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A Prospective Safety and Feasibility Study of Metered CryoSpray (MCS) for Patients with Chronic Bronchitis in COPD

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ABSTRACT

Background: No currently approved intervention counteracts airway metaplasia and mucus hypersecretion of Chronic Bronchitis (CB) in COPD. Metered Cryospray (MCS) delivering liquid nitrogen (LN_2) to the tracheobronchial airways ablates abnormal epithelium and facilitates healthy mucosal regeneration. The objective of this study was to evaluate the feasibility, efficacy and safety of MCS in CB.

Methods: Patients with a FEV1, 30-80% of expected, taking optimal medication were recruited. Primary outcomes: feasibility – completion of treatments; efficacy – 3-month change in St George's Respiratory Questionnaire (SGRQ); safety – incidence of adverse events (AEs). Secondary outcomes: lung function, exercise capacity, additional patient-reported outcomes (PROs).

Results: 35 patients, 19 male/16 female, aged 47-76 years, GOLD grade I (3), II (10) and III (22), underwent staggered LN_2 treatments to the tracheobronchial tree.

34 patients completed three treatments, each lasting 34·3±12·1 minutes, separated by 4-6 weeks: one withdrew after the first treatment. Approximately 1800 doses of MCS were delivered.

Clinically meaningful improvements in PROs were observed at 3-months; ΔSGRQ -6·4 [95% CI -11.4, -1.3; p=0·01], COPD Assessment Test (CAT) -3·8 [95% CI -6.4, -1.3; p<0·01] and Leicester Cough Questionnaire (LCQ) 21·6 [95% CI 7.3, 35.9; p<0·01]. CAT changes were durable to 6-months (-3·4 [95% CI -5.9, -0.9; p=0·01]), SGRQ and LCQ to 9-months (-6·9 [95% CI -13.0, -0.9; p=0·03] and 13·4 [95% 2.1, 24.6; p=0·02], respectively).

At 12-months, 14 serious AEs were recorded in 11 (31·4%) subjects, 6 moderate (43%) and 8 severe (57%). 9 were respiratory-related: 6 exacerbations of COPD, 2 pneumonias, and 1, increased coughing, recovered without sequelae. None were serious device or procedure-related AEs.

Conclusion: MCS is safe, feasible and associated with clinically meaningful improvements in multidimensional PROs.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a complex inflammatory lung condition characterized by airflow limitation, cough, dyspnoea and impaired quality of life(1). Chronic bronchitis (CB) defined as chronic cough and sputum production occurring on most days for at least 3 months of two consecutive years(2), is a common clinical phenotype of COPD(3), and is associated with accelerated lung function decline(4-6), worse health-related quality of life(7-9), increased rate of exacerbations(7, 10, 11) and hospitalisations(5, 10) and reduced life expectancy(6, 12-14).

There is no currently approved therapy that reverses the airway metaplasia and mucus hypersecretion of CB in COPD and restores the integrity and functionality of the respiratory tract epithelium. However, a novel approach is suggested by the observation that selective cellular ablation preserving extracellular structures is followed by rapid replacement with healthy tissue(15, 16). Flash freezing at -196°C induces intracellular ice crystal formation, disrupting cellular structures but sparing the extracellular matrix, facilitating epithelial regrowth(17). The RejuvenAir® system (CSA Medical, Lexington, MA, USA) consists of a console that stores liquid nitrogen (LN_2) and a disposable catheter with a radial spray head inserted through the working channel of a flexible bronchoscope. Using a specially developed algorithm, programmed doses of LN_2 are delivered in a radial spray, termed Metered Cryospray™ (MCS), to the bronchial airways. It is designed to cryoablate abnormal epithelium and excessive mucous-producing goblet cells to a depth of 0·1 to 0·5 mm and a width up to 10mm(18). Re-epithelialization with healthy mucosa has been demonstrated within 48 hours of cryospray treatment, and with durability out to 106 days(19).

The objective of this study was to evaluate the feasibility, efficacy, and safety of MCS therapy to treat patients with CB in COPD. This report documents the outcomes at 12 months after the last delivered MCS treatment.

METHODS

This is a prospective, open label, single arm study of sequentially accrued subjects diagnosed with CB in COPD. The multicentre study was conducted in the United Kingdom, Netherlands, and Canada and was approved by the respective Competent Authorities, Institutional Review Boards or Ethics Committees at each site and all participating subjects provided written informed consent. The trial is registered at clinicaltrials.org (NCT02483637). We recruited patients between the ages of 47 and 76 years with an established diagnosis of CB in COPD (defined as chronic cough and sputum production occurring on most days for at least 3 months of two consecutive years) who had ceased smoking for at least 2 months prior to enrolment, had not experienced a respiratory exacerbation in the past 6 weeks but were persistently symptomatic despite guideline approved therapy. The extensive inclusion and exclusion criteria were designed to maximise patient safety (table S1).

Phases of study

Treatments were conducted in 2 phases, which are detailed in the online supplement (section 2·1). Phase A was a preliminary assessment of feasibility and safety and confirmation of healing, including in this phase only, endobronchial biopsies in a small contingent of subjects undergoing their first (of three) treatments, before completing the programme of whole lung treatments in Phase B. (Figure 1).

Between March and August of 2016, 11 subjects completed Phase A. Following receipt of a satisfactory report on the findings by the Data Safety Monitoring Board (DSMB), an additional

twenty-four subjects were enrolled and underwent the three scheduled MCS treatments: 12-month follow-up was completed in February of 2019.

Study procedures

Baseline and follow-up assessments

Demographics, medical history including cough and sputum production, smoking history, urine pregnancy test for females of child bearing potential, lung function (spirometry and body plethysmography), high resolution computed tomography (HRCT), six-minute walk test (6MWT), plasma fibrinogen, and patient reported outcomes (PROs including St George's Respiratory Questionnaire, SGRQ; Leicester Cough Questionnaire, LCQ; COPD Assessment Test, CAT; Visual Analogue Score, VAS; modified Medical Research Council dyspnoea sale, mMRC – described in online supplement, section 2·2) were recorded. Subjects satisfying all the inclusion criteria proceeded to treatment.

Follow-up evaluations were conducted in person at 3-, 6-, 9-, and 12-months after completion of the final MCS treatment.

Device and procedure

The RejuvenAir® System is a cryosurgical device that delivers metered doses of medical grade LN_2 from a dewar stored in a console to a catheter emitting a radial spray at its tip. Details of the device and procedure have been published(17) and are outlined in the online supplement (sections 2·3 and 2·4). General anaesthesia, sedative and associated medications were administered as per institutional guidelines and routine clinical practice.

The first treatment delivered MCS to the right lower lobe and main stem bronchus, the second to the left lower lobe and main stem bronchus, and the third to both upper lobes, any residual main stem bronchus, and the distal end of the trachea. Precautionary measures were employed to avoid barotrauma and asphyxia: before each spray the cuff of the endotracheal tube was deflated, and the ventilator disconnected briefly. One-hour post-procedure a chest x-ray was obtained to exclude pneumothorax. Notwithstanding, the right middle lobe was omitted from the procedure on account of the perceived increased risk of barotrauma in a small structure. Endobronchial biopsies were obtained from the right lower lobe in the initial 11 patients at baseline and at day 60.

Intervals of 30 to 45 days were imposed between each of the three MCS treatment sessions and progression to the next treatment was contingent on the subject remaining stable without evidence of a recent acute exacerbation.

Study outcome measures

Primary Outcomes

The primary feasibility endpoint was the completion of all three MCS treatments. The primary safety endpoint was the incidence, seriousness and relatedness of adverse events experienced during the study. The primary efficacy endpoint was the change from baseline to 3 months in the SGRQ-total score.

Secondary Outcomes

Secondary endpoints included changes in FEV1 (ml), six-minute walk test distance (meters), and additional patient-reported outcomes (CAT, LCQ, VAS and mMRC scores).

Statistical analyses

The sample size of 35 subjects was based on an 80% statistical power using a one-sided test at the 0·05 significance level assuming a mean change of -4 points and standard deviation of 7 in total SGRQ score at 3-months relative to baseline.

Categorical data are presented as a percentage (%). Continuous data are summarized as mean ± SD / 95% confidence interval (95% CI) or median (interquartile range, IQR) depending on the distribution of the data. A 2-tailed paired t-test or a Wilcoxon matched-pairs signed rank test was used to compare these groups, respectively.

To evaluate and control for the potential effects of covariate factors on treatment outcomes, the change in SGRQ-total score from baseline to 3 months was assessed using the method of least squares from an ANCOVA model incorporating baseline GOLD stage, number of MCS treatments across the three treatments (i.e. <50 cryosprays versus >50 cryosprays), and study phase.

Statistical significance was set at $p<0·05$ and analysis was performed using SPSS version 24·0 (IBM, Chicago, IL, USA).

RESULTS

Results are presented for each follow-up visit to 12-months after the completion of the last MCS treatment.

Demographics

49 COPD subjects were screened and 35 (16 females and 19 males) were enrolled in the study. Their mean age was 67·2 ± 7·0 years and BMI 26·9 ± 5·2 kg/cm^2. Their GOLD grades were I (8·5%), II (28·5%) and III (63%). The mean pack year of smoking was 56·4 ± 35·1 years. (table 1).

At baseline, all subjects were taking at least one pulmonary medication. The most frequently used were inhaled beta-2 agonists (51·4%), anti-cholinergics (51·4%) and corticosteroids (48·6%). Fewer patients were taking prophylactic antibiotic (31·4%) and mucolytic agents (17·1%) (table 1).

A total of 34 patients (97·1%) attended the 3-month follow-up, 30 (85·7%) the 6- and 9-month follow-ups, and 31 (88·6%) were evaluated at the 12-month visit: three (8·6%) withdrew consent and one subject expired (2·9%) from unrelated complications of ischaemic heart disease during this period.

Primary Outcomes

Primary feasibility analysis

All subjects received general anaesthesia during the bronchoscopy procedure. The mean oxyhaemoglobin saturation on room air was 98·4 ± 1·0% at the start of treatment and 97·1 ± 1·9% at the end of treatment.

The mean number of sprays (±SD) delivered was 17·3 ± 4·6, 17·6 ± 2·1 and 26·2 ± 5·8 for the MCS treatments 1, 2, and 3, respectively: 20.3 ± 6.0 for all treatments. The percentage of full dose sprays was 87·7%, 85·3% and 84·3% for Treatments 1, 2, and 3, respectively: 85·8% for all treatments. The mean duration of each treatment session was 34·3 ± 12·1 minutes. (table S2). Device observations

(i.e. console readouts indicating the cause of spray delivery failure) were recorded in 29 subjects: the majority, 95%, were related to the catheter and 5% to the console. Catheters were replaced as necessary. None of the device observations were associated with any adverse events (AEs).

All subjects were fit for discharge on the same day. Two had pre-planned stays for unrelated events. Chest x-rays were performed in all but one subject after Treatment 1 (2·9%). There were no reports of pneumothoraces following any of the MCS treatment procedures.

34 subjects (97·1%) completed all three MCS treatments – one withdrew consent after experiencing a mild COPD exacerbation following the initial MCS procedure.

