M, Jackson DM, Richards IM. The action of sodium cromoglycate on 'C' fibre endings in the dog lung. *British Journal of Pharmacology* 1980; **70***:*11-13.

58. Hemmati AA, Nazari Z, Motlagh ME, Goldasteh S. The role of sodium cromolyn in treatment of paraquat-induced pulmonary fibrosis in rat. *Pharmacol Res* 2002; **46**:229-234.

59. Harchegani AL, Hemmati AA, Nili-Ahmadabadi, Darabi B, Shabib S. Cromolyn sodium attenuates paraquat-induced lung injury by modulation of proinflammatory cytokines. *Drug Res* 2017; **67**:283-288.

60. Choi JS, Kim JK, Yang YJ, et al. Identification of cromolyn sodium as an anti-fibrotic agent targeting both hepatocytes and hepatic stellate cells. *Pharmacol Res* 2015; **102**:176-183.

61. Polaniyandi Selvaraj S, Watanabe K, Ma M, et al. Involvement of mast cells in the development of fibrosis in rats with postmyocarditis dilated cardiomyopathy. *Biol Pharm Bull* 2005; **28**:2128-2132.

62. Veerappan A, Reid AC, O'Conner N, et al. Mast cells are required for the development of renal fibrosis in the rodent unilateral ureteral obstruction model. *Am J Physiol Renal Physiol* 2012; **302**:F192-204.

63. Chen L, Schementi ME, Ranzer MJ, Wilgus TA, DiPietro LA. Blockade of mast cell activation reduces cutaneous scar formation. *PLOS One* 2014; **9**:e85226.

64. Moroni M, Porta C, Gualtieri G, Nastasi G, Tinelli C. Inhaled sodium Cromoglycate to treat cough in advanced lung cancer patients. *Br J Cancer* 1996; **74**:309-311.

65. Veerappan A, O'Conner NJ, Brazin J, et al. Mast cells: a pivotal role in pulmonary fibrosis. *DNA Cell Biol* 2013; **32**:206-218.

66. Doherty MJ, Mister R, Pearson MG, Calverly PMA. Capsaicin induced cough in cryptogenic fibrosing alveolitis. *Thorax* 2000; **55**:1028-1032.

Legend for Tables

Table 1. Data presented as mean ± SD unless otherwise stated. BMI = Body Mass Index; IPF = Idiopathic Pulmonary Fibrosis; CIC = Chronic Idiopathic Cough; NA = Not applicable; * = Pirfenidone or Nintedanib; VAS = Visual Analog Scale; LCQ = Leicester Cough Questionnaire; K-BILD = King's Brief Interstitial Lung Disease.

Table 2. IPF = Idiopathic Pulmonary Fibrosis; CIC: Chronic Idiopathic Cough; LS = Least Square; CI = Confidence Interval. Data presented for combined periods.

Table 3. IPF = Idiopathic Pulmonary Fibrosis; CIC: Chronic Idiopathic Cough; LCQ = Leicester Cough Questionnaire; LS = Least Square; CI = Confidence Interval.

Table 4. IPF = Idiopathic Pulmonary Fibrosis; CIC: Chronic Idiopathic Cough; VAS = Visual Analogue Scale; SD = Standard Deviation; LS = Least Square; CI = Confidence Interval.

Table 5. IPF = Idiopathic Pulmonary Fibrosis; K-BILD = King's Brief Interstitial Lung Disease Questionnaire; LS = Least Square; SD = Standard Deviation; CI = Confidence Interval.

Notes: K-BILD = worst score 15, best score 105.

Table 6. IPF = Idiopathic Pulmonary Fibrosis; CIC = chronic idiopathic cough; N = total number of subjects; n = number of subjects experiencing at least one adverse event. AEs occurring in at least 2 participants.

Legend for Figures

Figure 1. V= visit; ↑= Clinic visit. Primary outcome= Study Day 14.

Figure 2. IPF = Idiopathic pulmonary fibrosis; AE= Adverse event; EAS= Efficacy analysis set; PP= Per-protocol.

Figure 3. CIC = Chronic Idiopathic Cough; AE= Adverse event; EAS= Efficacy analysis set; PP= Per-protocol.

