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Short Communication

Evaluating the alginate oligosaccharide (OligoG) as a therapy for Burkholderia cepacia complex cystic fibrosis lung infection

Rianald Fischer[a], Carsten Schwarz[b], Rebecca Weiser[c], Eshwar Mahenthiralingam[c], Knut Smerud[d], Nils Meland[d], Hugo Flaten[e], Philip D Rye[e,*]

[a] *Pneumologisches Studienzentrum München-West, München, Germany*
[b] *Division of Cystic Fibrosis, CF Center Westbrandenburg, Campus Potsdam, Potsdam, Germany*
[c] *Cardiff University, School of Biosciences, Cardiff, Wales, UK*
[d] *SMERUD, Karenslyst alle 6, 0278 Oslo, Norway*
[e] *AlgiPharma AS, Industriveien 33, 1337 Sandvika, Norway*

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ABSTRACT

OligoG has previously shown potentiation of aztreonam against *Burkholderia cepacia* complex (Bcc) through biofilm disruption. A randomized, double-blind, placebo-controlled cross-over design was used to evaluate safety and efficacy of inhaled OligoG as a therapy for Bcc-infected CF patients taking aztreonam. Subjects received OligoG (1050 mg daily) or matching placebo for 28-days. Of 14 subjects completing the study, 8 showed a mean decrease in total bacterial CFU's (0.82 log10) after OligoG treatment. There was a reduction in mean Bcc CFU's (2.19 log10) after OligoG treatment but this was not statistically significant. Rheology analysis showed improvements in phase-angle after OligoG, but there was no statistically significant improvement in lung function parameters. Six out of 12 QoL summary scores showed relative improvement after OligoG treatment compared to placebo. There was a favourable safety profile for OligoG. Potential for reducing Bcc warrants further investigation of OligoG for the treatment of infection in CF.

1. Introduction

The *Burkholderia cepacia* complex (Bcc) comprises greater than 20 species of bacteria [1] that are found in the natural environment including, *B. cenocepacia, B. multivorans, B. vietnamiensis, B. dolosa,* and *B. cepacia*. This group are notorious drug resistant/insensitive opportunistic pathogens and a major cause of morbidity and mortality in cystic fibrosis (CF) patients [2]. While infection control policies and patient awareness [3–5] has highlighted the challenges and led to more effective clinical management measures, infection with Bcc remains a significant clinical challenge in CF [6,7]. Strict infection control measures, including segregation of infected patients have become the standard although such measures may have negative psychosocial effects among infected patients [8]. Although these infection control measures have had benefit in reducing transmission, Bcc infection nevertheless remains a significant and life-threatening pathogen in CF patients inducing a rapid clinical decline. The Bcc species *B. multivorans* is now the most common *Burkholderia* seen in CF [9], and like all Bcc species it has intrinsic antimicrobial resistance [10] and may be associated with poor clinical outcome in certain individuals [11]. Overall, CF patients with Bcc have a very limited range of therapeutic options and can be subject to cohort segregation measures to inhibit transmission, but which may also lead to reduced access to multidisciplinary care. More recent studies also show that despite these segregation measures, new cases of unrelated *B. cepacia* complex infections continue to occur, which supports an environmental origin of infection [9]. These infections are not only difficult to treat due to inherent resistance of the bacteria to antibiotics but have also been recognised as the cause of Cepacia syndrome which is a fatal pneumonia accompanied with septicaemia [2].

OligoG is a new oligosaccharide drug in development to facilitate mucus clearance and combat bacterial infections in CF. Results from *ex vivo* and *in vivo* studies [12–14] indicate that OligoG is able to restore the mucus phenotype in terms of consistency and viscosity, enabling improved mucociliary function and mucus clearance. Although these parameters did not show a statistically significant improvement in the intention-to-treat (ITT) population in a recent phase 2b study (EudraCT No.: 2014-000844-13; [15]), there was improvement in viscosity, elasticity and phase angle in expectorated sputum in a modified ITT population at 14 days of treat-

* Corresponding author.
E-mail address: phil.rye@algipharma.com (P.D. Rye).

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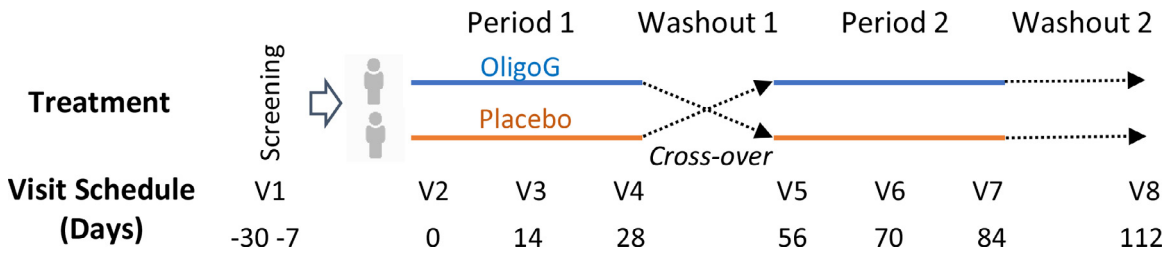


Fig. 1. Cross over trial design and visit schedule.

ment and in younger patients (<25 years) at the end of treatment (28 days), which supports what has previously been reported for the mechanism of action of OligoG [16–18]. In addition to these mucoactive properties, *in vitro* and *in vivo* studies have shown that OligoG is able to potentiate antibiotics against a wide range of multidrug-resistant bacteria [19], likely through biofilm disruption of key respiratory pathogens [18,20–24]. OligoG has also been shown to reduce virulence factors and bacterial communication in biofilm development [20,25,26]. OligoG also showed notable potentiating effects on aztreonam [19,21]. The combined mucoactive and antimicrobial properties support the rationale to explore the potential for OligoG treatment as a therapy for *Burkholderia cepacia* complex CF lung infection.

