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Inhaled dry powder alginate oligosaccharide in cystic fibrosis: a randomised, double-blind, placebo-controlled, crossover phase 2b study

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ABSTRACT

Background: OligoG is a low molecular-weight alginate oligosaccharide that improves the viscoelastic properties of cystic fibrosis (CF) mucus and disrupts biofilms, thereby potentiating the activity of antimicrobial agents. The efficacy of inhaled OligoG was evaluated in adult patients with CF.

Methods: A randomised, double-blind, placebo-controlled multicentre crossover study was used to demonstrate safety and efficacy of inhaled dry powder OligoG. Subjects were randomly allocated to receive OligoG 1050 mg per day (10 capsules three times daily) or matching placebo for 28 days, with 28-day washout periods following each treatment period. The primary end-point was absolute change in percentage predicted forced expiratory volume in 1 s (FEV_1) at the end of 28-day treatment. The intention-to-treat (ITT) population (n=65) was defined as randomised to treatment with at least one administration of study medication and post-dosing evaluation.

Results: In this study, 90 adult subjects were screened and 65 were randomised. Statistically significant improvement in FEV_1 was not observed in the ITT population. Adverse events included nasopharyngitis, cough and pulmonary exacerbation. The number and proportions of patients with adverse events and serious adverse events were similar between OligoG and placebo group.

Conclusions: Inhalation of OligoG-dry powder over 28 days was safe in adult CF subjects. Statistically significant improvement of FEV_1 was not reached. The planned analyses did not indicate a significant treatment benefit with OligoG compared to placebo. *Post hoc* exploratory analyses showed subgroup results that indicate that further studies of OligoG in this patient population are justified.

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Inhalation of OligoG-DPI over 28 days was shown to be safe in adult CF subjects. Statistically significant improvement of FEV_1 was not reached. *Post hoc* subgroup analyses support mechanism of action for OligoG and warrant further prospective studies. https://bit.ly/2PHq6Z0

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This study is registered at www.clinicaltrials.gov with identifier number NCT02157922. Individual participant data that underlie the results reported in this article after deidentification (text, tables, figures and appendices) will be made available to researchers who provide a methodologically sound proposal for subsequent meta-analysis of trial data up to 36 months following publication. Requests can be made to the corresponding author.

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Introduction

Despite recent advances in the treatment of cystic fibrosis (CF) with drugs that directly improve cystic fibrosis transmembrane conductance regulator (CFTR) function, there is still an unmet medical need for new therapeutics that enhance the clearance of airway secretions, decrease chronic infection and inflammation, and reduce the treatment burden currently experienced by people with CF [1, 2]. Existing mucolytic treatments either hydrate CF sputum, *e.g.* hypertonic saline, or degrade macromolecules that contribute to its abnormal viscoelastic properties, *e.g.* dornase alfa. OligoG represents a potential alternative way to improve airway clearance in CF, with additional potential to combat bacterial infections. It is a low molecular weight alginate oligosaccharide derived from the stem of the brown seaweed, *Laminaria hyperborea*. OligoG has been shown in previous *in vitro* studies to alter the viscoelastic properties of mucin/alginate gels, mucin/DNA gels and CF sputum [3, – 5]. Extensional and shear rheology analyses showed that OligoG treatment resulted in marked reductions in both elastic (G′) and viscous response (G′′) of CF sputum compared to controls and a significant reduction in the elastic response, G′, and viscous response, G′′ [4]. In addition, potentiation of rhDNase I (a mucolytic used in the treatment of CF) was observed when used in conjunction with OligoG, showing statistically significant differences in both G′ and G″ [4]. Fourier-transform infrared spectroscopy analysis of CF sputum confirmed an interaction with mucin glycans that supports the changes in viscoelastic properties of CF sputum in the presence of OligoG [5]. Results of sputum rheology in a clinical phase 2A study of nebulised OligoG (www.clinicaltrialsregister.eu EudraCT number: 2010-023090-19), as well as *ex vivo* studies [5, 6], indicated that OligoG normalises mucus biophysical properties (*i.e.* viscosity) without degradation or cleavage of polymers within CF mucus. Furthermore, studies in a CF mouse model demonstrated that treatment with OligoG significantly reduced the accumulation of mucin, normalising the mucosal phenotype and improving long-term survival [7]. These observations and biophysical changes suggest that mucociliary function and mucus clearance could be improved by OligoG, which in turn might lead to better lung function and reduced frequency of pulmonary exacerbations.

In addition to its mucoactive properties, *in vitro* studies have shown that OligoG is able to potentiate antibiotics against a wide range of multidrug-resistant bacteria [8], probably by disrupting bacterial biofilms [9]. The disruption of biofilm by OligoG was observed in a dose-dependent manner over 24 h, with up to a 2.5-log reduction in *Pseudomonas aeruginosa* in infected mouse lungs [10]. The same study also demonstrated that the presence of OligoG showed a significant reduction in minimum biofilm eradication concentration for colistin from 512 $\mu g \cdot mL^{-1}$ to 4 $\mu g \cdot mL^{-1}$ after 8 h. A later study confirmed these findings while also demonstrating disruption of *P. aeruginosa* microcolony formation [11], to which increased tolerance to antibiotics has been attributed. OligoG also reduces the expression of both the las and rhl components in *P. aeruginosa* quorum sensing, which is a key mechanism influencing virulence factors and bacterial communication in biofilm development [12, 13]. In addition to reducing bacterial virulence, OligoG has also been shown to inhibit hyphal formation in *Candida albicans* and reduce invasion and growth of fungal pathogens such as *C. albicans* and *Aspergillus* spp. [14, 15].

Based on these pre-clinical data and the improved sputum rheology observed in the previous phase 2A study (EudraCT number: 2010-023090-19) [16], it was expected that an increased dose of OligoG combined with an improved lung distribution pattern could be achieved with a dry powder formulation [4, 17] which would provide a more effective delivery mechanism without imposing a higher treatment burden on the patients. At the time of the study, pre-clinical rodent toxicity studies were limited to 4-week repeat-dose inhalation exposure. These studies confirmed an exemplary safety profile, but restricted the duration of exposure in this clinical trial to a 28-day treatment regimen.

The primary aim of the study was to demonstrate superiority of OligoG compared to placebo determined by the absolute changes in forced expiratory volume in 1 s (FEV_1) at the end of 28 days' treatment.

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Methods

We conducted a phase 2B randomised, double-blind, placebo-controlled, multicentre crossover study to assess the efficacy and safety of inhaled alginate oligosaccharide (OligoG) in subjects with CF. The study was performed at 18 centres in Denmark, Germany, Norway, Sweden and the UK between December 30, 2014 (first patient first visit) and December 16, 2016 (last patient last visit). The study was conducted in full accordance with the Declaration of Helsinki 1964 (amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013) and International Conference on Harmonisation guidelines for good clinical practice, and according to applicable laws and regulations for clinical research in the involved countries. A clinical trial application was submitted to national competent authorities and ethics committees before commencement of the trial, as applicable according to local regulations.

