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Original Article

Antisense oligonucleotide eluforsen is safe and improves respiratory symptoms in F508DEL cystic fibrosis

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ABSTRACT

Background: Eluforsen is an antisense oligonucleotide designed to bind to the mRNA region around the F508-encoding deletion and restore the cystic fibrosis transmembrane conductance regulator (CFTR) protein function in the airway epithelium. We assessed the safety and tolerability, pharmacokinetics and exploratory measures of efficacy of inhaled eluforsen in cystic fibrosis (CF) patients homozygous for the F508del-*CFTR* mutation.

Methods: This randomised, double-blind, placebo-controlled, dose escalation 1b study recruited adult CF subjects with a $FEV_1 > 70\%$ predicted in four single ascending dose cohorts and four multiple ascending dose cohorts. Primary objectives were safety and tolerability. Secondary endpoints included pharmacokinetics, percent predicted forced expiratory volume in 1 s ($ppFEV_1$), and Cystic Fibrosis Questionnaire-Revised (CFQ-R) Respiratory Symptom Score (RSS).

Results: Single and multiple doses of inhaled eluforsen up to 50 mg were safe and well tolerated. A maximum tolerated dose was not established. Systemic exposure was low in all cohorts and lung function remained stable throughout the study. Three of four eluforsen-treated groups in the MAD study demon-

Abbreviations: AEs, Adverse events; CF, Cystic fibrosis; CFQ-R, Cystic Fibrosis Questionnaire; CFTR, Cystic fibrosis conductance regulator; CI, Confidence interval; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; MAD, Multiples ascending dose; NPD, Nasal potential difference; PK, Pharmacokinetics; $ppFEV_1$, Percent predicted forced expiratory volume in 1 s; SAD, Single ascending dose; RSS, Respiratory Symptom Score.

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strated an improvement in CFQ-R RSS at end of treatment with adjusted mean change from baseline values ranging from 6.4 to 12.7 points. In comparison, there was a mean decrease of 6.5 points in the placebo group from baseline to end of treatment.
Conclusions: Inhaled eluforsen up to 50 mg dosed 3 times per week for 4 weeks was safe and well tolerated, showed low systemic exposure, and demonstrated improvement in CFQ-R RSS, a relevant measure of clinical benefit in CF patients.

1. Introduction

Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene [1], a key anion transporter that hydrates airway mucus. The most common CF-causing mutation is a deletion of three nucleotides leading to the loss of a phenylalanine at position 508 of the protein (p.Phe508del [F508del hereafter]). Nearly 90% of patients with CF have an F508del-encoding mutation (F508del-*CFTR* hereafter), and over half of these individuals are homozygous for the mutation [2,3].

Substantial progress has been made in the development of disease-modifying therapies for CF using small molecules that directly target CFTR [4–7]. However, CFTR-enhancing therapies with broader applicability and greater efficacy are still needed. Recently, molecular therapies that target RNA have been successfully advanced as a novel treatment option targeting mutations in diseases including Duchenne's muscular dystrophy and spinal muscular atrophy [8,9]. Eluforsen,[1] previously known as QR-010, is an antisense 33-mer RNA oligonucleotide with a phosphorothioate backbone and full 2'-*O*-methylation, designed to bind specifically to the mRNA region around the F508-encoding deletion and to restore CFTR protein function in the airway epithelium. Preclinical data showed that eluforsen improved CFTR function as measured by in vitro functional assays using primary bronchial epithelial cultures from F508del-*CFTR* homozygous donors and nasal potential difference (NPD) in F508del-*CFTR* CF mice [10], and a recent clinical study showed changes in NPD reflecting improved sodium and chloride currents following intranasal administration of eluforsen to F508del-*CFTR* homozygous subjects [11]. Based on these data, eluforsen is being developed as a novel RNA-based therapy for patients with CF carrying the F508del-*CFTR* mutation. The aim of this study was to assess the safety and tolerability of inhaled (i.e., nebulized) eluforsen in subjects with CF who are homozygous for the F508del-*CFTR* mutation. Efficacy endpoints were also explored, although the study was not powered for hypothesis testing.

2. Methods

2.1. Study design and participants

This was a dose-escalation phase 1b study, consisting of 4 single ascending dose (SAD) cohorts and 4 multiple ascending dose (MAD) cohorts (supplementary Fig. S1), conducted at 23 centres across 10 countries in North America and Europe. The trial is registered with clinicaltrials.gov as NCT02532764. The protocol and informed consent form were approved by institutional review boards or independent ethics committees at each investigational site. Each subject provided written informed consent at screening prior to undergoing any protocol-related procedures. Eligible participants were aged 18–60 years, had a confirmed diagnosis of CF (sweat chloride >60 mmol/L) with confirmation of *CFTR* gene mutations homozygous for the F508del mutation, a forced expiratory volume in 1 s, reported as percent predicted ($ppFEV_1$), that was ≥70% of the predicted normal value. Further eligibility criteria are shown in the online supplement. Subjects were randomised to a current enrolling cohort. If both a SAD and a MAD cohort were enrolling concurrently, the subject could elect participation specifically in an open SAD or MAD cohort. Subjects were randomised at a 3:1 ratio (eluforsen to placebo) within each dosing cohort.

2.2. Analysis populations

The safety population included all subjects who received at least one dose of eluforsen or placebo. The per-protocol population included all randomised subjects in the MAD cohorts of the safety population who received at least 10 of the 12 doses of either eluforsen or placebo.

