Randomised, phase 1/2a trial of ION-827359, an antisense oligonucleotide inhibitor of ENaC

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Shareable abstract (@ERSpublications)
Single and multiple doses of ION-827359 were well tolerated in this trial of healthy volunteers and pwCF. This study demonstrates that mRNA expression in the lung can be regulated after treatment with an inhaled antisense oligonucleotide. https://bit.ly/4d4MVzh

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Abstract

Background Hyperactivity of epithelial sodium channel (ENaC) with increased sodium absorption is a feature of cystic fibrosis (CF). ION-827359 is a 2.5-generation antisense oligonucleotide targeted to reduce ENaC protein. This study evaluated ION-827359 safety, pharmacokinetics and pharmacodynamics.

Methods In this three-part phase 1/2a, double-blind, randomised study, healthy volunteers received single doses of placebo or ION-827359 (3, 10, 37.5 or 100 mg; Part 1) or multiple doses of placebo or ION-827359 (5×10 mg, 5×37.5 mg, 5×75 mg or 10×37.5 mg; Part 2). People with CF (pwCF) received multiple doses of placebo or ION-827359 (5×10 mg, 5×37.5 mg, 5×75 mg and 5×100 mg; Part 3). Treatments were administered *via* Pari eFlow© mesh nebuliser. The primary outcome was safety; pharmacokinetic and pharmacodynamic parameters were also assessed.

Results 64 healthy volunteers and 34 pwCF were enrolled. ION-827359 was well tolerated with an acceptable safety profile. There were no clinically relevant changes in laboratory values, ECG or vital signs. Systemic drug exposure was low (plasma half-life ∼2 weeks). Multiple doses of ION-827359 were associated with dose-dependent reductions in ENaC mRNA in bronchial epithelium. After multiple dosing, forced expiratory volume in 1 s was slightly higher in pwCF receiving ION-827359 (+2.9% with ION-827359 100 mg *versus* placebo; p=0.27).

Conclusions The tolerability and safety of ION-827359 appear favourable at this stage of investigation. Reduction in ENaC mRNA supports mechanistic efficacy at the doses and regimens tested, and supports further investigation of ION-827359 in pwCF.

Introduction

In people with cystic fibrosis (pwCF), the loss of CFTR function in the lung results in reduced chloride and bicarbonate transport, which causes airway surface liquid (ASL) depletion and accumulation of mucus. This makes the airway susceptible to persistent infections, sustained inflammation and progressive airway obstruction, and increases risk of death [1–3]. Until recently, treatment for CF primarily focused on prevention and control of infection *via* antibiotics, mucolytics and postural drainage [3–5]. However, new medications that improve CFTR function are now available, such as CFTR potentiators (*e.g.*, ivacaftor) and CFTR correctors (*e.g.*, elexacaftor, tezacaftor, lumacaftor). These agents, which are mostly used in combination with each other, effectively reduce inflammation, improve lung function and quality of life, decrease exacerbation frequency and improve nutritional status [6–11].

Despite combination CFTR modulator therapies being highly effective in clinical trials, individual patient response to modulators is variable and data from a real-world study suggest pwCF can still experience

slow decline in lung function and progression of lung disease [12]. Furthermore, a subset of pwCF have mutations that are not amenable to treatment with CFTR modifiers (*e.g.*, nonsense mutations). Thus, there remains an unmet need for alternative therapeutic options for pwCF.

The epithelial sodium channel (ENaC) is present on the apical surface of airway epithelial cells and is responsible for sodium ion (Na^+) and fluid absorption, which is itself essential for volume regulation of ASL [13]. The introduction of amiloride as a small molecule ENaC inhibitor represented a ground-breaking step in therapeutic ENaC suppression for CF. Clinical evidence has confirmed the efficacy of amiloride as an ENaC blocker in pwCF, providing substantial insights into its potential therapeutic advantages [13]. Hyperactivation of ENaC due to CFTR dysfunction, especially when augmented by proteolytic activation by bacterial and host-derived proteases [14–16], contributes to the dehydration of ASL in CF [2, 16]. ION-827359 is a 2.5-generation antisense oligonucleotide (ASO) drug targeted to the fourth intronic region of the α-ENaC pre-mRNA. The hybridisation of ION-827359 to the cognate pre-mRNA results in RNase H1-mediated degradation of ENaC pre-mRNA, preventing production of ENaC mRNA and protein. Maximal antisense-mediated reduction of target mRNA levels is typically >90% of control levels in sensitive tissues [17, 18] and correlates directly with a subsequent reduction in target protein [17–20]. In a mouse model of CF-like lung disease, delivery of ASOs that target mRNAs encoding each of the three ENaC subunits directly into the lung improved disease phenotypes, suggesting that targeting ENaC subunits could be an effective approach for the treatment of CF [20].

The aim of the current study was to evaluate the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of single and multiple nebulised doses of ION-827359 in healthy volunteers and pwCF.

