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Original Article

Long-term treatment with oral N-acetylcysteine: Affects lung function but not sputum inflammation in cystic fibrosis subjects. A phase II randomized placebo-controlled trial☆

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Abstract

Purpose: To evaluate the effects of *oral* N-acetylcysteine (NAC), which replenishes systemic glutathione, on decreasing inflammation and improving lung function in CF airways.
Methods: A multicenter, randomized, double-blind proof of concept study in which 70 CF subjects received NAC or placebo orally thrice daily for 24 weeks. Endpoints: primary, change in sputum human neutrophil elastase (HNE) activity; secondary, FEV_1 and other clinical lung function measures; and safety, the safety and tolerability of NAC and the potential of NAC to promote pulmonary hypertension in subjects with CF.
Results: Lung function (FEV_1 and $FEF_{25–75\%}$) remained stable or increased slightly in the NAC group but decreased in the placebo group ($p = 0.02$ and 0.02). Log_{10} HNE activity remained equal between cohorts (difference 0.21, 95% CI −0.07 to 0.48, $p = 0.14$).
Conclusions: NAC recipients maintained their lung function while placebo recipients declined (24 week FEV_1 treatment effect = 150 mL, $p < 0.02$). However no effect on HNE activity and other selected biomarkers of neutrophilic inflammation were detected. Further studies on mechanism and clinical outcomes are warranted.

Keywords: Cystic fibrosis; Clinical trial; Anti-oxidant; Glutathione; Lung function

1. Introduction

CF is characterized by chronic oxidative stress and inflammation [1–3]. The hallmarks of lung disease in CF include massive neutrophil influx, hyper viscous mucus, progressive airways obstruction, tissue damage, and chronic infection of the airway by various pathogens [4,5]. Anti-inflammatory therapies can benefit people with CF [6,7]. However, current anti-inflammatory treatments are associated with significant adverse effects including gastrointestinal bleeding, hyperglycemia, and osteoporosis, limiting their long-term use [8,9].

Glutathione (GSH), the primary intracellular antioxidant, is synthesized by the liver from dietary cysteine sources. It regulates intra- and extra-cellular oxidative metabolism and inflammatory processes and influences many neutrophil functions. It is present at low levels in plasma but can be secreted into certain extracellular compartments, when needed. Thus, GSH levels in the epithelial lining fluid of the lung (ELF) normally are 400 times greater than in plasma [10].

In CF patients, GSH levels in whole blood, blood neutrophils [11], lymphocytes [12], and ELF [13] are markedly decreased. This profound GSH depletion is believed to affect neutrophil recruitment to the lungs of CF patients and may contribute to the exuberant inflammatory response described in these patients [11,14]. Orally administered NAC is readily absorbed via the intestinal epithelium where it is immediately modified to liberate cysteine, which is transported to the liver, the main site for GSH synthesis. For this reason, NAC is used to treat GSH depletion due to acetaminophen overdose. All cells have the capability to synthesize GSH, but GSH transport throughout the body also occurs via intracellular compartments, including neutrophils and lymphocytes.

We previously performed a 12-week, single center, double blind, randomized, placebo-controlled pilot study evaluating the safety and efficacy of oral NAC for treatment of airway inflammation in 19 CF subjects. These pilot data indicated that oral NAC therapy increased GSH levels in the peripheral blood neutrophils (measured by FACS) and reduced both neutrophil count and HNE activity in sputum, yet no significant effect on lung function was measured over the short term [11]. In a meta-analysis of four multi-center studies by Mayer–Hamblett, et al., sputum HNE activity and forced expiratory volume in 1 s (FEV_1) showed a strong inverse correlation [15]. Thus it logically followed that, with a longer, well-randomized study, the secondary clinical benefits on lung function expected from long-term control of airways inflammation might be elicited. The present trial was designed primarily to determine whether a 6-month course of high-dose oral NAC treatment would exert anti-inflammatory effects and secondarily improve lung function in subjects with CF as measured by HNE activity and FEV_1, respectively.