2.3. Procedures

Subjects in a SAD cohort received a single 6.25, 12.5, 25, or 50 mg dose of eluforsen or placebo via oral inhalation (nebulization). Subjects in a MAD cohort received a dose of 6.25, 12.5, 25, or 50 mg eluforsen or placebo via nebulization 3 times weekly for 4 weeks, for a total of 12 doses per subject. The dosing regimen was based on the human equivalent dose and maximum recommended starting dose derived from nonclinical studies, and the doses were administered using the eFlow® Nebulizer System (PARI Pharma GmbH, Starnberg, Germany) at the study sites. Pulmonary function was assessed via spirometry, using American Thoracic Society/European Respiratory Society standards. Patient-reported outcomes were measured in the MAD cohorts using the Cystic Fibrosis Questionnaire-Revised (CFQ-R) Respiratory Symptom Score (RSS). At visits where the CFQ-R was administered, subjects completed this questionnaire prior to study drug administration and/or any other assessments. Sweat was collected by pilocarpine iontophoresis using a method according to CTN/TDN protocols and chloride concentrations measured in an accredited local laboratory. Eluforsen concentrations were measured in serum, urine, and sputum for pharmacokinetic evaluation.

2.4. Outcomes and measurements

The primary endpoint was the incidence and severity of adverse events (AEs) and the occurrence of dose-limiting toxicities in each dose cohort from baseline through end of study. Changes in efficacy measures including $ppFEV_1$, CFQ-R RSS, and sweat chloride were assessed, and C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) measured using standard clinical laboratory techniques. For the CFQ-R RSS, subjects reported their symptoms over the 2 weeks prior to the study visit. Subjects completed the CFQ-R RSS at the beginning of the study visit, before any other clinical examinations, to reduce biases associated with knowledge of clinical status [12].

[1] International Nonproprietary Name (INN), eluforsen, is under review.