2. Methods

OligoG was administered for 28 days as dry powder for inhalation (DPI). The study was performed at 2 centres in Germany between 18 Feb 2015 (First Patient First Visit) and 10 May 2016 (Last Patient Last Visit). The study was conducted in full accordance with the, 64th WMA General Assembly, Fortaleza, Brazil, October 2013, amendment to the Declaration of Helsinki 1964, ICH guidelines for GCP and with laws, ethics committees and regulations for clinical research in the involved countries. Inclusion criteria included a confirmed diagnosis of CF defined by clinical features and sweat chloride test (≥60 mmol/L) by pilocarpine iontophoresis, or a genotypic confirmation of CFTR mutation. Adult patients (aged ≥18 years) must have had a Bcc lung infection diagnosis in their medical history, defined by at least two positive microbiological cultures in expectorated sputum within the last 12 months prior to Visit 1. Patients were also required to take inhaled aztreonam three times daily (TID) in a 4 weeks on/off cycle treatment regimen or a continuous intake regimen for at least 4 weeks before screening Visit. For on/off cycles, the Screening Visit (Day −28 to Day −7) was scheduled to take place in the "off" phase. Randomization Visit (Day 0) was the first day "on" to harmonize the aztreonam inhalation period with the IMP intake period. At screening, the FEV1 had to be between 40% and 100% of the predicted normal value according to the Global Lung Function Initiative normative equations at screening [27]. All patients provided informed consent. Full inclusion/exclusion criteria are listed in Table S1 Supplemental data.

Eligible participants were randomized (1:1) to receive OligoG 1050 mg per day (10 capsules TID) and a matching placebo of lactose in randomized 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 Fig. 1. The study medication was administered using a dry powder monodose inhaler (MIAT S.p.A., Milano, Italy). The individual patient treatment assignments were blinded to all study site personnel and those involved in the monitoring or management of the study. Efficacy variables included microbiological measurements in expectorated sputum (primary explorative endpoint), absolute changes in FEV_1, 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 sputum rheology, and Quality-of-Life measured by the Cystic Fibrosis Questionnaire Revised (CFQ-R) [27].

Culture microbiology to quantify viable bacterial load in sputum was performed at a central lab (Synlab AG, Munich, Germany) and samples were processed within 28 h of collection. Numerical counts for total *Burkholderia cepacia* complex species, and *Pseudomonas aeruginosa* were performed on selective media OFPBL (oxidative-fermentative polymyxin B-bacitracin-lactose) and MacConkey agar plates, respectively. Total bacteria counts were determined from Columbia agar plates.

Culture independent microbiology analyses of total Bcc load were performed to account for the combined viable and non-viable bacteria in sputum samples. Expectorated sputum samples were collected at six visit time points across the trials for culture-independent microbiological investigations: V1, V2, V4, V5, V7 and V8. Sputum samples were stored and shipped at −80 °C from clinical sites to the analytical lab for DNA extraction within 4 weeks. At each time point two sputum samples were taken within 2 h from each patient where possible (paired samples). One sputum sample from each time point was used for the culture-independent investigations reported here as bacterial diversity profiles were found to be highly similar between paired samples [28]. Microbiology and rheology methods are outlined in Supplemental data, while the DNA extraction and qPCR were performed essentially as described [28].

Whole blood samples were collected from all subjects for plasma OligoG concentrations by liquid chromatography (Agilent 1200 LC Systems) with tandem mass spectrometric detection (Agilent 6460 Triple Quad LC-MS/MS detector). The planned sample size was determined pragmatically due to very low prevalence and was not based on any sample size calculation. The primary analysis was performed on the Bcc *rpoD* gene copy number, as measured from the sputum molecular microbiology. Patients with no measurements post baseline in one or both treatment periods were excluded from the model. The mean of the three log transformed replicate values at each visit were used in the analysis; copy number values of 0 were ignored in the calculation of the mean. If the copy number was 0 for all replicates at a visit, a value of 0 was imputed for the log10 value. For the log transformed values at the end of each treatment period, a linear model using SAS PROC MIXED was constructed, 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. Visit 2 is defined as baseline for period 1; Visit 5 is defined as baseline for period 2. If the Visit 2 value was missing, the Visit 1 value was used as baseline if available. Also centre and centre × treatment effects was included in the model if statistically significant on a 10% level.

3. Results

Seventeen patients were assessed for eligibility, and 15 patients randomized to receive treatment (Fig. 2), with 7 patients random-

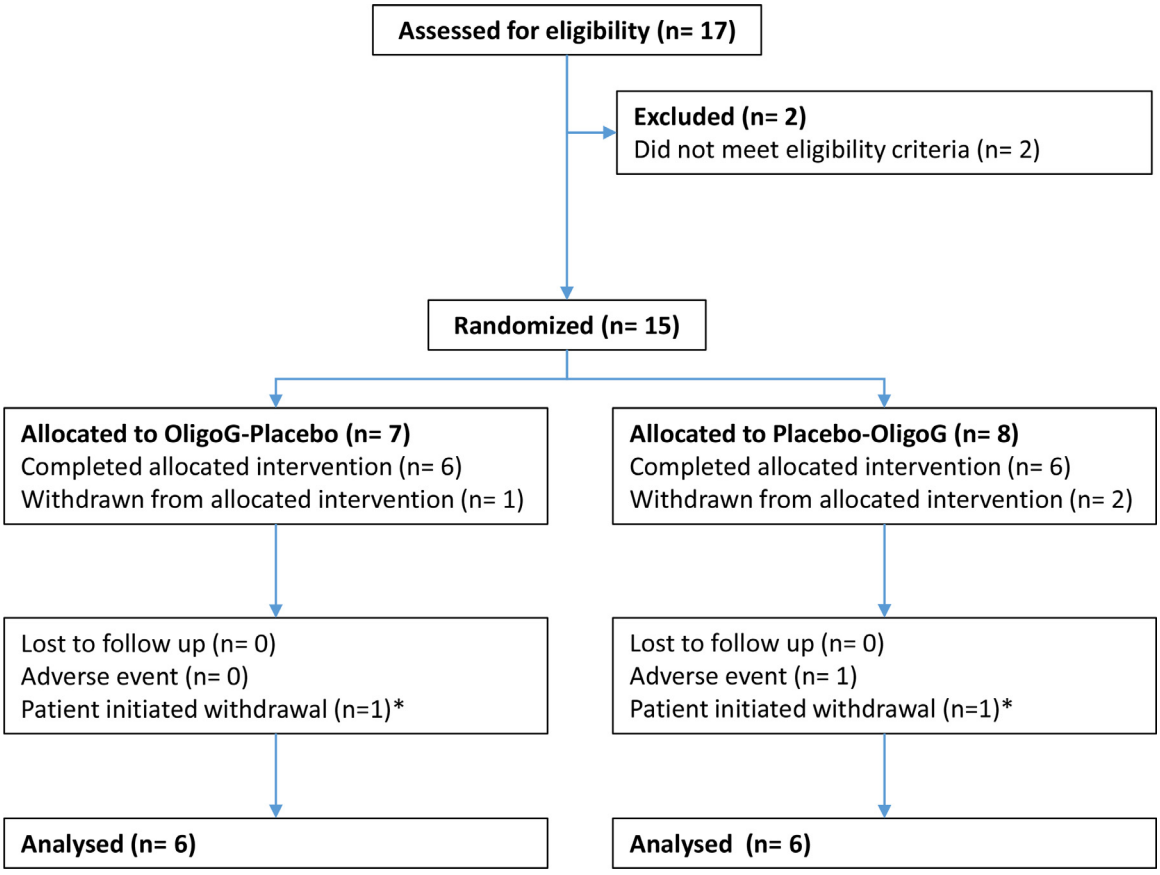


Fig. 2. Study flow chart. *Subjects withdrew for personal reasons.

