CLINICAL STUDY REPORT

A randomised, double-blind, placebo-controlled cross-over study of inhaled alginate oligosaccharide (OligoG) for 28 days in subjects with Cystic Fibrosis using aztreonam due to chronic colonization with *Burkholderia spp.*

Protocol code:	SMR-2591
EudraCT no:	2014-002125-35
Investigational Product:	OligoG CF-5/20 – Guluronic acid rich oligosaccharide derived from alginate polysaccharide (short-form OligoG)
Indication:	Cystic fibrosis
Development Phase:	II
Study Initiation Date:	FPFV: 18 Feb 2015
Study Completion Date:	LPLV: 10 May 2016
Report Completion Date:	Final version 1.0: 05 JUL 2018
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GCP STATEMENT

This study was conducted in compliance with Good Clinical Practices, according to the ICH Harmonised Tripartite Guideline.

CONFIDENTIALITY STATEMENT

2. SYNOPSIS

Title of Study:

A randomised, double-blind, placebo-controlled cross-over study of inhaled alginate oligosaccharide (OligoG) for 28 days in subjects with Cystic Fibrosis using aztreonam due to chronic colonization with *Burkholderia spp.*

Co-ordinating Investigator:

Prof. Dr. Rainald Fischer, Pneumologische Praxis Pasing, München-Pasinig, Germany.

Study Centre(s):

- Site 27610: Prof. Dr. Rainald Fischer, Pneumologische Praxis Pasing, München-Pasing, Germany.
- Site 27611: Dr. Carsten Schwarz, Christiane Herzog Zentrum, Charité Berlin, Germany.

Publication (reference):

N/A

Studied Period (years):	**Clinical Phase:**
Date of first enrolment: 18 Feb 2015 Date of last patient last visit: 10 May 2016	Phase II

Objectives:

Primary:
To explore the efficacy of inhaled OligoG in reducing the microbial burden of *Burkholderia spp*. as measured in expectorated sputum samples.

Secondary:
To explore the effect of inhaled OligoG on various efficacy variables such as lung function, Quality-of-Life, rheology and other microbiological outcome measures.
To evaluate the safety, tolerability and subject compliance with treatment.

Methodology:

Study design:
This was a randomised, placebo-controlled, cross-over, multi centre study of inhaled alginate oligosaccharide (OligoG) for 28 days in subjects with Cystic Fibrosis using inhaled aztreonam due to chronic colonization with *Burkholderia spp*. The study was explorative. The primary objective of the study was to explore the efficacy of OligoG in reducing the microbial burden of *Burkholderia spp*. as measured in expectorated sputum samples. A total of 17 patients were screened at two centres in Germany. Of the screened patients 16 were given a test administration of study drug. A total of 15 patients were randomised and included in the study. Of the randomised patients, 12 successfully completed two 28 days treatment periods with OligoG-Placebo (or vice versa), separated by a 4 weeks washout. During the treatment periods, the patients inhaled 3 times 10 capsules per day with a total daily dose of 1050 mg dry powder for inhalation.

Number of Subjects (total and for each dosage):

A total of 17 patients were screened across both sites in Germany. A total of 15 patients were randomised equally to the treatment sequence OligoG/placebo or placebo/OligoG. There were 3 patients withdrawn and a total of 12 patients completed the study.

Diagnosis and Criteria for Inclusion:

Inclusion Criteria:

In order to participate in the study subjects must meet all of the following inclusion criteria:

1. Male or female with a confirmed diagnosis of cystic fibrosis defined by:
    a. Clinical features consistent with the diagnosis of CF [31]; and sweat chloride ≥60 mmol/L by pilocarpine iontophoresis;
    or
    b. Genotypic confirmation of CFTR mutation
2. Aged 18 years or older
3. Expected ability and willingness to provide sputum samples for microbiological evaluation throughout the study either spontaneously or induced by means of using inhaled hypertonic saline.
4. Chronic colonization with *Burkholderia spp.* defined as at least two positive microbiological cultures in expectorated sputum within the last 12 months prior to Visit 1.
5. Use of inhaled aztreonam three times daily 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) should take place in the "off" phase. Randomization Visit (Day 0) should take place the first day "on" to harmonize the aztreonam inhalation period with the IMP intake period.
6. At Screening (Visit 1), no clinical or laboratory findings suggestive of significant pulmonary illness, other than CF, which in the opinion of the investigator would preclude participation in the study. In case lab values exceed 3x the upper limit, the subject will be excluded, as per exclusion criterion 10, below.
7. Forced Expiratory Volume in one second (FEV1) must, at Screening (Visit 1), be greater than 25% of the predicted normal value following adjustment for age, gender, and height according to the Global Lung Initiative [28].
8. Female subjects of child bearing potential and male subjects participating in the study who are sexually active must use acceptable contraception. Female subjects documented as being of non-child-bearing potential are exempt from the contraceptive requirements. For the purpose of this study acceptable contraception is defined as:
    oral, injected or implanted hormonal methods of contraception; or
    placement of an intrauterine device (IUD) or intrauterine system (IUS);
    or
    barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository
9. Provision of written informed consent.

Exclusion Criteria

In order to participate in the study subjects must not meet any of the following exclusion criteria:

1. Changes in underlying therapy (e.g., chest physiotherapy, bronchodilators, NSAIDs, pancreatic enzyme preparations, nutritional supplements and DNase within the 14 days prior to Day 0 (Visit 2). Subjects must be willing to remain on the same underlying stable therapy regimens for the duration of the study until the final follow-up visit at Day 112 (Visit 8).
2. Changes in physiotherapy technique or schedule within 14 days prior to Day 0 (Visit 2).
3. Concomitant use of inhaled mannitol or hypertonic saline within 7 days prior to Day 0 (Visit 2).
4. Concomitant use of inhaled antibiotics other than aztreonam.
5. Pulmonary exacerbation within 28 days of Screening (Visit 1).
6. Lactose intolerance/milk allergy. A skin test for milk allergy will be performed for lactose intolerance unknowns at screening. Subjects who have previously received inhaled formulations containing lactose without any allergic or tolerance issues are allowed to proceed without a skin test. For subjects demonstrating a positive skin prick test for milk allergy but have no problems eating milk and lactose products, the decision to exclude will be at the investigator's discretion.

7. On-going acute illness. Subjects must not have needed an outpatient visit, hospitalization or required any change in therapy for other pulmonary disease between Screening (Visit 1) and Day 0 (Visit 2).
8. History of, or planned organ transplantation.
9. Active allergic bronchopulmonary aspergillosis (ABPA) in the last 12 months prior to Screening (Visit 1), defined as having received pharmacological treatment for ABPA.
10. Clinically significant abnormal findings or any value ≥ 3 x the upper limit of normal on haematology or biochemistry
11. Subjects unable to perform pulmonary function tests according to the ATS/ERS criteria.
12. Pregnant or breast-feeding women. A negative urine pregnancy test must be demonstrated in females of child-bearing potential at Screening (Visit 1).
13. Subjects who have participated in any clinical trial within the 28 days (or shorter than 5 half-lives of the investigational drug) prior to Screening (Visit 1).
14. Subjects with documented or suspected clinically significant, alcohol or drug abuse as per Investigator's discretion.
15. Current malignant disease (with the exception of basal cell carcinoma; BCC).
16. Any serious or active illness incl. psychiatric diseases, which in the opinion of the Investigator, would interfere with subject treatment, assessment, or compliance with the protocol.
17. Subjects not willing/able to follow the study instructions.
18. Resistance to aztreonam.
19. Intolerance to aztreonam or any of its excipients.

Test Product, Dose, Mode of Administration, Batch No.:
OligoG was supplied as a dry powder for inhalation (DPI), approximately 48 mg (35 mg OligoG) in hydroxypropylmethyl cellulose (HPMC) capsules delivered via the Miat Monodose Inhaler, ten capsules three times daily (1050 mg daily dose).

The OligoG product was from the following batch code: PMBN14053.

Duration of Treatment:
OligoG and placebo was each inhaled for 28 days.

Reference Therapy, Dose, Mode of Administration, Batch No.:
Placebo (Lactose) was supplied as a DPI, approximately 48 mg in HPMC capsules delivered via the Miat Monodose Inhaler, ten capsules three times daily.