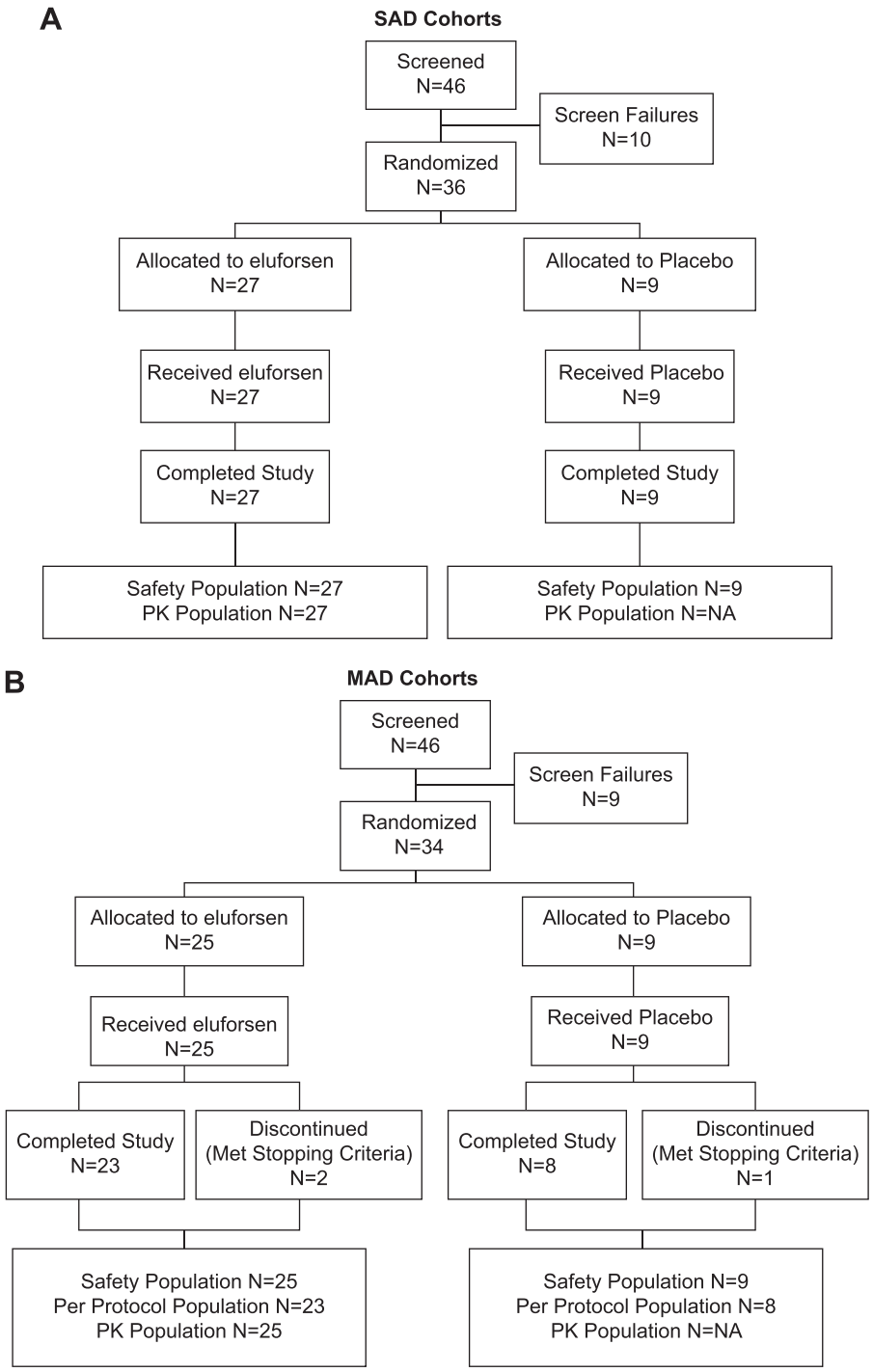

Fig. 1. Trial profile. Patient disposition for the SAD (a) and MAD (b) cohorts. The safety population includes all subjects who received at least one dose of eluforsen or placebo. The per protocol population included all randomised subjects (MAD) that received at least 10 out of 12 doses of eluforsen or placebo. The PK population included all subjects who received at least 1 dose of eluforsen and who had measurable concentrations for the purpose of modelling. MAD, multiple ascending dose; PK, pharmacokinetics; SAD, single ascending dose.

2.5. Statistical analysis

For each endpoint, the actual value, absolute change from baseline, and percentage change from baseline (for $ppFEV_1$) were summarized descriptively over time by treatment group. Differences in $ppFEV_1$ and CFQ-R RSS versus placebo were evaluated using a mixed model analysis with repeated time measures on the change from baseline as outcome variable and including treatment, baseline value, time, and interaction between time and treatment as covariates. Adjusted means and corresponding 95% confidence intervals (CI) are presented. All analyses were pre-specified in the Statistical Analysis Plan (see supplementary material for additional information). To explore the impact of the baseline status, subgroups of subjects with $ppFEV_1 < 90$ and $\geq 90\%$ at baseline were considered for $ppFEV_1$ and CFQ-R RSS analyses.

3. Results

3.1. Subject disposition and demographics

Subjects were recruited between August 2015 and July 2017. As shown in Fig. 1a, 36 eligible subjects with CF were enrolled in

Table 1
Baseline and demographic characteristics (MAD cohorts safety population).

	Placebo (n = 9)	Eluforsen 6.25 mg (n = 6)	Eluforsen 12.5 mg (n = 6)	Eluforsen 25 mg (n = 7)	Eluforsen 50 mg (n = 6)
Age (years)	26 (18; 38)	23 (19; 26)	28 (19; 41)	32 (21; 46)	23 (19; 30)
Sex, n (%)					
Male	3 (33)	3 (50)	3 (50)	2 (29)	4 (67)
Female	6 (67)	3 (50)	3 (50)	5 (71)	2 (33)
BMI (kg/m^2)	21 (19; 24)	24 (20; 27)	23 (19; 26)	24 (21; 26)	21 (18; 25)
Predicted FEV_1 (%)	86.7 (70.7; 99.7)	90.7 (75.3; 115.6)	89.0 (74.4; 108.2)	79.9 (69.2; 94.9)	84.7 (73.7; 110.7)
Sweat chloride (mmol/L)	105.3 (93.0; 123.0)	101.1 (84.0; 126.0)	91.0 (61.0; 110.0)	99.7 (83.0; 109.0)	98.4 (91.0; 107.5)
Pseudomonas aeruginosa, n (%)					
Yes	5 (56)	1 (17)	0 (0)	0 (0)	0 (0)
No	4 (44)	5 (83)	6 (100)	7 (100)	6 (100)
CF-related diabetes, n (%)					
Yes	1 (11)	1 (17)	3 (50)	5 (71)	3 (50)
No	8 (89)	5 (83)	3 (50)	2 (29)	3 (50)
CFQ-R RSS	77.8 (66.7; 88.9)	74.1 (61.1; 88.9)	72.2 (55.6; 94.4)	72.2 (61.1; 83.3)	78.7 (55.6; 88.9)

Data are mean (range) unless otherwise specified.
BMI, body mass index; CF, cystic fibrosis; CFQ-R RSS, Cystic Fibrosis Questionnaire-Revised Respiratory Symptom Score; FEV_1, forced expiratory volume in 1 s; MAD, multiple ascending dose.

Table 2
Overall summary of adverse events in the MAD cohorts (safety population).

	Placebo (N = 9) n (%)	Eluforsen 6.25 mg (N = 6) n (%)	Eluforsen 12.5 mg (N = 6) n (%)	Eluforsen 25 mg (N = 9) n (%)	Eluforsen 50 mg (N = 6) n (%)	Total Eluforsen (N = 27) n (%)
Subjects with at least one AE	9 (100.0)	5 (83.3)	5 (83.3)	6 (85.7)	5 (83.3)	21 (84.0)
Number of AEs	55	36	23	13	14	86
Subjects with at least one:						
Drug-related AE	6 (66.7)	3 (50.0)	4 (66.7)	2 (28.6)	3 (50.0)	12 (48.0)
Device-related AE	2 (22.2)	0	0	0	0	0
Serious AE	1 (11.1)	0	0	0	1 (16.7)	1 (4.0)
AE leading to treatment discontinuation	1 (11.1)	0	0	0	1 (16.7)	1 (4.0)
Subjects with AEs by severity						
Mild	5 (55.6)	5 (83.3)	4 (66.7)	5 (71.4)	1 (16.7)	15 (60.0)
Moderate	3 (33.3)	0	1 (16.7)	0	4 (66.7)	5 (20.0)
Severe	1 (11.1)	0	0	1 (14.3)	0	1 (4.0)
Life threatening	0	0	0	0	0	0
Death	0	0	0	0	0	0