Table 1
Demographics information for ITT population consisted of gender, ethnic group, age at informed consent, height and weight. Data are presented as n, n(%), mean±SD or minimum/median/maximum. The baseline demographic characteristics were similar in the two treatment sequences.

	OligoG - Placebo	Placebo - OligoG	Total
Number of subjects	7	8	15
Male	3 (42.9)	1(12.5)	4 (26.7)
Female	4 (57.1)	7(87.5)	11 (73.3)
Caucasian/European ethnic group	7(100)	8(100)	15(100)
Age at Informed consent (years)	31.4 (7.4) 24/31/42	38.3 (15.5) 23/31.5/71	35.1 (12.5) 23/31/71
Height (cm]	170.4 (5.6) 162 / 170 / 180	161.6 (4 8) 154/161/168	165.7 (6.7) 154/167/180
Body weight (kg]	57.3 (4.2) 52 / 59 / 61.3	54.5(12.0) 38 / 51.5 / 78.3	55.8(9.1) 38 / 55 / 78.3
BMI (kg/m2]	19.75 (1.71) 17.58 / 20.48 / 21.87	20.81 (4.41) 16.02 / 19.69 / 30.59	20.32 (335) 16.02 / 20.31/ 30.59

ized to receive OligoG in the first treatment period and placebo in the second treatment period, while 8 patients were randomized to receive placebo in the first treatment period and OligoG in the second treatment period. Each treatment period was 28 days followed by 28 days wash-out. Patient demographics are summarized in Table 1. One patient was an early withdrawal resulting in incomplete culture microbiology data and was excluded from the interpretation of these data.

Microbiology (characteristics at screening)

A total of 15 patients were randomized in this study, although one patient was an early withdrawal at V3 resulting in incomplete culture microbiology data and was excluded from this interpretation. All 14 remaining subjects showed bacterial growth in sputum as determined by culture at Screening, but only 8 subjects showed culturable Bcc at Screening despite previous documented history of chronic colonization with Bcc. In addition, the 6 subjects that were negative for culturable Bcc at Screening did not grow Bcc at any of the other time points during the trial (Table 2). Culture-independent microbiological analysis using qPCR was performed on 13 of the 14 subjects and identified that all had evidence of Bcc lung infection at Screening, although total abundances varied (0.76–8.55 $\log_{10}$ Bcc/g sputum).

Via conventional microbiology, Bcc was identified as *B. cepacia* complex (undefined species) in 13 subjects and *B. multivo-*

Table 2
The *Burkholderia* species identity and total abundance in Screening samples as determined by culture-independent and culture-dependent methods.

Patient	Burkholderia species (molecular ID)	Burkholderia species (culture ID)	Total abundance (log10-Bcc/g sputum)	Total viable Bcc (log10 CFU/ml sputum)
27610-002		B. cepacia	7.74	6.82
27610-005	B. cenocepacia		8.55	0
27610-006		Unknown	8.01	0
27611-002			7.36	0
27610-001		B. cepacia	5.96	5.6
27610-004		B. multivorans	8.33	6.82
27610-007	B. multivorans	Unknown	7.50	0
27610-008			8.30	3.78
27610-009		B. cepacia	6.96	6.02
27611-006			6.41	5.19
27610-003		Unknown	0.76	0
27610-011	Unknown[a]	B. cepacia	5.44	5.83
27611-005		Unknown	3.69	0
27611-001[b]	Not performed	B. cepacia	Not performed	6.45

[a] Unknown. No ID was obtained either due to a negative PCR result (molecular ID) or no growth (culture ID).
[b] Patient 27611-001 was not included in the culture-independent investigations.

rans in 1 subject (Table 2). Further follow up by molecular identification methods for 10 subjects revealed that 4 subjects were colonised with *B. cenocepacia* and 6 were colonised with *B. multivorans* (Table 2). For the remaining 3 subjects that underwent culture-independent analysis, no Bcc species ID could be obtained (Table 2). Interestingly, sputum samples colonised with *B. cenocepacia* trended towards higher total abundances as determined by qPCR (mean=7.92, range=7.36–8.55 $\log_{10}$ Bcc/g sputum), whereas those colonised with *B. multivorans* had a broader range of values (mean=7.24, range=5.96–8.33 $\log_{10}$ Bcc/g sputum) (Table 2). The culture-independent results could not be linked to the culture microbiology findings as three of the four patients colonised with *B. cenocepacia* did not have culturable Bcc (Table 2).

Microbiology (OligoG and placebo treatment)

Total bacterial counts were performed using culture microbiology to determine CFU per ml of sputum for each treatment. Of the 14 subjects completing the study, 8 showed a mean decrease in total bacterial CFU's (0.82 log10) after OligoG, although there was no difference in the overall means before and after OligoG or placebo treatments (Fig. 3). Total Bcc counts were performed using both culture microbiology and qPCR. Although there was no marked difference in the overall means as determined by qPCR (Fig. 4), there was a reduction in total Bcc counts as measured by culture microbiology: After OligoG treatment there was a reduction of log10 2.19 compared to a reduction of log10 1.29 after placebo treatment (Fig. 5). When comparing the OligoG and placebo treatment periods the difference showed an effect in favour of OligoG but this was not statistically significant ($p = 0.164$). The reduction in Bcc counts observed after OligoG treatment appeared to be independent of susceptibility to aztreonam with 4 of the 8 subjects having an MIC >256 mg/L. The absence of culturable Bcc significantly impacted the power of this already small study. All 13 subjects included in the culture-independent analysis had evidence of Bcc by qPCR at Screening and during the treatment periods. These 13 subjects did not include one of the patients that had culturable Bcc at Screening (27611-001). It was not possible to determine the impact of the different Bcc species on the responsiveness of patients to OligoG treatment. Due to low patient numbers, it was not possible to show statistical significance in the ITT or per-protocol populations at a 5% level between the active and placebo groups. Nevertheless, these findings suggest that treatment with OligoG could reduce the microbial burden of Bcc spp. in CF mucus.