Methods

Study design

This phase 1/2a, double-blind, randomised, placebo-controlled, multicentre, dose-escalation study (ClinicalTrials.gov identifier, NCT03647228) comprised three parts: single ascending doses and then multiple ascending doses in healthy volunteers, followed by multiple ascending doses in subjects with CF. A central ethics committee in each country reviewed and approved the trial protocol and informed consent forms. All enrolled participants provided written informed consent before study participation.

Participants

Healthy males or females of non-childbearing potential, aged 18–50 years at the time of informed consent, and males and females aged at least 18 years with a confirmed diagnosis of CF (by sweat chloride test and/or genetics) with a forced expiratory volume in 1 s (FEV_1) at least 50% of predicted, with stable CF disease as judged by study investigators, were eligible for the study. A complete list of inclusion and exclusion criteria is provided in the supplementary material.

Treatment and methodology

ION-827359 or placebo was given *via* a PARI eFlow© mesh nebuliser. In all cohorts, subjects were randomised in a 6:2 ratio to ION-827359 or placebo (phosphate-buffered saline solution for inhalation). In the single-dose healthy volunteer cohort, subjects received ION-827359 3 mg, 10 mg, 37.5 mg or 100 mg or matching placebo. The post-treatment evaluation period was from Day 2 to Day 30. In the multiple-dose healthy volunteer cohort, subjects received five doses of placebo or ION-827359 10 mg, 37.5 mg or 75 mg on Days 1, 4, 8, 15 and 22 or 10 doses of placebo or ION-827359 37.5 mg on Days 1, 3, 5, 8, 10, 12, 15, 17, 19 and 22. pwCF received five doses of placebo or ION-827359 10 mg, 37.5 mg, 75 mg or 100 mg on Days 1, 4, 8, 15 and 22. For the multiple-dose cohorts, the post-treatment evaluation period was Day 29 through Day 113.

End-points

Safety

The primary end-point was safety and tolerability, as measured by the number of subjects with at least one treatment-emergent adverse event (TEAE). Other safety end-points were adverse events, vital signs, physical examination findings, clinical laboratory tests, electrocardiogram (ECG), serial spirometry and use of concomitant medication.

Pharmacokinetics and pharmacodynamics

Noncompartmental analysis of ION-827359 PK was performed on individual subject data using Phoenix WinNonlin Version 8.3 (Pharsight Corp., Mountain View, CA, USA).

Bronchial brushings and bronchoalveolar lavage (BAL) fluid samples were obtained during fiberoptic bronchoscopy from the multiple-dose healthy volunteer cohort (before the first dose and within 48 h of the last dose). BAL was performed by wedging the bronchoscope into either the right middle lobe or lingula and infusing 100 mL of normal saline. The saline was gently extracted after minimal dwell time and collected for analysis.

PD end-points were change in cell count, differential and inflammatory biomarkers (interleukin (IL)-6, IL-10, IL-8, tumour necrosis factor (TNF) and interferon gamma–induced protein 10 (IP-10)) in BAL, and change in ENaC mRNA, as determined by quantitative polymerase chain reaction (qPCR) from bronchial brushings.

Spirometry parameters, including FEV_1 and forced vital capacity (FVC), were determined in pWCF, in accordance with American Thoracic Society/European Respiratory Society guidelines [21].

Sample size

The sample size was based on prior experience to ensure the safety, tolerability, PK and preliminary PD of ION-827359 would be adequately assessed while minimising unnecessary subject exposure. The planned enrolment was 96 subjects (32 subjects per cohort).

Statistical analysis

The safety population included all randomised subjects who received at least one dose of study medication. The per-protocol population included all randomised subjects in multiple-dose cohorts who received protocol-specified study medication. The PK population included all randomised subjects who received at least one dose of ION-827359 and had at least one evaluable PK sample collected and analysed with a reportable result.

Noncompartmental ION-827359 PK parameters were determined using the plasma data set from each individual subject. The amount and percentage of administered dose excreted in urine as ION-827359 following 24-h collection were determined. Plasma and urine PK parameters were summarised using descriptive statistics.

PD analyses were conducted in the safety and per-protocol populations from multiple-dose cohorts. Change and per cent change from baseline in BAL cell count, differential and inflammatory biomarkers, and ENaC mRNA levels were summarised using descriptive statistics and compared between the ION-827359 and pooled placebo groups using analysis of variance (ANOVA). If data departed substantially from normality, the Wilcoxon rank sum test and the Hodges–Lehmann estimator with corresponding confidence limits were used to assess treatment difference.

TEAEs, coded by system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA), Version 21.1, were analysed using descriptive statistics and reported as the number and percentage of subjects experiencing each TEAE, and based on severity and relationship to study treatment. Absolute values, change and per cent change from baseline were determined for laboratory tests, vital signs and ECG data. Change and percent change from baseline in spirometry and diffusing capacity assessments, and change and per cent change from Day 1 pre-dose values for spirometry assessments, were summarised using descriptive statistics and compared between ION-827359 and pooled placebo using ANOVA. If data departed substantially from normality, the Wilcoxon Rank Sum test and the Hodges–Lehmann estimator with corresponding confidence limits were used to assess treatment differences. Owing to the exploratory nature of this study, p-values and 95% CIs were not adjusted for multiplicity. All analyses were performed using SAS version 9.4.