AE, adverse event; MAD, multiples ascending dose.

the SAD cohorts and completed the study. Thirty-four eligible subjects were enrolled in the MAD cohorts (Fig. 1b). Of these 34 subjects, 3 subjects in the MAD cohorts (1 in the 50 mg group, 1 in the 25 mg group, and 1 in the placebo group) discontinued from the study due to the protocol-defined stopping criterion of a decrease in $ppFEV_1$ of >15% from baseline, which happened prior to receiving 10 of 12 planned doses, and were excluded from the per-protocol population. None of these events were reported as AE as subjects were asymptomatic. A summary of baseline characteristics (Table 1, MAD cohorts; supplementary Table S1, SAD cohorts) reveals typical clinical features of the classical CF phenotype with early or mild disease.

3.2. Safety

Single and multiple doses of eluforsen up to 50 mg via nebulization were well tolerated during the study. No dose-limiting toxicities were identified; thus, a maximum tolerated dose was not established. No serious AEs were reported in the SAD cohorts, and 3 serious AEs were reported in 2 subjects in the MAD cohorts (respiratory tract infection and urinary calculus in a subject in the 50 mg group and abdominal pain in a subject in the placebo group); both were assessed as unrelated to study drug. Most AEs in the study were mild-to-moderate, with 2 subjects in the MAD cohorts reporting 1 severe AE each (hypoglycaemia in a subject in the 25 mg group and abdominal pain in a subject in the placebo group); again, both were assessed as unrelated to study drug. No subjects in the SAD cohorts discontinued from treatment or withdrew from the study due to AEs. In the SAD cohorts, AEs were most frequently reported in the nervous and gastrointestinal systems, and in the MAD cohorts, AEs were most frequently reported in the respiratory system, general disorders, and infection and infestation (Tables 2 and 3, supplementary Table S2).

3.3. Pharmacokinetics

Following single doses, eluforsen was undetectable in serum at doses up to 25 mg; at a dose of 50 mg, systemic exposure was low and highly variable, with peak serum concentrations occurring between 0.5 and 2.0 h post dose (supplementary Fig. S2). Following single dose administration, there were no detectable eluforsen concentrations in the urine. In sputum, quantifiable concentrations of eluforsen following single doses were seen across all doses with no apparent relationship to dose (supplementary Fig. S3).

Following multiple doses, systemic exposure to eluforsen remained low and serum pharmacokinetics profiling could only be performed for the 50 mg dose. At Week 4, systemic exposure was similar to that following a single dose, with peak serum concentrations occurring between 1.0 and 2.0 h, indicating lack of accumulation. After the first dose in the eluforsen 50 mg MAD cohort, the mean (standard deviation) C_{max} was 4.5 (3.4) ng/mL and AUC_{last} was 19.0 (15.6) ng.hr/mL, with median T_{max} at 0.5 h. After Week 4, Dose 12, the mean (standard deviation) C_{max} was 3.6 (2.7) ng/mL and AUC_{last} was 17.0 (11.4) ng.hr/mL, with median T_{max} at 1.0 h. Following multiple dose administration, there were no detectable eluforsen concentrations in the urine. There were quantifiable concentrations of eluforsen in sputum following multiple doses administration of eluforsen across all doses (6.25–50 mg) and at most time points, suggesting that all subjects were continually exposed to eluforsen over the multiple dose study duration.

Table 3
Adverse events reported in more than one eluforsen treated subject, by MedDRA System Organ Class and Preferred Term for the MAD cohorts (safety population).

System organ class preferred term	Placebo (N = 9) n (%)	Eluforsen 6.25 mg (N = 6) n (%)	Eluforsen 12.5 mg (N = 6) n (%)	Eluforsen 25 mg (N = 7) n (%)	Eluforsen 50 mg (N = 6) n (%)	Total Eluforsen (N = 25) n (%)
Respiratory, thoracic and mediastinal disorders						
Cough	2 (22.2)	3 (50.0)	1 (16.7)	0	2 (33.3)	6 (24.0)
Sputum increased	3 (33.3)	2 (33.3)	1 (16.7)	1 (14.3)	0	4 (16.0)
Nasal congestion	1 (11.1)	2 (33.3)	0	0	1 (16.7)	3 (12.0)
Wheezing	0	3 (50.0)	0	0	0	3 (12.0)
Oropharyngeal pain	0	1 (16.7)	0	1 (14.3)	0	2 (8.0)
General disorders and administration site conditions						
Fatigue	0	2 (33.3)	1 (16.7)	1 (14.3)	0	4 (16.0)
Pyrexia	1 (11.1)	0	1 (16.7)	2 (28.6)	0	3 (12.0)
Infections and infestations						
Oral candidiasis	0	0	1 (16.7)	1 (14.3)	0	2 (8.0)
Respiratory tract infection	0	0	0	0	2 (33.3)	2 (8.0)
Rhinitis	0	0	2 (33.3)	0	0	2 (8.0)
Gastrointestinal disorders						
Diarrhoea	0	1 (16.7)	0	1 (14.3)	0	2 (8.0)
Investigations						
Forced expiratory volume decreased	2 (22.2)	0	0	0	2 (33.3)	2 (8.0)
Musculoskeletal and connective tissue disorders						
Arthralgia	1 (11.1)	0	1 (16.7)	1 (14.3)	0	2 (8.0)

MAD, multiple ascending dose; MedDRA, Medical Dictionary for Regulatory Activities.