Lung function

Baseline lung function FEV1 prior to OligoG treatment or placebo was 49.9% and 50.3% respectively (Table 3). Statistically significant improvement was not reached for FEV1 (Table 3), or any of the lung function parameters.

Quality of life

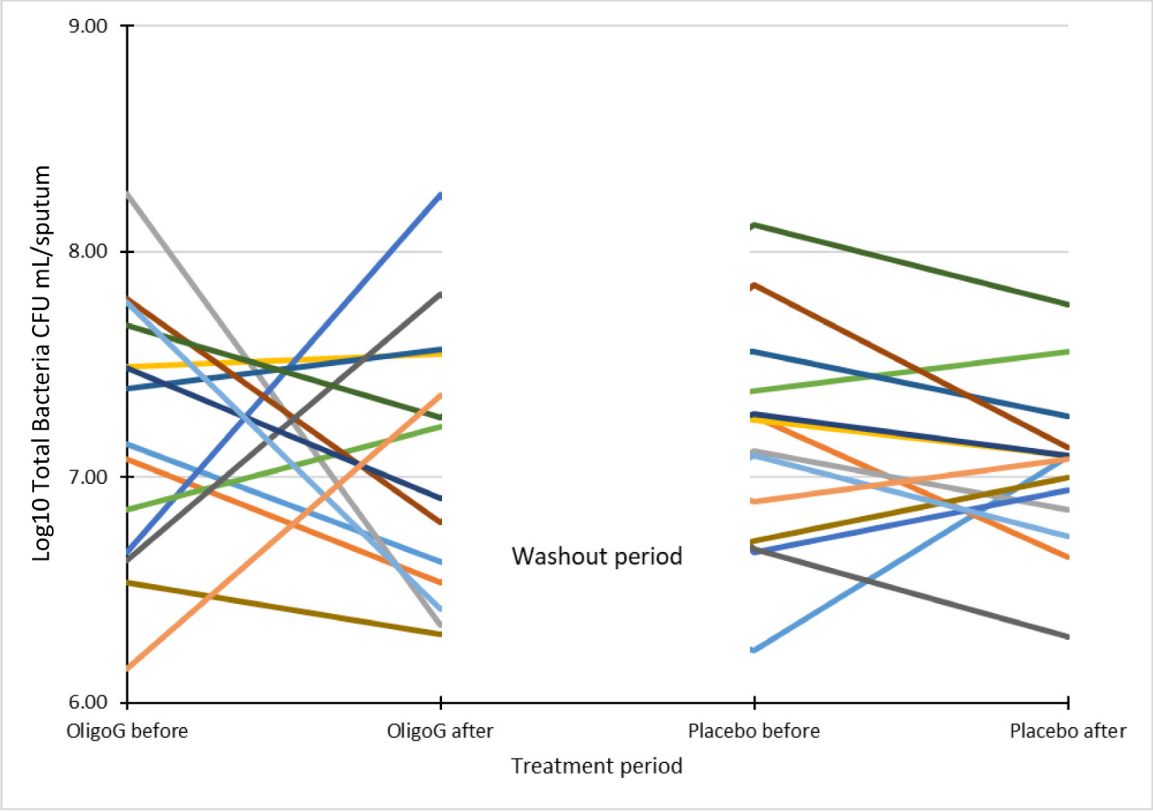
Analysis of QoL measurements in this exploratory study did not show significant effects with the active drug (Table S2 Supplementary data). However, 6 out of the 12 QoL summary scores (Physical, Vitality, Social, Eat, Health, Weight) showed a minor relative improvement in performance after OligoG treatment with mean end of treatment scores of 65.22 after OligoG treatment compared to 60.85 after placebo treatment, although neither showed a significant improvement from baseline. QoL scores are ranged from 0 to 100, with the higher scores indicating better health.

Rheology

Viscosity, elasticity and phase angle in expectorated sputum, showed an improvement after OligoG treatment. The analysis of the phase angle showed an improvement in the active group at the end of treatment with an increase in phase angle at 10 Hz (Table 4) indicating that the sputum viscosity is reduced and more liquid after treatment with OligoG. This confirms observations from *ex vivo* studies, although the small dataset did not permit an analysis for statistical significance.

Safety

The numbers and proportions of patients with adverse events (AE) were similar during OligoG and placebo treatment; Most of the events were of grade 1 (mild) severity in all treatment sequences and treatment periods (Table 5). There was only one SAE (Serious Adverse Event) in the study (haemoptysis) that occurred during OligoG treatment. The investigator considered this to be possibly related to the study drug. Accordingly, this was classified as a SUSAR (suspected, unexpected, serious, adverse drug reaction) and as such was reported immediately to Regulatory Authorities. No serious AEs (SAEs) occurred during placebo treatment. No



Total Bacteria		Log_{10} total bacteria (cfu/mL)						
		OligoG				Placebo		
		n	Mean	SD	SE	Mean	SD	SE
	Before treatment	14	7.21	0.59	0.16	7.15	0.50	0.13
	After treatment	14	7.07	0.60	0.16	7.04	0.36	0.10