Results

Study population

Healthy volunteers were enrolled at a single centre in the UK; pwCF were enrolled at six centres in the UK and Germany. The first subject was enrolled on 13 December 2018, and the last subject completed the study on 13 October 2020. Of 275 subjects screened, 98 met the inclusion and exclusion criteria and were randomised to treatment (figure 1). These included 32 out of 98 (33%) healthy volunteers in the single-dose cohort, 32 out of 98 (33%) healthy volunteers in the multiple-dose cohort and 34 out of 98 (35%) pwCF. One pwCF withdrew from the study before any treatment was given. Volunteers and patients were white, except for one Afro-Caribbean pwCF who received placebo (table 1). All pwCF were receiving treatment with other medications. Nearly all were using a β_2-agonist. Combinations of a long-acting β_2-agonist and inhaled corticosteroid were used by seven out of nine (78%) patients treated

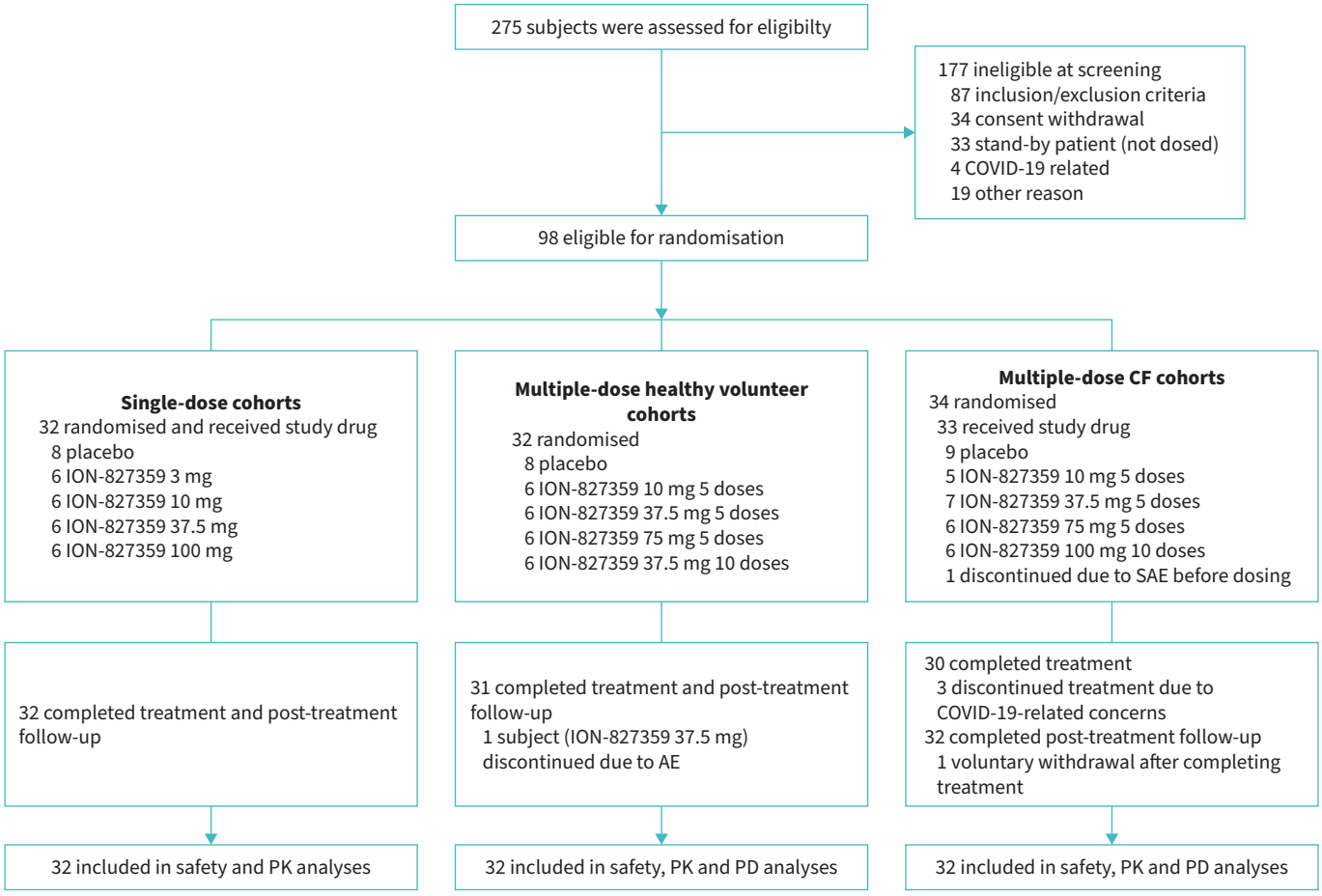

FIGURE 1 Patient flow chart. AE: adverse event; CF: cystic fibrosis; SAE: serious adverse event; PK: pharmacokinetics; PD: pharmacodynamics.