Figure ~~3~~4. IPF = Idiopathic pulmonary fibrosis; Error bars represent Mean ± SEM. Data presented in Efficacy Analysis Set.

Figure ~~4~~5. IPF = Idiopathic pulmonary fibrosis; LS = Least Square; CI = Confidence Interval.

Data in Efficacy Analysis Set. Data presented as change in log transformed data using mixed-effect model.

Figure ~~5~~6. IPF = Idiopathic pulmonary fibrosis; Data in Efficacy Analysis Set.

Figure ~~6~~7. CIC = Chronic Idiopathic Cough; LS = Least Square; CI = Confidence Interval.

Table 1. Baseline Demographics and Clinical Characteristics

Clinical Characteristics	IPF Cohort	CIC Cohort
Number	24	27
Age (range, years)	67 ± 6 (56 to 79)	62 ± 11 (23 to 73)
Gender	15 Male/9 Female	6 Male/21 Female
Ethnicity: Caucasian (n, %)	22 (92%)	25 (93%)
BMI (kg/m^2)	29.2 ± 3.8	27.7 ± 5.7
Time since IPF Diagnosis (years)	4.1 ± 3.3 (1 to 13)	NA
Duration of Chronic Cough (years)	5.6 ± 4.2 (1 to 16)	9.9 ± 9.8 (2 to 43)
Currently using IPF therapy* (n, %)	13 (54%)	NA
Cough Severity (VAS, mm)	61.5 ± 13.0	70.5 ± 15.3
Cough Specific Quality of Life (LCQ)	12.9 ± 3.2	10.5 ± 2.6
Disease Specific Quality of Life (K-BILD) – Total Score	56.2 ± 10.5	NA
Psychological domain	56.6 ± 13.2	NA
Breathlessness and Activities domain	44.6 ± 18.1	NA
Chest Symptoms domain	62.7 ± 20.8	NA

Data presented as mean ± SD unless otherwise stated. BMI = Body Mass Index; IPF = Idiopathic Pulmonary Fibrosis; CIC = Chronic Idiopathic Cough; NA = Not applicable; * = Pirfenidone or Nintedanib; VAS = Visual Analog Scale; LCQ = Leicester Cough Questionnaire; K-BILD = King's Brief Interstitial Lung Disease.

Table 2a. Change from Baseline in Log Transformed Daytime Cough Frequency Using Mixed Model in IPF Cohort (combined periods)

	PA101 (N=23)	Placebo (N=23)
Day 7		
LS mean estimate	0.78	1.08
95% CI	0.64, 0.96	0.87, 1.33
Day 7 – Between Group Comparison (PA101 / Placebo)		
Ratio of LS Means	0.73	
p-value	0.0329	
95% CI	0.54, 0.97	
Day 14		
LS Mean Estimate	0.70	1.05
95% CI	0.55, 0.90	0.81, 1.36
Day 14 – Between Group Comparison (PA101 / Placebo)		
Ratio of LS Means	0.67	
p-value	0.0241	
95% CI	0.48, 0.94	

Table 32b. Change from Baseline in Log Transformed Daytime Cough Frequency Using Mixed Model in CIC Cohort (combined periods)

	PA101 (N=25)	Placebo (N=27)
Day 7		
LS mean estimate	0.94	0.72
95% CI	0.74, 1.19	0.57, 0.91
Day 7 – Between Group Comparison (PA101 / Placebo)		
Ratio of LS Means	1.30	
p-value	0.06	
95% CI	0.99, 1.71	
Day 14		
LS Mean Estimate	0.86	0.68
95% CI	0.59, 1.26	0.47, 0.98
Day 14 – Between Group Comparison (PA101 / Placebo)		
Ratio of LS Means	1.27	
p-value	0.31	
95% CI	0.78, 2.06	