Study participants

Inclusion criteria were adults (aged ⩾18 years) with a confirmed diagnosis of CF, including typical clinical features and a sweat chloride level of ⩾60 mmol·L^{-1} and/or a confirmation of two CF-causing CFTR mutations. Patients must have had evidence of *P. aeruginosa* lung infection in their medical history (based on positive sputum or cough swabs over the past 12 months; supplementary table S1) and an FEV_1 of 40–100% predicted normal value according to the Global Lung Function Initiative normative equations at screening [18]. A subset of patients who had FEV_1 >60% pred were included in a substudy (at qualified study sites) that utilised the multiple-breath nitrogen washout technique to measure the lung clearance index (LCI). Females of child-bearing potential and sexually active males were required to use contraception as defined per protocol throughout the study. Eligible patients were requested, when possible, to remain on their stable therapy, including physiotherapy, for the duration of the study, including no change in the 14 days prior to baseline measurements. Inhaled n-acetylcysteine was not allowed during the entire study period; inhaled mannitol or hypertonic saline were not allowed from 7 days prior to and throughout each treatment period. Concomitant antibiotics were permitted including cycled tobramycin (TOBI), colistin and/or aztreonam, but patients who had recently initiated cycled therapies should have had at least two complete cycles in the months preceding enrolment. Patients with alternating TOBI and colistin cycles were started on an "off-TOBI" period at day 0 (visit 2); patients with alternating colistin or TOBI and aztreonam cycles were started on an "off-TOBI" or an "off-colistin" period at day 0 (visit 2). Patients on cycled aztreonam were started concurrently with an "on-aztreonam" cycle. Concomitant use of all other marketed antibiotic agents was permitted, providing patients were willing to remain on the same regimens within the 28 days immediately prior to day 0 (visit 2) and for the entire duration of the study (until the follow-up visit, day 112).

Patients could not have experienced any pulmonary exacerbations within the 28 days prior to screening, nor could there be any positive microbiological finding of *Burkholderia* spp. or a history of allergic bronchopulmonary aspergillosis within 12 months before enrolment. Further exclusion criteria included known lactose intolerance or hypersensitivity to any component of the study medication, any ongoing acute illness or hospitalisation between screening and first study drug administration, and CFTR modulator therapy (ivacaftor was approved when study was performed). All patients provided informed consent before any study procedures. Full inclusion/exclusion criteria are listed in supplementary table S1.

Study design and assessments

Eligible participants were randomised (1:1) to receive OligoG 1050 mg per day (10 capsules three times daily) and a matching placebo of lactose in randomised treatment sequences. Each treatment period lasted 28 days followed by a 28-day washout period. A final follow-up safety visit was scheduled 4 weeks after the final washout. The design and visit schedule are outlined in figure 1. The study medication was administered using a dry-powder monodose inhaler (MIAT, Milan, Italy).

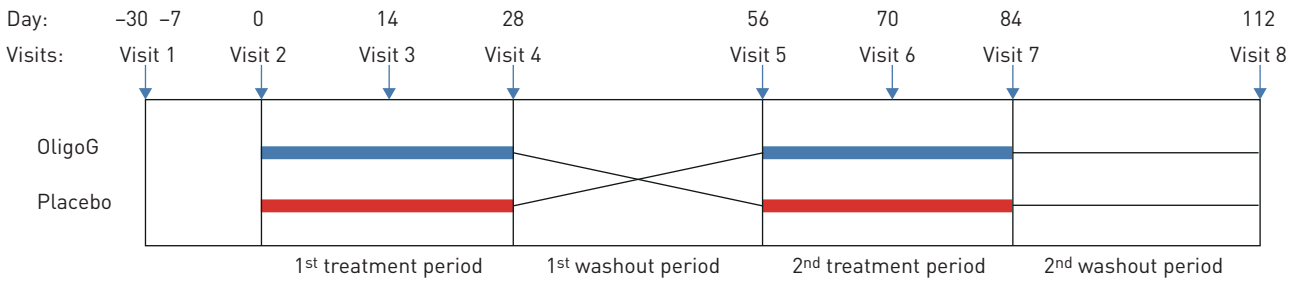


FIGURE 1 Trial design and visit schedule.

Since the selected OligoG dose was higher than those used previously, the first 12 patients followed a dose-titration scheme for the first 3 days, starting with a total of 10 capsules on day 1 (*i.e.* 350 mg once daily), followed by a total of 20 capsules on day 2 (*i.e.* 700 mg twice daily) and as final dosage a total of 30 capsules on day 3 (*i.e.* 1050 mg three times daily). An interim review of safety and tolerability data during this dose escalation was performed by the data and safety monitoring board (DSMB), which led to approval for ongoing enrolment at this dose without dose titration.

The randomisation list was generated by a statistician not involved in the study operations, using SAS software (version 9.3; SAS Institute, Cary, NC, USA). All study site personnel, as well as personnel involved in the monitoring or management of the study, were blinded to the individual patient treatment assignments. The study sponsor and the DSMB were blinded to individual patient treatment assignments for the duration of the entire study. If required, the DSMB would have been allowed to be unblinded in order to facilitate prompt analysis of any safety/tolerance issues that may have been raised.

Study efficacy variables included absolute changes in FEV_1 (primary end-point), vital capacity (VC), forced vital capacity (FVC), forced expiratory flow at 25–75% of FVC ($FEF_{25–75\%}$), FEV_1/FVC ratio, peak expiratory flow (PEF), changes in mucociliary and cough clearance (MCC), LCI, microbiological measurements including *P. aeruginosa* density in expectorated sputum, sputum rheology, quality of life measured by the Cystic Fibrosis Questionnaire Revised (CFQ-R) [19].

Mucociliary and cough clearance

In this study, three sites with MCC capabilities (Copenhagen, Denmark; Southampton and Glasgow, UK) were selected and trained to follow a study-specific standard operating procedure similar to published methods [20, 21]. MCC was measured by γ-scintigraphy after the inhalation of technetium-labelled albumin colloid particles. A cobalt-57 transmission scan was used to define lung boundaries and regions of interest. Radioactive fiducial markers placed over the spine were used to assist image alignment. Technetium-99m-labelled albumin colloid was delivered using a controlled breathing pattern (500 mL tidal volume; 500 $mL{\cdot}s^{-1}$ inspiratory flow rate; 30 $breaths{\cdot}min^{-1}$) with a standardised aerosol delivery set-up at each site. Dynamic acquisition of serial 2-min images were obtained for 60 min while asking the subject to minimise spontaneous coughing to assess cilia-driven mucus clearance. 60 voluntary huff coughs were performed between 60 and 90 min of the scanning period to assess cough-driven mucus clearance. A static image obtained 24 h after isotope inhalation was obtained as an additional assessment of composite whole-lung clearance. Blinded scans were analysed at a central reading site (University of North Carolina, Chapel Hill, NC, USA).

Microbiology

Standard culture microbiology to quantify viable bacterial load in sputum was performed at a central lab (Synlab, Munich, Germany).

Lung clearance index

LCI was performed at selected sites on subjects with mild to moderately impaired lung function (FEV_1 >60% pred) using the Exhalyser D nitrogen washout (Eco Medics, Duernten, Switzerland) according to standard operating procedures [22]. Traces were assessed for quality and scored by the European Cystic Fibrosis Society Clinical Trial Network LCI core facility based at the Royal Brompton Hospital (London, UK).

Sputum rheology

Rheological analyses were performed on sputum samples over a range of frequencies (0.1–10 Hz) relevant to biological process in the airway. Analyses involved measuring the frequency-dependent complex shear modulus (G*), and subsequent calculation of the loss tangent tan (δ) or phase angle, of sputum samples using the small amplitude oscillatory shear (SAOS) technique [23]. In SAOS the deformation of the sample is studied in terms of its rheological response to a series of imposed oscillatory stress waveforms over a range of frequencies. The results of the measurements are reported as the two components of G*: the shear elastic modulus G′, and the (viscous) loss modulus G″.