Table 4
CFQ-R Respiratory Symptom Score (MAD per-protocol population).

	Placebo (N = 8)	Eluforsen 6.25 mg (N = 6)	Eluforsen 12.5 mg (N = 6)	Eluforsen 25 mg (N = 6)	Eluforsen 50 mg (N = 5)
Baseline, N	8	6	6	6	5
Observed mean value	77.8	74.1	72.2	70.4	76.7
(i) Range	66.7; 88.9	61.1; 88.9	55.6; 94.4	61.1; 83.3	55.6; 88.9
Day 15, N	8	6	6	6	5
Adjusted mean change (SE)	−5.09 (3.59)	−4.13 (4.10)	8.95 (4.12)	7.76 (4.15)	−0.80 (4.51)
95% CI for adjusted mean change	−12.48; 2.30	−12.58; 4.32	0.46; 17.43	−0.79; 16.31	−10.09; 8.48
p value	0.1686	0.3242	0.0395	0.0733	0.8604
Difference vs placebo (SE)		0.96 (5.46)	14.03 (5.49)	12.85 (5.55)	4.29 (5.73)
95% CI for difference		−10.28; 12.20	2.72; 25.35	1.43; 24.27	−7.52; 16.10
p value		0.8616	0.0171	0.0290	0.4614
Day 33, N	8	6	6	6	5
Adjusted mean change (SE)	−6.48 (4.29)	6.43 (4.92)	12.65 (4.93)	7.76 (4.96)	−3.02 (5.40)
95% CI for adjusted mean change	−15.31; 2.35	−3.70; 16.55	2.50; 22.80	−2.45; 17.97	−14.14; 8.09
p value	0.1434	0.2030	0.0166	0.1300	0.5803
Difference vs placebo (SE)		12.91 (6.53)	19.13 (6.56)	14.24 (6.60)	3.46 (6.87)
95% CI for difference		−0.54; 26.35	5.62; 32.64	0.64; 27.83	−10.69; 17.60
p value		0.0592	0.0074	0.0408	0.6193
Day 54, N	7	6	6	6	5
Adjusted mean change (SE)	−10.31 (5.87)	−10.24 (6.56)	0.61 (6.57)	5.91 (6.59)	−4.13 (7.20)
95% CI for adjusted mean change	−22.39;1.77	−23.76; 3.28	−12.93; 14.15	−7.67; 19.49	−18.96; 10.69
p value	0.0911	0.1314	0.9265	0.3788	0.5709
Difference vs placebo (SE)		0.07 (8.81)	10.92 (8.83)	16.22 (8.87)	6.18 (9.26)
95% CI for difference		−18.07; 18.21	−7.27; 29.11	−2.04; 34.48	−12.91; 25.26
p value		0.9936	0.2277	0.0794	0.5111

Data are from a mixed model analysis of change from baseline. Differences from placebo are shown as adjusted mean change.
CI, confidence interval; CFQ-R, Cystic Fibrosis Questionnaire-Revised; MAD, multiple ascending dose; SE, standard error of the mean.
The bold is to highlight the different assessment times.

3.4. Cystic fibrosis questionnaire—revised respiratory symptom score

Eluforsen treatment was associated with an improvement in CFQ-R RSS scores (adjusted mean change from baseline) in the MAD per-protocol population receiving 6.25, 12.5, or 25 mg (Table 4, Fig. 2). Improvement was seen at Day 15 in the 12.5 and 25 mg dose groups and was sustained to 4 weeks post dose at the 25-mg dose level, suggesting durability of treatment and a dose response. The differences from placebo compared with the 12.5 and 25 mg doses were statistically significant (p = 0.007 and 0.041 at Day 33, respectively). No improvement in CFQ-R RSS scores relative to baseline was observed for the highest dose group (50 mg).

A similar pattern of improvement was seen in the subgroup of subjects with <90% ppFEV$_1$ at baseline, with a larger treatment effect consistent with a population that is more symptomatic at study entry with greater room for clinical improvement (supplementary Table S3). In subjects with ≥90% ppFEV$_1$ at baseline, a smaller but still statistically significant treatment effect was observed in the 12.5 and 25 mg doses at Day 33, as well as at Day 15 in the 12.5 mg dose, but the differences compared to placebo were not statistically significant (supplementary Table S4).

