Supporting Information

A new class of safe oligosaccharide polymer therapy to modify the mucus barrier of chronic respiratory disease

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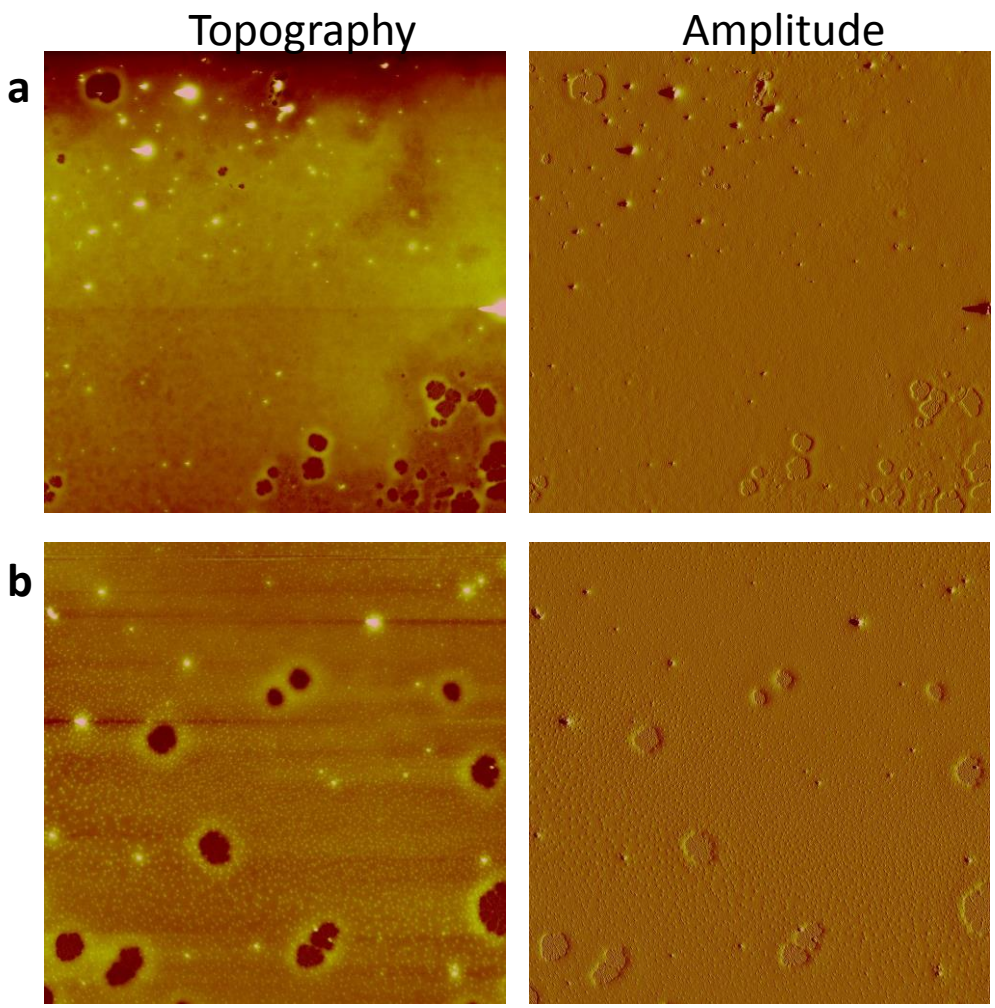
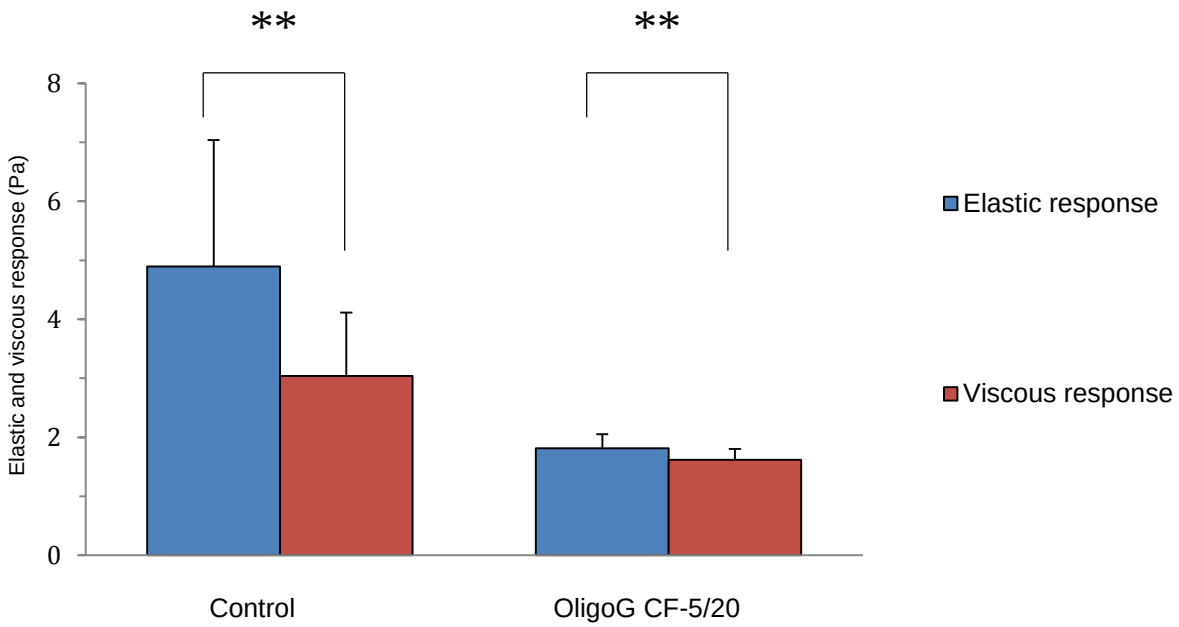
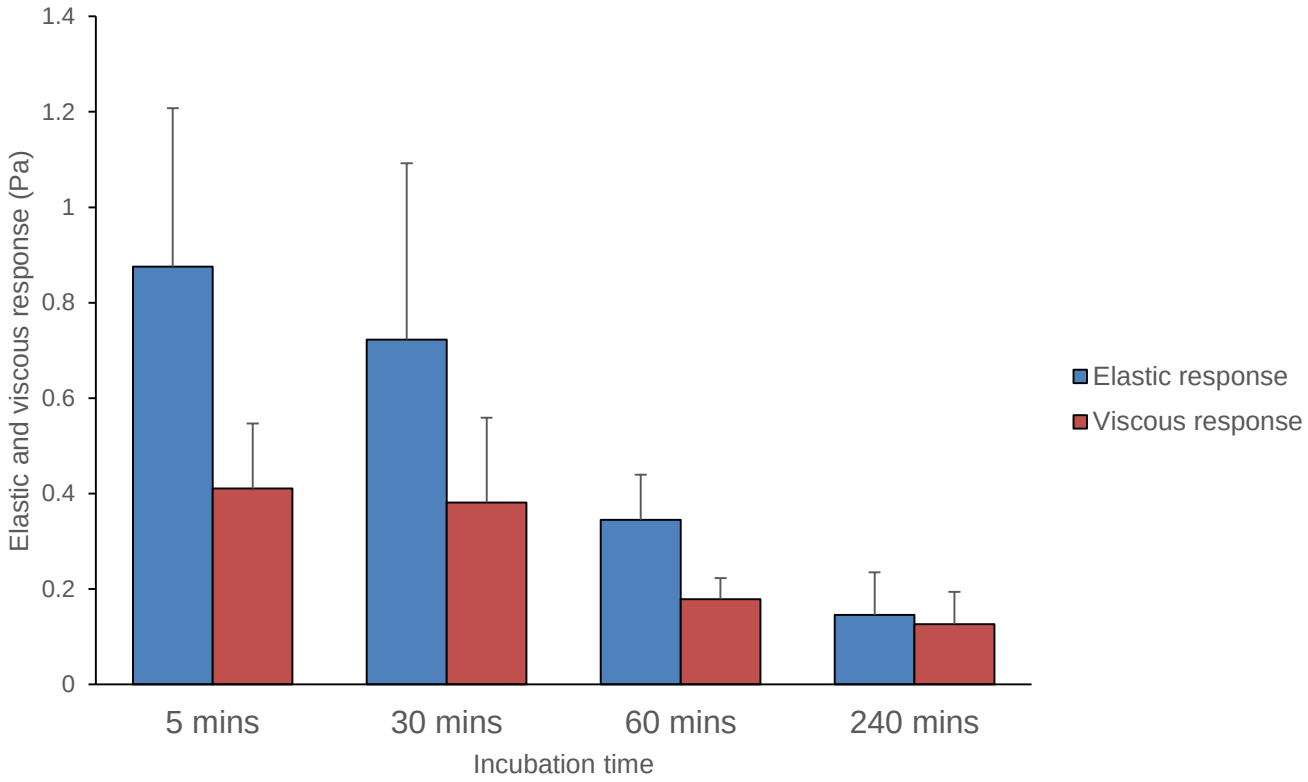