with placebo and nine out of 24 (38%) of those treated with ION-827359. Ivacaftor was used by nine out of 33 (27%) patients (placebo, four out of nine (44%); ION-827359, five out of 24 (21%)), ivacaftor with tezacaftor or lumacaftor was used by nine out of 33 (27%) subjects (placebo, three out of nine (33%); ION-827359, six out of 24 (25%)), inhaled DNase was used by 18 out of 33 (55%) subjects (placebo, three out of nine (33%); ION-827359, 15 out of 24 (63%)), and hypertonic saline was used by two out of 33 (6%) subjects (ION-827359, two out of 24 (8%)).

Safety

Administration of ION-827359 *via* nebuliser was well tolerated. The rate of TEAEs was similar after inhalation of ION-827359 and placebo (table 2). The majority of TEAEs were of mild to moderate severity; two severe TEAEs were reported for two pwCF receiving ION-827359 10 mg (infective pulmonary exacerbation of CF) and 100 mg (hypoglycaemic seizure). The most common drug-related TEAEs were headache and cough. Drug-related cough occurred in three out of 24 (13%) healthy volunteers and headache in two out of 24 (8%) pwCF who received ION-827359 (table 2).

No participants in any group discontinued study treatment due to a drug-related TEAE. No deaths or adverse events of concern occurred. One serious TEAE of infective pulmonary exacerbation was reported for a pwCF; this was considered unrelated to study drug treatment.

No abnormal trends in laboratory values were identified. No clinically significant changes or patterns were identified at any dose for chemistry, haematology, complement or urinalysis parameters in any cohort. In particular, no evidence was found of changes in serum potassium or 24-h urinary potassium excretion after treatment with ION-827359 compared with placebo. Also, no clinically significant changes in vital signs, ECG, body weight or body mass index occurred during the study. No subject had a >15% reduction in FEV_1 after drug administration (*i.e.*, no drug-induced bronchospasm occurred).

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TABLE 1 Demographic characteristics of study participants at baseline

		Treatment group					
	All subjects	**All placebo**	**All ION-827359**	**ION-827359 3 mg**	**ION-827359 10 mg**	**ION-827359 37.5 mg**	**ION-827359 100 mg**
Single-dose cohort (healthy volunteers)							
Subjects n	32	8	24	6	6	6	6
Age years	36±10 (23–50)	36±6 (27–44)	36±10 (23–50)	40±10 (23–49)	30±6 (25–40)	35±11 (23–49)	39±10 (26–50)
Male, n (%)	30 (94)	8 (100)	22 (92)	5 (83)	6 (100)	5 (83)	6 (100)
BMI kg·m^{-2}	26.7±3.4 (21.6–34.0)	26.9±1.4 (25.3–29.3)	26.6±3.9 (21.6–34.0)	25.9±1.6 (24.7–29.0)	25.3±4.4 (21.6–33.1)	26.3±4.5 (22.8–32.5)	29.0±4.1 (24.6–34.0)
Ethnicity, n (%)							
Hispanic or Latino	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other	32 (100)	8 (100)	24 (100)	6 (100)	6 (100)	6 (100)	6 (100)
Race, n (%)							
White	32 (100)	8 (100)	24 (100)	6 (100)	6 (100)	6 (100)	6 (100)
	All subjects	**All placebo**	**All ION-827359**	**ION-827359 5x 10 mg**	**ION-827359 5x 37.5 mg**	**ION-827359 5x 75 mg**	**ION-827359 10x 37.5 mg**
Multiple-dose cohort (healthy volunteers)							
Subjects n	32	8	24	6	6	6	6
Age years	38±7 (21–50)	38±8 (2–50)	39±7 (21–50)	42±6 (33–50)	33±7 (21–41)	39±3 (36–40)	41±10 (23–48)
Male, n (%)	29 (91)	8 (100)	21 (88)	5 (83)	6 (100)	6 (100)	4 (67)
Ethnicity, n (%)							
Hispanic or Latino	1 (3)	0 (0)	1 (4)	0 (0)	0 (0)	1 (17)	0 (0)
Other	31 (97)	8 (100)	23 (96)	6 (100)	6 (100)	5 (83)	6 (100)
Race, n (%)							
White	32 (100)	8 (100)	24 (100)	6 (100)	6 (100)	6 (100)	6 (100)
BMI kg·m^{-2}	26.7±3.4 (20.2–34.1)	25.7±3.2 (22.8–31.6)	27.1±3.4 (20.2–34.1)	28.6±3.3 (23.9–33.1)	26.0±3.2 (22.9–31.2)	25.5±3.5 (20.2–28.9)	28.3±3.4 (24.8–34.1)
	All subjects	**All placebo**	**All ION-827359**	**ION-827359 5× 10 mg**	**ION-827359 5× 37.5 mg**	**ION-827359 5× 75 mg**	**ION-827359 5× 100 mg**
Multiple-dose cohort (patients with CF)							
Subjects n	33	9	24	5	7	6	6
Age years	32±9 (20–57)	28±7 (20–39)	33±9 (21–57)	29±6 (21–37)	37±12 (23–57)	30±7 (22–41)	34±8 (24–45)
Male, n (%)	16 (48)	3 (33)	13 (54)	4 (80)	2 (29)	3 (50)	4 (67)
Ethnicity, n (%)							
Hispanic or Latino	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other	33 (100)	9 (100)	24 (100)	5 (100)	7 (100)	6 (100)	6 (100)
Race, n (%)							
White	32 (97)	8 (89)	24 (100)	5 (100)	7 (100)	6 (100)	6 (100)
Afro-Caribbean	1 (3)	1 (11)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BMI kg·m^{-2}	23.2±3.1 (16.1–31.4)	22.9±3.5 (19.4–29.7)	23.3±3.1 (16.1–31.4)	23.8±3.5 (19.3–29.2)	24.1±3.7 (21.3–31.4)	22.1±3.1 (16.1–24.6)	23.1±2.1 (19.1–25.1)