Primary safety analysis

All subjects experienced at least one AE (table S3). A total of 251 were reported from enrolment to the completion of the 12-month follow-up evaluation. (tables S4). The majority were classified as respiratory-related (52·6%). Of these, 91 (36·3%) were attributed to the underlying COPD. (tables S5).

Six non-serious device-related adverse events (2·4%) were reported in four (11·4%) subjects, one episode of bronchospasm during treatment and five exacerbations of COPD occurring 1·0 (0, 3·5) day after treatment and lasting 15·0 (10·5, 31·0) days. These events were graded mild (n=2) or moderate (n=4) and all resolved without sequelae. (table S6). There were 40 adverse events attributed to the procedure in 21 (60%) subjects: none were serious. (tables S3 and S4).

14 serious adverse events (SAEs; 5·6%) were reported in 11 (31·4%) subjects, six moderately so (43%), eight severe (57%) (see online supplement section 2·5). Nine were respiratory-related: Six exacerbations of COPD, two pneumonias, and one, increased coughing. The other incidents recorded were gastritis/a duodenal ulcer, urosepsis, and in one subject pulmonary embolus, rectal bleeding and finally ischaemic heart disease 243 days after completing all three MCS treatments. This subject was a 77-year old Caucasian female with GOLD grade 2 COPD, who underwent a coronary revascularisation complicated by pancreatitis, cardiac arrest and multiple organ failure which proved fatal.

None of the SAEs were deemed related to the device or the procedure by the Principal Investigator (PI) or the Data Safety Monitoring Board (DSMB) (table S7).

The exacerbation rate from treatment 1 to 12-months was 1·84 per patient year (PPY). Stratification according to GOLD grades II and III demonstrated rates of 1·29 and 2·10, respectively. (table S8). Higher baseline SGRQ-total scores were significantly associated with higher exacerbation frequency (p=0.02).

There were no reports of unanticipated adverse device effects or pneumothoraces during the study.

Primary efficacy analysis

The primary endpoint, the mean change in total SGRQ score (ΔSGRQ-total) from baseline to 3-months, was statistically significant and clinically meaningful (≥4 points) at -6·4 [95% CI -11.4, -1.3; p=0·01](20), and unaffected by covariables including baseline GOLD grade, number of cryosprays administered and study phase (ANCOVA: $p<0.05$). (figure 2 and table 2).

Secondary Outcomes

Lung function and exercise capacity

Over the 12-month follow-up period, FEV_1 declined modestly: -96·5mls [95% CI -169.0, -23.9; p=0·01]. There were no statistically significant changes in airways resistance observed. (table 2).

The mean change in 6MWT at 9-months, 24·3 meters [95% CI -0.4, 49.0; p=0·05], was just short of that to achieve the MCID, 26 meters (21), but at 12-months had decreased to 8·5 meters [95% CI -19.4, 36.5; p=0·54). (table 2).

Patient-reported outcomes

The minimal clinically important difference (MCID) of -4 points in the total SGRQ score was met during the 12-month follow-up. The total SGRQ was driven by 'symptoms' and 'impact' domains and endured at 9-months: -6·9 [95% CI -13.0, -0.9; p=0·03]. (figure 2 and table 2).

Mean change in CAT was statistically significant and clinically meaningful at 3- and 6-months(22): -3·8 [95% CI -6.4, -1.3; p<0·01] and -3·4 [95% CI -5.9, -0.9; p=0·01], respectively. At 12-months, the MCID of -2 was met but was not statistically significant: -2·0 [95% CI -4.7, 0.6; p=0·12]. (figure 3 and table 2).

The mean change in LCQ score was statistically significant and far exceeded the MCID of +1·3(23) at 3-, 6, and 9-months: 21·6 [95% CI 7.3, 35.9; p<0·01], 21·6 [95% CI 8.3, 34.9; p<0·01], and 13·4 [95% CI 2.1, 24.6; p=0·02], respectively. At 12-months, the LCQ score exceeded the MCID but was not statistically significant: 9·1 [95% CI -4.1, 22.3; p=0·17].

Mean change in VAS on activity was statistically significant at 6-months: -10·3 [95% CI -18.7, -1.9; p=0·02]. There were no statistically significant improvements in mMRC over 12-months.

On post-hoc analysis, those individuals who had worse baseline SGRQ total scores (i.e. > 50 points) experienced substantially greater improvements at 3-, 6-, 9-, and 12-months, respectively: ΔSGRQ total scores of -9·8 [95% CI -15.9, -3.8], -15·4 [95% CI -22.6, -8.2], -13·5 [95% CI -20.7, -6.3], and -10·9 [95% CI -16.4, -5.4] (p<0·01 at all timepoints) (figure 4 and table S9) – not attributable to regression to the mean on ANCOVA analysis (p=0.29); ΔCAT scores of -5·2 [95% CI -8.4, -2.1; p<0·01), -5·4 [95% CI -8.6, -2.3; p<0·01], -2·2 [95% CI -6.2, 1.8; p=0·27) and -4·0 [95% CI -7.2, -0.8; p=0·02]; ΔLCQ scores of 36·3 [95% CI 20.1, 52.5], 35·0 [95% CI 17.4, 52.6], 26·2 [95% CI 12.7, 39.6], and 23·5 [95% CI 10.2, 36.9] (p<0·01 at all timepoints); ΔVAS on activity of -10·6 [95% CI -21.4, 0.3; p=0·06], -15·8 [95% CI -27.6, -4.1; p=0·01]. -11·9 [95% CI -25.5, 1.7; p=0·08], and -10·9 [95% CI -22.0, 0.2; p=0·05) (table S10).

Bronchoscopy outcomes

The presence of mucus at each bronchoscopy was documented as none, mild, moderate, and severe: Treatment 1 – 0%, 49%, 37%, 14%; Treatment 2 – 9%, 35%, 41%, 15%, Treatment 3 – 0%, 65%, 29%, 6%, respectively.

Microbiology samples obtained for gram stain (bacteria, mycobacteria, and fungi) were evaluated: Treatment 1 – 22.9%, 0%, 14.3%, Treatment 2 – 26.5%. 2.9%, 23.5%, Treatment 3 – 20.6%, 8.8%, 23.5%, respectively.

128 endobronchial biopsies from 11 subjects were analysed, including 52 baseline and 57 post-treatment (at day 60). There were no definitive histological differences observed.

DISCUSSION

We have shown that metered cryospray (MCS) administered to patients with chronic bronchitis (CB) in COPD produced statistically significant and clinically meaningful improvements in patient-

reported outcomes (PROs) at 3-months. The reduction in total SGRQ score was driven by 'symptoms' and 'impact' domains and was durable at 9-months. The 'symptoms' domain includes the assessment of cough and sputum production, which the RejuvenAir® system is designed to ameliorate, and has been suggested as a robust descriptor of the chronic bronchitic phenotype prone to exacerbations(24). The reduction in SGRQ-total score was accompanied by clinically relevant gains in CAT and LCQ scores at 6- and 9-months, respectively, reinforcing the beneficial impact of MCS treatment on multidimensional disease-specific and treatment-responsive PROs evaluating cough and sputum production. Subjects with poorer baseline health status (defined as a total SGRQ score of >50 points) experienced substantially greater benefits in these domains that persisted out to 12-months and which may inform future patient selection.

The use of MCS therapy was safe and feasible. All but one subject completed the three treatments and the ratio of full dose sprays exceeded 84% at each of the procedures. None of the device observations resulted in an AE and the majority were resolved by replacing the catheter. All patients were fit for discharge on the day of their treatment. The treatment was safe: with 2·4% of AEs related to the device and 15·9% to the procedure, all were mild or moderate - and resolved without sequelae. There was no serious device or procedure-related SAEs. The RejuvenAir® system is intended to induce a regenerative endobronchial tissue effect by 1) destroying abnormal surface epithelium with mucin-producing goblet cell hyperplasia, 2) promoting normal ciliated bronchial epithelium regrowth without globlet cell hyperplasia, and 3) reducing chronic inflammation and associated airway constriction. The modest decline in FEV1 observed might reflect the epithelial-focused nature of this treatment to airways that have since remodelled on a background of natural disease progression(25).

Most of the safety events were related to natural progression of their disease or unrelated medical disorder. Post-treatment exacerbation frequency increased with GOLD grade, consistent with the experiences of others in the literature(26). From completion of treatment 1 to 12 months, the exacerbation rates of subjects classified as GOLD grades II and III were 1·29 and 2·10 per patient year (PPY), respectively. These rates compare favourably to those reported in untreated similarly-matched individuals: 2·68 PPY in GOLD grade II and 3·43 PPY in GOLD grade III(27). Higher baseline total SGRQ score was associated with an increased exacerbation rate and this mirrors a large dataset of 12,043 patients in whom a higher SGRQ total score predicted increased risk of an adverse COPD outcome (exacerbations, hospitalisation, or death)(28). A reduction in SGRQ achieved using the RejuvenAir MCS treatment may translate to a reduction in COPD exacerbations, particularly in more symptomatic individuals(24), though this is speculative.

The study had some limitations. In the interest of risk adversity, there was a prolonged interval of 9·4 (8·7, 10·8) months between the first and third treatments in the initial 11 (phase A) patients, which may have influenced the efficacy of the therapy and skewed the overall 12-month outcomes, potentially diluting the effects on PROs demonstrated in this study. Multiple validated, nevertheless subjective, disease-specific instruments, SGRQ, CAT, and LCQ were necessary to characterise complex symptoms such as cough, sputum production, breathlessness and health-related quality of life and their responses to a therapeutic intervention that could not be achieved using any one physiological correlate(29). The sample size was small, the treatment was unblinded, and a control group was lacking. Moreover, there were no consistent historical data on pre-treatment exacerbation rates. Lastly, no definitive histological differences were observed between baseline and day 60 endobronchial biopsies and may reflect non-uniform sampling as cryothermic sites were not directly marked or grossly identifiable. The forceps biopsies were obtained from the right lower lobe segmental carina and were of varying quality with crush artefact. Furthermore, the samples were obtained from mucosal tissue at the carina where there tends to be fewer goblet cells. A more

standardised approach within a sham controlled study and sampling using endobronchial cryobiopsies has been initiated and should provide more informative results (ClinicalTrials.gov, NCT03892694).

Bronchial rheoplasty is an alternative novel bronchoscopic therapy using pulsed electric fields to ablate the mucosal lining and is currently under investigation(30). However, no comparable treatment option exists in the mainstream management of chronic bronchitis and current therapeutic modalities are principally pharmacological based. The effects of RejuvenAir MCS on health-related quality of life may be superior compared to mucolytics(31), prophylactic antibiotics(32), inhaled bronchodilators and steroid(33). Future studies including a randomized sham-controlled trial are advocated to confirm the benefits and durability of this treatment in a larger population of patients.

CONCLUSIONS

Treatment with the RejuvenAir system in individuals with CB in COPD is safe, feasible, well tolerated, and resulted in clinically and statistically meaningful improvements in multidimensional measures of cough, sputum production, breathlessness, and health-related quality of life. The safety and efficacy of this therapy will require confirmation by prospective randomised, sham-controlled trials.