Pharmacokinetics

Whole-blood samples were collected from all subjects for the quantification of plasma OligoG concentrations. Plasma concentrations of OligoG (DPn 10) were analysed by a liquid chromatography-tandem mass spectrometry method (Vitas, Oslo, Norway), using OligoG-C13 as an internal standard. OligoG was extracted from plasma using protein precipitation following enzymatic digestion. Analysis was performed by liquid chromatography (Agilent 1200 LC Systems; Santa Clara, CA, USA) with tandem mass spectrometric detection (Agilent 6460 Triple Quad LC-MS/MS detector).

Statistical analysis

For determination of sample size, it was assumed that the FEV_1 change from baseline to end of treatment would have a mean±SD value of 0.1±0.2 L during OligoG treatment and 0.0±0.2 L during placebo treatment. In addition, it was assumed that the correlation between the changes from baseline in the two treatment periods would be 0. With 66 subjects enrolled (33 in each treatment sequence), a two-sample t-test would have 80% power to detect a difference in FEV_1 between the treatments using a 5% level of significance. The FEV_1 values at the end of each treatment period were examined using a mixed model. It was expected that the model would have similar, but slightly higher power to detect a treatment difference than the two-sample t-test.

For the FEV_1 (the primary variable of the study) absolute values at the end of each treatment period, a linear model was made using SAS PROC MIXED, with treatment, treatment sequence and treatment period as fixed effects, patient as random effect and the baseline value in each treatment period as a covariate. Patients with no measurements post-baseline in one or both treatment periods were excluded from the model. Week 0 was defined as baseline for period 1; week 8 was defined as baseline for period 2. If the week 0 value was missing, the screening visit value was used as baseline, if available. Country and country×treatment effects were also included in the model if statistically significant on a 10% level. If model assumptions were not met, transformations of data or alternative analyses were attempted. If a significant sequence effect was found, this was interpreted as an indication of a carry-over effect and a separate analysis was performed using only the data from the first treatment period. Additionally, models were made for the percentage of predicted values. Effects were estimated with 95% confidence intervals; p-values <0.05 were considered statistically significant.

Subjects were included in the intention-to-treat population (ITT) if they were randomised to treatment, received at least one administration of study medication and had at least one post-dosing evaluation. The primary population for all analyses (including main safety analyses) is the ITT population. A total of 65 patients were included in the ITT population, while 25 out of the 90 patients enrolled were not randomised because they did not fulfil all inclusion criteria.

Initial analyses included in the clinical trial analysis plan on the ITT and the per-protocol populations suggested that the number of OligoG doses (as observed through patient compliance) influenced the efficacy outcome. Therefore, further unplanned *post hoc* analyses were performed in a modified (m)ITT population, which included subjects who were compliant to the study protocol without any major violations except for dose compliance; any level of treatment compliance was accepted in the ITT population. Study drug compliance was defined as the number of study medication capsules used divided by the scheduled number of study medication capsules. The per-protocol population consisted of 47 patients. Of the 65 patients in the ITT population, 18 were excluded from the per-protocol population due to low study drug compliance and/or missing visits.

For the spirometry parameters VC, FVC, $FEF_{25–75\%}$, FEV_1/FVC and PEF, analyses were performed in the same manner as for FEV_1. Models were made in which the end-of-treatment-period values were replaced by the week-2 and week-10 values, as defined in the trial protocol. For MCC, the average lung clearance through 60 min was analysed, similar to the primary end-point. Central and peripheral lung clearance through 60 min, and cough clearance at 60–90 min (whole lung, central lung and peripheral lung) were similarly analysed.

Supplementary analyses were performed on end-of-treatment data where no values were carried forward, *i.e.* for every analysis parameter, all patients with missing data at either week 0, 4, 8 or 12 were excluded. For MCC, retention/clearance and deposition parameters and time intervals not mentioned above were summarised with descriptive statistics. Lung clearance index (LCI), quality of life by CFQ-R, sputum rheology and microbiological measurements were summarised with descriptive statistics.

Retrospective analysis of pulmonary exacerbations

Patients who had completed the study per protocol and were included at sites in the UK or Germany were eligible for an additional retrospective data collection focusing on the number of pulmonary exacerbations. This retrospective assessment of pulmonary exacerbation frequencies, hospitalisations and antibiotic treatments was performed in subjects during the 6 months before and after study participation. The definition of pulmonary exacerbation used in the study was based upon criteria on the CF Foundation Therapeutics Development Network Coordinating Center (Seattle Children's Hospital, Seattle, WA, USA) [24].

For evaluation of a potential long-term benefit of OligoG treatment, the proportion (p1) of patients with pulmonary exacerbations within 6 months after the end of treatment were compared to the proportion (p0) within 6 months prior to study participation. The null hypothesis, p0=p1, was assessed with McNemar's test both for the two treatment sequences combined and for each treatment sequence separately. For each patient, the difference between the number of exacerbations in the 6 months after the

end of randomised treatment and the number of exacerbations in the 6 months prior to study participation was calculated. The Wilcoxon signed-rank test was used to test whether the number of pulmonary exacerbations per patient before and after treatment had the same distribution. Furthermore, to evaluate the severity of pulmonary exacerbations, the proportion of patients with hospitalisations due to pulmonary exacerbations, as well as the proportion of patients that had received antibiotic treatments due to pulmonary exacerbations were compared within 6 months before and after OligoG treatment. These were compared in the same manner as the proportions of patients with exacerbations. The number of hospitalisations per patient and the number of antibiotic treatments per patient were analysed in the same manner as the number of exacerbations per patient.

Results

A total of 90 patients were screened for the study. 65 patients were randomised to receive treatment (figure 2). 32 patients were randomised to receive OligoG in the first treatment period, while 33 patients received placebo first. Patient demographics are summarised in table 1. The baseline demographic characteristics were similar in the two treatment sequences.

Efficacy

Lung function

The primary outcome parameter was the absolute change in FEV_1. The primary analysis was performed on the ITT population. Statistically significant improvement in FEV_1 was not observed in the ITT population (supplementary table S2). The treatment difference OligoG–placebo was −1.3% (95% CI −3.8–1.2%); p=0.31. In other words, the treatment difference was found to be 1.3% units in favour of placebo, and the difference was not statistically significant. Relative change in FEV_1 showed a pronounced drop in FEV_1 at day 14, followed by recovery to baseline by day 28 (figure 3).