2. Methods

2.1. Design

The study was a 24-week safety and efficacy randomized, multi-center, double-blinded, placebo-controlled trial (Fig. 1). Our study commenced with an initial safety cohort to ascertain

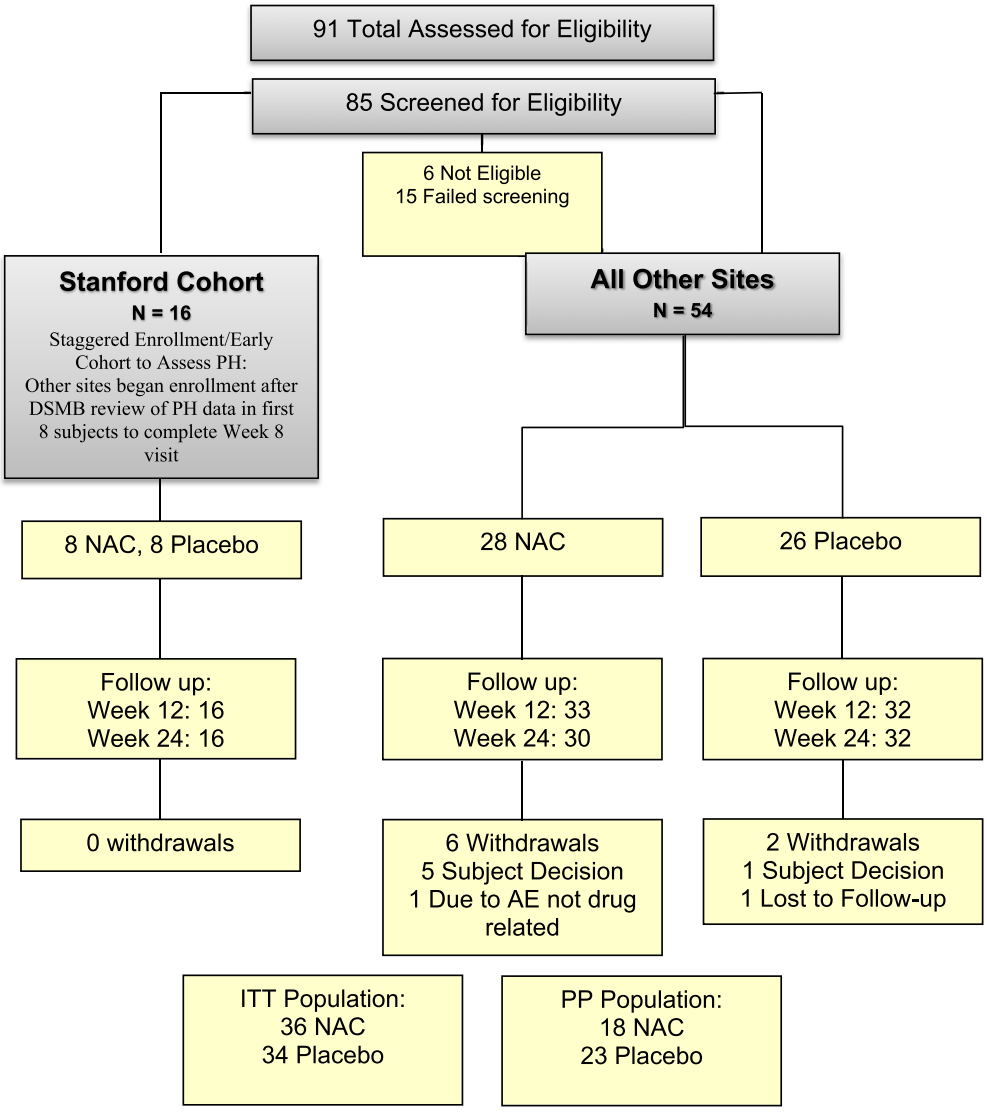

Fig. 1. Flow diagram of participants.

whether NAC at doses utilized in this trial could cause pulmonary arterial hypertension (PH) because Palmer and colleagues had previously reported that chronic, systemic administration of either NAC or SNOAC (S-nitroso-acetylcysteine) caused hypoxia-mimetic PH in a murine model [16]. Because of this possible safety concern, sixteen subjects at Stanford University were enrolled to the initial safety cohort, and their data used to evaluate the potential for NAC to cause PH in CF subjects.