RESEARCH IN CONTEXT

Evidence before this study

Cryo-ablation of respiratory epithelium followed by rapid regeneration of healthy tissue observed in animal models has encouraged exploration of the technique in patients with chronic bronchitis in COPD. Currently approved therapies address symptoms only. We searched PubMed and Embase for all studies (up to November 1, 2019) of bronchoscopic treatment of chronic bronchitis with liquid nitrogen cryospray to identify case reports, series, and clinical trials in which the RejuvenAir® liquid nitrogen cryospray system was used. Search terms used were “bronchoscopic lung volume reduction”, “liquid nitrogen cryospray”, “lung volume reduction coils”, “chronic bronchitis”, and “chronic obstructive pulmonary disease”. There were no language restrictions. We identified one study in 16 patients with lung cancer undergoing lobectomy that showed use of the RejuvenAir® system was technically feasible and seemed to be safe. We identified no previous trials that assessed safety or efficacy of RejuvenAir® for the treatment of chronic bronchitis.

Added value of this study

Findings from this study of RejuvenAir® liquid nitrogen cryospray indicates that it is safe and feasible in patients with chronic bronchitis. The therapy has resulted in clinically and statistically meaningful improvements in multidimensional measures of cough, sputum production, breathlessness, and health-related quality of life at 3, 6 & 9 months. The effects of RejuvenAir® on health-related quality of life may be superior than those of mucolytics, prophylactic antibiotics, inhaled bronchodilators and steroids.

Implications of all the available evidence

Future studies including a randomized sham-controlled trial are advocated to confirm the benefits and the durability of MCS treatment in a larger population of patients.

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Author contributions

1. **J L Garner** – 1st author; wrote first manuscript draft together with P L Shah; recruited, treated and followed-up patients.
2. **T Shaipanich** – contributed to writing / revisions of manuscript; recruited, treated and followed-up patients.
3. **J E Hartman** - contributed to writing / revisions of manuscript; recruited and followed-up patients.
4. **C M Orton** - contributed to writing / revisions of manuscript; recruited and followed-up patients.
5. **C Caneja** - contributed to writing / revisions of manuscript; recruited and followed-up patients.
6. **K Klooster** - contributed to writing / revisions of manuscript; recruited and followed-up patients.
7. **J Thornton** - contributed to writing / revisions of manuscript; treated patients.
8. **D D Sin** - contributed to writing / revisions of manuscript; recruited, treated and followed-up patients.
9. **D J Slebos** - contributed to writing / revisions of manuscript; recruited, treated and followed-up patients.
10. **P L Shah** – senior author; wrote first manuscript draft together with J L Garner; recruited, treated and followed-up patients.

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CSA Medical

STATEMENT OF ETHICS

Subjects have given their written informed consent to participate in the trial, and the study protocol was approved by each research institution's committee on human research.

Figure 1 (legend). Study protocol flowchart. Each treatment is separated by 30-45 days

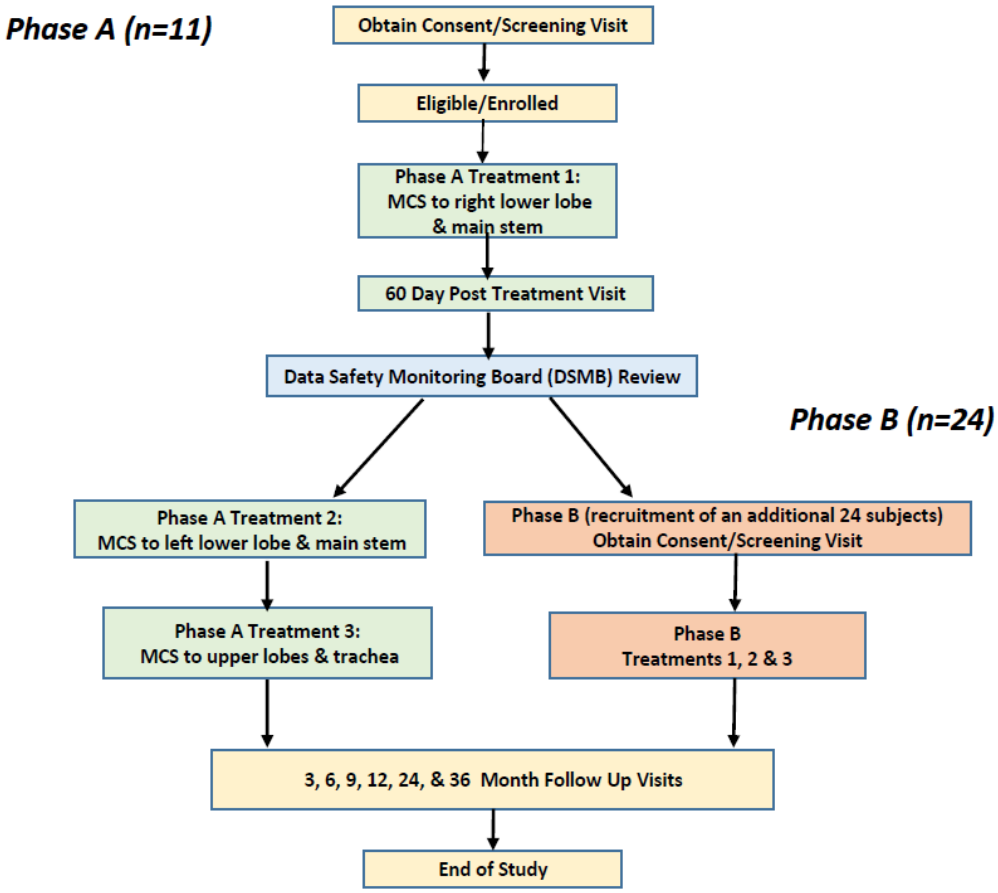


Figure 2 (legend). Mean changes in patient-reported outcomes (PROs) over 12 months: a) St George's Respiratory Questionnaire (SGRQ)-total score; b) SGRQ-symptoms score; c) SGRQ-impacts score; d) SGRQ-activity score. *indicates statistical significance compared to baseline set at p<0.05.

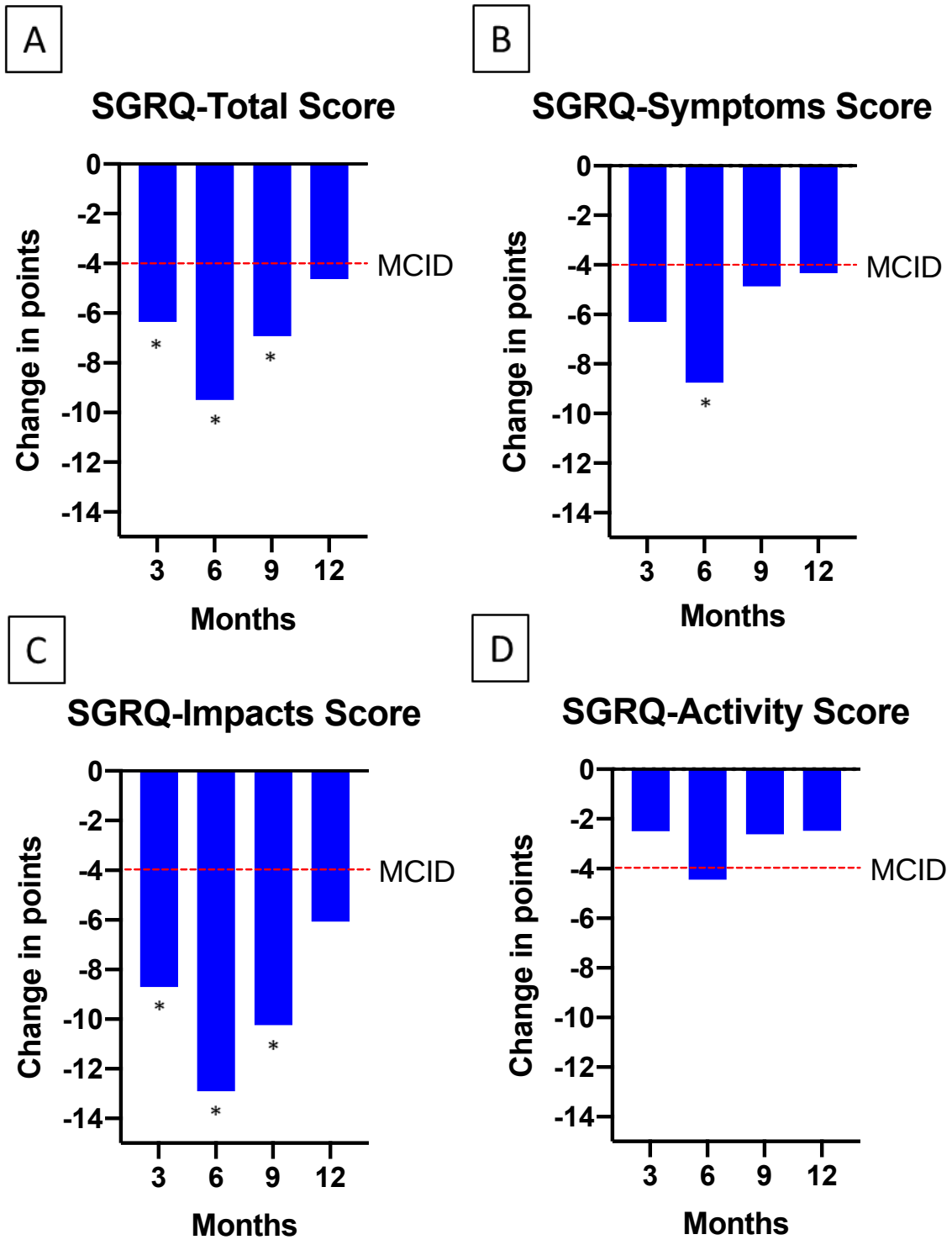


Figure 3 (legend). Mean changes in patient-reported outcomes (PROs) over 12 months: a) COPD Assessment (CAT) score; b) Leicester Cough Questionnaire (LCQ) score; c) modified Medical Research Council (mMRC) dyspnea score; and d) Visual Analogue Score (VAS)-activity. *indicates statistical significance compared to baseline set at p<0.05.