Table 43a. Cough Specific Quality of Life (LCQ) in IPF Cohort

	PA101 (N=23)	Placebo (N=23)
Baseline		
Mean ± SD	13.7 ± 3.6	13.6 ± 3.5
Day 7		
Mean ± SD	14.4 ± 3.2	14.0 ± 3.3
Change from Baseline	0.7	0.3
Day 14		
Mean ± SD	14.8 ± 3.3	13.4 ± 3.7
Change from Baseline	1.1	0.01
Day 14 – Between Group Comparison		
LS Mean Difference (PA101 - Placebo)	1.1	
p-value	0.09	
95% CI	-0.18, 2.33	

Table 53b. Cough Specific Quality of Life (LCQ) in CIC Cohort

Cough Quality of Life (LCQ)	PA101 (N=25)	Placebo (N=27)
Baseline		
Mean ± SD	10.9 ± 3.4	11.1 ± 3.1
Day 7		
Mean ± SD	12.4 ± 3.9	12.3 ± 3.9
Change from Baseline	1.4	1.2
Day 14		
Mean ± SD	12.6 ± 4.5	12.0 ± 4.1
Change from Baseline	1.6	1.0
Day 14 – Between Group Comparison		
LS Mean Difference (PA101 - Placebo)	0.5	
p-value	0.42	
95% CI	-0.80, 1.80	

Table 4a. Cough Severity (VAS, mm) in IPF Cohort

	PA101 (N=23)	Placebo (N=23)
Baseline		
Mean ± SD	54.8 ± 20.6	55.3 ± 23.8
Day 7		
Mean ± SD	52.5 ± 20.7	51.2 ± 26.5
Change from Baseline	-2.3	-3.4
Day 14		
Mean ± SD	44.3 ± 26.7	53.3 ± 26.6
Change from Baseline	-10.6	-2.0
Day 14 – Treatment Comparison		
LS Mean Difference (PA101 - Placebo)	-8.5	
p-value	0.13	
95% CI	-19.65, 2.70	

Table 4b. Cough Severity (VAS, mm) in CIC Cohort

Cough Severity (VAS, mm)	PA101 (N=25)	Placebo (N=27)
Baseline		
Mean ± SD	65.4 ± 20.8	71.2 ± 12.2
Day 7		
Mean ± SD	60.3 ± 21.8	63.3 ± 19.8
Change from Baseline	-5.3	-8.1
Day 14		
Mean ± SD	57.8 ± 22.8	61.2 ± 23.8
Change from Baseline	-7.7	-10.0
Day 14 – Treatment Comparison		
LS Mean Difference (PA101 - Placebo)	-0.4	
p-value	0.92	
95% CI	-9.37, 8.58	

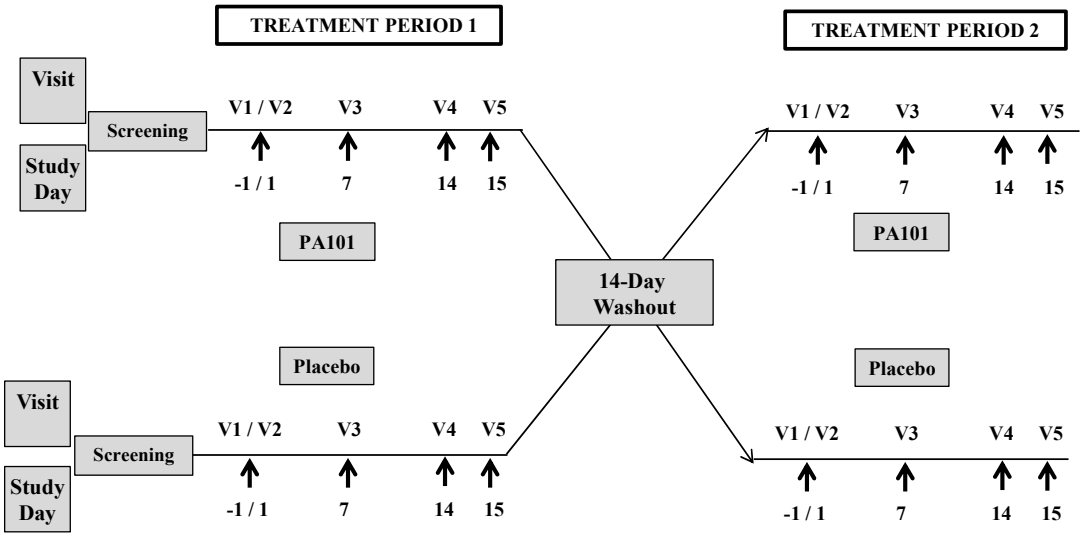
Table 5. Disease-Specific Quality of Life (K-BILD) in IPF Cohort