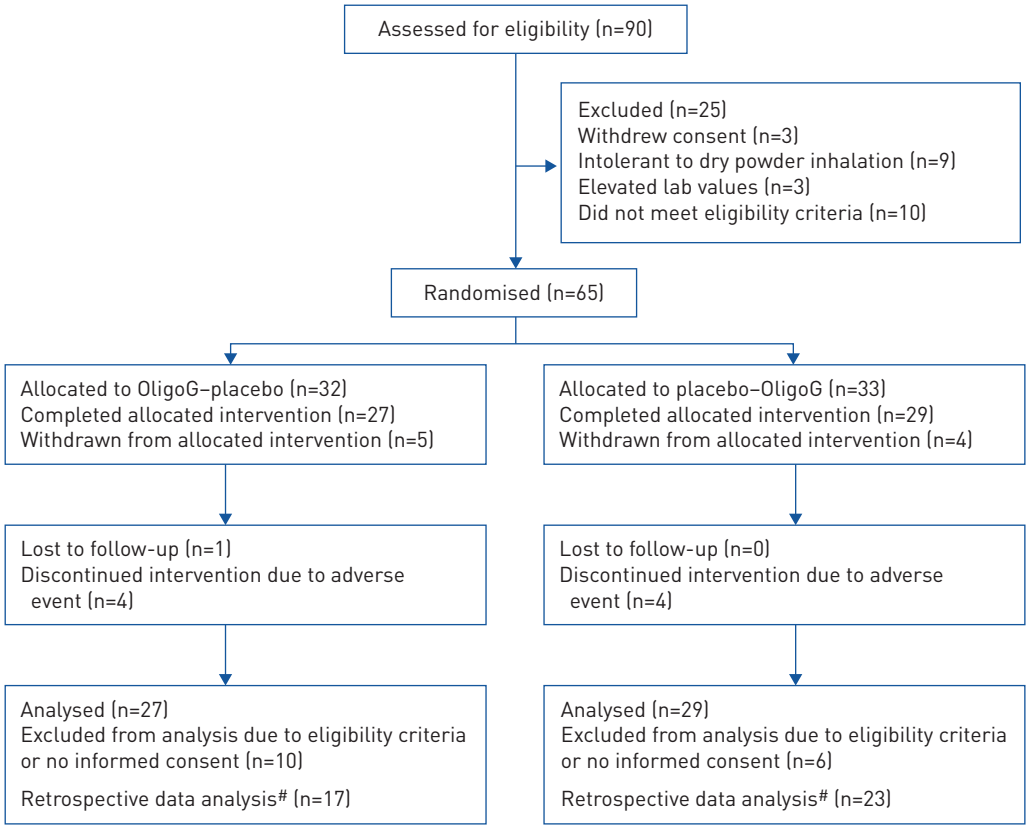


FIGURE 2 Study flow chart. The 12 patients who either withdrew consent or were intolerant to the dry powder inhalation had a drop in forced expiratory volume in 1 s or severe cough during the test inhalation. #: patients were included in the retrospective data analysis population if they completed the study in Germany or the UK and gave informed consent for retrospective collection of data for pulmonary exacerbation frequencies, hospitalisations, intravenous antibiotic treatments and spirometry assessments in the 6 months prior to visit 1 and 6 months post-study treatment visit 7. A total of 40 patients were included in the retrospective data analysis population.

TABLE 1 Demographic information

	OligoG–Placebo	Placebo–OligoG	Total
Subjects	32	33	65
Male	15 (46.9)	16 (48.5)	31 (47.7)
Female	17 (53.1)	17 (51.5)	34 (52.3)
Caucasian/European ethnic group	32 (100)	33 (100)	65 (100)
Age at informed consent years	31.8±10.3	35.3±8.9	33.5±9.7
	19/31/59.3	19/36/57	19/33/59.3
Height cm	170.6±10.1	170.4±8.2	170.5±9.1
	156.5/169.0/191	154/172/184	154/170/191
Body weight kg	67.4±14.4	65.8±13.3	66.6±13.8
	45.7/64.8/103	42/62/91	42/63.3/103
BMI kg·m^{-2}	23.07±4.02	22.46±3.17	22.76±3.59
	17.63/22.40/34.33	17.26/22.23/29.13	17.26/22.30/34.33
FEV_1 L	2.437±0.789	2.106±0.558	2.269±0.697
	1.42/2.260/4.33	1.20/2.060/3.51	1.20/2.120/4.33
FEV_1 % predicted	64.3±15.8	57.5±12.2	60.9±14.4
	39/61.0/97	39/57.2/84	39/58.4/97

Data are presented as n, n (%), mean±SD or minimum/median/maximum. The baseline demographic characteristics were similar in the two treatment sequences. All subjects were of Caucasian/European origin. BMI: body mass index; FEV_1: forced expiratory volume in 1 s.

Mucociliary and cough clearance

A total of 14 patients from three sites, underwent MCC assessments. However, only 12 patients completed all planned MCC assessments. MCC data obtained at the two pre-treatment baseline visits were analysed to characterise the reproducibility of key MCC outcomes in this multisite study. Correlation between the paired baseline MCC rates (Ave90Clr) was high (R^2=0.73; p<0.001) as was that between the central: peripheral (C/P) particle deposition ratios (R^2=0.73; p<0.001), suggesting that these data are robust and did not suffer from carry-over effects. While no change in the Ave90Clr or cough clearance was observed

a)

Period	Interval	Subjects n	OligoG Mean±SD	Subjects n	Placebo Mean±SD	Difference n	OligoG–Placebo Mean±SD
1	Day 0–14	27	−2.20±14.00%	27	3.10±7.00%	54	−5.40±11.10%
	Day 0–28	27	−0.60±13.00%	28	0.10±7.10%	55	−0.80±10.40%
	Day 0–56	27	0.50±11.10%	27	−2.50±8.40%	54	3.20±9.90%
2	Day 56–70	27	−3.50±13.60%	27	1.70±10.70%	54	−5.20±12.20%
	Day 56–84	26	0.50±11.30%	26	3.00±11.00%	52	−2.50±11.20%
	Day 56–112	27	0.20±11.70%	25	−0.60±11.50%	52	0.80±11.60%

b)

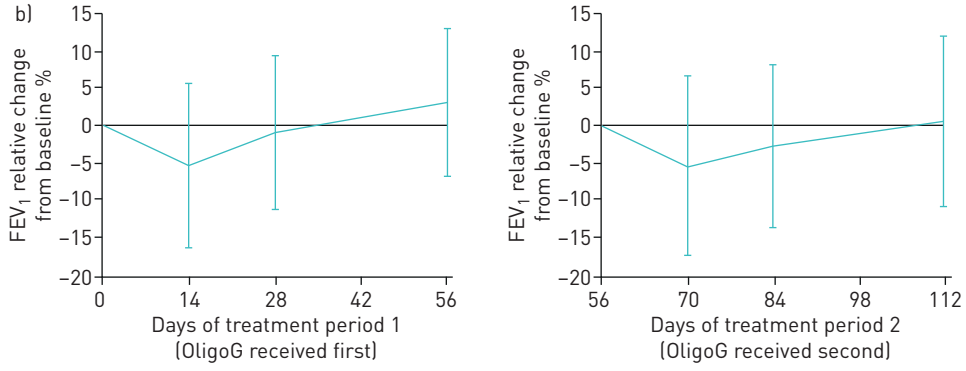


FIGURE 3 Relative changes of forced expiratory volume in 1 s (FEV_1) in the intention-to-treat (ITT) population. a) FEV_1 relative changes from baseline, ITT population, patients with more than one missing value excluded; b) mean±SD difference OligoG–placebo in relative change from baseline in FEV_1 in the ITT population.