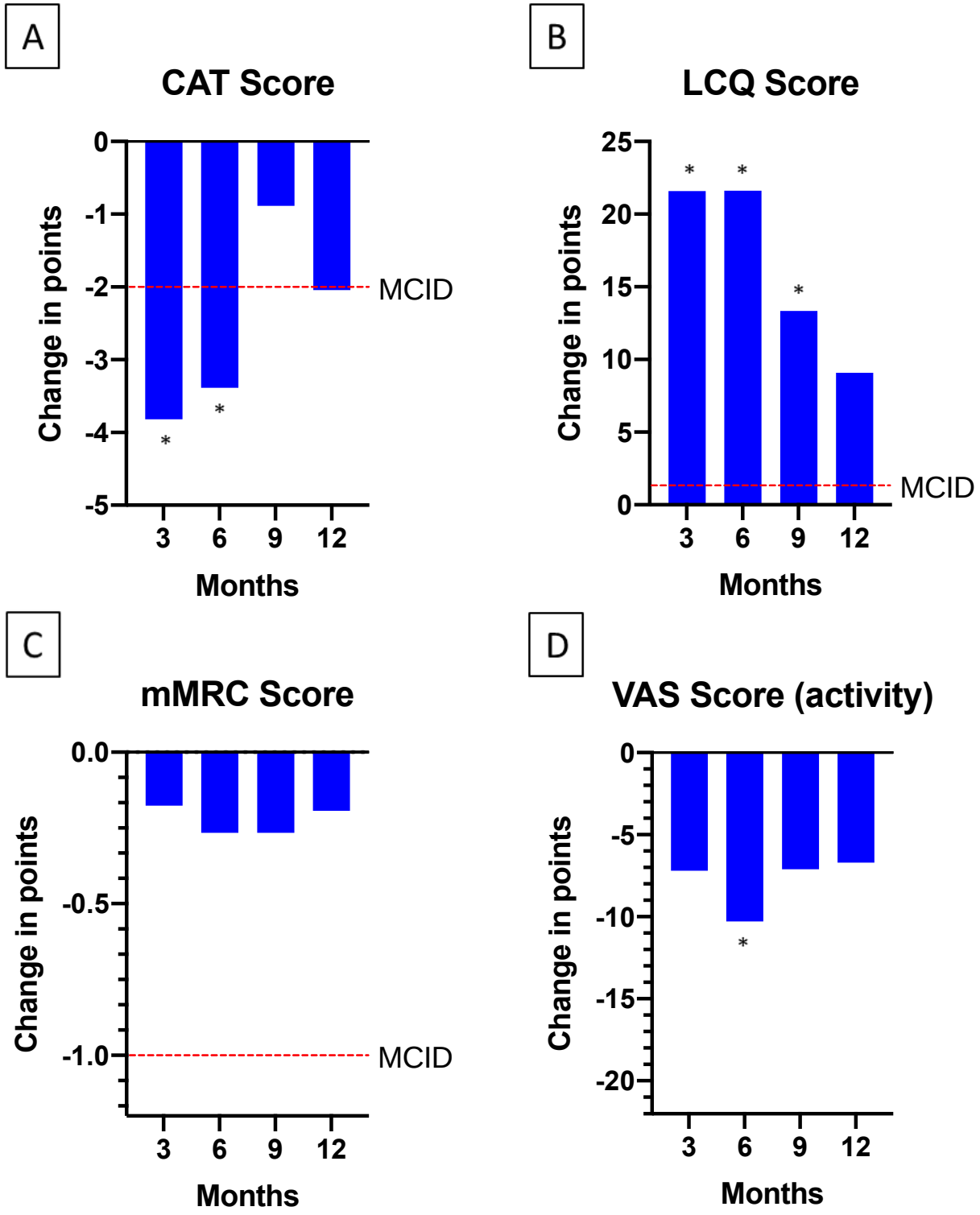


Figure 4 (legend). Mean changes in the total St George's Respiratory Questionnaire (SGRQ) total and domain scores over 12 months in those individuals with baseline total SGRQ scores of > 50 points: a) SGRQ-total score; b) SGRQ-symptoms score; c) SGRQ-impacts score; d) SGRQ-activity score. *indicates statistical significance compared to baseline set at p<0.05.

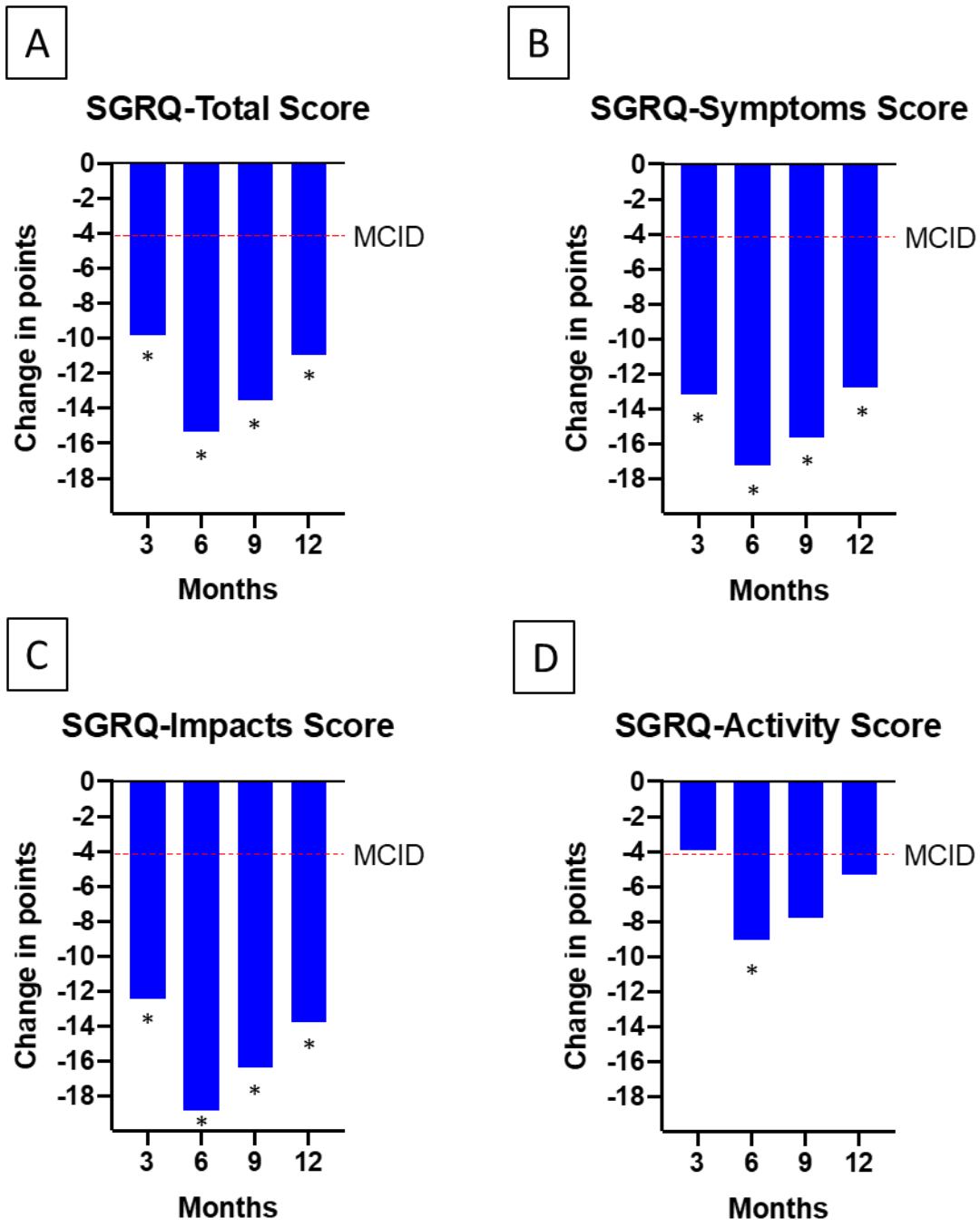


Table 1 (legend): Baseline characteristics of patients.

		n	value
Demographics			
Age, years		35	67.2 ± 7.0
Gender (male), %:		19	54.3%
BMI, kg/m^2		35	26.9 ± 5.2
Pack years		35	45 (33, 68)
Co-morbidities:		35	2 (1, 4)
GOLD grade	I	3	8.5%
	II	10	28.5%
	III	22	63.0%
Baseline medications			
Beta-agonist		18	51.4%
Anticholinergic		18	51.4%
Corticosteroid		17	48.6%
Mucolytic		6	17.1%
Antibiotic		11	31.4%
Lung function			
FEV_1, L		35	1.4 ± 0.5
FEV_1, % predicted		35	50.2 ± 14.5
FVC, L		35	3.6 ± 1.0
FVC, % predicted		35	103.6 ± 16.9
FEV1/FVC, % predicted		35	38.5 ± 10.1
FIV1, L		25	3.2 ± 0.9
Raw, kPA/L/s		27	0.6 ± 0.3
Exercise capacity			
6MWD, m		35	400.6 ± 86.8
Symptoms			
mMRC		35	2 (2, 3)[†]

CAT[§]		34	22.7 ± 7.1
SGRQ	total	35	59.2 ± 18.9
	symptoms		66.5 ± 20.5
	impacts		48.3 ± 22.4
	activity		74.1 ± 19.0
LCQ		23	85.0 ± 27.7
VAS[§]	rest	34	36.1 ± 28.7
VAS[§]	activity	34	68.6 ± 23.9
Mortality Score			
BODE Index		35	3 (2, 4)[†]
Inflammatory marker			
Plasma fibrinogen, mg/dL		35	341.1 ± 72.5

Categorical data are presented as a percentage (%). Numeric data are presented as mean ± SD or median (IQR). [§]Pre-treatment 1 data used. BMI, Body Mass Index; BODE index = Body mass index, airflow Obstruction, Dyspnoea, and Exercise capacity; CAT, COPD Assessment Test; FEV_1, Forced Expiratory Volume in 1 second; FIV1, Forced Inspiratory Volume in 1 second; FVC, Forced Vital Capacity; IC, Inspiratory Capacity; LCQ, Leicester Cough Questionnaire; mMRC, modified Medical Research Council dyspnoea scale; PO_2, Partial pressure for oxygen; RAW, airways resistance; RV, Residual Volume; SGRQ, St George's Respiratory Questionnaire; TLC, Total Lung Capacity; TL_{CO}, Transfer factor for carbon monoxide; VAS, Visual Analogue Score; VC, Vital Capacity; 6MWD, Six-Minute Walk Distance.

Table 2 (legend): Changes in clinical characteristics over 12-months.

	3-month		6-month		9-month		12-month	
	value	*p-value*	value	*p-value*	value	*p-value*	*value*	*p-value*
Lung function								
ΔFEV_1, ml	-33.2 ± 166.9 (95% CI -91.5 to 25.0)	0.25					-96.5 ± 197.7 (95% CI -169.0 to -23.9)	**0.01**
ΔFEV_1, %	-0.7 ± 5.7 (95% CI -2.7 to 1.3)	0.45					-2.4 ± 6.5 (95% CI -4.8 to 0.0)	0.05
ΔFVC, ml	-125.9 ± 330.4 (95% CI -241.2 to -10.6)	**0.03**					-191.3 ± 483.7 (95% CI -368.7 to -13.9)	**0.04**
ΔFVC, %	-3.1 ± 9.5 (95% CI -6.4 to 0.2)	0.06					-2.8 ± 13.0 (95% CI -7.6 to 2.0)	0.24
ΔFEV1/FVC, %	0.3 ± 10.6 (95% CI -3.5 to 4.0)	0.89					-0.9 ± 3.6 (95% CI -2.2 to 0.4)	0.18
ΔFIV1, ml	-175.8 ± 389.5 (95% CI -340.3 to -11.4)	**0.04**					-66.2 ± 371.1 (95% CI -235.1 to 102.7)	0.42
ΔVC, L	1.2 ± 6.6 (95% CI -1.4 to 3.9)	0.35					-0.1 ± 0.4 (95% CI -0.3 to 0.1)	0.49