	PA101 (n=23) (Mean ± SD)	Placebo (n=21) (Mean ± SD)
Baseline		
Total Score	55.4 ± 9.2	57.0 ± 11.7
Psychological	55.5 ± 13.0	57.7 ± 13.3
Breathlessness & Activities	44.1 ± 15.2	45.0 ± 20.9
Chest	61.8 ± 19.9	63.5 ± 21.7
Day 14		
Total Score	56.7 ± 9.1	55.1 ± 10.0
Psychological	57.8 ± 9.1	55.0 ± 13.7
Breathlessness & Activities	44.3 ± 18.7	44.5 ± 17.9
Chest	69.3 ± 14.3	60.8 ± 19.3
Within Group Change from Baseline		
Total Score	0.5 ± 1.3	-1.7 ± 1.3
Psychological	1.3 ± 1.9	-2.6 ± 1.9
Breathlessness & Activities	0.1 ± 2.2	-0.4 ± 2.2
Chest	6.3 ± 2.7	-2.4 ± 2.7
Between Group Difference (LS Mean)		
Total Score	2.2 (95% CI -0.76, 5.19) (p=0.11)	
Psychological	3.9 (95% CI 0.37, 7.40) **(p=0.032)**	
Breathlessness & Activities	0.5 (95% CI -7.24, 8.21) (p=0.87)	
Chest	8.7 (95% CI 1.38, 15.99) **(p=0.027)**	

Table 6. Treatment Emergent Adverse Events

	IPF Cohort		CIC Cohort	
Incidence by System Organ Class and Preferred Term	**PA101 (N=23)**	**Placebo (N=23)**	**PA101 (N=25)**	**Placebo (N=27)**
	n (%)	**n (%)**	**n (%)**	**n (%)**
Respiratory System				
Cough	3 (13.0)	4 (17.4)	3 (12.0)	3 (11.1)
Dyspnea	1 (4.3)	2 (8.7)	1 (4.0)	1 (3.7)
Oropharyngeal pain	1 (4.3)	0	2 (8.0)	3 (11.1)
Pharyngeal hypoaesthesia	0	0	2 (8.0)	0
Other Systems				
Headache	3 (13.0)	2 (8.7)	1 (4.0)	1 (3.7)
Dizziness	1 (4.3)	2 (8.7)	0	1 (3.7)
Tremor	0	0	2 (8.0)	0
Diarrhea	2 (8.7)	1 (4.3)	0	0
Defecation urgency	2 (8.7)	0	0	0
Dry mouth	2 (8.7)	0	3 (12.0)	0
Fatigue	1 (4.3)	2 (8.7)	1 (4.0)	1 (3.7)
Flushing	2 (8.7)	0	0	0

Figure 1. Study design



V= visit; ↑= Clinic visit. Primary outcome= Study Day 14.