3.5. Percent predicted forced expiratory volume in 1 s

In the SAD and the MAD per-protocol population, eluforsen was not associated with any meaningful change from baseline in ppFEV$_1$ nor any improvement compared to placebo (Fig. 3; supplementary Tables S5 and S6). However, in the subgroup of subjects with <90% ppFEV$_1$ at baseline, which is a more symptomatic population with greater room for clinical improvement, improvements from baseline and relative to placebo were seen for subjects in the 6.25 and 12.5 mg MAD dose groups (Fig. 3; Table 5). The 6.25 mg

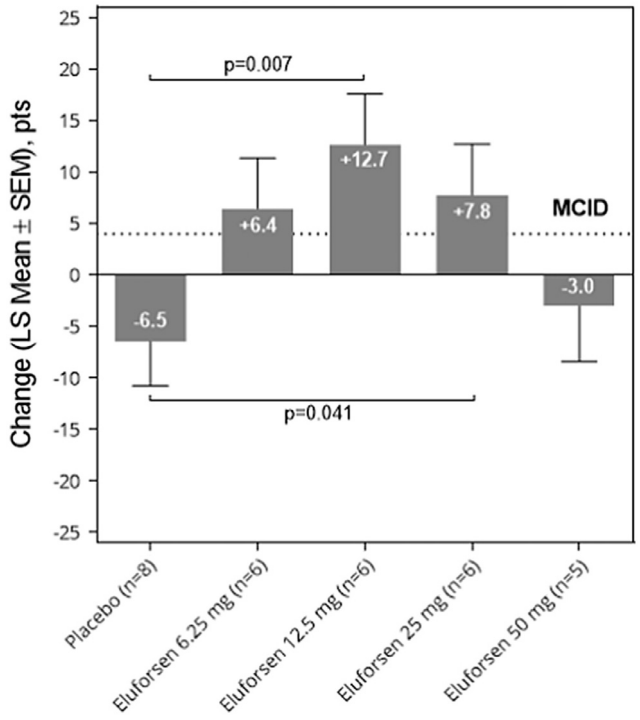

Fig. 2. Mean change from baseline CFQ-R RSS at Day 33 (MAD per-protocol population). Data are from a mixed model analysis of change from baseline. Differences from baseline indicated as adjusted mean change. Cystic Fibrosis Questionnaire-Revised (CFQ-R); CI, confidence interval; LS, least squares; MAD, multiple ascending dose; MCID, minimal clinically important difference; Respiratory Symptom Score (RSS).

dose showed an adjusted mean change from baseline $ppFEV_1$ value of −0.3% (95% CI −5.1; 4.5) on Day 15, 4.2% (95% CI −4.5; 12.9) on Day 26, and 6.0% (95% CI −0.8; 12.8) on Day 33. For the 12.5 mg dose these values were 4.5% (95% CI 0.4; 8.7) on Day 15, 7.2% (95% CI −0.4; 14.7) on Day 26, and 3.8% (95% CI −2.1; 9.7) on Day 33, whereas no improvements were observed in the 25 mg and 50 mg MAD dose groups, and the placebo group showed decreased adjusted mean change from baseline values for $ppFEV_1$ (−3.4% [95% CI −7.6; 0.8] on Day 15, −3.8% [95% CI −11.3; 3.8] on Day 26, and −4.2% [95% CI −10.1; 1.7] on Day 33). No improvements from baseline were seen for any dose among subjects with ≥90% $ppFEV_1$ at baseline (supplementary Table S7).

3.6. Sweat chloride

Eluforsen treatment did not result in a significant change compared to placebo in sweat chloride (supplementary Fig. S4).

3.7. C-reactive protein and erythrocyte sedimentation rate

After a single dose, eluforsen was not associated with a significant improvement in CRP or ESR at either Day 2 or Day 8 (supplementary Tables S8 and S10). After multiple doses of eluforsen, there were no consistent changes from baseline in mean CRP or ESR at any time point (supplementary Tables S9 and S11). No subjects had clinically meaningful changes in CRP or ESR values during the study.

4. Discussion

The data from this study showed that inhaled eluforsen in single and multiple doses (12 doses over 4 weeks) up to 50 mg administered to homozygous F508del CF patients with baseline lung function defined as $FEV_1 \geq 70\%$ was safe and well tolerated. The pattern of treatment emergent adverse event incidence was consistent with what is expected for the CF population [21]. Throughout the study the mean level of lung function ($ppFEV_1$) of this CF