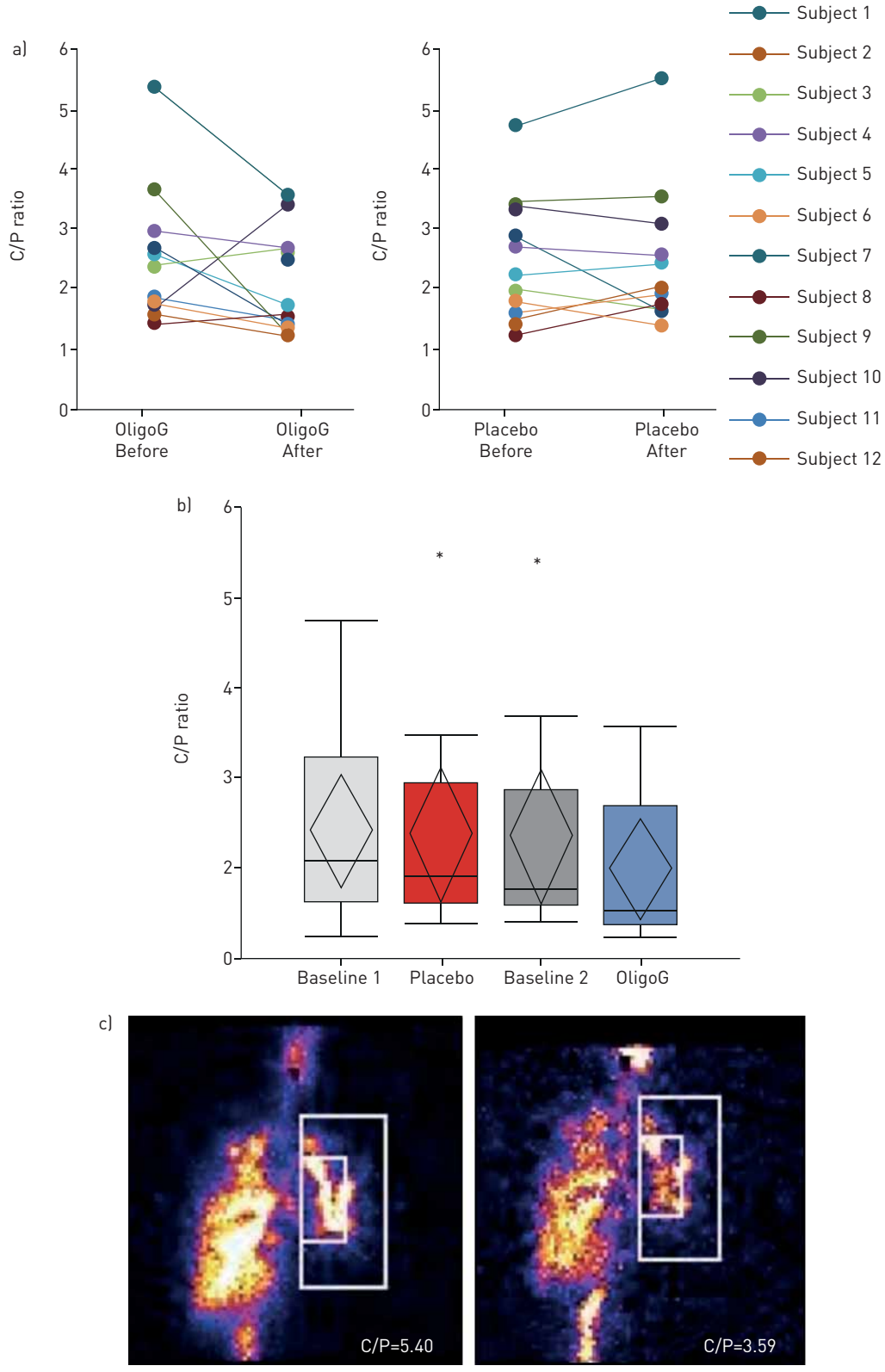


FIGURE 4 Central/peripheral (C/P) deposition per subject. a) Scintigraphic imaging pre- and post-4 weeks' treatment shows reduced C/P ratio with OligoG as compared to placebo; b) box plots for C/P ratio of isotope deposition at each study visit; c) example of C/P analysis from baseline and post-OligoG deposition in a person with cystic fibrosis. A trend toward more peripheral deposition was observed following OligoG treatment.

after OligoG treatment, a trend towards a more peripheral deposition pattern after OligoG when compared to the preceding baseline was observed (C/P 2.46±1.15 *versus* 1.98±0.87; p=0.09, Wilcoxon test), that was not seen during placebo treatment (figure 4 and supplementary table S3).

Lung clearance index

A total of 11 patients with FEV_1 between 60% and 100% at screening also had LCI assessments at seven study sites. Due to tests not being performed at certain visits (6%) or failing to meet quality criteria (14% of test occasions), only three patients had LCI values available from all six visits. Subject treatment differences are summarised for the ITT and per-protocol populations, and no statistically significant treatment differences were seen (supplementary table S4). Due to the low numbers of patients who underwent LCI testing, no conclusions as to effect of treatment on LCI could be drawn.

Quality of life

No differences were seen between OligoG and placebo for eight out of the nine quality-of-life domains (physical, role, emotion, social, body image, eating, treatment burden, health perceptions) and two of the symptom scales (weight, digestion). For the respiratory scale and vitality life domain, baseline adjusted mean and median scores were higher with placebo than with OligoG after both 2 and 4 weeks of treatment, suggesting that study subjects considered OligoG treatment was not beneficial for these quality-of-life parameters (supplementary table S5).

Sputum rheology

For sputum rheology analysis in the ITT population, no meaningful differences in rheological properties of the sputum were observed between the two treatment groups at 0.1, 1 and 10 Hz (supplementary table S6).

Microbiology

The low sample size available for sputum culture analysis (n=6–15, dependent on treatment and visit) was the result of a delayed start in sputum collection. No effect of OligoG on bacterial density and type (mucoid *P. aeruginosa*, non-mucoid *P. aeruginosa* and *Staphylococcus aureus*) in sputum cultures was observed. There was a reduction in mean CFUs for total bacteria after OligoG treatment (figure 5), although this was not reflected in the mean *P. aeruginosa* counts compared to the lactose placebo (figure 6). Furthermore, the placebo showed almost 1 log reduction in *P. aeruginosa* CFUs (figure 6).

Safety

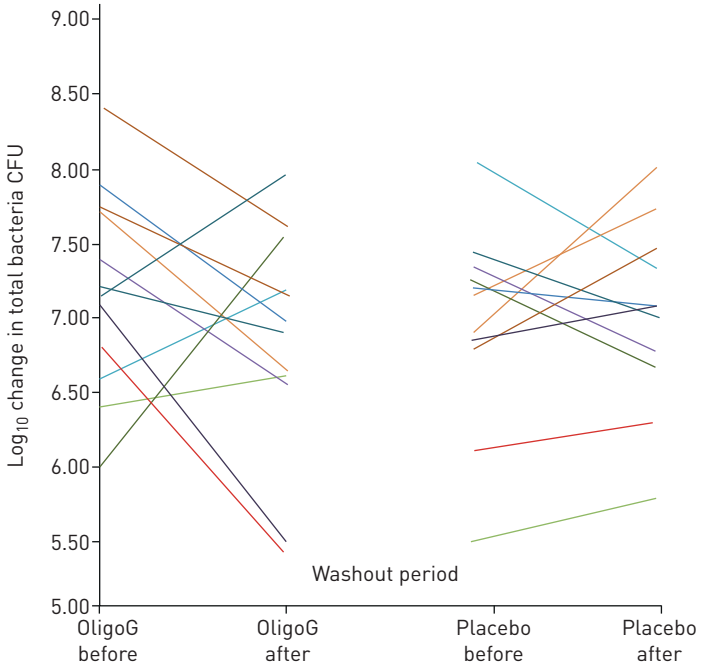
The numbers and proportions of patients with adverse events during the study were similar during OligoG and placebo treatment; 52 (83%) patients experienced one or more adverse events during OligoG treatment compared to 51 (84%) patients during placebo treatment. Most of the reported adverse events were probably related to the underlying CF disease. Seven (11%) patients experienced one or more serious adverse events (SAEs) during OligoG treatment, while eight (13%) patients had one or more SAEs during placebo treatment. The most frequent SAE was pulmonary exacerbations. Most events were of grade 1 (mild) severity in all treatment sequences and treatment periods. There were two grade 4 events in the study: one appendicitis and one elevated potassium. Both events occurred during the first washout period in the placebo–OligoG treatment sequence. No large treatment differences were seen for the safety and tolerability variables between OligoG and placebo, although dyspnoea was identified in 12 events for OligoG compared to four for placebo; these were of low-grade severity. The most common adverse events were nasopharyngitis, cough and pulmonary exacerbations of infective origin (table 2).