ΔRAW, kPA/L/s		0.1 ± 0.3 (95% CI -0.1 to 0.2)	0.28					0.0 ± 0.2 (95% CI -0.1 to 0.2)	0.33
Exercise capacity									
Δ6MWD, m		1.1 ± 55.4 (95% CI -18.6 to 20.7)	0.91	20.3 ± 72.0 (95% CI -6.6 to 47.2)	0.13	24.3 ± 65.0 (95% CI -0.4 to 49.0)	0.05	8.5 ± 76.2 (95% CI -19.4 to 36.5)	0.54
Symptoms									
ΔmMRC		0 (IQR: -1, 0)	0.29[†]	0 (IQR -1, 0)	0.10[†]	0 (IQR -1, 0)	0.16[†]	0 (IQR -1, 0)	0.30[†]
ΔCAT[§]		-3.8 ± 7.1 (95% CI -6.4 to -1.3)	**<0.01**	-3.4 ± 6.8 (95% CI -5.9 to -0.9)	**0.01**	-0.9 ± 7.7 (95% CI -3.8 to 2.0)	0.53	-2.0 ± 7.2 (95% CI -4.7 to 0.6)	0.12
ΔSGRQ	*Total score*	-6.4 ± 14.4 (95% CI -11.4 to -1.3)	**0.01**	-9.5 ± 15.7 (95% CI -15.4 to -3.6)	**<0.01**	-6.9 ± 16.2 (95% CI -13.0 to -0.9)	**0.03**	-4.6 ± 15.1 (95% CI -10.2 to 0.9)	0.10
	Symptoms	-6.3 ± 22.1 (95% CI -14.0 to 1.4)	0.10	-8.8 ± 19.6 (95% CI -16.1 to -1.4)	**0.02**	-4.9 ± 21.9 (95% CI -13.1 to 3.3)	0.23	-4.3 ± 21.5 (95% CI -12.2 to 3.5)	0.27
	Activity	-2.5 ± 15.0 (95% CI -7.7 to 2.7)	0.34	-4.4 ± 17.5 (95% CI -11.0 to 2.1)	0.17	-2.6 ± 17.9 (95% CI -9.3 to 4.1)	0.43	-2.5 ± 14.8 (95% CI -7.9 to 3.0)	0.36
	Impacts	-8.7 ± 16.7 (95% CI -14.5 to -2.9)	**<0.01**	-12.9 ± 17.9 (95% CI -19.6 to -6.2)	**<0.01**	-10.2 ± 18.4 (95% CI -17.1 to -3.4)	**<0.01**	-6.1 ± 20.0 (95% CI -13.4 to 1.3)	0.10
ΔLCQ		21.6 ± 32.2	**<0.01**	21.6 ± 29.2	**<0.01**	13.4 ± 24.1	**0.02**	9.1 ± 29.0	0.17

		(95% CI 7.3 to 35.9)		(95% CI 8.3 to 34.9)		(95% CI 2.1 to 24.6)		(95% CI -4.1 to 22.3)	
∆VAS[§]	*Rest*	-3.6 ± 31.5 (95% CI -14.8 to 7.5)	0.51	-2.7 ± 25.5 (95% CI -12.2 to 6.9)	0.57	-1.1 ± 31.1 (95% CI -12.8 to 10.5)	0.85	-0.4 ± 25.4 (95% CI -9.7 to 8.9)	0.93
	Activity	-7.2 ± 22.2 (95% CI -15.0 to 0.7)	0.07	-10.3 ± 22.4 (95% CI -18.7 to -1.9)	**0.02**	-7.1 ± 25.2 (95% CI -17.3 to 1.9)	0.13	-6.7 ± 21.4 (95% CI -14.6 to 1.2)	0.09
Mortality Score ∆BODE Index		-0.1 ± 1.1 (95% CI -0.5 to 0.3)	0.54					0.1 ± 1.4 (95% CI -0.4 to 0.6)	0.61
Inflammatory marker ∆Fibrinogen, mg/dL		45.2 ± 84.5 (95% CI 15.2 to 75.1)	**<0.01**					29.3 ± 65.2 (95% CI 4.5 to 54.1)	**0.02**

Numeric data are presented as mean ± SD / 95% confidence interval (95% CI) or median (IQR). Two-tailed t-test or Wilcoxon matched-pairs signed rank test[†] were used, respectively, to calculate statistical significance between groups. [§]Pre-treatment 1 data used. BMI, Body Mass Index; BODE index = Body mass index, airflow Obstruction, Dyspnoea, and Exercise capacity; CAT, COPD Assessment Test; FEV_1, Forced Expiratory Volume in 1 second; FIV1, Forced Inspiratory Volume in 1 second; FVC, Forced Vital Capacity; IC, Inspiratory Capacity; LCQ, Leicester Cough Questionnaire; mMRC, modified Medical Research Council dyspnoea scale; PO_2, Partial pressure for oxygen; RAW, airways resistance; RV, Residual Volume; SGRQ, St George's Respiratory Questionnaire; TLC, total lung capacity; TL_{CO}, transfer factor for carbon monoxide; VAS, Visual Analogue Score; VC, Vital Capacity; 6MWD, six-minute walk distance.

ONLINE SUPPLEMENT

Section 1

Table S1 (legend): Inclusion and Exclusion criteria.

Inclusion Criteria
- Males and females ≥40 to ≤75 years of age. - Subject is able to read, understand, and sign a written Informed Consent in order to participate in the Study. - Subject has been optimally treated according to Gold treatment guidelines without successful resolution of chronic bronchitis and agrees to continue maintenance pulmonary/COPD medications for the duration of the study. - Diagnosis of chronic bronchitis (CB) and chronic obstructive pulmonary disease (COPD) for a minimum of two years. (Chronic Bronchitis is defined clinically as chronic productive cough for 3 months in each of 2 successive years in a patient in whom other causes of productive cough have been excluded.) - Pre-procedure post bronchodilator FEV1 of greater than or equal to 30% and less than or equal to 80% of predicted within 3 months of enrolment. - Smoking history of at least 10 pack years. - Non-smoking for a minimum of 2 months prior to consent and agrees to continue not smoking for the duration of the study. - Subject is able to adhere to and undergo 3 (4 if in Phase A) bronchoscope procedures that includes lung biopsies and multiple MCS treatments in the opinion of the investigator or per hospital guidelines. (Only Phase A subjects receive biopsies).
Exclusion Criteria
- Subject has had an acute pulmonary infection or pneumonia within prior 6 weeks of study bronchoscopy. - Subject has had a CB and/or COPD exacerbation (requiring steroids and/or antibiotics) within 6 weeks prior to study bronchoscopy, as defined by their treating physician. - Subject has clinically significant bronchiectasis or other respiratory disease other than chronic bronchitis and COPD; subject with chronic cough of other pathogenesis, in particular cardiac cause. Subject with the following conditions should not undergo bronchoscopy: untreatable or life-threatening arrhythmias, inability to adequately oxygenate the patient during the procedure, or subject has acute respiratory failure with hypercapnia (unless intubated and ventilated) or has high grade tracheal stenosis. - Diagnosis of asthma with an onset before 30 years of age. - Subject has bullous emphysema characterized as large bullae >30 millimetres on CT; or subject has stenosis in the tracheobronchial system, tracheobronchomegaly, trachea-bronchomalacia, amyloidosis or cystic fibrosis. If a CT is not available in the past 12 months, the Principle Investigator may use the baseline HRCT in lieu of the CT. - Subject has had a transplant. - Subject has the inability to walk >140 meters. - Subject has PaC02 >8kPa, or a PaO2<7kPa at room air. - Subject has a RVSP >45mmHg or a LVEF<45% on 2D-cardiac echo. - Subject has a known mucosal tear, requires treatment to the Right Middle Lobe or has undergone lung surgery: pneumonectomy, lobectomy, bullectomy, lung volume reduction surgery. - Subject has had a prior lung device procedure, including emphysema stent(s) implanted, lung coils, valves, lung denervation or other devices for emphysema. - Subject is unable to temporarily discontinue use of anticoagulant therapy: warfarin, Coumadin, LMWH, heparin, clopidrogel (or equal). - Subject is on >10 mg of prednisolone/day. - Subject has a serious medical condition, such as: uncontrolled coagulopathy or bleeding disorder, congestive heart failure, uncontrolled angina, myocardial infarction in the past year, renal failure, liver disease, cerebrovascular accident within the past 6 months, uncontrolled diabetes uncontrolled hypertension, autoimmune disease or uncontrolled gastric reflux. - Subject is pregnant, nursing, or planning to get pregnant during study duration. - Subject has or is receiving chemotherapy or active radiation therapy within the past 6 months or is expected to receive chemotherapy during participation in this study. Subject life expectancy is less than one year. - Subject is or has been in another clinical investigational study within 6 weeks of baseline. - Subject has known sensitivity to medication required to perform bronchoscopy (such as lidocaine, atropine, and benzodiazepines).
CB, Chronic Bronchitis; COPD, Chronic Obstructive Pulmonary Disease; CT, Computed Tomography; FEV1, Forced Expiratory Volume in 1 second; kPA, kilopascal; LVEF, Left Ventricular Ejection Fraction; LMWH, Low Molecular Weight Heparin; MCS, Metered CryoSpray; mg, milligram; mm Hg, millilitre of mercury; $PaCO_2$, Partial pressure of carbon dioxide; PaO_2, Partial pressure of oxygen; RVSP, Right Ventricular Systolic Pressure; 2D, 2-Dimensional.

Table S2 (legend): Procedural details.

	Treatment 1	Treatment 2	Treatment 3	Mean of Treatments
Mean treatment duration (minutes)	33.3 ± 11.8	31.4 ± 11.5	38.3 ± 12.3	34.3 ± 12.1
Mean number of metered cryosprays (MCS)	17.3 ± 4.6	17.6 ± 2.1	26.2 ± 5.8	20.3 ± 6.0
Mean number of Full Doses (sprays)	15.2 ± 4.5	14.9 ± 2.5	21.8 ± 5.4	17.3 ± 5.3
Mean number of Partial Doses (sprays)	2.2 ± 2.5	2.6 ± 2.4	4.4 ± 4.2	3.1 ± 3.3
Mean percentage of Full Doses (%)	87.7	85.3	84.3	85.8
Mean percentage of Partial Doses (%)	12.3	14.7	15.7	14.2

Table S3 (legend): Subject overview of adverse events over 12-months.

Adverse Event (AE) categorisation	N	%
Subjects experiencing any AE	35	100
Subjects experiencing a Serious AE	11	31.4
Subjects experiencing a Device-related AE*	4	11.4
Subjects experiencing a Serious Device Related AE*	0	0
Subjects experiencing a Procedure Related AE*	21	60.0
Subjects experiencing a Serious Procedure Related AE*	0	0
Subjects experiencing a Severe AE**	6	17.1
Subjects experiencing an AE leading to discontinuation***	1	2.9

* = AE is related if: relation to device / procedure is reported 'Possibly', 'Probably' or 'Causal Relationship'.
** = AE is severe if: severity is reported 'Severe'.
*** = AE led to discontinuation if: reason for early withdrawal at End of Study is 'Adverse Event' or 'Death'.

Table S4 (legend): Categorization of adverse events over 12-months.