Figure 2. Trial consort flow diagram in IPF Cohort

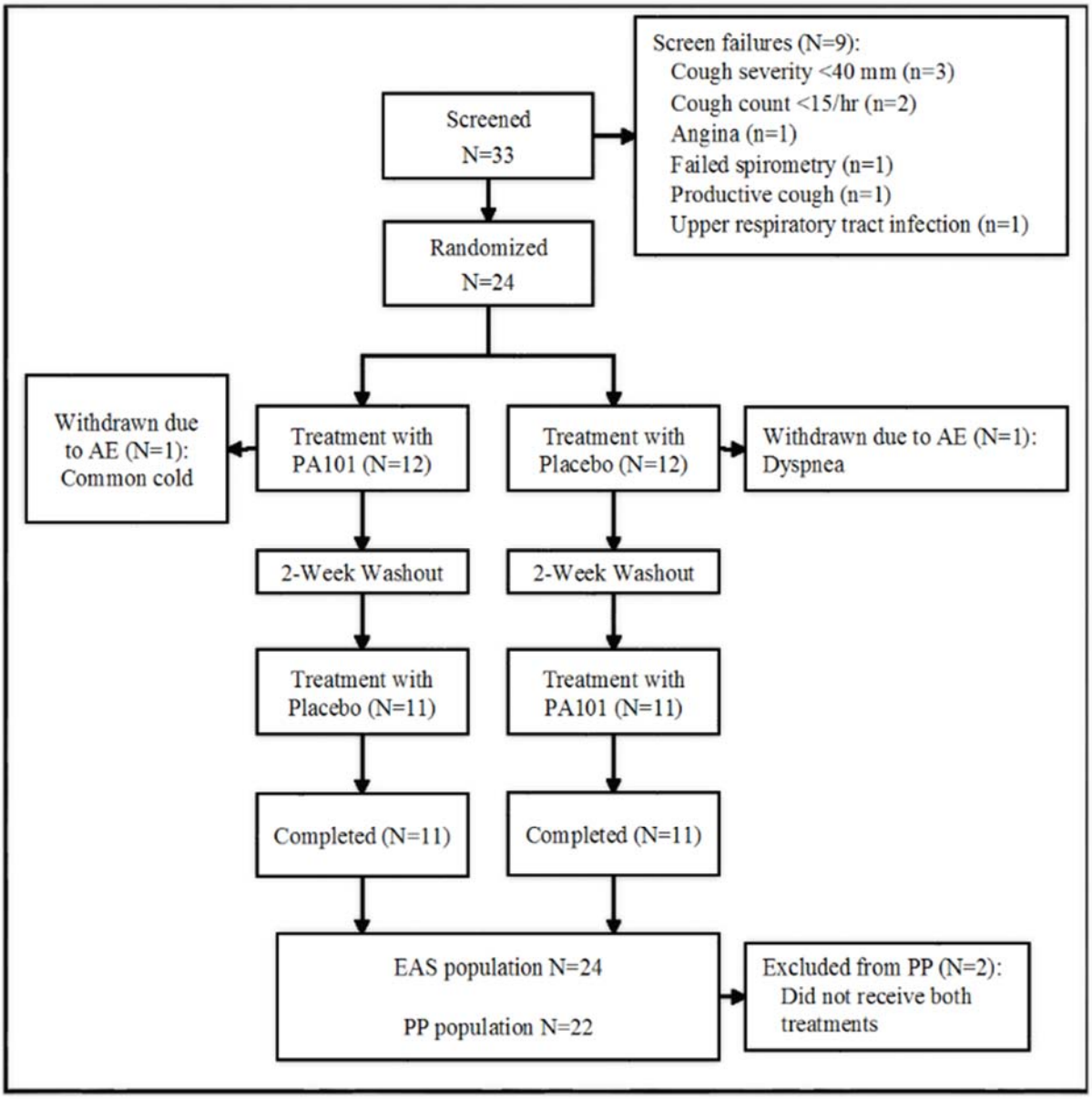


Figure 3. **Trial consort flow diagram in CIC Cohort**

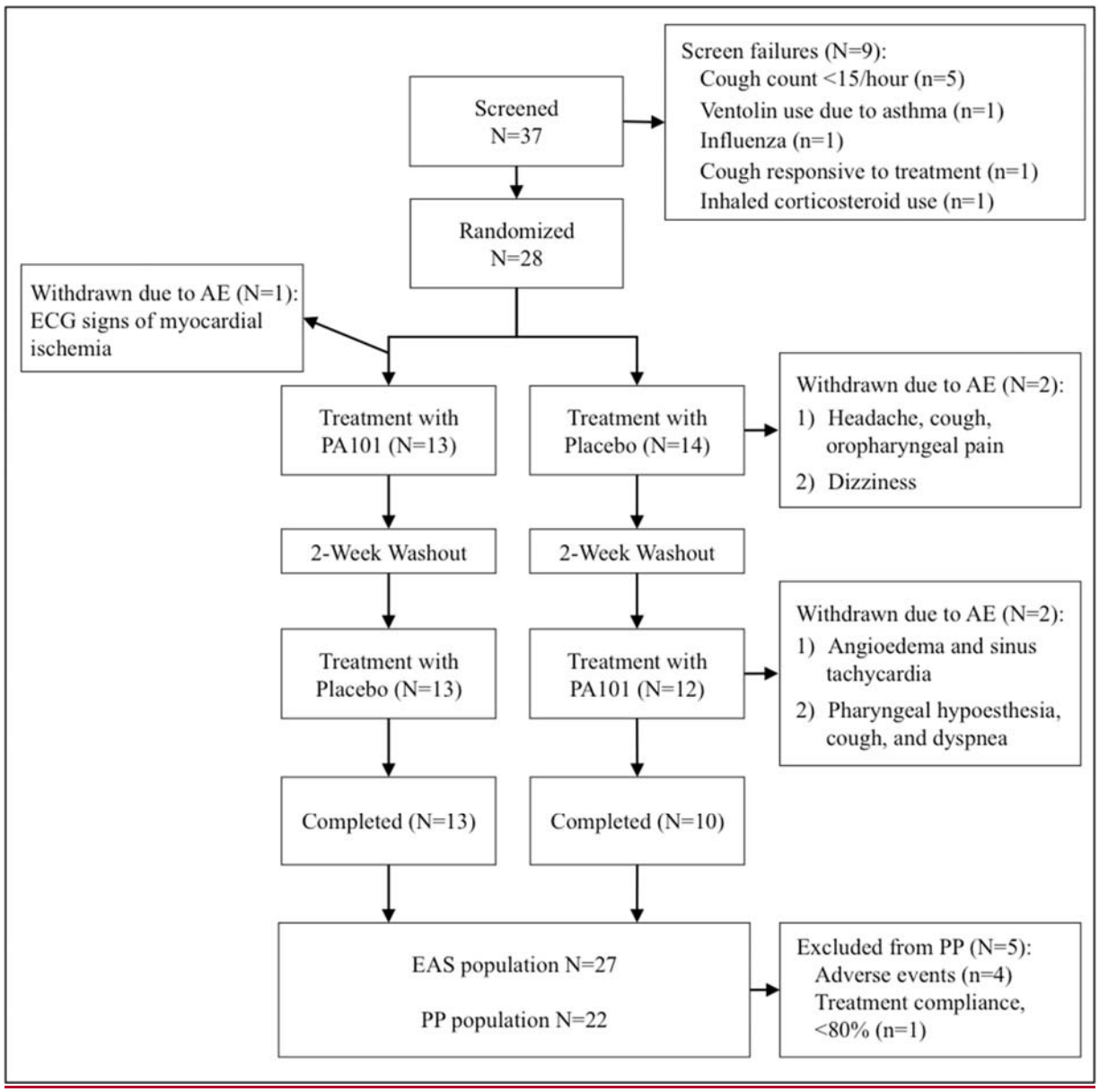