Supporting Information Figure 1. Atomic force microscopy imaging (20 µm) of (a) pig gastric mucin (0.1% PGM) (b) PGM with OligoG CF-5/20 (0.001%), z-scale 9 nm.



Supporting Information Figure 2. Shear rheology showing changes in elastic response (*G'*) and viscous response (*G''*) of 2% OligoG CF-5/20 treatment of sputum samples compared to water treated controls measured at 10 Hz (n=21)**.**



Supporting Information Figure 3. Shear rheology showing changes in elastic response (*G'*) and viscous response (*G''*) of 2% OligoG CF-5/20 treatment of sputum samples following 5, 30, 60 and 240 mins of incubation at 37°C (0.16 Hz; n=3)**.**

Supporting Information Table 1. Patient data, including antibiotic and rhDNase I (if applicable) regimen at time of sampling.

Patient No.	Age (Yrs)	Sex	rhDNase I	Antibiotic regimen at time of sampling			Recent sputum microbiology	FEV_1 (%)
				IV	Oral	Inhaled		
1[a]	17	F	Y	Tobramycin Meropenem	Flucloxacillin Azithromycin	Colistin (alternate month with) Tobramycin	*P. aeruginosa*	1·10 L (37%)
2	31	M	N	Colistin Meropenem Chloramphenicol		Colistin (alternate month with) Tobramycin	*P. aeruginosa*	1·85 L (51%)
3	28	M	Y	Tobramycin Ceftazidime			*P. aeruginosa*	1·93 L (54%)
4	39	M	Y		Azithromycin Flucloxacillin	Colistin (alternate month with) Tobramycin	*P. aeruginosa* *S. aureus*	1·85 L (50%)
5	30	F	N	Tobramycin Aztreonam	Azithromycin	Colistin (alternate month with) Tobramycin	*Pseudomonas* sp. *P. aeruginosa*	1·93 L (65%)
6	20	M	Y	Tobramycin Meropenem	Azithromycin	Colistin	*Pseudomonas* sp.	0·80 L (20%)
7	18	M	Y	Tobramycin Ceftazidime	Flucloxacillin Azithromycin	Colistin (alternate month with) Tobramycin	*P. aeruginosa*	1·30 L (43%)

[a]Sputum from Patient 1 was used for the longitudinal study.
P. aeruginosa, Pseudomonas aeruginosa; S. aureus, Staphylococcus aureus

Supporting Information Table 2. Atom names, types and partial charges used for the guluronate residues (OligoG).

Atom Name	Atom Type	Partial Charge
C1	CT	0.3135
C2	CT	0.164
O2	OH	-0.3819
C3	CT	0.1172
O3	OH	-0.3872
C4	CT	0.1236
O4	OS	-0.4405
C5	CT	0.1584
O5	OS	-0.4105
C6	CT	0.2175
O6A	OS	-0.6366
O6B	OS	-0.6366
H1	HC	0.0746
H2	HC	0.237
H3	HO	0.066
H4	HC	0.1807
H5	HO	0.0696
H6	HC	0.0775
H7	HC	0.0937

Supporting Information Table 3. Atom names, types and partial charges used for the Threonine amino acid with core one sugars attached at the glycosylation site

Atom Name	Atom Type	Partial Charge
CG2	CT	0.065
HG22	HC	-0.0025
HG23	HC	-0.0025
HG21	HC	-0.0025
OG1	O	-0.091
C	C	0.181
CA	CT	0.08
HA	H1	-0.003
N	N	0.323
CB	C	0.1235
HB	H4	-0.0025
C1	C	0.1235
H1	H4	-0.0035
C2	C	0.0795
H21	H4	-0.0025
N2	N	0.3225
H2	H	0.0005
C7	C	0.08
O7	O2	-0.095
C8	CT	0.065
H82	HC	-0.0025
H83	HC	-0.0025

Cont.		
H81	HC	-0.0025
O	O	-0.352
C5	C	0.1235
H5	H4	-0.0025
C6	CT	0.066
H61	HC	-0.0025
H62	HC	-0.0025
O6	OH	-0.0955
H63	HO	0.0235
C4	CT	0.08
H41	H1	-0.0025
O4	OH	-0.095
H42	HO	0.0235
C3	C	0.1235
H3	H4	-0.0025
O3	O	-0.091
C5A	C	0.08
C6A	CT	0.066
H6A	H1	-0.003
H84	H1	-0.0025
O6A	OH	-0.095
H89	HO	0.0235

Cont.		
OA	O	-0.091
C1A	C	0.1235
H1A	H4	-0.0025
O1A	OH	-0.095
H85	HO	0.0235
C2A	CT	0.08
H2A	HC	-0.0025
O2A	OH	-0.095
H86	HO	0.0235
C3A	CT	0.076
H3A	HC	-0.0025
O3A	OH	-0.095
H87	HO	0.0235
C4A	CT	0.11
H4A	H1	-0.0025
O4A	OH	-0.095
H88	HO	0.0205
O5	O	-0.091
H	H	-0.0025

Supporting Information Table 4. Atom names, types and partial charges used for the Serine amino acid with core one sugar attached at the glycosylation site

Atom Name	Atom Type	Partial Charge
OG1	O	-0.091
C	C	0.181
CA	CT	0.08
HA	H1	-0.003
N	N	0.323
CB	C	0.1235
HB1	H4	-0.0006
HB2	H4	-0.0006
C1	C	0.1235
H1	H4	-0.0035
C2	C	0.0795
H21	H4	-0.0025
N2	N	0.3225
H22	H	0.0005
C7	C	0.08
O7	O2	-0.095
C8	CT	0.065
H82	HC	-0.0025
H83	HC	-0.0025
H81	HC	-0.0025
O	O	-0.352
C5	C	0.1235
O5	O	-0.0025
H5	H4	0.066
C6	CT	-0.0025