Adverse Event (AE) categorisation		**N**	**%**
Serious	*No*	237	94.4
	Yes	14	5.6
Causality			
	Concomitant or previous medication	8	3.2
	Disease under study	123	49.0
	Medical history	37	14.7
	Other	83	33.1
Relationship to device*			
	Not related	245	97.6
	Possibly	5	2.0
	Probably	1	0.4
Relationship to study procedure*			
	Not related	205	81.7
	Unlikely	6	2.4
	Possibly	30	12.0
	Probably	10	4.0
Severity**			
	Mild	114	45.4
	Moderate	129	51.4
	Severe	8	3.2
Outcome			
	Death	1	0.4
	Ongoing	21	8.4
	Resolved with sequelae	15	6.0
	Resolved without sequelae	214	85.3

* = AE is related if: relation to device or procedure is reported 'Possibly', 'Probably' or 'Causal Relationship'.
** = AE is severe if: severity is reported 'Severe'

Table S5 (legend): Individual classification of adverse events (AEs) over 12-months.

Adverse Event (AE) - System Organ Classification (listed alphabetically)	**Total AEs**		**Total Subjects**	
	N	**%**	**N**	**%**
Ear and labyrinth disorders	1	0.4	1	2.9
Hypoacusis	1	0.4	1	2.9
Vertigo				
Gastrointestinal disorders				
Abdominal pain upper	1	0.4	1	2.9
Diarrhoea	2	0.8	2	5.7
Gastrointestinal disorder	1	0.4	1	2.9
Glossodynia	1	0.4	1	2.9
Nausea	4	1.6	4	11.4
Peptic ulcer	1	0.4	1	2.9
Rectal haemorrhage	1	0.4	1	2.9
Rectal ulcer	1	0.4	1	2.9
Toothache	1	0.4	1	2.9
General disorders				
Chest discomfort	3	1.2	2	5.7
Chest pain	4	1.6	4	11
Fatigue	4	1.6	4	11
Pain	1	0.4	1	2.9
Peripheral swelling	1	0.4	1	2.9
Hepatobiliary disorders				
Bile duct obstruction	1	0.4	1	2.9
Infections and infestations				
Bacterial infection	1	0.4	1	2.9
Cellulitis	2	0.8	2	5.7
Cystitis	2	0.8	2	5.7
Haemophilus infection	1	0.4	1	2.9
Influenza	3	1.2	3	8.6
Nasopharyngitis	5	2.0	3	8.6
Pneumonia	6	2.4	6	17
Pseudomonas infection	1	0.4	1	2.9
Respiratory tract infection	1	0.4	1	2.9
Rhinitis	1	0.4	1	2.9
Sinusitis	2	0.8	2	5.7
Staphylococcal infection	1	0.4	1	2.9
Upper respiratory tract infection	1	0.4	1	2.9
Urinary tract infection	3	1.2	2	5.7
Urosepsis	1	0.4	1	2.9
Injury, poisoning and procedural complications				
Fall	1	0.4	1	2.9
Procedural hypotension	1	0.4	1	2.9
Rib fracture	1	0.4	1	2.9
Skin abrasion	1	0.4	1	2.9
Wound complication	1	0.4	1	2.9
Investigations				
Bacterial test positive	1	0.4	1	2.9
Blood potassium decreased	1	0.4	1	2.9
Blood sodium decreased	1	0.4	1	2.9
Moraxella test positive	1	0.4	1	2.9
Mycobacterium test positive	2	0.8	2	5.7

Adverse Event (AE) - System Organ Classification (listed alphabetically)	Total AEs		Total Subjects	
	N	%	N	%
Pseudomonas test positive	1	0.4	1	2.9
Sputum culture positive	1	0.4	1	2.9
Streptococcus test positive	1	0.4	1	2.9
Vitamin D decreased	1	0.4	1	2.9
Metabolism and nutrition disorders				
Hypoglycaemia	2	0.8	1	2.9
Musculoskeletal and connective tissue disorders				
Arthralgia	2	0.8	2	5.7
Back pain	1	0.4	1	2.9
Bursitis	1	0.4	1	2.9
Dupuytren's contracture	1	0.4	1	2.9
Joint swelling	2	0.8	2	5.7
Muscle spasms	1	0.4	1	2.9
Musculoskeletal discomfort	1	0.4	1	2.9
Musculoskeletal pain	3	1.2	3	8.6
Neck pain	1	0.4	1	2.9
Pain in extremity	1	0.4	1	2.9
Polymyalgia rheumatica	1	0.4	1	2.9
Neoplasms benign, malignant				
Malignant melanoma	1	0.4	1	2.9
Skin cancer	1	0.4	1	2.9
Nervous system disorders				
Balance disorder	1	0.4	1	2.9
Headache	1	0.4	1	2.9
Migraine	1	0.4	1	2.9
Morton's neuralgia	1	0.4	1	2.9
Nervous system disorder	1	0.4	1	2.9
Psychiatric disorders				
Anxiety	1	0.4	1	2.9
Renal and urinary disorders				
Chronic kidney disease	1	0.4	1	2.9
Dysuria	1	0.4	1	2.9
Urinary retention	2	0.8	2	5.7
Reproductive system and breast disorders				
Breast mass	1	0.4	1	2.9
Pelvis prolapse	1	0.4	1	2.9
Respiratory, thoracic and mediastinal disorders				
Bronchospasm	1	0.4	1	2.9
Chronic obstructive pulmonary disease	91	36.3	32	91.4
Cough	5	2.0	4	11.4
Dyspnoea	8	3.2	7	20.0
Epistaxis	2	0.8	2	5.7
Hyperventilation	1	0.4	1	2.9
Increased viscosity of bronchial secretion	1	0.4	1	2.9
Lung consolidation	2	0.8	2	5.7
Oropharyngeal pain	1	0.4	1	2.9
Pulmonary embolism	1	0.4	1	2.9
Pulmonary mass	8	3.2	8	22.9
Rhinorrhoea	4	1.6	4	11.4
Sputum increased	3	1.2	3	8.6
Wheezing	4	1.6	2	5.7

Adverse Event (AE) - System Organ Classification	Total AEs		Total Subjects	
(listed alphabetically)	N	%	N	%
Skin and subcutaneous tissue disorders				
Eczema	1	0.4	1	2.9
Erythema	1	0.4	1	2.9
Surgical and medical procedures				
Cholecystectomy	1	0.4	1	2.9
Hip arthroplasty	1	0.4	1	2.9
Knee operation	1	0.4	1	2.9
Tooth extraction	1	0.4	1	2.9
Vascular disorders				
Aortic aneurysm	2	0.8	2	5.7
Hypertension	1	0.4	1	2.9
Hypotension	3	1.2	3	8.6
Thrombophlebitis	1	0.4	1	2.9
Total	**251**	**100**	**35**	**100**

Table S6 (legend): Device-related events.

Subject	Adverse Event	Severity	Duration (days)	Outcome	Device	Procedure
47-011	Exacerbation of COPD	Mild	7	Resolved without sequelae	Possibly	Possibly
47-011	Bronchospasm	Moderate	0	Resolved without sequelae	Probable	Probable
47-012	Exacerbation of COPD	Moderate	15	Resolved without sequelae	Possibly	Possibly
47-014	Exacerbation of COPD	Moderate	37	Resolved without sequelae	Possibly	Possibly
47-014	Exacerbation of COPD	Moderate	25	Resolved without sequelae	Possibly	Possibly
47-034	Exacerbation of COPD	Mild	14	Resolved without sequelae	Possibly	Possibly

Table S7 (legend): Serious adverse events over 12-months.

Subject ID	Description of SAE	Duration (days)	Outcome	Severity	Related to device	Related to procedure
46-003	Cough increased	72	Resolved without sequelae	Moderate	Not Related	Not Related
46-004	COPD exacerbation	9	Resolved without sequelae	Moderate	Not Related	Not Related
46-005	COPD exacerbation	6	Resolved without sequelae	Moderate	Not Related	Not Related
46-006	COPD exacerbation	39	Resolved without sequelae	Moderate	Not Related	Not Related
47-002	Peptic ulcer	117	Resolved without sequelae	Severe	Not Related	Not Related
47-008	Pulmonary embolus	4	Resolved with sequelae	Severe	Not Related	Not Related
47-008	Chest pain	39	Death	Severe	Not Related	Not Related
47-008	Rectal bleeding	1	Resolved without sequelae	Severe	Not Related	Not Related
47-014	COPD exacerbation	10	Resolved without sequelae	Severe	Not Related	Not Related
47-017	Pneumonia	12	Resolved without sequelae	Severe	Not Related	Not Related
47-034	COPD exacerbation	29	Resolved without sequelae	Severe	Not Related	Not Related
51-003	Pneumonia	170	Resolved without sequelae	Severe	Not Related	Not Related
51-006	COPD exacerbation	4	Resolved without sequelae	Moderate	Not Related	Not Related
51-006	Urosepsis	19	Resolved without sequelae	Moderate	Not Related	Not Related

SAE = Serious Adverse Event

Table S8 (legend): Exacerbation rates over 12-months.

Time period	N	Per patient year
Whole cohort		
T1 to 3-months	49	2.00
T1 to 6-months	59	2.04
T1 to 9-months	65	1.81
T1 to 12-months	83	1.84
T3 to 3-months	22	2.50
T3 to 6-months	34	2.09
T3 to 9-months	45	1.98
T3 to 12-months	62	1.96
GOLD grade II		
T1 to 3-months	17	1.89
T1 to 6-months	18	1.72
T1 to 9-months	19	1.46
T1 to 12-months	21	1.29
T3 to 3-months	7	2.32
T3 to 6-months	9	1.50
T3 to 9-months	12	1.43
T3 to 12-months	14	1.19
GOLD grade III		
T1 to 3-months	32	2.03
T1 to 6-months	41	2.29
T1 to 9-months	46	2.01
T1 to 12-months	62	2.10
T3 to 3-months	15	2.44
T3 to 6-months	25	2.41
T3 to 9-months	33	2.24
T3 to 12-months	48	2.28

GOLD, Global initiative for Obstructive Lung Disease; T1, Treatment 1; T3, Treatment 3.

Table S9 (legend): Changes in St George's Respiratory Questionnaire total scores over 12-months stratified according to disease severity thresholds.