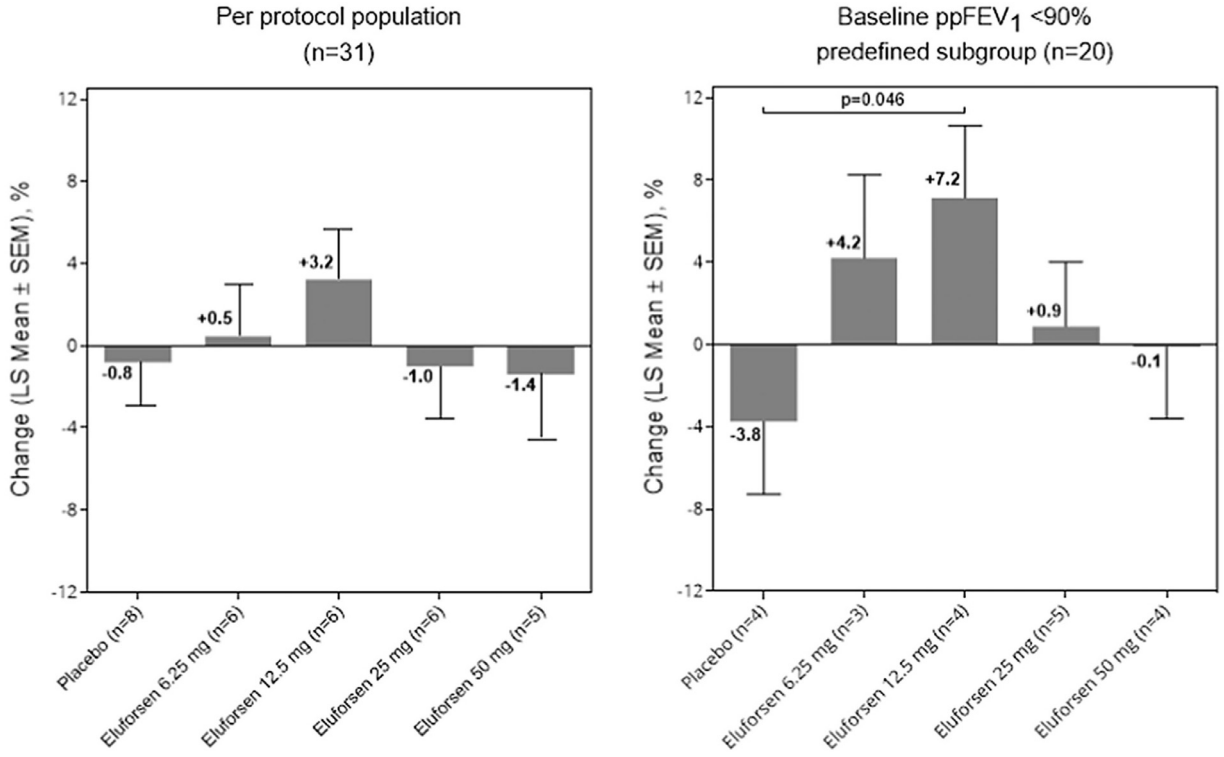

Fig. 3. Mean change from baseline at Day 26 in percent predicted forced expiratory volume in 1 s (MAD in the per-protocol population [left] and in the subgroup with baseline ppFEV1 < 90% [right]). Data are from a mixed model analysis of change from baseline. Differences from placebo are shown as adjusted mean change. CI, confidence interval; LS, least squares; MAD, multiple ascending dose; $ppFEV_1$, percent predicted forced expiratory volume in 1 s.

Table 5
Percent predicted forced expiratory volume in 1 s (MAD per-protocol population; subgroup with baseline ppFEV1 < 90%).

	Placebo (N = 4)	Eluforsen 6.25 mg (N = 3)	Eluforsen 12.5 mg (N = 4)	Eluforsen 25 mg (N = 5)	Eluforsen 50 mg (N = 4)
Baseline, N	4	3	4	5	4
(ii) Observed mean value	75.7	81.1	80.5	78.9	79.3
(iii) Range	70.7; 87.8	75.3; 89.3	74.4; 87.6	69.2; 89.6	73.7; 87.0
Day 15, Pre-dose, N	4	3	4	5	4
Adjusted mean change (SE)	−3.40 (1.95)	−0.29 (2.22)	4.54 (1.92)	−3.45 (1.71)	−1.01 (1.91)
95% CI for adjusted mean change	−7.58; 0.79	−5.05; 4.47	0.43; 8.65	−7.11; 0.22	−5.11; 3.09
p value	0.1036	0.8991	0.0329	0.0633	0.6048
Difference vs placebo		3.11 (2.99)	7.93 (2.76)	−0.05 (2.59)	2.38 (2.74)
95% CI for difference		−3.30; 9.52	2.01; 13.86	−5.61; 5.51	−3.48; 8.25
p value		0.3157	0.0123	0.9849	0.3983
Day 26, Pre-dose, N	4	3	4	5	4
Adjusted mean change (SE)	−3.76 (3.52)	4.21 (4.05)	7.15 (3.51)	0.89 (3.13)	−0.10 (3.50)
95% CI for adjusted mean change	−11.31; 3.80	−4.48; 12.90	−0.37; 14.67	−5.83; 7.60	−7.61; 7.41
p value	0.3044	0.3163	0.0607	0.7806	0.9768
Difference vs placebo		7.97 (5.39)	10.90 (4.98)	4.65 (4.71)	3.65 (4.97)
95% CI for difference		−3.59; 19.52	0.21; 21.59	−5.46; 14.75	−7.01; 14.31
p value		0.1613	0.0461	0.3410	0.4745
Day 33, N	4	3	4	5	4
Adjusted mean change (SE)	−4.18 (2.76)	6.01 (3.16)	3.81 (2.74)	−0.68 (2.44)	−0.97 (2.73)
95% CI for adjusted mean change	−10.10; 1.74	−0.78; 12.80	−2.06; 9.68	−5.92; 4.56	−6.83; 4.89
p value	0.1522	0.0783	0.1858	0.7855	0.7286
Difference vs placebo		10.19 (4.22)	7.99 (3.91)	3.50 (3.69)	3.21 (3.89)
95% CI for difference		1.13; 19.25	−0.39; 16.37	−4.40; 11.41	−5.13; 11.55
p value		0.0301	0.0601	0.3580	0.4224

Analysis was performed using a mixed model with repeated time measures on the change from baseline $ppFEV_1$ as outcome variable and including treatment, baseline $ppFEV_1$ value, time and interaction between time and treatment as covariates. An unstructured covariance matrix was used.
CI, confidence interval; FEV_1, forced expiratory volume in 1 s; MAD, multiple ascending dose; SE, standard error of the mean.
The bold is to highlight the different assessment times.

population (overall mean baseline $ppFEV_1$ of 89%) was stable in all treatment groups. The low level of systemic exposure seen following both single and multiple doses of eluforsen is consistent with the low level of systemic AEs and the absence of effects on sweat chloride and markers of inflammation (including CRP and ESR) in this study.