Values are mean±SD (range) or number of patients (%). BMI: body mass index; CF: cystic fibrosis.

TABLE 2 Summary of treatment-emergent adverse events (TEAEs)

	Any TEAE	Any drug-related TEAE[#]	Specific drug-related TEAEs[#]	
			Headache	Cough
Single-dose cohort (healthy volunteers)				
Placebo (n=8)	3 (38)	0 (0)	0 (0)	0 (0)
ION-827359				
3 mg (n=6)	1 (17)	0 (0)	0 (0)	0 (0)
10 mg (n=6)	3 (50)	2 (33)	1 (17)	0 (0)
37.5 mg (n=6)	3 (50)	0 (0)	0 (0)	0 (0)
100 mg (n=6)	1 (17)	0 (0)	0 (0)	0 (0)
Multiple-dose cohort (healthy volunteers)				
Placebo (n=8)	4 (50)	1 (13)	0 (0)	0 (0)
ION-827359				
5× 10 mg (n=6)	3 (50)	0 (0)	0 (0)	0 (0)
5× 37.5 mg (n=6)	0 (0)	0 (0)	0 (0)	0 (0)
5× 75 mg (n=6)	3 (50)	1 (17)	0 (0)	0 (0)
10× 37.5 mg (n=6)	6 (100)	3 (50)	0 (0)	3 (50)
Multiple-dose cohort (patients with CF)				
Placebo (n=9)	7 (78)	1 (11)	0 (0)	0 (0)
ION-827359				
5× 10 mg (n=5)	3 (60)	1 (20)	0 (0)	1 (20)
5× 37.5 mg (n=7)	6 (86)	2 (29)	1 (14)	0 (0)
5× 75 mg (n=6)	3 (50)	3 (50)	0 (0)	0 (0)
5× 100 mg (n=6)	6 (100)	1 (17)	1 (17)	0 (0)

Data are presented as number of patients (%). CF: cystic fibrosis. [#]: considered by the investigator to be related or possibility related to the study treatment.

Pharmacokinetics

After inhaled administration, ION-827359 was rapidly absorbed into the systemic circulation, with a t_{max} of 3–8 h (table 3). Mean C_{max} and total exposure (area under the curve) (AUC) were dose dependent but were greater than dose proportional over the studied dose range of 3–100 mg (table 3). After reaching C_{max}, mean plasma concentrations of ION-827359 declined over time in a biphasic manner, with an initial rapid distribution phase that dominated the plasma clearance, followed by a slower elimination phase with estimated half-life of ∼2 weeks (table 3).

There was no or little increase in mean plasma C_{max} or AUC after repeated inhalations compared with the first dose in healthy volunteers and patients with CF (table 4). PwCF showed more rapid absorption to the systemic circulation (table 4). Although plasma C_{max} was slightly (⩽20%) higher in pwCF compared with healthy volunteers, plasma total exposure (AUC) and trough levels (C_{min}) at equivalent doses were 20%–30% lower in pwCF *versus* healthy volunteers.

Only a small fraction of the administered dose (<0.05% after a single or first dose and <0.5% after the last multiple dose) was excreted in the urine of healthy volunteers and pwCF (table 3).

Pharmacodynamics

Multiple doses of ION-827359 were associated with dose-dependent reductions in ENaC mRNA levels in healthy volunteers, with a significantly greater reduction from baseline in the group treated with ION-827359 75 mg compared with placebo ($p<0.05$; figure 2). In this group, the mean change in ENaC mRNA from baseline to Day 23 was −55.6% (median −65.3%). The reduction in ENaC mRNA expression became more pronounced as ION-827359 trough levels increased (figure 3).