2.2. Study centers and participant selection criteria

The trial was conducted at 11 accredited CF Foundation care centers in the United States between November 4, 2008 and June 30, 2011. It was coordinated by the CF Therapeutics Development Network Coordinating Center in Seattle, Wash. Seventy patients with CF, ranging from 9 to 59 years of age, were enrolled; 16 were from the CF Center at Stanford University, and 54 were from 10 other CF Centers in the United States. Patients were eligible for enrollment if they had CF (according to the conventional criteria), were clinically stable, had stable mild-moderate lung disease forced expiratory volume in 1 s (FEV_1) $\geq$ 40% and $\leq$ 85% predicted for age, ability to tolerate sputum induction with 3% hypertonic saline, and followed restrictions on consumption of anti-oxidants, antibiotics, and anti-inflammatory medications. Informed consent was obtained from all the subjects, and the Institutional Review Board at each center approved the study.

2.3. Randomization and blinding

An adaptive randomization strategy was used to stratify according to baseline FEV_1% predicted (moderate: 40% $\leq$ FEV_1 $\leq$ 60% vs. mild: 60% < FEV_1 $\leq$ 85%), age (pediatric: 7 to 17 years vs. adult: $\geq$18 years), gender, and indicators for chronic oral and inhaled antibiotic and chronic ibuprofen use. Randomization assignments and a series of blinded drug kit

numbers were generated by PPD, Inc. Kits were distributed to each center and were assigned with the use of a centralized secure randomization system at the coordinating center. All study personnel and participants were blinded to treatment assignment. The randomization codes for each participant were revealed to the researchers once recruitment, data collection, and data analyses were completed.

2.4. Treatments

NAC (PharmaNAC® 900 mg effervescent tablets in blister packs, ensuring protection from oxidation) and the identically packaged placebo were supplied by BioAdvantex Pharma, Inc. (Mississauga, ON, Canada). All participants were instructed to take 1 tablet dissolved in liquid 3 times a day for 24 weeks. Participants were monitored for adverse events and side effects. Regular medical care of the patients was under the direction of the primary CF care teams. The use of corticosteroids, non-steroidal anti-inflammatory drugs, and acetaminophen was specifically limited (see online supplement). The study drug was discontinued due to any of the following reasons: the occurrence of severe adverse effects or hypersensitivity to the study drug, noncompliance with the protocol, a positive urine pregnancy test, subject withdrawal of consent, or loss to follow-up. If a subject discontinued the use of study drug (NAC or placebo) for any reason or if a subject withdrew from the study, they were asked to undergo evaluation at an early termination visit and to complete all remaining scheduled visits and procedures. Compliance was monitored by the number of tablets dispensed and returned. Overall, the subjects in both cohorts were well-adherent to the study medication regimen (i.e., consumed $\geq$90% of study drug), but 45 protocol violations occurred, 19 of which involved prohibited use of antibiotics, anti-inflammatory, or anti-oxidant medications (11 NAC recipients, 8 placebo). The remaining violations pertained to visits that occurred out of the window, or delayed specimen processing, etc. (Table E3, online supplement).

2.5. Clinical and safety evaluations

Medical history, physical examination, spirometry, oximetry, liver function tests and sputum induction, and, at Stanford, Echocardiogram (ECHO) and carbon monoxide diffusing capacity (DLCO), were obtained at the screening visit (day 0). If liver enzyme tests were within inclusion criteria, subjects were randomized and study kit was assigned. Clinical evaluations, blood, urine testing and spirometry were performed at days 0 (randomization), week 12, and week 24 (end of active treatment phase). Follow-up telephone contact occurred on weeks 6, 18, and 25. Adverse events and concomitant medications were recorded at each contact point. Both the Cystic Fibrosis Quality of Life Questionnaire and the CF Respiratory Symptom Diary [17,18] were administered to all participants and parents/guardians on day 0 and weeks 12 and 24.