Figure 4. **Changes in Objective Daytime Cough Frequency in IPF Cohort**

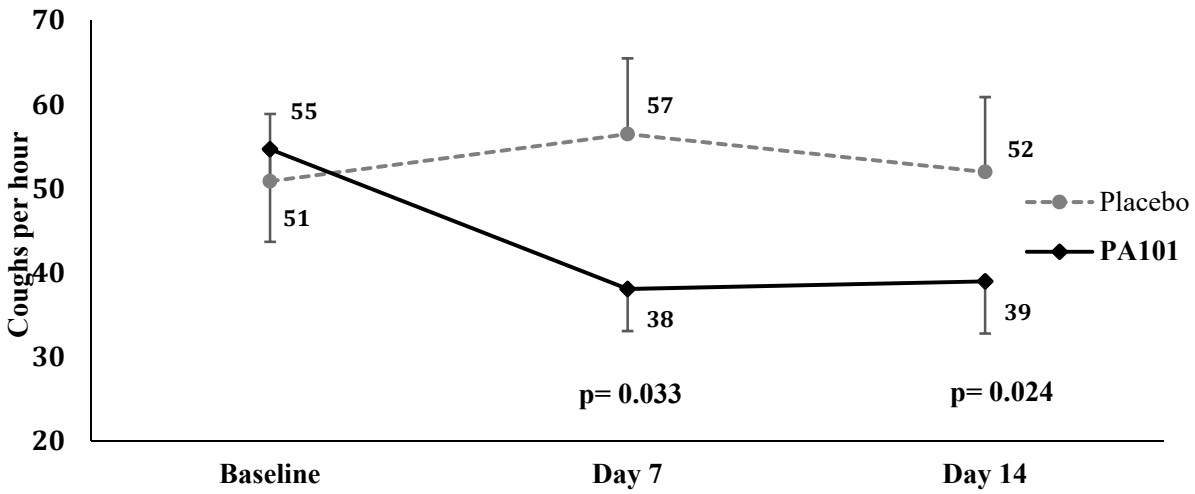


Figure 45. Change from Baseline in 24-Hour Cough Frequency in IPF Cohort

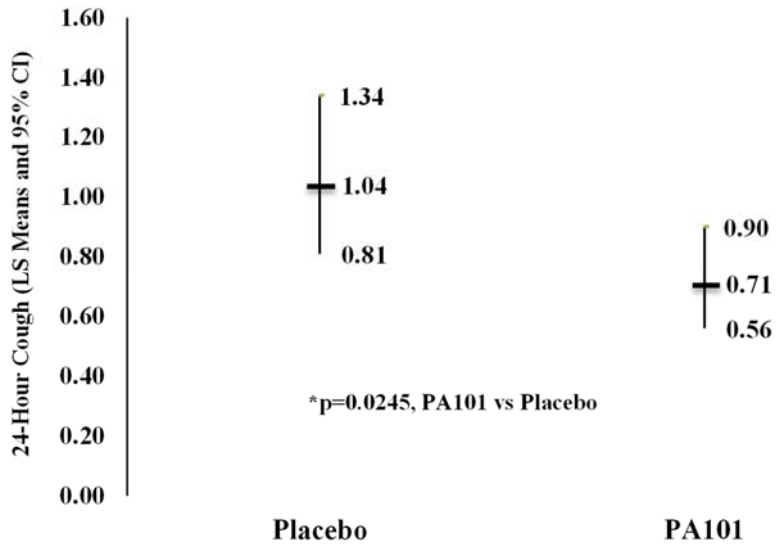