Bronchoalveolar lavage

No changes were observed in any parameter assessed in BAL fluid (cells or cytokines) after treatment with any dose of ION-827359. Cell counts and differential and inflammatory biomarker levels in BAL fluid at baseline and Day 23 in the multiple-dose healthy volunteer cohort are shown in supplementary figure S1. In addition, no notable differences occurred in diffusing capacity over time or as change from baseline between the placebo and ION-827359 groups in the multiple-dose volunteer and CF patient cohorts.

TABLE 3 Plasma pharmacokinetic parameters after single doses of ION-827359 in healthy volunteers

	ION-827359			
	3 mg	10 mg	37.5 mg	100 mg
Healthy volunteers n	6	6	6	6
C_{max} ng·mL^{-1}	0.914 (32.4)	6.19 (53.2)	35.9 (10.3)	150 (35.1)
t_{max} h	4.00 (2.98, 6.02)	3.01 (2.02, 4.15)	6.00 (1.50, 6.07)	6.00 (4.00, 8.00)
$AUC_{0–24h}$ ng·h·mL^{-1}	12.0 (42.9)	71.4 (48.8)	445 (81.6)	2289 (37.1)
$AUC_{0–168h}$ ng·h·mL^{-1}	15.9 (51.0)	88.2 (47.7)	545 (76.1)	2972 (38.6)
AUC_{last} ng·h·mL^{-1}	11.8 (45.9)	73.4 (54.3)	560 (78.8)	3092 (38.4)
$AUC_{0-\infty}$ ng·h·mL^{-1}	14,7 (45.0)	79.3 (51.1)	590 (82.6)	3280 (37.5)
$\%AUC_{extr}$ %	16.2 (62.2)	5.49 (125)	3.38 (153)	5.46 (33.8)
CL/F L·h^{-1}	NC	NC	45.0 (60.0)[¶]	38.5 (28.0)[¶]
$CL_{0–24h}$/F L·h^{-1}	0.270 (14.5)[+]	0.128 (46.6)[§]	0.0943 (85.1)[§]	0.0507 (29.9)[+]
$t_{1/2\lambda z}$[#] days	NC	NC	12.0 (21.8)[¶]	9.06 (32.6)[§]

Data are presented as geometric mean (geometric mean % coefficient of variation), apart from t_{max}, which is shown as median (range). C_{max}: maximum plasma concentration; t_{max}: time to maximum plasma concentration; $AUC_{0–24h}$: area under the concentration–time curve from 0 to 24 h; $AUC_{0–168h}$: area under the concentration–time curve from 0 to 168 h; $AUC_{0\text{-}last}$: area under the concentration–time curve from the first to the last quantifiable measurement; $AUC_{0-\infty}$: area under the concentration–time curve from the first to the last quantifiable concentration with extrapolation to infinity; $\%AUC_{extr}$: percentage of extrapolated AUC from T_{last} to infinity, expressed as percentage of $AUC_{0-\infty}$; CL/F: apparent plasma clearance after inhaled study drug administration (actual dose/$AUC_{0-\infty}$); NC: not calculated; $CL_{0–24h}$/F: apparent plasma clearance from time zero to 24 h after inhaled study drug administration (actual dose/$AUC_{0–24h}$); $t_{1/2\lambda z}$: apparent terminal elimination half-life. [#]: $t_{½\lambda z}$ was not included in descriptive statistics if r-square was <0.8 or there were <3 data points in the elimination phase because λz cannot be accurately defined; [¶]: n=3; [+]: n=4; [§]: n=5.

Lung function

The mean baseline FEV_1 across cohorts was 2.61 L (68.9% predicted) and mean FVC was 4.06 L (88.6% predicted) (supplementary table S1). There was no significant change from baseline in FEV_1 in any multiple-dose treatment group (figure 4). However, a dose-dependent trend in FEV_1 response to ION-827359 was observed for pwCF, with attenuation of the decline in FEV_1 over time from baseline to Day 29 (2.9% difference for ION-827359 *versus* placebo, p<0.27) (figure 4). Changes from baseline in FVC are shown in table 4.

Discussion

This study provides the first human data on the ASO drug ION-827359. The results showed that treatment with single and multiple inhaled doses was safe and well tolerated in healthy volunteers and pwCF. There was evidence of a mechanistic effect with significant and dose-dependent reduction in ENaC mRNA. PK parameters after inhaled administration showed rapid absorption, low systemic exposure and no drug accumulation after multiple doses.

For all subjects in this study there was a low incidence of TEAEs that were considered to be related to the study treatment. Most of the events were of mild or moderate severity, and the most common were headache and cough, although these only occurred in a low percentage of participants. All vital signs and laboratory parameters were clinically stable during the study. In particular, no hyperkalaemia was detected after treatment with ION-827359. Hyperkalaemia was previously reported with an earlier ENaC blocker (GS-9411) [22].