The Stanford safety cohort began first and underwent additional evaluations on weeks 4, 8, 12, and 24 for potential pulmonary hypertension. After 8 subjects at Stanford had completed their Week 8 visit, interim safety analysis was completed by the Data Safety Monitoring Committee (DSMC) by review of a composite of tests (see online supplement) to detect PH. At that point, the 10 remaining sites began enrollment. A second interim analysis for safety was performed after half of all subjects had completed 12 weeks of therapy. Blood, urine, ECHO and DLCO were obtained for the Stanford cohort on day 0 and weeks 4, 8, 12, and 24. For the entire subject population, creatinine, hypoxia-inducible factor 1α (HIF-1α), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and liver function and hematologic analyses were performed at day 0 and weeks 12 and 24. We found no evidence of PH upon high-dose oral NAC treatment over 24 weeks, which supports the safe, long-term use of this drug in CF.

2.6. Analysis of outcome measures

The primary objective of the study was to determine if NAC treatment was associated with a change in $\log_{10}$ HNE activity in sputum from day 0 to day 168. Secondary efficacy outcomes included change in spirometry indices, change in incidence and number of sinus and pulmonary exacerbation, time to first pulmonary and/or sinus exacerbation, time to first new or increased use of antibiotics, change in the neutrophil count measured in sputum, change in concentration of interleukin-8 (IL-8) measured in sputum and plasma, change in concentration of glutathione (GSH) measured in whole blood,[26] change in weight, change in quality of life indices, frequency and severity of treatment-emergent AEs, changes in clinical laboratory parameters and vital signs, incidence of abnormal clinical laboratory measures, vital signs and physical exam results. Complete data for primary endpoint analysis was provided by 26 NAC recipients (72% of the cohort), and 30 (88%) of the placebo recipients. Complete data for secondary endpoint (FEV1) analysis was provided by 30 NAC recipients (83% of the cohort), and 32 (94%) of the placebo recipients. All analyses reported are performed per the intention-to-treat principle.

It is important to explain why lung function parameters were not utilized as the primary outcome measure in this study. It is rare that FEV_1 or FVC are utilized as measures of treatment efficacy in Phase 2 trials because of the impracticality this measure imposes on trial design [19]. Based on the natural history of lung function changes in CF, power calculations demonstrate that ~400 subjects would need to be enrolled and studied over a 6-month period of time if lung function decline was to be halved over the course of the study. Alternatively, a cohort with 80 subjects would in principle require a length of 4 years of repeated measures to detect efficacy of the treatment [20].

[26] Whole blood GSH rather than intracellular levels measured by FACS in neutrophils was the measure utilized. FACS methodology requires same-day analysis and highly specialized procedures and expertise. This was not feasible across the 11 centers involved in this study, which is often a major constraint imposed by the nature of multi-center trials.

The forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1) and forced mid-expiratory flow rate ($FEF_{25–75\%}$) and the respective calculated percent of predicted were measured based on standardized equations [21,22]. Pulmonary function testing was performed in accordance with American Thoracic Society standards [23]. The presence of a pulmonary or sinus exacerbation was established using previously published criteria [24]. Sputum and plasma levels of IL-8 levels and HNE activity were determined by the Therapeutics Development Network Inflammatory Markers Core Laboratory using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn) and a spectrophotometric enzymatic activity assay, respectively [25]. GSH levels in whole blood were determined by using high performance liquid chromatography. This method was chosen of necessity, as it is the most reliable method in which samples could be collected across multiple centers without deterioration of the analyte. Note: this was not the same method utilized in our single center pilot study.

2.7. Sample size estimation

Other investigations of anti-inflammatory interventions in CF have not shown a significant difference in FEV_1 with the notable exception of a 4-year study of high-dose ibuprofen in 85 CF patients [6]. The data from this well-controlled study eventually demonstrated a benefit in slowing the usual decline in lung function by 50% in the treatment group, but the slowed decline of FEV_1 did not statistically manifest until the end of the 4-year study time period [6,8]. Thus, and given the previously noted inverse correlation of FEV_1 with HNE activity, change in HNE activity was the biomarker selected as the primary outcome measure. This allowed a feasible Phase 2 study in which a minimum of 64 enrolled subjects were required in order to detect a significant decrease in the activity of HNE in the sputum of CF subjects after treatment for 6 months (see below). Based upon previous data [11], assuming a 1:1 randomization, a Type I error of 0.05, a two-sided test, and a SD of change in HNE activity (log_{10} scale) over 6 months of 0.7, complete data on 32 per group (64 total) would result in 80% power to detect a 70% reduction in HNE activity.