Pharmacokinetics

Plasma concentrations of OligoG were in the range of 0.5–8.98 $\mu g \cdot mL^{-1}$ (table 3) and did not show any signs of systemic accumulation: mean plasma concentration at 14, 28 and 56 days (28 days after completing treatment) was 1.57, 1.32 and 0.00 $\mu g \cdot mL^{-1}$, respectively. Furthermore, there was no detectable OligoG in plasma at day 56 in any of the patients who received OligoG in period 1 (day 56 levels were not measured in period 2).

Patient compliance

Study drug compliance for the ITT population in terms of proportion of scheduled study drug capsules used per treatment period (*i.e.* number of study medication capsules used divided by the scheduled number of study medication capsules) is summarised in table 4; the majority of patients had a treatment compliance of >80%. The compliance for patients randomised to placebo in the first treatment period was higher than the compliance for patients randomised to placebo in the second treatment period, which was similar to the compliance seen in the two treatment periods using OligoG. All patients with treatment



Total bacteria	CFU in sputum $log_{10} \cdot g^{-1}$							
	OligoG				Placebo			
	Subjects n	Mean	SD	SE	Subjects n	Mean	SD	SE
Before treatment	12	7.20	0.68	0.20	12	6.99	0.65	0.19
After treatment	12	6.84	0.77	0.22	12	7.07	0.63	0.18

FIGURE 5 Total bacterial density (log_{10} change in CFUs) in sputum samples from individual patients before and after treatment with OligoG or placebo.

compliance <80% were excluded from the per-protocol population, but included in the mITT population (see *post hoc* analyses).

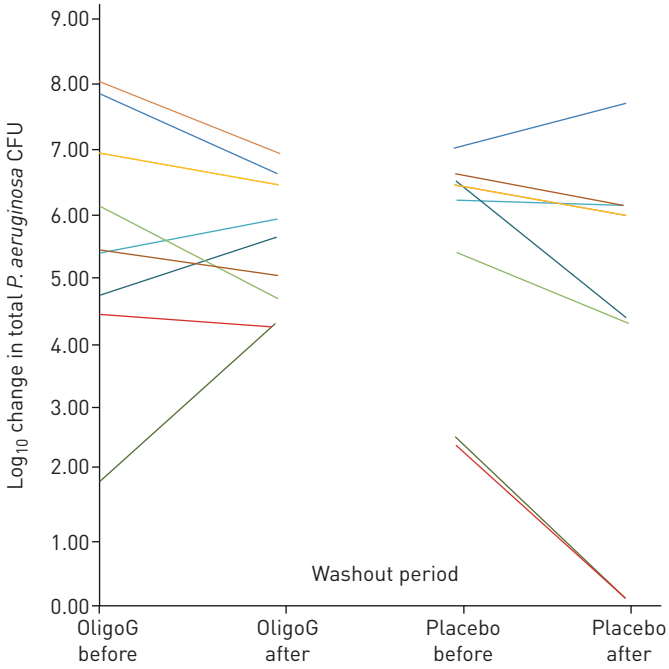
Retrospective study on pulmonary exacerbations

In the retrospective pulmonary exacerbations study, 17 SAEs were reported in the 6 months that followed the study treatment; all of them unrelated to, or with an unlikely relationship to the study treatment. No significant differences were found in numbers of patients with hospitalisations or antibiotic treatments due to pulmonary exacerbations pre- *versus* post-study. Thirty-four pulmonary exacerbations were reported in the pre-treatment period *versus* 24 in the post-treatment period of OligoG. This represents a mean reduction of 0.25 pulmonary exacerbations per patient, or a 29% reduction in pulmonary exacerbations (p=0.06).

Post hoc *analyses*

Lung function

Initial analyses included in the clinical trial analysis plan on the ITT and the per-protocol populations, suggested the dosing of OligoG influenced the outcome in efficacy: 45% of trial participants had taken fewer than the prescribed number of capsules per treatment period. Therefore, further unplanned *post hoc* analyses were performed in a mITT population (n=54 based on having had no protocol violations except dose compliance). Positive trends were shown in subgroup analyses for those with the following clinical features: 1) taking cyclic inhaled tobramycin; 2) using continuous inhaled antibiotics and <100% OligoG compliance; and 3) younger age (⩽25 years). Data at the end of treatment (day 28) and after 4 weeks of washout (day 56) were assessed to describe the sustained effect on FEV_1. OligoG treatment in the setting of concomitant cyclic inhaled tobramycin (synchronised in the off-cycle) was associated with a relative



Total *P. aeruginosa*	CFU in sputum $log_{10}·g^{-1}$							
	OligoG				Placebo			
	Subjects n	Mean	SD	SE	Subjects n	Mean	SD	SE
Before treatment	10	5.58	1.83	0.58	10	5.61	1.69	0.54
After treatment	10	5.54	0.96	0.30	10	4.67	2.64	0.83

FIGURE 6 Total *Pseudomonas aeruginosa* density (log_{10} change in CFUs) in sputum samples from individual patients before and after treatment with OligoG or placebo.

TABLE 2 Summary of subjects with treatment-emergent adverse events by preferred term, with grade of severity, for OligoG or placebo

	OligoG	Grade				Placebo	Grade			
	Events n	1	2	3	4	Events n	1	2	3	4
Diarrhoea	4	2	2	0	0	0	0	0	0	0
Chest pain	2	2	0	0	0	5	3	2	0	0
Infective pulmonary exacerbation of cystic fibrosis	8	1	2	5	0	7	1	1	5	0
Nasopharyngitis	14	11	3	0	0	11	10	1	0	0
Respiratory tract infection	5	1	3	1	0	5	1	3	1	0
Blood potassium increased	3	3	0	0	0	5	3	0	1	1
White blood cell count increased	4	3	1	0	0	1	0	1	0	0
Bronchial obstruction	4	2	2	0	0	2	0	2	0	0
Cough	9	8	1	0	0	12	8	2	2	0
Dyspnoea	12	10	2	0	0	4	4	0	0	0
Haemoptysis	5	3	2	0	0	8	6	2	0	0
Oropharyngeal pain	4	3	1	0	0	1	1	0	0	0
Sputum increased	6	5	1	0	0	5	5	0	0	0
Wheezing	4	3	1	0	0	2	2	0	0	0
Total	84	57	21	6	0	68	44	14	9	1

TABLE 3 Plasma pharmacokinetic values for OligoG in the intention-to-treat population

	Subjects	OligoG value μg·mL^{-1}	
Treatment period 1, days of treatment with OligoG			
14	29	1.57±1.864	0/1.18/8.98
28	28	1.328±1.023	0/1.205/3.54
Treatment period 2, days of treatment with OligoG			
14	29	1.563±1.223	0/1.36/4.41
28	28	1.317±1.187	0/1.105/4.04
Total days of treatment with OligoG			
14	58	1.567±1.557	0/1.31/8.98
28	56	1.322±1.098	0/1.165/4.04

Data are presented as n, mean±SD or minimum/median/maximum.