The initial distribution phase for ION-827359 after nebulised administration was characterised by rapid absorption from the lungs combined with an initial distribution phase, mainly reflecting extensive distribution to tissues from plasma. The slower terminal elimination phase is expected to mean that drug levels are in equilibrium with the major tissues of disposition (*i.e.*, lungs) and thus reflects drug accumulation and slow elimination of ION-827359 from tissues. Furthermore, generally comparable plasma concentration–time profiles were observed after first and last dose in the multiple-dose cohorts, which is consistent with the occurrence of little or no plasma accumulation and time-invariant plasma kinetics.

Plasma total exposure (AUC) and trough levels (C_{min}) after administration of equivalent ION-827359 doses were 20%–30% lower in pwCF compared to healthy volunteers, suggesting that changes in the airways due to CF may reduce the disposition and systemic absorption of ION-827359. It has been

TABLE 4 Plasma pharmacokinetic parameters after multiple doses of ION-827359 in healthy volunteers and patients with cystic fibrosis

	ION-827359 5× 10 mg	ION-827359 5× 37.5 mg	ION-827359 5× 75 mg	ION-827359 10× 37.5 mg
Healthy volunteers				
Volunteers n	6	6	6	6
C_{max} ng·mL^{-1}				
Day 1	6.5 (59.7)	50.1 (47.4)	134.0 (52.8)	28.5 (66.8)
Day 22	5.5 (57.6)	50.0 (39.3)	134.0 (64.3)	28.8 (70.9)
t_{max} h				
Day 1	4.0 (3.0–8.0)	6.0 (4.0–6.0)	4.0 (2.1–6.0)	6.0 (4.2–8.0)
Day 22	5.0 (3.0–8.0)	8.0 (4.0–8.0)	6.0 (4.0–6.0)	3.5 (3.0–6.0)
$AUC_{0–24h}$ ng·h·mL^{-1}				
Day 1	81.8 (54.7)	679.0 (38.8)	1836.0 (52.0)	432.0 (62.8)
Day 22	73.2 (62.5)	705.0 (39.2)	1788.0 (59.8)	401.0 (67.1)
$AUC_{0–168h}$ ng·h·mL^{-1}				
Day 1	NC	NC	NC	NC
Day 22	124.0 (62.7)	1198.0 (42.4)	2876.0 (63.1)	854.0 (49.8)
$CL_{0–24h}$/Fc L·h^{-1}				
Day 1	123.0 (54.7)	55.2 (38.8)	40.9 (52.0)	86.8 (62.8)
Day 22	137.0 (52.5)	53.2 (39.2)	42.0 (59.8)	93.5 (67.1)
CL_{ss}/F L·h^{-1}				
Day 1	NC	NC	NC	NC
Day 22	80.9 (62.7)	31.3 (42.4)	26.1 (63.1)	43.9 (49.8)
Vz/F L				
Day 1	NC	NC	NC	NC
Day 22	NC	13 352.0 (26.6)[¶]	16 532.0 (94.8)[+]	37 719.0 (82.6)[¶]
$t_{1/2\lambda z}$ days[#]				
Day 1	NC	NC	NC	NC
Day 22	NC	16.6 (25.3)[¶]	16.5 (2.5)[+]	16.5 (44.9)[¶]
C_{min} ng·mL^{-1}				
Day 1	NC	NC	NC	NC
Day 22	0.145 (44.1)[+]	0.694 (48.3)	1.32 (52.1)	1.56 (29.4)
	ION-827359 5× 10 mg	**ION-827359 5× 37.5 mg**	**ION-827359 5× 75 mg**	**ION-827359 5× 100 mg**
Patients with CF				
Patients n	5	7	6	6
C_{max} ng·mL^{-1}				
Day 1	8.1 (97.5)	56.0 (103.0)	159.0 (81.3)	216.0 (60.3)
Day 22	7.3 (134.0)	62.8 (80.0)	115.0 (58.6)	222.0 (59.9)
t_{max} h				
Day 1	1.58 (0.5–5.98)	2.0 (1.5–6.0)	2.0 (1.2–4.0)	2.0 (0.9–3.0)
Day 22	1.53 (1.0–2.0)	1.8 (1.0–2.2)	2.0 (1.6–2.1)	2.0 (1.0–3.1)
$AUC_{0–24h}$ ng·h·mL^{-1}				
Day 1	61.9 (90.0)	535.0 (64.6)	1493.0 (105.0)	1882.0 (64.4)
Day 22	56.2 (157.0)	513.0 (64.2)	1190.0 (86.3)	2103.0 (37.8)
$AUC_{0–168h}$ ng·h·mL^{-1}				
Day 1	NC	NC	NC	NC
Day 22	77.7 (187.0)	753.0 (60.2)	1964.0 (84.9)	3344.0 (37.8)
$CL_{0–24h}$/Fc L·h^{-1}				
Day 1	162.0 (90.0)	70.0 (64.6)	50.2 (105.0)	53.1 (64.4)
Day 22	179.0 (157.0)	73.1 (64.2)	63.0 (86.3)	47.5 (37.8)
CL_{ss}/F L·h^{-1}				
Day 1	NC	NC	NC	NC
Day 22	129.0 (187.0)	49.8 (60.2)	38.2 (84.9)	29.9 (37.5)
Vz/F L				
Day 1	NC	NC	NC	NC
Day 22	NC	34 226.0 (NA)[¶]	11 601.0 (NA)[¶]	18 494.0 (70.9)[§]
$t_{1/2\lambda z}$ days[#]				
Day 1	NC	NC	NC	NC
Day 22	NC	24.4 (NA)[¶]	9.3 (NA)[¶]	17.7 (42.9)[§]
C_{min} ng·mL^{-1}				
Day 1	NC	NC	NC	NC
Day 22	0.2 (NA)[¶]	0.4 (36.1)[+]	1.2 (82.4)	2.2 (80.8)