2.8. Statistical methods

T-tests, Fisher's exact tests and log-rank tests were used to test continuous, dichotomous and time-to-event variables for differences between the NAC and placebo treatment groups. Secondary analyses used linear, logistic, or Cox proportional hazards regression models to adjust for baseline values and the factors used to stratify the randomization. All reported analyses are reported per CONSORT guidelines for presentation of outcome data (i.e., estimated effect size and its precision (95% confidence interval)) and followed the intention-to-treat principle. All randomized and evaluable subjects as randomized are included. The per-protocol population (PP) is the secondary analysis population for the efficacy analyses and is supportive of the ITT analyses. SAS version 9.2 (SAS Institute Inc., Cary, NC) and R version 2.11.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for all statistical analyses.

2.9. Research participants

Of the 85 participants screened for this study, 70 (74%) were randomized: 36 to NAC and 34 to placebo. Six participants in the NAC group and 2 in the placebo group were withdrawn or lost to follow-up. All but 1 participant in the placebo group, who was lost to follow-up before the day 28 visit, were included in the efficacy analysis (Fig. 1). The baseline characteristics and randomization strata were similar between treatment groups (Table 1). FEV1% predicted was less than 60 in 40% of subjects, 27% of subjects were under 18 years of age, 67% used azithromycin chronically, and none used ibuprofen regularly.

3. Results

3.1. NAC recipients had sustained baseline lung function after 6 months of treatment in the absence of decrease in HNE activity

We detected a potentially substantial clinical benefit in the secondary outcome, as the NAC cohort maintained baseline FEV_1 and FEF 25–75% throughout the 24-week period while 4–6% declines in these measures occurred in the placebo cohort (Table 2, Fig. 2). HNE activity levels in the two groups were similar at baseline, mean (SD) = 2.16 (0.66) and 2.21 (0.61) log μg/mL NAC and placebo, respectively. There was no significant change in HNE activity from baseline to week 24 (difference between NAC and placebo of 0.21, 95% CI −0.07 to 0.48, p = 0.14). There were also no differences detected between treatment groups in other indices of inflammation (absolute neutrophil count, sputum elastase activity, IL-8 in either sputum or plasma) or oxidation status (glutathione in whole blood) (Table 2). Calculated differences are potentially

Table 1
Baseline characteristics of participants by treatment group.

Characteristic	NAC (N = 36) N (%)	Placebo (N = 34) N (%)	Total (N = 70) N (%)	p-Value
Female	16 (44)	19 (56)	35 (50)	0.3388
Genotype				
ΔF508/ΔF508	18 (50)	16 (47)	34 (49)	0.4629
ΔF508/other	12 (33)	13 (38)	25 (36)	
Other/other	2 (6)	4 (12)	6 (9)	
Not done	4 (11)	1 (3)	5 (7)	
Age				
7–17 years	9 (25)	10 (29)	19 (27)	0.6783
≥18 years	27 (75)	24 (71)	51 (73)	
Azithromycin	23 (64)	24 (71)	47 (67)	0.5509
AZLI or TOBI	23 (64)	17 (50)	40 (57)	0.2406
FEV1 (% pred)				
40%–<60%	15 (42)	13 (38)	28 (40)	0.7696
60%–85%	21 (58)	21 (62)	42 (60)	
All subjects FEV_1 [mean (SD)]	62.9 (13.4)	63.8 (13.2)	63.3 (13.2)	

Table 2
Summary data for changes in primary and selected secondary endpoints from week 0 to week 24.