TABLE 4 Study drug compliance

	Subjects	Proportion of capsules used	
		<80%	>80%
OligoG–placebo			
Period 1: OligoG	32	6 (19)	26 (81)
Period 2: placebo	28	6 (21)	22 (79)
Placebo–OligoG			
Period 1: placebo	33	3 (9)	30 (91)
Period 2: OligoG	31	6 (19)	25 (81)

Data are presented as n or n (%).

TABLE 5 Exploratory subgroup analyses of relative change in forced expiratory volume in 1 s (FEV_1) from baseline to 28 days of treatment

	Subjects n	Estimate	FEV_1# %	SE	p-value
Modified ITT	54	−0.0153	−1.5	0.0201	0.4492
Modified ITT with OligoG compliance ⩾100%	29	−0.0728	−7.3	0.0243	0.0059
Modified ITT with OligoG compliance <100%	25	0.0552	5.5	0.0295	0.0738
Modified ITT age ⩽25 years	13	0.0437	4.4	0.0448	0.3505
Modified ITT age >25 years	41	−0.0449	−4.5	0.0235	0.0630
Modified ITT on tobramycin	16	0.0972	9.7	0.0235	0.0010
Modified ITT not on tobramycin	38	−0.0560	−5.6	0.0233	0.0217
Modified ITT on aztreonam	14	−0.0332	−3.3	0.0390	0.4123
Modified ITT not on aztreonam	40	−0.0135	−1.4	0.0233	0.5670
Modified ITT on antibiotics and with OligoG compliance <100%	24	0.0623	6.2	0.0297	0.0476
Modified ITT on continuous inhaled antibiotics	40	0.0067	0.7	0.0218	0.7625
Modified ITT on continuous inhaled antibiotics and with OligoG compliance <100%	19	0.0867	8.7	0.0335	0.0190
Modified ITT on tobramycin and with OligoG compliance <100%	10	0.1092	10.9	0.0341	0.0125
Modified ITT with BMI <20 kg·m^{-2} at screening	15	0.0604	6.0	0.0429	0.1826
Modified ITT with BMI ⩾20 kg·m^{-2} at screening	39	−0.0439	−4.4	0.0223	0.0560
Modified ITT with FEV_1 <55% predicted at screening	20	−0.0226	−2.3	0.0275	0.4226
Modified ITT with FEV_1 55–70% predicted at screening	18	−0.0006	−0.1	0.0314	0.9850
Modified ITT with FEV_1> 70% of predicted at screening	16	−0.0082	−0.8	0.0465	0.8630

Estimates of treatment differences from mixed models. ITT: intention-to-treat population; BMI: body mass index. #: percentage change in FEV_1 compared to placebo. Tobramycin treatment was always synchronised in the off-cycle.

TABLE 6 Exploratory subgroup analyses of relative change in forced expiratory volume in 1 s (FEV_1) from baseline to 56 days

	Subjects n	Estimate	FEV_1# %	SE	p-value
Modified ITT	56	0.0331	3.3	0.0259	0.2074
Modified ITT with study drug compliance ⩾100%	29	−0.0137	−1.4	0.0250	0.5869
Modified ITT with study drug compliance <100%	27	0.0854	8.5	0.0449	0.0690
Modified ITT age ⩽25 years	13	0.0953	9.5	0.0718	0.2115
Modified ITT age >25 years	43	−0.0072	−0.7	0.0259	0.7827
Modified ITT on tobramycin	15	0.1585	15.8	0.0522	0.0095
Modified ITT not on tobramycin	41	−0.0058	−0.6	0.0271	0.8330
Modified ITT on aztreonam	13	0.0836	8.4	0.0505	0.1264
Modified ITT not on aztreonam	43	0.0361	3.6	0.0306	0.2447
Modified ITT on antibiotics and with study drug compliance <100%	24	0.1101	11.0	0.0460	0.0257
Modified ITT on continuous inhaled antibiotics	40	0.0591	5.9	0.0295	0.0521
Modified ITT on continuous inhaled antibiotics and with study drug compliance <100%	19	0.1282	12.8	0.0501	0.0204
Modified ITT on tobramycin and with study drug compliance <100%	9	0.1996	20.0	0.0850	0.0512
Modified ITT with BMI <20 kg·m^{-2} at screening	16	0.1214	12.0	0.0646	0.0810
Modified ITT with BMI ⩾20 kg·m^{-2} at screening	40	0.0169	1.7	0.0258	0.5171
Modified ITT with FEV_1 <55% predicted at screening	21	0.0398	4.0	0.0469	0.4066
Modified ITT with FEV_1 55–70% predicted at screening	19	0.0166	1.7	0.0332	0.6230
Modified ITT with FEV_1 >70% predicted at screening	16	0.0621	6.2	0.0576	0.2992

Estimates of treatment differences from mixed models. ITT: intention-to-treat population; BMI: body mass index. #: percentage change in FEV_1 compared to placebo. Tobramycin treatment was always synchronised in the off-cycle.

improvement in FEV_1 of 9.7% at day 28 (p=0.001; table 5) and 15.9% at day 56 (p=0.01; table 6). Patients on continuous inhaled antibiotics and <100% compliance to OligoG also showed an 8.7% improvement in FEV_1 at day 28 (p=0.02), and 12.8% at day 56 (p=0.02). There was a trend towards improvement in FEV_1 which did not reach statistical significance, after OligoG treatment in those patients who took less than the full dose of the drug (<100% compliance), with 5.5% improvement in FEV_1 at day 28 (p=0.07), and 8.5% improvement in FEV_1 at day 56 (p=0.07). OligoG treatment in younger patients (⩽25 years) did not show significant effects, although a 4.4% increase in FEV_1 was noted at 28 days, which increased to 9.5% at 56 days.

In the ITT population there was a pronounced drop in FEV_1 at day 14, followed by recovery to baseline by day 28 (figure 3). This was not observed in those patients who were subsequently identified to have taken less than the full dose of OligoG (<100% compliance), as determined by the proportion of study drug capsules used per treatment period (figure 7).

Rheology

Rheology measurements of viscosity, elasticity and phase angle in expectorated sputum did not show a statistically significant improvement in the ITT population (supplementary table S6). Additional *post hoc* analyses were performed on the ITT population for sputum rheology at 0.1 and 1 Hz which were not planned in the study protocol, but added during the reporting phase of the study. Phase angle values at 0.1 Hz and 1 Hz for the ITT population where patients were excluded if they had one missing value per treatment period are shown in table 7. These results show a continuous trend during OligoG treatment of increased phase angle scores indicative of more fluid-like sputum from day 0 to day 56. The placebo scores were more variable during the treatment period, but generally similar scores were found at day 0 and day 56, suggesting no overall effect on sputum rheology from the placebo. There was a statistically significant improvement observed at 0.1 Hz (p=0.03) in the modified ITT population at 14 days of treatment. A similar finding was observed at 0.1 Hz for the modified ITT population on tobramycin (p=0.05), which may reflect the combined effects of OligoG on rheology, biofilm disruption and antibiotic potentiation.