Figure ~~5~~6. Responder Analysis – Daytime Cough Frequency in IPF Cohort

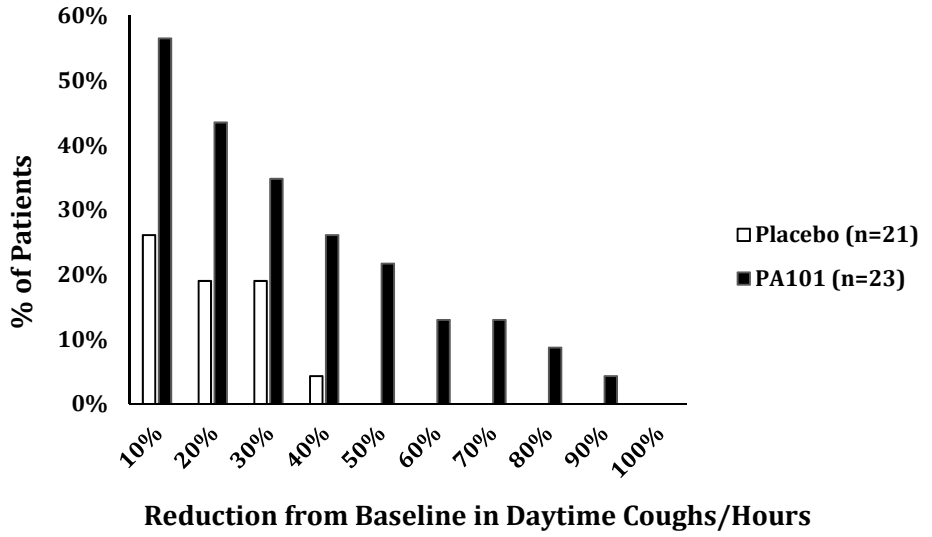


Figure ~~6~~7. Change from Baseline in 24-Hour Cough Frequency in CIC Cohort