Although the study was not designed to allow for a robust assessment of efficacy, there was an encouraging signal of efficacy on the clinical outcome measure CFQ-R RSS in this population of homozygous F508del CF subjects with preserved lung function. In three of the eluforsen dose groups (6.25, 12.5, and 25 mg) there was a mean improvement above the minimal clinically important difference of 4.0 [13], with adjusted mean change from baseline values on Day 33 (i.e., approximately 1 week post last dose) ranging from 6.4 to 12.7 points, and difference from placebo values ranging from 12.9 to 19.1 points in the per protocol group. In the subgroup of subjects with baseline lung function of <90% $ppFEV_1$, this improvement was more apparent, with adjusted mean change from baseline values on Day 33 ranging from 8.4 to 15.6 points and difference from placebo values ranging from 20.2 to 27.3 points. These changes in the RSS domain of the CFQ-R are of similar magnitude as those reported in CFTR modulator trials [5,6], and for patients in recovery from pulmonary exacerbations [14]. Overall, the CFQ-R RSS data suggest that eluforsen resulted in clinically relevant improvements in respiratory symptoms in this study, despite the mild disease status of the patients recruited, and the difficulty in general to demonstrate symptom improvement in people with CF due to their tendency of underreporting the severity of their symptoms compared to people with other respiratory conditions [15].

In the exploratory evaluation of lung function as assessed by spirometry ($ppFEV_1$,) a trend of improved lung function was only observed in the subgroup of subjects with baseline lung function of <90% $ppFEV_1$; for the 6.25 mg dose group (n = 3), the adjusted mean change from baseline in $ppFEV_1$ on Day 26 was 4.2 [95% CI −4.5; 12.9], while the difference versus placebo (n = 4) was 8.0 [95% CI −3.6; 19.5], and for the 12.5 mg dose group (n = 4), the adjusted mean change from baseline in $ppFEV_1$ on Day 26 was 7.2 [95% CI −0.4; 14.7], while the difference versus placebo was 10.9 [95% CI 0.21; 21.6]. Although this sensitivity analysis is based on a limited number of subjects in the subgroup, these data suggest that spirometry has limited sensitivity in detecting early eluforsen-induced improvements in lung function in CF subjects with a baseline $ppFEV_1 > 90\%$. Assessment of lung clearance index may have been a better choice to assess lung function of eluforsen, especially in this short duration study with CF subjects with mild disease, as lung clearance index allows assessment of lung function in the smaller airways and has shown to be more sensitive than spirometry in patients with better preserved lung function [7,16,17]. Improvements in the CFQ-R RSS and $ppFEV_1$ were not seen in the highest dose groups (25 mg and 50 mg), and suggest a bell-shaped dose response curve. Such a phenomenon was also observed in a phase 2 study of lumacaftor/ivacaftor in CF for $ppFEV_1$ and CFQ-R RSS and confirmed in a phase 3 study [18,19], and has been observed with other therapies, for example in the therapeutic targeting of angiogenesis, and may be explained by complex biological effects [22]. While the sample size in the current study is very small for making any strong conclusions about FEV_1 benefit, the exploratory findings are encouraging, and more importantly there is no negative impact to airflow. Larger studies with sufficient power are warranted to fully reveal any potential therapeutic benefit.

This study showed that repeated administration of inhaled eluforsen over a period of 4 weeks to homozygous F508del CF subjects with relatively preserved lung function was safe and well tolerated, and resulted in an encouraging signal of therapeutic benefit. To our knowledge, this is the first clinical study with an antisense oligonucleotide demonstrating improvement in a relevant measure of clinical benefit in people with CF. These data add to previously reported data with eluforsen showing improved CFTR activity in F508del animal models [10,20] and an early proof of concept clinical study showing that intranasal administration of eluforsen improved CFTR function in F508del-*CFTR* homozygous subjects as assessed by NPD [11].

Therapeutic agents targeting RNA such as antisense oligonucleotides are a growing area of research, and with the recent success of other oligonucleotide approaches in genetic disorders, translation of these novel technologies into CF treatment may

bring a new therapeutic class to the field [8,9]. The results from this phase 1 dose escalation study indicate that eluforsen is suitable for further development and provide support for additional larger controlled studies using the inhaled pulmonary route of delivery of eluforsen to assess therapeutic benefit in patients homozygous for the F508del-*CFTR* mutation.

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Author contributions

Author contributions were as follows. NP-L, JSE and NH contributed to the design of the study. PD, TP, MC, KDB, CS, and CE contributed to the collection of data. FB, MB and SM contributed to the statistical analysis plan. All authors contributed to the interpretation of data and drafting and revising the manuscript, and approved the final version.

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Conflict of interest statement

WdH, JP and NT are full-time employees of ProQR Therapeutics. NP-L and NH were full-time employees of ProQR Therapeutics during design development, execution and reporting of this study, and SM was full-time employee of ProQR Therapeutics during execution and reporting of this study. FB and MB are or were statistical consultants paid by ProQR Therapeutics. KDB report grants related to the submitted work. KDB and JSE report grants outside the submitted work. KDB and JSE report activity on advisory boards during the conduct of the study and outside the submitted work. PD, KDB and JSE received fees for consultancy. CS, MC and TP have no disclosures.

Appendix A. Supplementary data

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