		3-month		6-month		9-month		12-month	
		value	*p-value*	value	*p-value*	value	*p-value*	*value*	*p-value*
Baseline SGRQ-Total Score > 50 points		n=24		n=20		n=20		n=21	
ΔSGRQ	*Total score*	-9.8 ± 14.4 (95% CI -15.9 to -3.8)	**<0.01**	-15.4 ± 15.3 (95% CI -22.6 to -8.2)	**<0.01**	-13.5 ± 15.4 (95% CI -20.7 to -6.3)	**<0.01**	-10.9 ± 12.1 (95% CI -16.4 to -5.4)	**<0.01**
	Symptoms	-13.2 ± 19.0 (95% CI -21.2 to -5.2)	**<0.01**	-17.2 ± 16.7 (95% CI -25.0 to -9.4)	**<0.01**	-15.6 ± 16.9 (95% CI -23.5 to -7.7)	**<0.01**	-12.8 ± 17.9 (95% CI -20.9 to -4.6)	**<0.01**
	Activity	-3.9 ± 15.0 (95% CI -10.2 to 2.4)	0.21	-9.0 ± 17.2 (95% CI -17.1 to -1.0)	**0.03**	-7.8 ± 18.3 (95% CI -16.4 to 0.7)	0.07	-5.3 ± 16.1 (95% CI -12.7 to 2.0)	0.15
	Impacts	-12.5 ± 17.7 (95% CI -19.9 to -5.0)	**<0.01**	-18.9 ± 18.7 (95% CI -27.6 to -10.1)	**<0.01**	-16.4 ± 19.3 (95% CI -25.4 to -7.3)	**<0.01**	-13.8 ± 16.9 (95% CI -21.5 to -6.1)	**<0.01**
Baseline SGRQ-Total Score < 50 points		n=10		n=10		n=10		n=10	
ΔSGRQ	*Total score*	2.0 ± 10.9	0.58	2.3 ± 8.4	0.41	6.3 ± 7.2	**0.02**	8.6 ± 12.1	0.05

		(95% CI -5.8 to 9.8)		(95% CI -3.7 to 8.3)		(95% CI 1.1 to 11.4)		(95% CI 0.0 to 17.3)	
	Symptoms	10.2 ± 20.8	0.15	8.2 ± 13.3	0.09	16.6 ± 13.4	**<0.01**	13.4 ± 17.6	**0.04**
		(95% CI -4.6 to 25.0)		(95% CI -1.4, 17.7)		(95% CI 7.0 to 26.2)		(95% CI 0.8 to 26.0)	
	Activity	0.9 ± 15.3	0.86	4.8 ± 14.8	0.33	7.8 ± 12.1	0.07	3.5 ± 9.9	0.29
		(95% CI -10.0 to 11.8)		(95% CI -5.8 to 15.4)		(95% CI -0.8 to 16.4)		(95% CI -3.5 to 10.6)	
	Impacts	0.3 ± 9.9	0.92	-1.0 ± 7.3	0.68	2.0 ± 7.1	0.39	10.2 ± 16.3	0.08
		(95% CI -6.8 to 7.4)		(95% CI -6.2 to 4.3)		(95% CI -3.1 to 7.1)		(95% CI -1.5 to 21.8)	
Baseline CAT Score > 10 points		n=31		n=28		n=28		n=29	
∆SGRQ	*Total score*	-6.2 ± 14.9	**0.03**	-10.0 ± 16.2	**<0.01**	-7.6 ± 16.5	**0.02**	-5.5 ± 15.1	0.06
		(95% CI -11.7 to -0.7)		(95% CI -16.3 to -3.7)		(95% CI -14.0 to -1.3)		(95% CI -11.2 to 0.3)	
	Symptoms	-5.7 ± 22.2	0.16	-8.7 ± 20.3	**0.03**	-5.7 ± 22.2	0.19	-5.8 ± 21.3	0.16
		(95% CI -13.8 to 2.4)		(95% CI -16.6 to -0.8)		(95% CI -14.3 to 3.0)		(95% CI -13.9 to 2.3)	
	Activity	-2.4 ± 15.4	0.39	-4.8 ± 18.0	0.17	-3.1 ± 18.4	0.39	-2.0 ± 15.2	0.48
		(95% CI -8.0 to 3.2)		(95% CI -11.7 to 2.2)		(95% CI -10.2 to 4.1)		(95% CI -7.8 to 3.8)	
	Impacts	-8.7 ± 17.4	**0.01**	-13.6 ± 18.3	**<0.01**	-11.0 ± 18.8	**<0.01**	-7.4 ± 19.5	0.05
		(95% CI -15.1 to -2.3)		(95% CI -20.7 to -		(95% CI -18.3 to -3.7)		(95% CI -14.9 to	

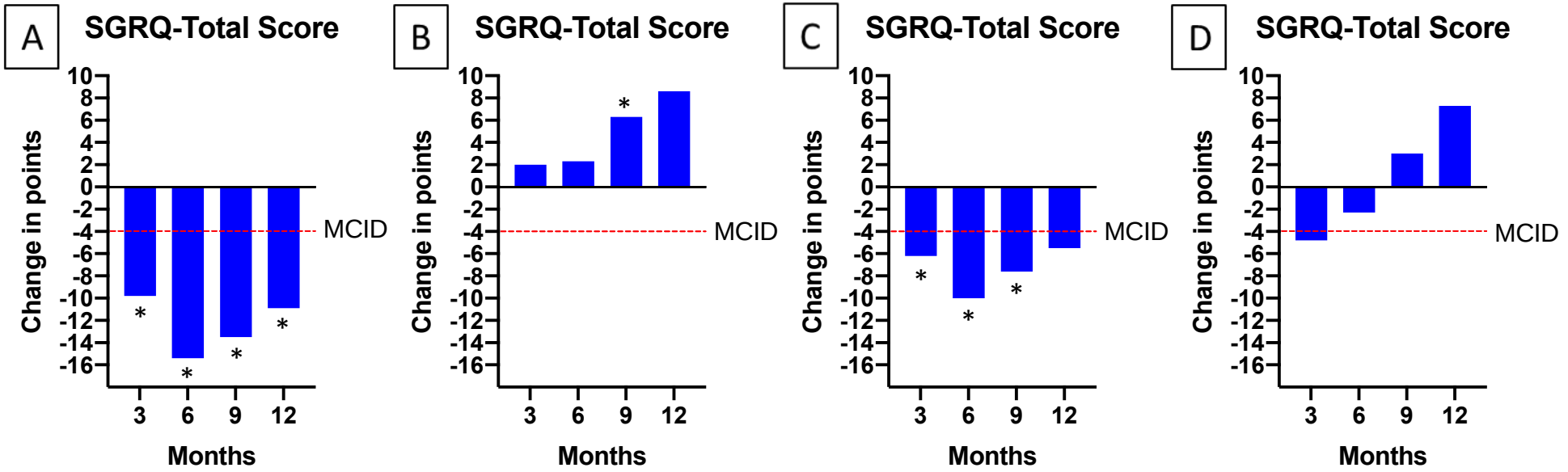
				6.5)				0.0)
Baseline CAT Score < 10 points		n=2		n=2		n=2		n=2	
∆SGRQ	*Total score*	-4.8 ± 6.9	0.50	-2.3 ± 3.5	0.53	3.0 ± 5.2	0.56	7.3 ± 11.4	0.53
		(95% CI -66.8 to 57.1)		(95% CI -33.6 to 29.1)		(95% CI -43.7 to 49.7)		(95% CI -95.1 to 109.7)	
	Symptoms	0.7 ± 10.3	0.94	-9.7 ± 5.2	0.23	6.2 ± 18.2	0.72	16.5 ± 13.7	0.34
		(95% CI -91.5 to 92.9)		(95% CI -56.8 to 37.4)		(95% CI -157.6 to 169.9)		(95% CI -107.0 to 140.0)	
	Activity	-8.9 ± 12.9	0.51	0.4 ± 9.1	0.96	3.5 ± 4.7	0.48	-8.7 ± 4.0	0.20
		(95% CI -125.2 to 107.4)		(95% CI -81.6 to 82.4)		(95% CI -38.4 to 45.5)		(95% CI -44.4 to 26.9)	
	Impacts	-3.9 ± 2.5	0.27	-2.6 ± 0.5	0.09	0.3 ± 2.0	0.86	13.9 ± 21.2	0.52
		(95% CI -26.0 to 18.1)		(95% CI -7.3 to 2.1)		(95% CI -18.0 to 18.6)		(95% CI -176.2 to 203.9)	
Baseline GOLD grade of 3		n=22		n=19		n=19		n=20	
∆SGRQ	*Total score*	-6.9 ± 14.4	**0.04**	-10.7 ± 15.5	**<0.01**	-8.6 ± 16.6	**0.04**	-6.3 ± 13.1	0.05
		(95% CI -13.3 to -0.5)		(95% CI -18.2 to -3.3)		(95% CI -16.6 to -0.6)		(95% CI -12.4 to -0.1)	
	Symptoms	-9.4 ± 23.4	0.07	-9.2 ± 20.2	0.06	-7.2 ± 23.0	0.19	-7.1 ± 20.1	0.13
		(95% CI -19.8 to 0.9)		(95% CI -18.9 to 0.6)		(95% CI -18.3 to 3.9)		(95% CI -16.5 to 2.3)	

	Activity	-1.5 ± 14.3 (95% CI -7.9 to 4.9)	0.63	-4.7 ± 16.2 (95% CI -12.5 to 3.1)	0.22	-2.8 ± 16.1 (95% CI -10.5 to 5.0)	0.46	-1.0 ± 10.2 (95% CI -5.8 to 3.8)	0.66
	Impacts	-9.2 ± 16.2 (95% CI -16.4 to -2.1)	**0.01**	-14.7 ± 17.6 (95% CI -23.2 to -6.3)	**<0.01**	-12.4 ± 20.2 (95% CI -22.1 to -2.7)	**0.02**	-9.0 ± 18.0 (95% CI -17.4 to -0.5)	**0.04**
<u>Baseline GOLD grade of 2</u>		n=10		n=9		n=9		n=9	
ΔSGRQ	*Total score*	-5.1 ± 16.4 (95% CI -16.8 to 6.7)	0.35	-8.0 ± 18.5 (95% CI -22.2 to 6.2)	0.23	-5.7 ± 17.0 (95% CI -18.7 to 7.4)	0.35	-2.8 ± 19.6 (95% CI -17.9 to 12.2)	0.68
	Symptoms	0.42 ± 20.9 (95% CI -14.5 to 15.3)	0.95	-8.5 ± 21.8 (95% CI -25.2 to 8.3)	0.28	-4.1 ± 21.0 (95% CI -20.3 to 12.0)	0.57	-2.3 ± 25.0 (95% CI -21.5 to 17.0)	0.79
	Activity	-3.5 ± 17.7 (95% CI -16.2 to 9.1)	0.54	-5.7 ± 22.3 (95% CI -22.8 to 11.4)	0.46	-3.8 ± 23.7 (95% CI -22.0 to 14.4)	0.64	-4.4 ± 23.6 (95% CI -22.5 to 13.8)	0.59
	Impacts	-8.0 ± 20.4 (95% CI -22.5 to 6.5)	0.25	-9.7 ± 20.6 (95% CI -25.5 to 6.1)	0.19	-7.7 ± 16.5 (95% CI -20.4 to 5.0)	0.20	-2.6 ± 23.1 (95% CI -20.4 to 15.1)	0.74

Numeric data are presented as mean ± SD or median (IQR). Two-tailed t-test or Wilcoxon matched-pairs signed rank test[†] were used, respectively, to calculate statistical significance between groups. [§]Pre-treatment 1 data used. BMI, Body Mass Index; BODE index = Body mass index, airflow Obstruction, Dyspnoea, and Exercise capacity; CAT, COPD Assessment Test; FEV_1, Forced Expiratory Volume in 1 second; FIV1, Forced Inspiratory Volume in 1 second; FVC, Forced Vital Capacity; GOLD, Global initiative for Obstructive Lung Disease; IC, Inspiratory Capacity; LCQ, Leicester Cough Questionnaire; mMRC, modified Medical Research Council dyspnoea scale; PO_2, Partial pressure for oxygen; RAW, airways resistance; RV, Residual Volume; SGRQ, St George's Respiratory Questionnaire; TLC, total lung capacity; TL_{CO}, transfer factor for carbon monoxide;

VAS, Visual Analogue Score; VC, Vital Capacity; 6MWD, six-minute walk distance.