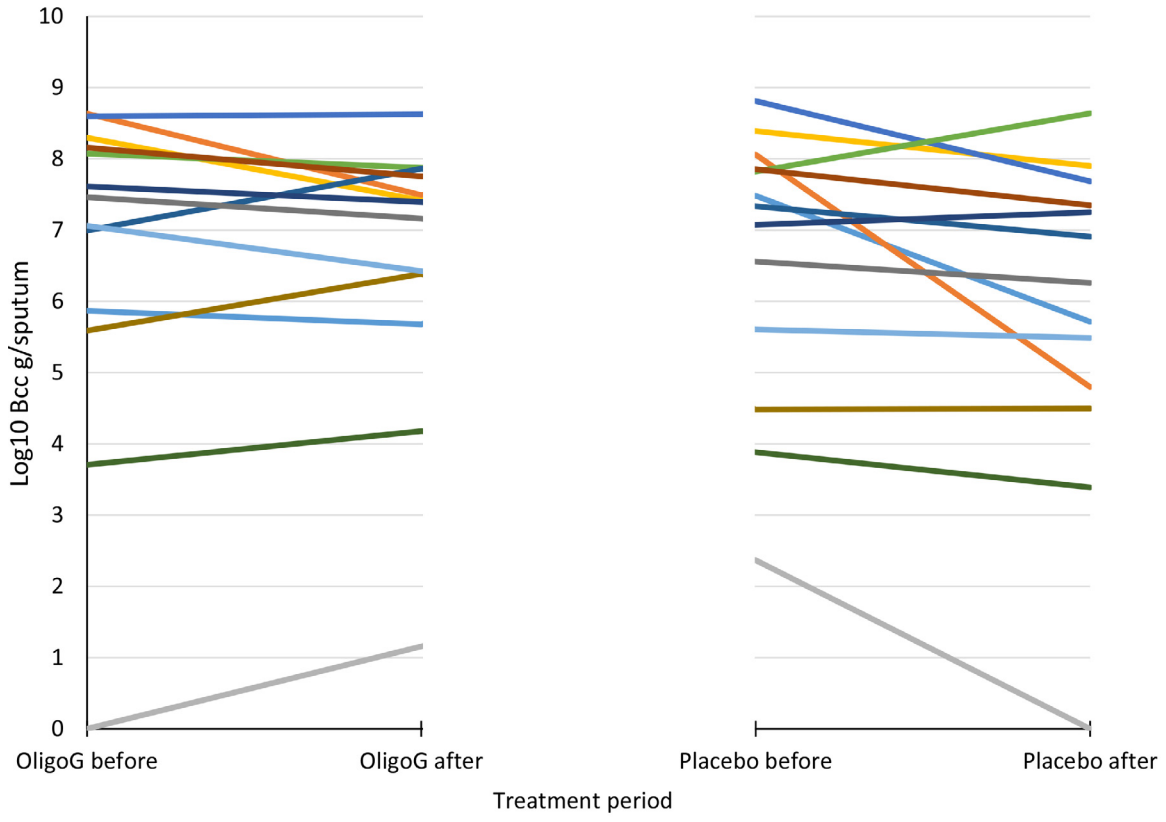
Fig. 3. Log10 counts for total bacteria (as determined by culture, cfu/mL) in sputum samples from individual patients before and after treatment with OligoG or placebo.

Table 3
FEV1 (% of predicted value) for ITT population.

		FCV1 {% of predicted)					Change from baseline					
		n	Mean	SD	Min	Median	Max	n	Mean	SD	Min	Median	Max
Treatment	Visit												
OligoG	Baseline	14	49.90%	17.30%	24%	50%	72%	0					
	Two weeks	13	50.00%	17.80%	20%	54%	73%	13	−1.10%	3.30%	−6%	−3%	5%
	End of treatment	14	48.90%	17.10%	22%	50%	72%	14	−1.00%	4.60%	−8%	−2%	10%
Placebo	Baseline	14	50.30%	17.50%	26%	49%	74%	0					
	Two weeks	14	51.10%	17.80%	22%	51%	75%	14	0.80%	3.60%	−8%	1%	7%
	End of treatment	14	51.10%	17.10%	24%	50%	74%	14	0.80%	3.60%	−5%	0*	10%

large treatment differences were seen in the laboratory, vital signs or ECG parameters. Only one out-of-range lab value (eosinophil count) was classified as clinically significant. Overall, OligoG appears safe, with a safety profile similar to placebo.

Plasma concentrations of OligoG were in the range of 0 to 3.33 µg/mL with a mean of 0.94 ug/mL after 14 days of treatment and 1.03 ug/mL after 28 days of treatment (Table S3 Supplementary data). There was no detectable OligoG in the plasma at the following visit timepoint 28 days after cessation of OligoG treatment. Study drug compliance was defined by the proportion of scheduled study drug capsules used per treatment period and the majority of patients had a treatment compliance of >75% in both treatment periods (Table S4 Supplementary data). Compliance was higher in the first treatment period for both OligoG and placebo, compared to the second treatment period. The compliance for patients randomized to OligoG in the first treatment period was higher than those randomized to OligoG in the second treatment period.



Total Bcc		Bcc in sputum (log10/g)						
		OligoG				Placebo		
		n	Mean	SD	SE	Mean	SD	SE
	Before treatment	13	6.62	2.44	0.68	6.59	1.95	0.54
	After treatment	13	6.57	1.99	0.55	5.84	2.31	0.64

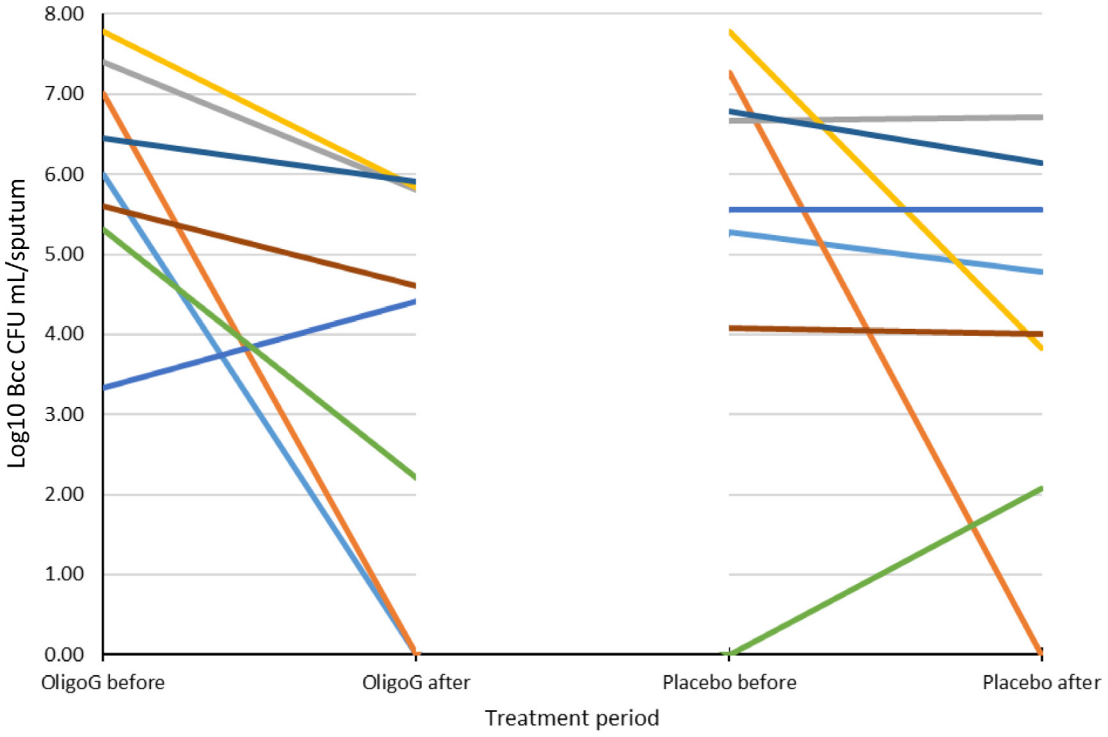
Fig. 4. Log10 counts for total Bcc (as determined by qPCR; gene copies/g sputum) in sputum samples from individual patients before and after treatment with OligoG or placebo.