Cont.		
H61	HC	-0.0025
H62	HC	-0.0955
O6	OH	0.0235
H63	HO	0.08
C4	CT	-0.0025
H41	H1	-0.095
O4	OH	0.0235
H42	HO	0.1235
C3	C	-0.0025
H3	H4	-0.091
O3	O	0.08
C5A	C	0.066
C6A	CT	-0.003
H6A	H1	-0.0025
H84	H1	-0.095
O6A	OH	0.0235
H89	HO	-0.091
OA	O	0.1235
C1A	C	-0.0025
H1A	H4	-0.095
O1A	OH	0.0235
H85	HO	0.08
C2A	CT	-0.0025
H2A	HC	-0.095
O2A	OH	0.0235

Cont.		
H86	HO	0.076
C3A	CT	-0.0025
H3A	HC	-0.095
O3A	OH	0.0232
H87	HO	0.17
C4A	CT	-0.0055
H4A	H1	-0.095
O4A	OH	0.02
H88	HO	-0.091
H	H	-0.0025

Supporting Information Table 5. Atom names, types and partial charges used for the Threonine amino acid with core two sugars attached at the glycosylation site

Atom Name	Atom Type	Partial Charge
CG2	CT	0.123
C	C	0.356
C1	C	0.24
C1A	C	0.24
C1B	C	0.24
C2	C	0.153
C2B	C	0.153
C2N	C	0.356
C3	C	0.24
C3A	CT	0.153
C3B	C	0.153
C4	CT	0.153
C4A	CT	0.153
C4B	CT	0.153
C5	C	0.24
C5A	C	0.153
C5B	C	0.24
C6	C	0.279
C6A	CT	0.125
C6B	CT	0.125
C7	C	0.356
C8	CT	0.123
CA	CT	0.153
CB	C	0.24
CME	CT	0.123

Cont.		
H	H	-0.012
H1	H4	-0.011
H12	HO	-0.012
H1A	H4	-0.012
H1M	HC	0.04
H2	H	-0.005
H21	H4	-0.012
H2B	H4	-0.012
H2M	HC	0.04
H2N	H	0.04
H3	H4	-0.012
H3B	H4	-0.012
H3M	HC	-0.012
H3O	HO	-0.012
H41	H1	-0.012
H42	HO	0.04
H4A	H1	-0.012
H4B	H1	-0.012
H4O	HO	0.04
H5	H4	0.04
H5B	H4	-0.012
H61	H4	-0.012
H62	H4	-0.012
H6A	H1	-0.012
H6B	HC	-0.012

Cont.		
H6F	HC	0.04
H81	HC	-0.012
H82	HC	-0.011
H83	HC	-0.012
H84	H1	-0.012
H85	HO	-0.012
H86	HO	-0.012
H87	HO	-0.012
H88	HO	0.04
H89	HO	0.04
HA	H1	-0.011
N	N	0.641
N2	N	0.126
N2B	N	0.127
O2N	O2	-0.709
O3	O	-0.188
O3B	OH	-0.197
O4	OH	-0.197
O4A	OH	-0.197
O4B	OH	-0.197
O5B	O	-0.187
O6	O	-0.188
O6A	OH	-0.197
O6B	OH	-0.196
O7	O2	-0.709

Cont.		
OA	O	-0.188
OA1	O	-0.188
OG1	O	-0.188
C2A	CT	0.147
H2A	HC	-0.012
H3A	HC	-0.012
H48	H4	-0.012
HB	H4	-0.012
HG21	HC	-0.012
HG22	HC	-0.012
HG23	HC	-0.012
O	O	-0.709
O1A	OH	-0.197
O2A	OH	-0.197
O3A	OH	-0.197

Supporting Information Table 6. Atom names, types and partial charges used for the Serine amino acid with core two sugars attached at the glycosylation site

Atom Name	Atom Type	Partial Charge
C	C	0.356
C1	C	0.24
C1A	C	0.24
C1B	C	0.24
C2	C	0.154
C2B	C	0.153
C2N	C	0.356
C3	C	0.24
C3A	CT	0.153
C3B	C	0.153
C4	CT	0.153
C4A	CT	0.153
C4B	CT	0.153
C5	C	0.24
C5A	C	0.153
C5B	C	0.24
C6	C	0.279
C6A	CT	0.125
C6B	CT	0.125
C7	C	0.356
C8	CT	0.123
CA	CT	0.153
CB	C	0.24
CME	CT	0.123
C	C	0.356

Cont.		
H	H	-0.012
H1	H4	-0.011
H12	HO	-0.012
H1A	H4	-0.012
H1M	HC	0.04
H2	H	-0.005
H21	H4	-0.012
H2B	H4	-0.012
H2M	HC	0.04
H2N	H	0.04
H3	H4	-0.012
H3B	H4	-0.012
H3M	HC	-0.012
H3O	HO	-0.012
H41	H1	-0.012
H42	HO	0.04
H4A	H1	-0.012
H4B	H1	-0.012
H4O	HO	0.04
H5	H4	0.04
H5B	H4	-0.012
H61	H4	-0.012
H62	H4	-0.012
H6A	H1	-0.012
H	H	-0.012