Variable	Treatment effect (95% CI)	p-Value
FEV_1 (% pred)	4.4 (0.83, 7.9)	0.02
FEV_1 (L)	0.15 (0.03, 0.28)	0.02
Sputum neutr. elastase activity (log_{10})	0.21 (−0.07, 0.48)	0.14
Sputum neutrophil count (log_{10})	2.6 (−12.1, 17.3)	0.73
Sputum IL-8 (log_{10})	0.19 (−0.03, 0.42)	0.09
Plasma IL-8 (log10)	−0.1 (−0.33, 0.14)	0.42
GSH in whole blood	64.2 (−177.6, 305.9)	0.60
Incidence of pulmonary exacerbation	−0.08 (−0.30, 0.14)	0.48
New use of antibiotics	0.08 (−0.14, 0.29)	0.50
CFQ-R respiratory domain	−0.34 (−6.3, 5.67)	0.91
CFRSD number of resp sx	−0.15 (−1.1, 0.8)	0.75

95% point wise confidence intervals (using t-distribution approximation) are included at each time point. Similar changes were measured in FEF 25–75% (see online supplement).

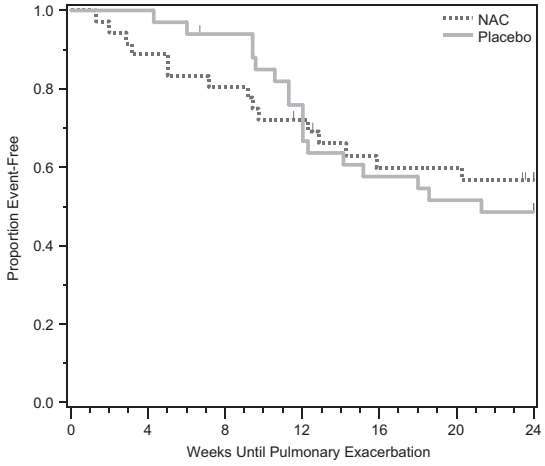

Fig. 3. Kaplan–Meier plot of time to pulmonary exacerbation. Censored subjects are denoted with 'tick' marks.

affected due to incomplete data on inflammatory indices including HNE activity obtained on 26 (72%) and 30 (88%) of the NAC and placebo recipients, respectively. Thus, the differences might have been detected had complete data been provided for the 32 subjects needed per power calculations.

3.2. Incidence of pulmonary exacerbations

Fig. 3 shows a Kaplan–Meier plot of time to pulmonary exacerbation for each treatment group. 32 subjects experienced between 1 and 3 pulmonary exacerbations. There were slightly fewer subjects with pulmonary exacerbations in the NAC group, however this difference was not significant (NAC, 15/36 = 42% vs. Placebo, 17/34 = 50%, Diff = 8%, 95% CI: 30 to 14%, p = 0.48). The numbers of subjects hospitalized, initiating oral antibiotics, initiating inhaled antibiotics, and initiating IV antibiotics were similar between groups (Table 2). Online supplemental data presents figures for time to antibiotic use, incidence of sinus exacerbations and Kaplan–Meier plot of time to any antibiotic use for each treatment group, which did not differ between the NAC and placebo groups.

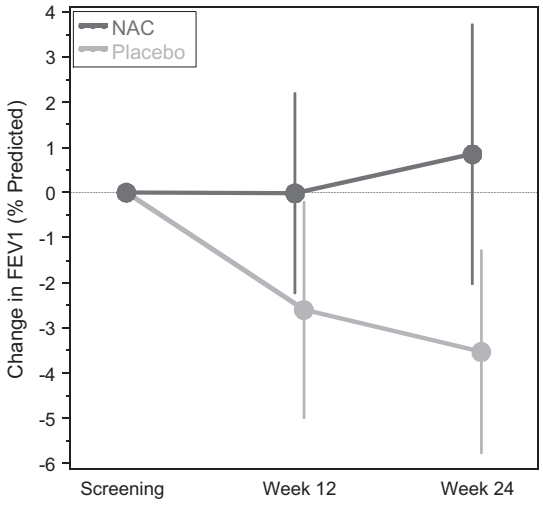

Fig. 2. Mean change from baseline in FEV_1 (L) over time by treatment group. The 95% confidence intervals are calculated using one-sample t-tests.