Figure S5 (legend): Changes in St George's Respiratory Questionnaire total scores over 12-months stratified according to disease severity thresholds: A) SGRQ-Total Score > 50; B) SGRQ-Total Score < 50; C) CAT score > 10; D) CAT score < 10; E) Baseline GOLD grade 3; F) Baseline GOLD grade 2.



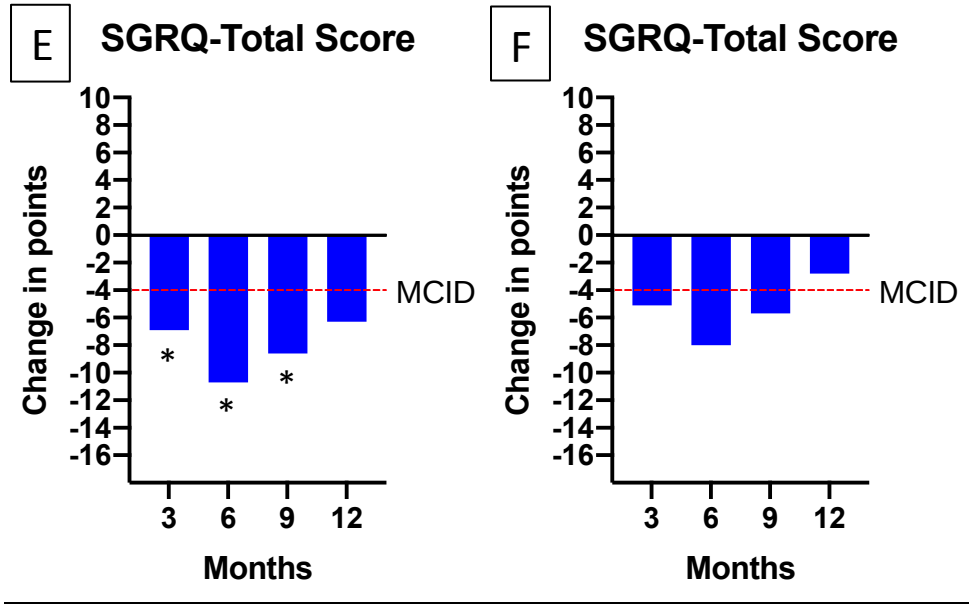

E
SGRQ-Total Score
Change in points
10
8
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
MCID
*
*
*
3
6
9
12
Months
F
SGRQ-Total Score
Change in points
MCID
3
6
9
12
Months

Table S10 (legend): Changes in Patient-Reported Outcomes over 12-months in those individuals with a baseline SGRQ total score > 50 points.

Baseline SGRQ-Total Score > 50 points	3-month		6-month		9-month		12-month	
	value	*p-value*	value	*p-value*	value	*p-value*	*value*	*p-value*
ΔCAT[§]	n=23 -5.2 ± 7.3 (95% CI -8.4 to -2.1)	**<0.01**	n=20 -5.4 ± 6.8 (95% CI -8.6 to -2.3)	**<0.01**	n=20 -2.2 ± 8.6 (95% CI -6.2 to 1.8)	0.27	n=21 -4.0 ± 7.0 (95% CI -7.2 to -0.8)	**0.02**
ΔLCQ	n=14 36.3 ± 28.1 (95% CI 20.1 to 52.5)	**<0.01**	n=13 35.0 ± 29.1 (95% CI 17.4 to 52.6)	**<0.01**	n=12 26.2 ± 21.1 (95% CI 12.7 to 39.6)	**<0.01**	n=13 23.5 ± 22.1 (95% CI 10.2 to 36.9)	**<0.01**
ΔmMRC	n=24 0 (-1, 0) (95.7% CI 0 to 0)	0.24	n=20 0 (-1, 0) (95.9% CI -1 to 0)	**0.04**	n=20 0 (-1, 0) (95.9% CI -1 to 0)	0.13	n=21 0 (-1, 0) (97.3% CI -1 to 0)	0.49
ΔVAS - Rest[§]	n=23 -10.0 ± 33.5 (95% CI -24.4 to 4.5)	0.17	n=20 -7.4 ± 29.9 (95% CI -21.4 to 6.6)	0.28	n=20 -4.2 ± 36.2 (95% CI -21.1 to 12.8)	0.61	n=21 -5.8 ± 28.3 (95% CI -18.6 to 7.1)	0.36
	n=23		n=20		n=20		n=21	

△VAS - Activity[§]	-10.6 ± 25.1 (95% CI -21.4 to 0.3)	0.06	-15.8 ± 25.0 (95% CI -27.6 to -4.1)	**0.01**	-11.9 ± 29.1 (95% CI -25.5 to 1.7)	0.08	-10.9 ± 24.3 (95% CI -22.0 to 0.2)	0.05

Numeric data are presented as mean ± SD or median (IQR). Two-tailed t-test or Wilcoxon matched-pairs signed rank test[†] were used, respectively, to calculate statistical significance between groups. [§]Pre-treatment 1 data used. CAT, COPD Assessment Test; LCQ, Leicester Cough Questionnaire; mMRC, modified Medical Research Council dyspnoea scale; VAS, Visual Analogue Score.

Section 2

2.1. Methodology - Phases of Study

Phase A

Between 8th March 2016 and the 30th August 2016, eleven subjects were allocated to receive a single treatment to the right lower lobe and main stem bronchus. Six endobronchial biopsies were collected from the first segmental and lobular bronchi immediately prior to MCS delivery. Bronchoscopy was performed again at 60+/-7 days, the biopsy sites identified, guided by photographic documentation, inspected, and the sampling regime repeated. Biopsies were evaluated for evidence of healing and healthy mucosal regeneration. The patients were assessed at the 3-month follow-up visit and the primary endpoint data submitted to the data safety monitoring board (DSMB). Subjects received a monthly telephone call to ascertain their wellbeing and health status until review and approval of Phase A data by the DSMB.

Phase B

Following receipt of a satisfactory report on the findings in Phase A by the DSMB on the 29th September 2016, the participants' schedules were completed with two further sessions, treatments to the left lower lobe and main stem bronchus, followed by treatments to both the upper lobes, any residual main stem bronchus and the distal end of the trachea. (The right middle lobe was not treated). Airways have been inspected at each procedure and video recordings made. Intervals of 30 to 45 days were imposed between sessions and progression to the next treatment was contingent on the subject remaining stable without evidence of a recent acute exacerbation. An additional twenty-four subjects were enrolled and have undergone their three scheduled treatments: the last subject entered on the 6th November 2017. 12-month follow-up was completed on the 14th February 2019.

2.2. Methodology - Patient-Reported Outcome (PRO) Assessment Tools

The St George's Respiratory Questionnaire (SGRQ) is a 50-item multidimensional instrument to measure quality of life in patients with airways obstruction and to quantify changes after therapy(1, 2). Scores are calculated for three domains: Symptoms (frequency and severity), Activities (that cause or are limited by breathlessness), and Impacts (psycho-social disturbance resulting from airways disease), that are combined to generate a total score. Scores range from 0 to 100, with higher scores indicating more severe limitation. An MCID of ≥4 is considered meaningful(3).

The COPD Assessment Test (CAT) is an 8-item multidimensional tool that evaluates the impact of the disease (cough, sputum, dyspnoea, chest tightness) on quality of life(4). CAT scores range from 0 to 40, with higher scores denoting more severe impact on an individual's life. An MCID of ≥2(5) and a triangulated MCID of ≥2.54(6) have been suggested.

The LCQ is a 19-item multidimensional instrument using a 7-point Likert response scale designed to assess the impact of cough on three domains: physical, psychological and social(7). Patients are asked to complete the questionnaire daily for two weeks prior to each follow-up visit. An MCID of ≥1.3 is considered meaningful(8).

The visual analogue scale (VAS) is a unidimensional psychometric measure for subjective characteristics or attitudes that cannot be objectively quantified. The patient's assessment of his or her current state for a given parameter is indicated with a mark on a linear scale representing the worst to the best outcomes. An MCID is not yet established.

The modified Medical Research Council (mMRC) dyspnoea scale(9) provides a simple means of categorising patients in terms of the disability associated with breathlessness due to COPD(10). A minimum clinically important difference (MCID) of ≥1 is considered meaningful(11).

2.3. Methodology - Device

The RejuvenAir® System is a cryosurgical device that delivers metered doses of medical grade liquid nitrogen from a dewar in a console to a catheter emitting a radial spray at its tip(12). A thermocouple at the distal end of the catheter tailors the dosage of spray to the diameter of the targeted airway. The single-use 5.3-French cryo-catheter, with an introducer, is inserted through the 2mm working channel of a standard therapeutic flexible video-bronchoscope with 4.4mm outer diameter (OD) to reach the targeted site and deliver the vaporised LN_2 at about -195°C(13) with a cooling energy of 25W(14). The spray location guide sheath fits over the shaft of the bronchoscope. 0.5cm graduations facilitate accurate deliveries of MCS incrementally throughout the airway tree.

2.4. Methodology – Procedure

The RejuvenAir® procedure is carried out in an operating room or bronchoscopy suite. Enrolled subjects received standard anaesthetic, sedative and associated medications per institutional guidelines and routine clinical practice for their bronchoscopy procedure.

As part of the pre-anaesthetic, glycopyrrolate could be administered to reduce airway secretions. The patient under general anaesthesia is intubated and ventilated with 100% oxygen. The airways are first suctioned clear of mucus using a separate large diameter flexible bronchoscope and the target lobe sampled for routine microbiology using a bronchial wash pre-treatment.

The 4.4mm OD bronchoscope with inserted cryo-catheter is introduced into the endotracheal tube and navigated to the target site as instructed on the console display touchscreen, the catheter extruded several centimetres and the MCS delivered. The catheter is then retracted incrementally by 1cm and at each station a further MCS released.

Treatment 1 delivered MCS to the right lower lobe and main stem bronchus, treatment 2 to the left lower lobe and main stem bronchus, and treatment 3 to both upper lobes, any residual main stem bronchus, and the distal end of the trachea. (The right middle lobe was not treated). Precautionary measures are employed to avoid barotrauma and asphyxia: The spray is emitted at a pressure of less than 1 psi but the expansion of vaporising LN_2 is 696-fold(13, 15). Before each spray the cuff of the endotracheal tube is deflated, and the ventilator disconnected briefly. One-hour post-procedure a chest x-ray is obtained to exclude barotrauma.

Intervals of 30 to 45 days were imposed between sessions and progression to the next treatment was contingent on the subject remaining stable without evidence of a recent acute exacerbation.

2.5. Methodology – Assessment of severity of adverse events (AEs):

a) Mild: Observations and symptoms requiring no intervention.
b) Moderate: Events leading to minimal non-invasive measures.
c) Severe:
    i. Not immediately life-threatening but necessitating hospitalisation
    ii. Life-threatening indicating urgent intervention
    iii. Fatal

ABBREVIATIONS

AE – Adverse Event

°C – Celsius

CAT – COPD Assessment Test

COPD – Chronic Obstructive Pulmonary Disease

DSMB – Data Safety Monitoring Board

LCQ – Leicester Cough Questionnaire

LN_2 – Liquid Nitrogen

MCS = Metered CryoSpray

MCID – Minimal clinically important difference

OD – Outer Diameter

SGRQ – St George's Respiratory Questionnaire

VAS – Visual Analogue Score

W – Watt

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