Table 4
Sputum rheology. Phase angle (0.1, 1.0 and 10 Hz) values and changes from baseline, ITT population.

	Phase angle 0.1 Hz					Phase angle 1.0 Hz					Phase angle 10.0 Hz			
	Subject n	Mean	SD	SE		Subject n	Mean	SD	SE		Subject n	Mean	SD	SE
OligoG					OligoG					OligoG				
0	13	22.80	4.20	1.16	0	13	19.46	5.21	1.44	0	13	51.15	43.82	12.15
14	13	20.60	5.78	1.60	14	13	19.30	6.46	1.79	14	13	62.56	43.21	11.98
28	12	20.27	4.67	1.35	28	12	17.88	3.82	1.10	28	12	81.02	25 56	7.38
56	11	21.93	4.30	1.30	56	11	19.15	3.73	1.12	56	11	67.63	42.92	12.94
Placebo					Placebo					Placebo				
0	14	23.24	8.22	2.20	0	14	19.88	6.22	1.66	0	14	67.78	43.93	11.74
14	14	22.76	4.59	1.23	14	14	19.88	3.55	0.95	14	14	74.40	41.35	11.05
28	14	24.65	4.73	1.26	28	14	20.13	4.94	1.32	28	14	75.66	39.04	10.43
56	13	21.86	5.65	1.57	56	13	19.24	4.36	1.21	56	13	56.72	35.57	9.87

Discussion

This was an exploratory clinical trial to evaluate the efficacy and safety of inhaled OligoG treatment in CF patients using aztreonam due to chronic colonization with Bcc spp. The study demonstrated that repeated inhalation of OligoG DPI was safe in adult CF patients. The primary endpoint of the study was to explore the efficacy of OligoG in reducing Bcc spp. as measured in expectorated sputum samples. While it was not possible to assign statistical significance to the primary endpoint a log10 2.19 reduction in total Bcc as determined by culture analysis suggest that inhaled OligoG might have some effect in reducing the microbial burden of Bcc spp. Differences between the culture and culture-independent analyses may reflect known challenges in culture microbiology, as



Total Bcc		Log_{10} Bcc (cfu/mL)						
		OligoG				Placebo		
		n	Mean	SD	SE	Mean	SD	SE
	Before treatment	8	6.11	1.42	0.50	5.59	2.10	0.74
	After treatment	8	3.92	2.02	0.71	4.30	1.90	0.67

Fig. 5. Log10 counts for Bcc (as determined by culture, cfu/mL) in sputum samples from individual patients before and after treatment with OligoG or placebo.

Table 5
Summary of subjects with treatment-emergent adverse events by preferred term, with grade of severity, for OligoG or placebo. There was only one grade 3 event in the study (haemoptysis) that occurred between visits 6 and 7 during OligoG treatment in the Placebo – OligoG treatment sequence.

Event	OligoG Events n	Grade				Placebo Events n	Grade			
		1	2	3	4		1	2	3	4
Bronchopneumonia	1	1	0	0	0	0	0	0	0	0
Infective pulmonary exacerbation of cystic fibrosis	1	0	1	0	0	1	0	1	0	0
Viral infection	1	1	0	0	0	1	0	1	0	0
Nasopharyngitis	2	2	0	0	0	3	3	0	0	0
Cough	1	1	0	0	0	5	4	1	0	0
Dyspnoea	3	3	0	0	0	2	1	1	0	0
Haemoptysis	1	0	0	1	0	1	1	0	0	0
Pulmonary haemorrhage	1	1	0	0	0	0	0	0	0	0
Eosinophil count increased	1	0	1	0	0	0	0	0	0	0
Oropharyngeal pain	0	0	0	0	0	2	2	0	0	0
Total	12	9	2	1	0	15	11	4	0	0

well as the quantification of live *versus* dead organisms unable to be resolved by qPCR on total sputum DNA. This highlights the challenges in evaluating antimicrobial therapies and understanding the impact of quiescent or dormant colonies in chronically infected airways.

Unfortunately, due to low patient number it was not possible to show statistical significance in the ITT or PP populations at a 5% level between the active and placebo groups. Interestingly, the two subjects showing the greatest reduction in Bcc cfu counts after OligoG treatment were identified by molecular analysis as positive for *B. multivorans* and *B. cenocepacia,* with aztreonam MIC values of 0.19 mg/L and >256 mg/L, respectively. This reduction in Bcc after treatment with OligoG supports previously published *in vitro* data that showed OligoG treatment of *B. cenocepacia* reduced the MIC of aztreonam from 1024 mg/L to less than 4 mg/L [19]. It is postulated that this effect of OligoG is mediated through its biofilm disruption properties, which in turn reduces the tolerance of Bcc to aztreonam.

Analysis of rheology data showed an effect of OligoG on sputum viscosity. The phase angle at 0.1 Hz and 1.0 Hz showed improved values in the active group at the end of treatment, indicating that the sputum viscosity was reduced and more liquid after treatment with OligoG, which is consistent with the reported mechanism of action [12,15,18].