3.3. Patient reported outcomes

Respiratory symptoms were recorded via the CF Respiratory Symptoms Diary (CFRSD) and the CF Quality of Life Questionnaire respiratory domain scale (CFQ-R). There were no significant differences reported between NAC and placebo groups by either measure.

3.4. Adverse events and PH outcomes

The Stanford cohort of 16 subjects served as an initial safety cohort to evaluate if long-term treatment with NAC was associated with the development of PH in subjects with CF. There was no evidence for the development of PH in these subjects by the following biomarker and clinical measurements: levels of plasma NAC, SNOAC, HIF-1α, VEGF, bFGF, cardiac echocardiogram and diffusion capacity for carbon monoxide. These indices were assessed at the first interim safety analysis by the Data Safety Monitoring Committee (DSMC) review of PH data after 8 subjects had completed week 8 visit. Review of the data revealed no evidence for the development of PH, thus the other sites began enrollment. A second interim analysis for safety performed after half of all subjects had completed 12 weeks of therapy similarly demonstrated no evidence that PH had developed in any study participant.

4. Conclusions

Herein, we report the results of a 24 week, multi-center, randomized, placebo-controlled, proof of concept trial in 70 CF subjects designed to test the hypothesis that oral NAC would primarily exert anti-inflammatory effects and secondarily improves lung function in subjects with CF as measured by sputum HNE activity and FEV_1, respectively. Even though

sputum HNE activity is a biomarker of neutrophilic inflammation that has been correlated inversely with FEV_1 in CF patients [15,26], our data did not demonstrate any change in sputum HNE activity levels in either cohort at any time point, nor did any of the secondary inflammatory indices of inflammation demonstrate any response to oral NAC administration. In contrast, our data demonstrate a clinically relevant outcome in the secondary outcome: long-term treatment with oral NAC prevents lung function deterioration in CF, as the NAC cohort maintained baseline FEV_1 and $FEF_{25-75\%}$ throughout the 24-week period, but fell in the placebo control at the expected rate over the 6-month study period, even though the study was not powered for the parameter. This effect was present even though the expected 70% reduction in sputum HNE activity was not measured (Table 2).

Our spirometric pulmonary function results are clinically meaningful. The CFF Registry reports that, on average, CF patients who have mild to moderate lung dysfunction lose between 2 and 4% of lung function per year [6]. In this study, the rate of decline of FEV_1% of the placebo group appears to be greater than that of the national average, but our results are consistent with placebo cohorts from recent clinical trials [19]. Our earlier pilot trial may have been too brief for the effect on FEV_1 to become manifest, since historically such parameters have not proved to be sensitive outcome measures in small or shorter-term clinical trials [20]. Dauletbaev and colleagues reported unchanged FEV_1 data in their 12-week pilot study comparing low- and high-dose oral NAC in 11 and 10 CF subjects, respectively, but this study was even more underpowered to detect lung function changes than ours and was of shorter duration [27]. Since, in its initial design, our study was underpowered to detect lung function changes, yet we obtained highly significant results, our study demonstrates findings that are clinically relevant and provide impetus for further investigation.

The anti-inflammatory effect we had hypothesized was not supported by the results obtained in this larger, better randomized study. By multiple analyses, the cohorts were well-balanced between the treatment groups (see online supplement), but the pilot study results were potentially affected by several factors: there was a significantly greater mean age in the NAC cohort at baseline and the placebo cohort had a higher incidence of pulmonary exacerbation episodes. In both studies, compliance with the study medication was at least 90%, but for the larger study, the primary outcome HNE activity measures were obtained at the beginning and the end of the study in only 72% of the subjects in the active group, decreasing the usable data for intention-to-treat analysis. This may have affected the ability to detect a treatment effect in the NAC cohort, since the study was powered to analyze data with groups of 32 subjects each.