Cont.		
H6B	HC	-0.012
H6F	HC	0.04
H81	HC	-0.012
H82	HC	-0.011
H83	HC	-0.012
H84	H1	-0.012
H85	HO	-0.012
H86	HO	-0.012
H87	HO	-0.012
H88	HO	0.03
H89	HO	0.04
HA	H1	-0.011
N	N	0.641
N2	N	0.122
N2B	N	0.127
O2N	O2	-0.709
O3	O	-0.188
O3B	OH	-0.197
O4	OH	-0.197
O4A	OH	-0.197
O4B	OH	-0.197
O5B	O	-0.187
O6	O	-0.188
O6A	OH	-0.197
H6B	HC	-0.012

Cont.		
O6B	OH	-0.196
O7	O2	-0.709
OA	O	-0.188
OA1	O	-0.188
OG1	O	-0.188
C2A	CT	0.247
H2A	HC	-0.012
H3A	HC	-0.012
H48	H4	-0.012
HB1	H4	-0.006
HB2	H4	-0.006
O	O	-0.709
O1A	OH	-0.197
O2A	OH	-0.197

Supporting Information Table 7. Additional angle measurements required for the running of GROMACS simulations of both Mucin and OligoG simulations

ijk	func	th0	cth
C O C	1	120.000	669.440
C H4 C	1	120.000	418.400
H4 C H4	1	120.000	292.880
OS CT OH	1	101.000	502.080
N* C O2	1	120.900	669.440
N C O2	1	120.900	669.440
HC CT OH	1	109.500	418.400
HC CT OS	1	109.500	418.400
C C CT	1	111.100	527.184
C CT C	1	111.100	527.184
C C C	1	111.100	527.184
C C N	1	111.200	669.440
H4 C N	1	120.000	418.400
C N C	1	121.900	418.400
N2 CT C	1	123.200	418.400
N2 CT CA	1	123.200	418.400
NA CA CA	1	123.200	418.400

Supporting Information Table 8. Additional dihedral angle measurements required for the running of GROMACS simulations of both Mucin and OligoG simulations

ijkl	func	phase	kd	pn
NC CB N* CB	4	180.00	4.60240	2
H5 N* CK NB	4	180.00	4.60240	2
NB CB CB C	4	180.00	4.60240	2
CA CA CA CA	4	180.00	4.60240	2
C CA NA CA	4	180.00	4.60240	2
NA NA CA NC	4	180.00	4.60240	2
NA CA CA CA	4	180.00	4.60240	2
N* CK OH HO	4	180.00	4.18400	2

Supporting Information Table 9. Summary of sampling for inhalation studies with $3H^-$ OligoG CF-5/20 in experimental Sprague-Dawley rats.

Dose group/ Treatment[#]	Samples	Additional samples	Additional samples from selected animals
1. IV (n=4) 2. OD (n=4)	Blood, plasma		
3. IV (n=4) 4. OD (n=4)	Urine/faeces	Blood, plasma, selected tissues/organs, carcass Blood, plasma, GI tract, carcass	Expired air (n=2)
5. IV (n=18)	Tissues and organs		

All animals received a dose of 5 mg kg^{-1} tritium-labelled OligoG; IV, intravenous; OD, oral dose.

Supporting Information Table 10. Study design for inhalation studies with OligoG CF-5/20 as a nebulized solution in experimental Sprague-Dawley rats.

Phase of study	Dose group/ Treatment[a]	Daily exposure duration (min)	OligoG CF-5/20 formulation ($mg^{-1}kg^{-1}day^{-1}$)	Non-aqueous component ($mg^{-1}kg^{-1}day^{-1}$)
A. Single dose	1. 6% OligoG	60	500.0	75.6
	2. 6% OligoG	120	997.5	150.8
	3. 6% OligoG	240	1,999.8	301.9
B. 7 days dosing	4. 6% OligoG	60	511.4	71.0
	5. 6% OligoG	240	2034.4	282.4
C. 14 days dosing	6. 6% OligoG	60	498.5	71.6
	7. 6% OligoG	240	1,984.3	284.8
	8. Vehicle only	240	1,916.7	0
D. 28 days dosing	9. 6% OligoG	120	494.4	71.6
	10. 6% OligoG	240	1,988.2	288.0

[a](n=10; 5 female, 5 male rats)

Supporting Information Table 11. Study design for multiple dosing of OligoG CF-5/20 as a dry powder for inhalation (DPI) in experimental Sprague-Dawley rats.

Group	Treatment	OligoG CF-5/20 dose (mg)		Exposure level ($mg^{-1}kg^{-1}day^{-1}$)		Exposure	Number of animals		
		Target	Achieved	Target	Achieved	Duration (mins)	Main	Recovery	Satellite[a]
1	Control	0	0	0	0	150	10	5	3
2	OligoG DPI	150	157	1.6	1.77	120	10	0	6
3	OligoG DPI	300	280	3.2	3.15	120	10	0	6
4	OligoG DPI	467	467	4.0	4.20	150	10	5	6

[a]Satellite animals used for toxicokinetic sampling only

Supporting Information Table 12. Summary of the macroscopic and microscopic findings for multiple dosing of OligoG CF-5/20 as a dry powder for inhalation (DPI) in experimental Sprague-Dawley rats.