There were no statistically significant differences found between OligoG and placebo at the end of treatment for the spirometry parameters. The study did not demonstrate an improvement in lung function of CF patients, and it was assumed that the small sample size prevented an appropriate statistical analysis of spirometry parameters. The extremely low prevalence of CF patients with *Bcc* infection precluded a larger study. The significant reduction in Bcc cfu counts in some patients but not others is intriguing and did not appear to correlate with aztreonam susceptibility. Clearly a larger study would help to determine if this was due to other factors such as concomitant medication, microbiota, or the infecting Bcc species It is not clear from this exploratory study if this impacted the response to treatment and it is known that clinical outcome with *B. cepacia* complex infected individuals is highly variable [11].

The safety profile of OligoG was also compared with placebo in this trial. Only one SAE in total was observed in this trial. This SAE occurred within the OligoG treatment phase and was judged by the investigator as possibly related to the study drug. However, the SAE was a deterioration of a pre-existing concomitant disease in the patient (increased haemoptysis) and hospitalization was only for observation; no treatment was necessary for the patient to recover. Furthermore, the numbers and proportions of patients with adverse events were similar during OligoG and placebo treatment. No clinically relevant treatment differences were seen in the laboratory, vital signs or ECG parameters and there were no deaths attributable to the active drug. Overall, it was concluded that OligoG as a DPI appears safe, with a safety profile similar to placebo.

A limitation in the study was the placebo composition having a high dose of lactose, which itself may have had some influence on lung microbiology: To minimize the risk of unblinding, the lactose placebo was administered in the same dose (1050 mg per day) as the dry powder formulation of the active drug. Retrospective analysis suggests that this high dose of lactose may not have been the best choice of placebo to evaluate the microbiological effects expected from OligoG in this study. The reasoning behind this interpretation is outlined elsewhere [15].

The small sample size was also a limitation of the study and reflects the low prevalence of Bcc infection. The prevalence varies between CF treatment centre and countries, but for example in the UK, prevalence of Bcc infection was 3.5% for 9053 CF patients [9]. While improved infection control measures are helping to reduce this, further clinical studies will require enrolment from a wider range of CF centres. Poor clinical outcome for Bcc infected individuals remains a major problem for which new therapeutics are still urgently required. An additional limitation of the study was the relatively high treatment burden imposed by the treatment regimen of 10 capsule inhalations TID. This dose was based on conclusions from the previous phase 2A study (EudraCT Number: 2010-023090-19) [29], which showed the dose was in the lower range of what would be expected to demonstrate efficacy. Nevertheless, further dose ranging studies are required to establish a more appropriate balance between efficacy and treatment burden.

In conclusion, the results did not reveal any safety concerns for adult CF patients following administration of 1050 mg OligoG per day DPI over a 28-day period and confirms early studies that the active drug is safe to use in this study population. Although there were no significant improvements in FEV1 or other lung function parameters in this exploratory study, the low baseline FEV of 50% in the study cohort may not allow for clear improvements in lung function to be observed in these adult individuals with severe disease. Nevertheless, there were interesting trends for OligoG treatment in reducing the microbial burden of Bcc infection in CF patients and highlights the need for further investigation.

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Declaration of Competing Interest

RF and CS were investigators during the study and their institutions received standard clinical trial support from AlgiPharma. Investigators received support for travel to investigator meetings. No investigator received any personal funding to participate in the study. HF, and PDR hold stocks in AlgiPharma.

CRediT authorship contribution statement

Rianald Fischer: Conceptualization, Investigation. **Carsten Schwarz:** Conceptualization, Investigation. **Rebecca Weiser:** Investigation, Methodology, Formal analysis, Writing – review & editing. **Eshwar Mahenthiralingam:** Investigation, Methodology, Formal analysis, Writing – review & editing. **Knut Smerud:** Conceptualization, Formal analysis. **Nils Meland:** Formal analysis, Investigation. **Hugo Flaten:** Formal analysis. **Philip D Rye:** Conceptualization, Methodology, Formal analysis, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2022.01.003.

References

[1] Jin Y, Zhou J, Zhou J, Hu M, Zhang Q, Kong N, Ren H, Liang L, Yue J. Genome-based classification of Burkholderia cepacia complex provides new insight into its taxonomic status. Biol Direct 2020;15:6. doi:10.1186/s13062-020-0258-5.
[2] Horsley A, Jones AM. Antibiotic treatment for Burkholderia cepacia complex in people with cystic fibrosis experiencing a pulmonary exacerbation. Cochrane Database Syst Rev 2012;10:CD009529.
[3] Festini F, Buzzetti R, Bassi C, Braggion C, Salvatore D, Taccetti G, Mastella G. Isolation measures for prevention of infection with respiratory pathogens in cystic fibrosis: a systematic review. J Hosp Infect Sep 2006;64(1):1–6.