The placebo product was from the following batch code: PMBN14051.

Criteria for Evaluation:

Efficacy:
Exploratory efficacy included changes in *Burkholderia spp.* density in sputum, changes in FEV1, sputum rheology, other lung function tests and Quality of Life from Baseline to the end of the treatment (and any other visit) and changes in microbiological sputum culture (qualitative and quantitative).

Safety:
The safety and tolerability data collected in this study included Adverse Events, rate of premature withdrawal from the study, clinical laboratory parameters (biochemistry and haematology), vital signs, ECG, spirometry, physical examinations, Pulmonary Exacerbation rates, use of rescue bronchodilators and concentration of OligoG in sputum and plasma.

Statistical Methods:
The methods for statistical analysis were described in the Clinical Trial Analysis Plan (CTAP), Version 1.0, 14.10.2016.

SUMMARY – CONCLUSIONS

Efficacy Results:

Microbiology Total bacterial load. 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 subjects showed some bacterial growth in sputum as determined by culture at screening. Six of these showed a mean increase (0.76 log10) in the total bacterial cfu counts after treatment with OligoG. The remaining 8 subjects showed a mean decrease (0.82 log10) in total bacterial cfu counts with OligoG treatment.
Microbiology Bcc. Only 8 subjects showed culturable Bcc. Two of these showed a mean increase (1.0 log10) in Bcc cfu counts after treatment with OligoG. The remaining 6 subjects showed a larger mean decrease (3.01 log10) in Bcc cfu counts after treatment with OligoG (ranging from 0.54 to 7.01). In particular two of these subjects, who also exhibited the highest reduction in Bcc cfu counts (7.01 and 6.00), showed no remaining Bcc after OligoG treatment. Although it is unclear if this constituted an eradication of Bcc in these patients, the result is nevertheless intriguing.
A further 6 subjects failed to show culturable Bcc, although these patients were identified as Bcc positive by molecular analysis. This significantly impacted the power of this already small study.
These tentative changes observed in the culture microbiology data were also reflected in a reduction in mean relative abundance of Bcc after OligoG treatment, as determined by molecular (non-culture) microbiology.
Despite these promising trends for OligoG effects compared to baseline, it was noted that the lactose placebo also showed a marked effect in Bcc reduction compared to baseline. Subsequent investigation has identified independent evidence that lactose inhibits *Burholderia spp* and *Pseudomonas aeruginosa* adhesion to lung epithelial cells and potentiates the activity of antibiotics [4, 5, 37, 38]. Additional studies have also identified a role for metabolites enhancing antibiotic susceptibility to antibiotics such as tobramycin [1, 19]. In order to minimize the risk of unblinding in the current Bcc cross-over trial, the lactose placebo was administered in the same dose (1410mg per day) as the dry powder formulation of the active drug. Retrospective analysis suggests that this high dose of lactose was an inappropriate choice of placebo in this particular study.
Lung function. Statistically significant improvement in the forced expiratory volume in one second (FEV1) was not reached in this challenging patient population. Analysis of other lung function parameters and QoL measurements in this exploratory study did not show significant effects with the active drug. However, 6 out of the 11 QoL summary scores showed a relative improvement in performance after OligoG treatment compared to placebo in mean changes from baseline.
Rheology. Rheology measurements of viscocity, elasticity and phase angle in expectorated sputum, showed an improvement after OligoG treatment. The analysis of the phase angle at 0.1 Hz and 1.0 Hz showed an improvement in the active group at the end of treatment, 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 Results:
There were no safety issues identified in this study and confirms early studies that the active drug is indeed safe to use in this study population.

Conclusions:
The study has confirmed a favourable safety profile for OligoG. There have been no deaths attributable to the active drug, and no significant treatment differences in laboratory, vital signs or ECG parameters. Although there were no significant improvements in FEV1 or other lung function parameters in this exploratory study, there were indications of efficacy in microbiology and reduction in microbial burden of Bcc.
The influence of lactose on lung microbiology, in particular interactions with Bcc, indicate that lactose in high concentrations as a placebo should be avoided.
Nevertheless, the findings from this trial demonstrate interesting trends for OligoG treatment of Bcc infection in CF patients and highlights the need for further investigation.