Group No. (Male/Female)	**1M**	**2M**	**3M**	**4M**	**1F**	**2F**	**3F**	**4F**
Achieved dose ($mg^{-1}kg^{-1}day^{-1}$)	**0**	**157**	**280**	**467**	**0**	**157**	**280**	**467**
Macropathology	Incidence							
Lungs								
Pale areas (Main)	0/10	0/10	1/10	1/10	0/10	0/10	0/10	2/10
Pale areas (Recovery)	0/5	-	-	0/5	0/4	-	-	1/5
Tracheobronchial lymph								
Enlarged (Main)	0/10	2/10	7/10	8/10	0/11	2/10	4/10	10/10
Enlarged (Recovery)	0/5	-	-	2/5	0/4	-	-	1/5
Histopathology	Incidence							
Lungs								
Diffuse alveolar macrophages/ macrophage debris (Main)	0/10	10/10	10/10	10/10	0/11	10/10	10/10	10/10
Diffuse alveolar macrophages/ macrophage debris (Recovery)	0/5	-	-	5/5	0/4	-	-	5/5
Tracheobronchial lymph								
Cellularity (Main)	0/10	2/10	6/10	8/10	0/11	3/10	4/10	10/10
Cellularity (Recovery)	1/4	-	-	1/5	1/4	-	-	1/5

Supporting Information Table 13. Summary of dosing for inhalation studies with OligoG CF-5/20 in healthy human volunteers (n=28).

Phase of study	Dose group/ Treatment	No. of volunteers
A. Single dose	1. 90 mg 6% OligoG CF5/20	2
	2. 0.9% NaCl placebo	2
B. 3 days OD[a]	1. 90 mg/day 6% OligoG CF5/20	6
	2. 0.9% NaCl (placebo)	2
C. 3 days OD	1. 270 mg/day 6% OligoG CF5/20	6
	2. 0.9% NaCl (placebo)	2
D. 3 days BID[b]	1. 540 mg/day 6% OligoG CF5/20	6
	2. 0.9% NaCl (placebo)	2

[a]OD, oral dose; [b]BID, twice daily.

Supporting Information Table 14. Scintigraphic study comparing pulmonary and extra pulmonary depositions using the dry powder formulation versus the nebulized solution on the distribution and deposition of OligoG CF-5/20 in the lungs of CF patients.

Parameter	Dry powder inhalation	Nebulised solution	*P*-value*
% dose in whole lung	**38.6 ± 12.8**	**17.1 ± 3.5**	**0.001**
% dose in central lung	11.3 ± 3.3	5.5 ± 1.2	0.002
% dose in peripheral lung	27.3 ± 9.9	11.6 ± 2.6	0.001
central to peripheral ratio index	0.4 ± 0.1	0.5 ± 0.1	0.117
% dose in mouth washing	2.3 ± 3.1	1.4 ± 1.0	0.341
% dose in oropharyngeal region	0.9 ± 0.8	10.9 ± 5.4	0.001
% dose in gastric region	8.1 ± 9.4	7.6 ± 2.8	0.853
% combined (mouth, oropharyngeal and gastric)	**11.3 ± 9.6**	**19.9 ± 7.1**	**0.033**

***P<0.05 was regarded as significant

Supporting Information- Methods

Sample incubation times. Incubation times for all experiments were ≤ 4 hours in accordance with the original *ex vivo* OligoG CF-5/20 rheological studies (Supporting Information Figure 3) which showed the greatest change in elastic or viscous response between 60 mins and 240 mins.

Molecular dynamic studies of mucin-OligoG interactions. The sequence studied, was composed of the repeating unit of the MUC5AC protein downloaded from NCBI (http://www.ncbi.nlm.nih.gov) and inputted into Hamby and Hirst's (2008)[1] glycosylation prediction tool. This 2-dimensional sequence was translated into a 3-dimensional structure using Acerlys Discovery Studio (ADS). Core 1 and 2 sugars were generated using Ambertools glycol tool protocols,[2] saved as *pdb* files and incorporated into the appropriate amino acid using the small ligand and mutate tools which are built into ADS. These sugars were incorporated into amino acids predicted to be glycosylated using the Hamby and Hirst's glycosylation prediction tool,[1] and at levels of mucin glycosylation observed in the CF lung.[3] The Alpha-L-Guluronate structure was downloaded in the form of the crystal structure, PDB ID 1J1N[4] and converted into a repeating unit using ADS. This repeating unit was elongated to make the OligoG structure file. MUC5AC and OligoG structures were then amalgamated into a single *pdb* file for simulation.

Partial charges for the sugars and OligoG molecules were calculated using Ambertools and the Amber99 force field as were atom types (Supporting Information Tables 2-6). Bond- and angle-parameters were assigned based upon chemically-similar, existing parameters with Ambertools (Supporting Information Tables 7-8). MD simulations were performed using GROMACS 4.5 software[5] and the Amber99 force-field. Structures were boxed and solvated using the GROMACS modules, editconf and genbox. The DNA molecule was placed in the center of a cubic box and solvated using single-point charge water molecules, SPC216. The box surrounding the molecule was approximately 36.47 nm^3 and filled with ~18,324 water molecules. To neutralize the system, an appropriate number of Na^+ ions were added to the box in place of the same number of water molecules. The Particle mesh Ewald (PME) method

was used to treat long-range electrostatic interactions and a 1.4 nm cut-off was applied to Lennard-Jones interactions. MD simulations were performed in a three-step process: (a) Energy minimization stage (EM); the EM process used was steepest descent, with a tolerance of 1000 $KJ^{-1}nm^{-1}$; (b) A pre-MD run (PR) stage of 25,000 steps at 0.002 ps per step. This simulation was run at 300 K; (c) The MD was run at 300 K for a total of 10 ns.