The discrepancy in the HNE activity measures between the pilot study and this multi-center trial may also relate as well to the fact that measurement of sputum elastase activity in CF is intrinsically variable within and between patients, and site differences in sputum induction efficiency and environmental factors can impose variability in the collection of sample. Therefore, HNE activity may be too insensitive for use as a biomarker in multi-center clinical trials. Confirming this, a placebo-controlled multicenter study showed no effect of one month exposure to high-dose ibuprofen on sputum HNE activity or other inflammatory biomarkers despite demonstrable long-term clinical benefits in controlled trials and observational data [28].

A recent publication by Griese and colleagues reports the results of a study utilizing *inhaled* GSH. The analyses determined that a statistically significant increase by 2.2% of FEV_1 from baseline over that of placebo was detected at 3 months, but was not sustained at the 6-month end of study analysis. Notably, the GSH subjects had higher rates than placebo subjects of chronic concomitant oral NAC (53% vs. 37%, respectively), inhaled fluticasone (40 vs. 20%) and oral ibuprofen use (14 vs. 7%), which theoretically should have skewed favor toward the GSH cohort. Inflammatory and oxidation indices were not reduced. Their study reflects the reasons we elected to explore the effects of *oral* doses of N-acetylcysteine rather than inhaled delivery of NAC, since the sulfhydryl group may interact with ROS present in the airways of CF subjects, and worsen the oxidative burden over the long run [29].

The mechanism of action of NAC in CF subjects as seen in our study remains to be elucidated, though we can speculate on various mechanisms. NAC, which supplies the cysteine required for GSH synthesis, may modulate CF airway disease via downstream biological mechanisms involving the pleiotropic roles GSH plays in cellular regulation and homeostasis. Our previous trial demonstrated that GSH stores in circulating neutrophils from CF subjects, as measured by flow cytometry, were significantly increased by oral NAC treatment, albeit not reaching normal levels [11]. In the present study, GSH measurements were performed on whole blood, not neutrophils. Thus, oral NAC may augment *intracellular* GSH to exert influence on oxidation, inflammatory pathways, DNA transcription, smooth muscle tone, and/or tissue fibroproliferative processes. An alteration in any of these could affect lung function [12,30,31,32].

Recent research has suggested that intriguing alternative mechanisms of action may involve effects of NAC upon CFTR function. In a study of the effects of NAC on cultured CF airway epithelial cells, Varelogianni et al. demonstrated a significant increase in chloride efflux, potentially via changes in redox potential that affect CFTR channel gating, so that reduction induced by increasing intracellular GSH enhances the duration and frequency rate of CFTR channel opening [33].

Luciani and colleagues demonstrated an autophagic pathway for intracellular trafficking dependent upon CFTR function that was restored by NAC treatment and allowed for normal CFTR maturation and trafficking to the cell surface [34,35].

Illustrating yet another potential mechanism, Chen and colleagues found a decrease in Nrf-2 (nuclear regulatory factor) dependent antioxidant responses in CF epithelia that result in an increase in steady-state hydrogen peroxide (H_2O_2) and contribute to the overproduction of the pro-inflammatory cytokines IL-6 and IL-8. Treatment with NAC ameliorated the excessive oxidant production and resultant cytokine over-expression [36].

Our study is the first long-term rigorously controlled clinical trial of high-dose oral NAC in CF subjects and we demonstrate

clinically meaningful benefit by effecting stabilization, if not gain, of lung function that may ultimately impact long-term survival. Multiple pre-clinical studies have provided evidence by which antioxidant therapy, particularly NAC, may act via several novel mechanisms and prove to be a valuable adjunct to standard CF therapies, particularly in light of recent studies with CFTR correctors and potentiats. A longer, larger Phase 3 trial utilizing FEV_1 as the primary outcome, with in vivo sweat iontophoresis or nasal potential difference biomarker measures as well as several cellular measures of oxidative processes as secondary outcomes, is warranted to further assess the potential clinical efficacy, confirm safety, and further explore the mechanism of action of high-dose oral NAC in CF.

Conflict of interest statement

R.T., C.K.C., L.A.H., and R.B.M. are listed as inventors on a provisional patent application covering NAC as a therapeutic agent for CF.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcf.2014.08.008.

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