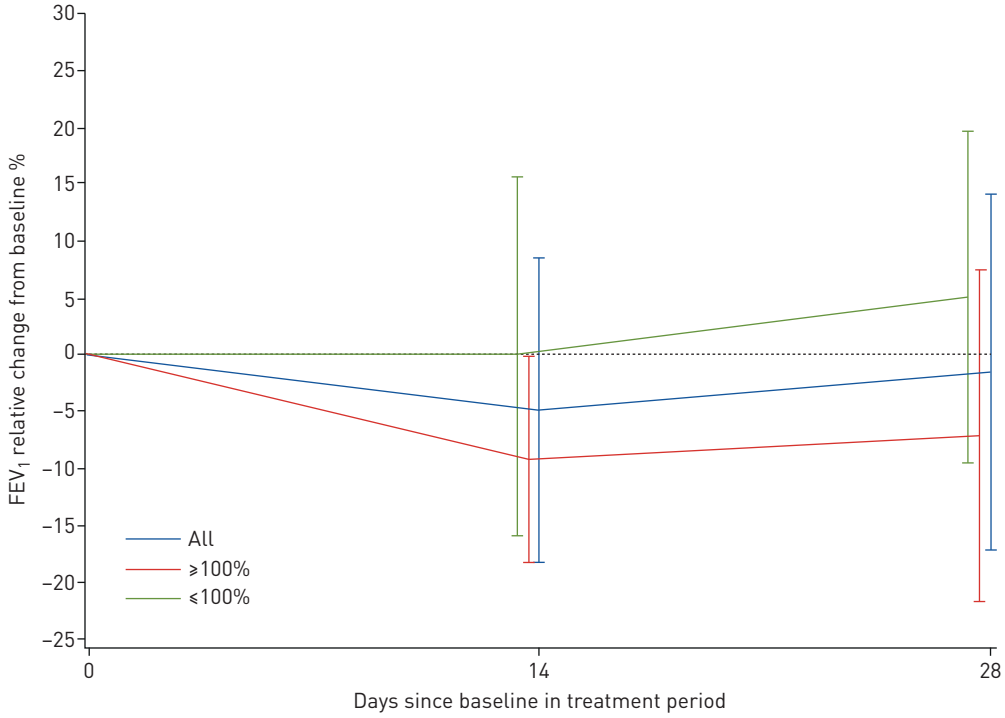


FIGURE 7 Relative change in forced expiratory volume in 1 s (FEV_1) from baseline showing within-patient difference OligoG–placebo for dose compliance, modified intention-to-treat population.

This effect of OligoG treatment was even more pronounced in patients aged <25 years who showed a marked improvement in the phase angle at 0.1 Hz (p=0.002) at the end of treatment (28 days), indicating that the sputum viscosity is reduced and more liquid present after treatment with OligoG.

Discussion

Statistically significant improvement in FEV_1 was not observed in the ITT population and this study did not meet the primary end-point. This study demonstrated that repeated inhalation of OligoG dry powder was generally safe in adult CF patients.

The relative change at day 14 followed by recovery to baseline by day 28 may be explained by the known mechanism of action of OligoG in reducing elevated mucus viscosity, especially since the drop in FEV_1 at week 2 was only a transitory phenomenon. This is thought to be related to the rapid increase in the mobility of mucus due to the calcium-binding effect of OligoG as previously described [6], which would

TABLE 7 Sputum rheology

	Phase angle 0.1 Hz				Phase angle 1.0 Hz			
	Subjects n	Mean	SD	SE	Subjects n	Mean	SD	SE
OligoG								
0	44	18.84	3.42	0.52	45	16.98	3.53	0.53
14	44	19.21	4.39	0.66	45	17.21	5.93	0.88
28	44	20.66	6.14	0.93	45	18.60	6.85	1.02
56	44	21.53	7.49	1.13	45	20.06	7.69	1.15
Placebo								
0	45	20.10	5.01	0.75	46	18.13	6.00	0.88
14	45	18.08	3.52	0.52	46	16.11	3.51	0.52
28	45	20.52	9.65	1.44	46	19.06	10.37	1.53
56	45	19.72	4.12	0.61	46	17.52	3.65	0.54

Post hoc analysis of phase angle (0.1 and 1 Hz) values in degrees, patients with no missing data per treatment period.

in turn trigger the release and possible swelling of stagnant mucus plugs. This could initially reduce pulmonary function parameters before the mucus was expectorated by the patients, and probably explains the observation of dyspnoea in OligoG compared to placebo treatment. This points towards the need to revise the dosing regimen. This notion is supported by the combined subgroup analyses that suggest the potential value for OligoG with a lower initial dose might potentially be less likely to lead to an initial drop in FEV_1, and therefore be more beneficial.

Although the viscosity, elasticity and phase angle in expectorated sputum did not show a statistically significant improvement in the ITT population, the improvement in the modified ITT population at 14 days of treatment and in younger patients (<25 years) at the end of treatment (28 days) supports what has previously been reported for the mechanism of action of OligoG [3–7].

A more peripheral deposition of radiolabelled particles following OligoG inhalation suggests that the smaller airways were more open after treatment. However, this effect was not reflected in a more effective clearance. This could be due to the fact that the MCC assay is best suited to capture effects on larger-airway cilia-driven clearance. An analysis of peripheral lung clearance, which is less confounded by deposition changes, showed a trend towards faster peripheral lung clearance with OligoG.

There was no difference in rates of pulmonary exacerbations between OligoG and placebo during this relatively short clinical trial. However, in the retrospective study, the trend towards fewer pulmonary exacerbations in the post-study period is an interesting observation, although it is far from clear whether this reduction was the direct result of OligoG or other treatment changes following the trial.

Post hoc analyses of defined subgroups were sufficiently intriguing to warrant further investigation of patients on inhaled anti-*P. aeruginosa* antibiotics combined with lower doses of OligoG. Nevertheless, it is important to note that recent clinical studies [32] have highlighted caution in placing too much emphasis on *post hoc* subgroup analyses: while the data presented in the current study might support the known mechanism of action of OligoG, further prospective clinical studies are clearly required to substantiate the potential for OligoG in CF.

One of the main limitations of the study was the placebo formulation. In order to minimise the risk of unblinding, the lactose placebo was administered in the same dose (1050 mg per day) and comparable particle size as the dry powder formulation of the active drug. Subsequent review suggests that this high dose of lactose may not have been the best choice of placebo to evaluate microbiological effects of OligoG, or the subsequent impact on lung function parameters. Indeed, the negative results from culture microbiology analysis were unexpected given the breadth of *in vitro* and *in vivo* data already highlighting the antimicrobial properties of OligoG [11–14, 25]. Further investigation identified independent evidence that lactose, present at high concentrations in the placebo used in the trial, inhibits the growth of *P. aeruginosa* and the adhesion of other respiratory pathogens (*e.g. Burkholderia* spp.) to lung epithelial cells and potentiates the activity of antibiotics [26–29]. Additional studies have also identified a role for metabolites, such as lactose, enhancing antibiotic susceptibility to antibiotics such as tobramycin [30, 31].

An additional limitation of the study was the selected dosing regimen of 10 capsules three times daily. This dose was based on conclusions from the previous phase 2A study (EudraCT Number: 2010-023090-19) [16], that indicated the dose was in the lower range of what would be expected to demonstrate efficacy: the dose was sufficient to affect the rheology of expectorated sputum, although not sufficient to result in significant changes in FEV_1. Although providing better compliance and reduced treatment burden compared to the nebulised formulation, the inhaled dry powder dose selected for the current study clearly proved to be a treatment burden for some patients, as indicated by the number of patients that were not compliant with taking the full number of capsules/doses. Considering these observations, future studies are required to re-evaluate the impact of dose, capsule size and capsule loading.

The results did not reveal any safety concerns for adult CF patients following administration of 1050 mg OligoG per day dry powder inhalation over a 28-day period and confirms the results of early-phase studies showing that the active drug has a favourable safety profile. Further phase 2B clinical studies in CF using lower doses of OligoG DPI are being planned (under the framework of HORIZON2020).

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