Fourier transform infrared spectroscopy of mucin-OligoG CF-5/20 interactions. Infrared (IR) spectra were obtained using a Bruker Alpha Fourier Transform IR (FTIR) instrument equipped with a platinum-attenuated total reflection (ATR) single reflection diamond-sampling module (Bruker Optics). The instrument was placed in a Captair Pyramid (Erlab) housed in a Concept 1000 workstation (Ruskinn Technology Ltd, Bridgend, Wales) and the equipment purged continuously overnight with a gentle flow of nitrogen. Sputum samples (3 µl) were then spotted directly onto the ATR sampling module and evaporated at room temperature under nitrogen flow. IR spectra were collected as an average of 24 scans per sample (at a wavenumber range 4000–450 cm^{-1}) at a resolution of 4 cm^{-1}, controlled by Optics User Software (OPUS) version 6.5 (Bruker Optics). The background spectrum was subtracted from the sample spectrum. Each sample spectrum was checked for a smooth baseline between 1750 and 2000 cm^{-1} to ensure no interference from water vapor. IR spectra were pre-processed using OPUS by subtracting a baseline between 1750 and 1485 cm^{-1}. Mean IR spectra were generated and a second derivative spectra was performed using OPUS and the R Statistical Programming Environment (www.R-project.org).

Pre-clinical studies with OligoG CF-5/20 in experimental animals. Initial studies with tritium-labelled OligoG CF-5/20 (Supporting Information Table 9) showed no toxicity, and rapid excretion in faeces and urine (after oral or IV administration respectively). Following this, inhalation studies with aerosolized OligoG CF-5/20 were performed in Sprague-Dawley rats using a modular, flow-past system. Aerosols of 6% (w/v) OligoG CF-5/20 in sterile water were generated using HEART® airjet nebulizers (Westmed, Tuscon, Arizona, USA). In the first series of experiments (Supporting Information Table 10), rats were assigned to 6% OligoG CF-5/20 dose for variable exposure times and screened for changes in body-weight, food

consumption, and respiratory function. In the second series of experiments (Supporting Information Table 11), daily inhalational dosing was performed with a 14 day off-test recovery period to assess the reversibility of any observed effects. Rats were screened for clinical, biochemical and pathological changes (Supporting Information Table 12).

Since the final drug formulation was to be a DPI (Dry Powder for Inhalation) a bridging study was required to ensure comparability with the previously used nebulized formulation. The DPI formulation of OligoG CF-5/20 was developed with the purpose of improving patient compliance and enabling increased doses to be administered. The product is manufactured in compliance with Good Manufacturing Practice as a spray dried powder with a particle size distribution for inhalation D_{10} ≤2.5 µm, D_{50} ≤5 µm, D_{90} ≤10 µm.

Therefore, Sprague-Dawley rats were dosed with placebo or OligoG DPI at three different doses by inhalation once daily for 4 weeks. Recovery from any potential effects was evaluated during a 4-week recovery period. The inhalation exposure system comprised a snout-only flow past inhalation exposure chamber, restraining tubes and a Rotating Brush Generator (RBG) mechanism to generate the test atmospheres. Separate exposure systems were used for each dose group. The mass aerosol concentration of the OligoG CF-5/20 formulation or Vehicle in the animal's breathing zone via the reference port was measured gravimetrically for all groups during each exposure period. From the concentration samplings the achieved dose levels for each group were calculated based on the following criteria:

$$\text{Dose (mg/kg/day)} = \frac{C \times RMV \times T}{\text{Body weight (kg)}}$$

where C is the aerosol concentration, RMV the Respiratory Minute Volume (L/min) and T the duration of exposure (min).

Animals were dosed once daily using a snout only inhalation exposure technique. Exposures to the OligoG CF-5/20 aerosols and the control vehicle were performed using a modular (3 tier) stainless steel flow past systems. The system allowed a continuous supply of test aerosol to be delivered to each animal; the biased flow ensured no re-breathing of the test atmosphere. Separate exposure chambers were used for the Vehicle control and the OligoG

CF-5/20 groups. The following parameters were investigated: toxicokinetics, clinical condition, body weight, food consumption, ophthalmoscopy, hematology (peripheral blood), blood chemistry, urinalysis, lung sampling, organ weight, macropathology and histopathology.

Clinical safety and efficacy testing of inhaled OligoG CF-5/20. The Phase I study was a single center, randomized, placebo controlled, dose escalation study to test the *in vivo* safety and tolerability of OligoG CF-5/20 in humans (www.clinicaltrials.gov, Identifier: NCT00970346).