[4] Ryan, et al. Nebulised anti-pseudomonal antibiotics for cystic fibrosis (Review). Cochrane Database Syst Rev 2003(3):CD001021 2003Art. No.
[5] Rowbotham NJ, Palser SC, Smith SJ, Smyth AR. Infection prevention and control in cystic fibrosis: a systematic review of interventions. Expert Rev Respir Med May 2019;13(5):425–34 Epub 2019 Mar 26. doi:10.1080/17476348.2019.1595594.
[6] Drevinek P, Mahenthiralingam E. Burkholderia cenocepacia in cystic fibrosis: epidemiology and molecular mechanisms of virulence. Clin Microbiol Infect 2010;16:821–30.
[7] Frost F, Shaw M, Nazareth D. Antibiotic therapy for chronic infection with Burkholderia cepacia complex in people with cystic fibrosis. Cochrane Database Syst Rev 2019 Jun 13;6:CD013079. doi:10.1002/14651858.CD013079.pub2.
[8] Lynch JP 3rd. Burkholderia cepacia complex: impact on the cystic fibrosis lung lesion. Semin Respir Crit Care Med Oct 2009;30(5):596–610 Epub 2009 Sep 16.
[9] Kenna DTD, Lilley D, Coward A, Martin K, Perry C, Pike R, Hill R, Turton JF. Prevalence of Burkholderia species, including members of Burkholderia cepacia complex, among UK cystic and non-cystic fibrosis patients. J Med Microbiol 2017;66:490–501.
[10] Rhodes KA, Schweizer HP. Antibiotic resistance in Burkholderia species. Drug Resist Updat 2016;28:82–90. doi:10.1016/j.drup.2016.07.003.
[11] Frangolias DD, Mahenthiralingam E, Rae S, Raboud JM, Davidson AG, Wittmann R, Wilcox PG. Burkholderia cepacia in cystic fibrosis. Variable disease course. Am J Respir Crit Care Med 1999;160:1572–7.
[12] Ermund A, Recktenwald CV, Skjåk-Braek G, Meiss LN, Onsøyen E, Rye PD, Dessen A, Myrset AH, Hansson GC. OligoG CF-5/20 normalizes cystic fibrosis mucus by chelating calcium. Clin Exp Pharmacol Physiol Jun 2017;44(6):639–47.
[13] Vitko M, Valerio DM, Rye PD, Onsøyen E, Myrset AH, Dessen A, Drumm ML, Hodges CA. A novel guluronate oligomer improves intestinal transit and survival in cystic fibrosis mice. J Cyst Fibros Nov 2016;15(6):745–51.
[14] Pritchard MF, Oakley JL, Brilliant CD, et al. Mucin structural interactions with an alginate oligomer mucolytic in cystic fibrosis sputum. Vib Spectrosc 2019;103:102932. doi:10.1016/j.vibspec.2019.102932.
[15] van Koningsbruggen-Rietschel S, Davies JC, Pressler T, et al. Inhaled dry powder alginate oligosaccharide in cystic fibrosis: a randomised, double-blind, placebo-controlled, crossover phase 2b study. ERJ Open Res 2020;6(4):00132-2020. doi:10.1183/23120541.00132-2020.
[16] Nordgård CT, Nonstad U, Olderøy MØ, Espevik T, Draget KI. Alterations in mucus barrier function and matrix structure induced by guluronate oligomers. Biomacromolecules 2014 Jun 9;15(6):2294–300.
[17] Nordgård CT1, Draget KI. Oligosaccharides as modulators of rheology in complex mucous systems. Biomacromolecules 2011 Aug 8;12(8):3084–90 Epub 2011 Jul 18. doi:10.1021/bm200727c.
[18] Pritchard MF, Powell LC, Menzies GE, Lewis PD, Hawkins K, Wright C, Doull I, Walsh TR, Onsøyen E, Dessen A, Myrvold R, Rye PD, Myrset AH, Stevens HN, Hodges LA, MacGregor G, Neilly JB, Hill KE, Thomas DW. A new class of safe oligosaccharide polymer therapy to modify the mucus barrier of chronic respiratory disease. Mol Pharm 2016 Mar 7;13(3):863–72 Epub 2016 Feb 16. doi:10.1021/acs.molpharmaceut.5b00794.
[19] Kahn S, Tøndervik A, Sletta H, Klinkenberg G, Emanuel C, Onsøyen E, Myrvold R, Howe RA, Walsh TR, Hill KE, Thomas DW. Overcoming drug resistance with alginate oligosaccharides able to potentiate the action of selected antibiotics. Antimicrob Agents Chemother Oct 2012;56(10):5134–41.
[20] Powell LC, Pritchard MF, Ferguson EL, Powell KA, Patel SU, Rye PD, Sakellakou SM, Buurma NJ, Brilliant CD, Copping JM, Menzies GE, Lewis PD, Hill KE, Thomas DW. Targeted disruption of the extracellular polymeric network of Pseudomonas aeruginosa biofilms by alginate oligosaccharides. NPJ Biofilms Microbiomes 2018 Jun 29;4:13 eCollection 2018. doi:10.1038/s41522-018-0056-3.
[21] Hengzhuang W, Song Z, Ciofu O, Onsøyen E, Rye PD, Høiby N. OligoG CF-5/20 disruption of mucoid pseudomonas aeruginosa biofilm in a murine lung infection model. Antimicrob Agents Chemother 2016 Apr 22;60(5):2620–6.
[22] Tøndervik A, Sletta H, Klinkenberg G, Emanuel C, Powell LC, Pritchard MF, Khan S, Craine KM, Onsøyen E, Rye PD, Wright C, Thomas DW, Hill KE. Alginate oligosaccharides inhibit fungal cell growth and potentiate the activity of antifungals against Candida and Aspergillus spp. PLoS ONE 2014 Nov 19;9(11):e112518 2014.
[23] Pritchard MF, Jack AA, Powell LC, Sadh H, Rye PD, Hill KE, Thomas DW. Alginate oligosaccharides modify hyphal infiltration of Candida albicans in an *in vitro* model of invasive human candidosis. J Appl Microbiol Sep 2017;123(3):625–36.
[24] Pritchard MF, Powell LC, Jack AA, Powell K, Beck K, Florance H, Forton J, Rye PD, Dessen A, Hill KE, Thomas DW. A low-molecular-weight alginate oligosaccharide disrupts pseudomonal microcolony formation and enhances antibiotic effectiveness. Antimicrob Agents Chemother 2017 Aug 24;61(9).
[25] Jack AA, Khan S, Powell LC, Pritchard MF, Beck K, Sadh H, Sutton L, Cavaliere A, Florance H, Rye PD, Thomas DW, Hill KE. Alginate oligosaccharide-induced modification of the lasI-lasR and rhlI-rhlR quorum-sensing systems in pseudomonas aeruginosa. Antimicrob Agents Chemother 2018 Apr 26;62(5):e02317–18 pii. doi:10.1128/AAC.02318-17.
[26] Powell LC, Pritchard MF, Emanuel C, Onsøyen E, Rye PD, Wright CJ, Hill KE, Thomas DW. A nanoscale characterization of the interaction of a novel alginate oligomer with the cell surface and motility of Pseudomonas aeruginosa. Am J Respir Cell Mol Biol Mar 2014;50(3):483–92.
[27] Quittner AL, Sawicki GS, McMullen A, Rasouliyan L, Pasta DJ, Yegin A, et al. Psychometric evaluation of the Cystic Fibrosis Questionnaire-Revised in a national sample. Qual Life Res 2012;21:1267–78 Epub 2012 Jun 27. doi:10.1007/s11136-011-0036-z.
[28] Weiser R, Rye PD, Mahenthiralingam E. Implementation of microbiota analysis in clinical trials for cystic fibrosis lung infection: experience from the OligoG phase 2b clinical trials. J Microbiol Methods Feb 2021;181:106133 Epub 2021 Jan 7. PMID: 33421446. doi:10.1016/j.mimet.2021.106133.
[29] Walshaw M, McElvaney G, Williams R, et al. A first-in-patient clinical trial demonstrates that inhaled alginate oligosaccharide (OligoG) is well tolerated in cystic fibrosis (CF) patients. J Cyst Fibros. J Cyst Fibros 2014;13(Suppl. 2, S58).