Healthy male subjects (28 in total) aged 18 to 65 years were assigned randomly to an intervention group (single- or multiple-dose) or control group (Supporting Information Table 13). Randomization was computer-generated and testing done by aerosol delivery system (Sidestream Plus/Portaneb, Phillips Respironics). OligoG CF-5/20 (6% solution) was prepared by spray drying and provided in 1.5 ml vials and inhaled at doses of 1.5 ml (90 mg/day) QD, 4.5 ml (270 mg/day) QD and 4.5 ml BID (540 mg/day), respectively for the three dosing cohorts. The medication was poured into the nebulizer cup for nebulization using the Sidestream-Plus and Portaneb (Phillips Respironics) aerosol delivery system. Matching placebo of saline (0.9% NaCl) was inhaled after nebulizing in the Sidestream Plus and Portaneb device. For each dose cohort, the same volume of active and placebo solution were administered, i.e. 1.5 ml QD, 4.5 ml QD or 4.5 ml BID. The placebo solution was indistinguishable to active OligoG CF-5/20.

Safety was monitored during the study, through pulmonary function tests, physical examination, vital signs, ECG, hematology and clinical chemistry. All adverse events reported by the subjects or observed by clinic staff were recorded in the Case Report Form (CRF). The treatment groups were compared with respect to the proportion of subjects experiencing one or more adverse events by the Fisher's exact test.

Clinical scintigraphy studies investigating lung deposition of radiolabelled OligoG. The scintigraphy study (Phase IIa) was an open label two-way randomized crossover study in 10 cystic fibrosis patients. Subjects received a single dose of OligoG CF-5/20 (dry powder for inhalation, DPI: 96 mg produced by spray drying) delivered by three

capsules via the Miat Monodose Dry Powder Inhaler, and a single dose of 1.5 mL (90 mg) aerosolized OligoG CF-5/20 (6% solution) delivered via the Sidestream-Plus nebulizer, separated by a 2-14 day washout period. Each treatment was radiolabelled with 10 MBq of ^{99m}Tc in total. The OligoG CF-5/20 was administered through a mouthpiece while the subject was tidal breathing in the upright position. Subjects were instructed to inhale to total lung capacity and hold for 5 to 10 seconds.

Sequential anterior and posterior images of the thorax/abdomen and lateral images of the head/neck were acquired. Images of the device hardware were acquired pre- and post-dose, using a Siemens E-Cam gamma camera with a 53.3 cm field of view and fitted with a low energy high-resolution collimator. Image analysis was performed using the WebLink software. Lung and extra-pulmonary deposition of radiolabel, including retention in the equipment, were characterized and assessed using paired t-tests (Supporting Information Table 13).

Rheological analysis of cystic fibrosis sputum. Samples of CF sputum (n=3) were divided and treated with 10% (v/v) distilled water (control) or 2% OligoG (incubated at 37°C for 4 h) to assess the extensional thinning behavior. A capillary break-up extensional rheometer was employed using two aligned 7 mm plates. Live recording was taken of a "step-strain" and the specimen fell at a time-lag. The extensional rheometer was manufactured 'in house' and the recording device was a FASTCAM ultima APX I2 (Photon Europe Ltd).

Further experiments were conducted employing shear rheology. Each sputum sample (n=23; from 7 patients) was divided into a control and 2% OligoG CF-5/20 treated experiment (Supporting Information Fig. 3a; see patient details in Supporting Information Table 13). Further analysis entailed collecting (n=9) samples longitudinally from a single patient (patient 1) and treating them with six treatment modalities: (i) distilled water control; (ii) 100 nM rhDNase I; (iii) 0.2% OligoG CF-5/20; (iv) 2% OligoG CF-5/20; (v) 100 nM rhDNase I and 0.2% OligoG CF-5/20; (vi) 100 nM rhDNase I and 2% OligoG CF-5/20. The final concentration of 2.5 μg ml^{-1} rhDNase I (equivalent to 100 nM) was based on previous published experiments.[6]

Treated samples were inverted gently (X 4) and incubated statically for 4 h at 37°C, prior to rheological analysis.

Samples were analyzed using an AR-G2 controlled stress rheometer (TA instruments, UK) fitted with a low inertia parallel-plate system (aluminium; 60 mm diameter) and peltier control (37°C) with a gap distance (400-1000 µm) as dictated by the volume of sample available. Values for overall resistance to deformation (complex modulus, *G**) were obtained by measuring the strain response to imposed oscillatory stress over 0.1 to 10 Hz, covering frequencies relevant to mucociliary clearance and ciliary beat.[7, 8] Analysis of the components of *G** provided values of the elastic and viscous response to imposed stress (*G'* and *G''*, respectively). The level of strain was set at a maximum of 2% (within the linear viscoelastic range of sputum). Control and 2% OligoG CF-5/20-treated samples were compared using Wilcoxon matched-pairs signed-ranks test and the longitudinal study analyzed using Dunnett multiple comparisons test in conjunction with analysis of variance (ANOVA) to compare the means (GraphPad Prism® Software, La Jolla, USA).

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