Data are presented as the geometric mean (geometric mean % coefficient of variation), apart from t_{max}, which is shown as median (range). C_{max}: maximum plasma concentration; t_{max}: time to maximum plasma concentration; $AUC_{0–24h}$: area under the concentration–time curve from 0–24 h; $AUC_{0–168h}$: area under the concentration–time curve from 0–168 h; NC: not calculated; $CL_{0–24h}$/Fc: apparent plasma clearance from time zero to 24 h after inhaled study drug administration (actual dose/$AUC_{0–24h}$); CL_{ss}/F: apparent plasma clearance at steady-state (actual dose/$AUC_{0–168h}$); Vz/F: apparent volume of distribution in the terminal phase; $t_{1/2\lambda z}$: apparent terminal elimination half-life; C_{min}: trough plasma concentration at steady state; CF: cystic fibrosis; NA: not applicable (data from two patients only). [#]: $t_{½\lambda z}$ was not included in descriptive statistics if r-square was <0.8 or there were <3 data points in the elimination phase because λz cannot be accurately defined; [¶]: n=2; [+]: n=3; [§]: n=5.

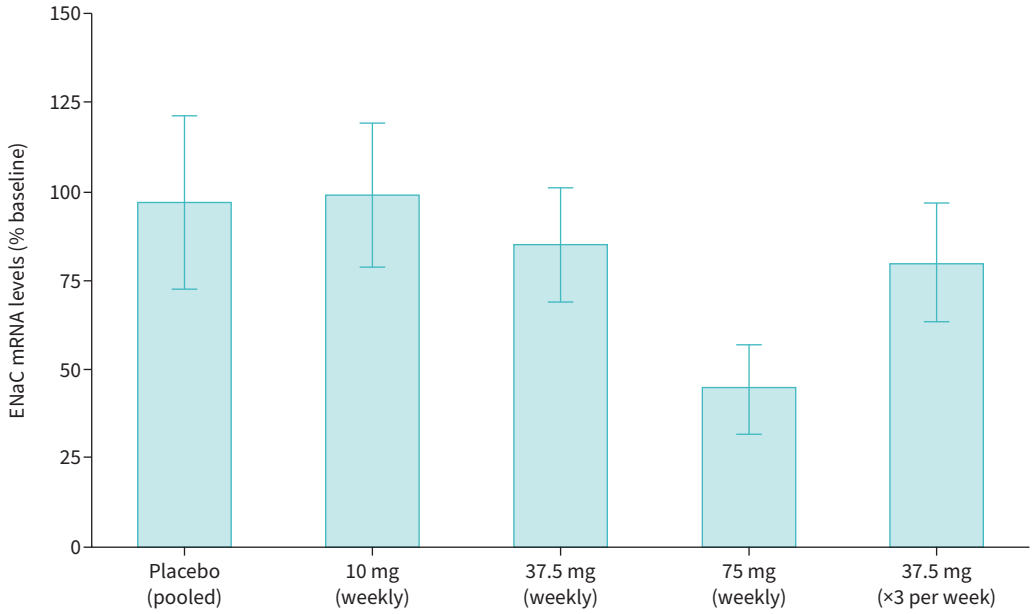

FIGURE 2 Epithelial sodium channel (ENaC) mRNA expression, shown as the mean±SE of the mean percentage of baseline (pretreatment screening), in cells from bronchial brushings at Day 23 after multiple doses of ION-827359 in healthy volunteers.

suggested that the failure of previous ENaC inhibitors to progress through clinical development may have been due to inadequate dosing and/or inadequate drug deposition after inhalation in pwCF because of the mucus plugging that is characteristic of this disease [23]. This creates both uneven distribution of inhaled compounds due to ventilation heterogeneity, as well as presents additional physical barriers to drug absorption and uptake in the form of a thickened mucus layer. Drug dosing needs to be adequate to overcome these issues, and although we cannot be certain to what extent mucus plugging is an issue for

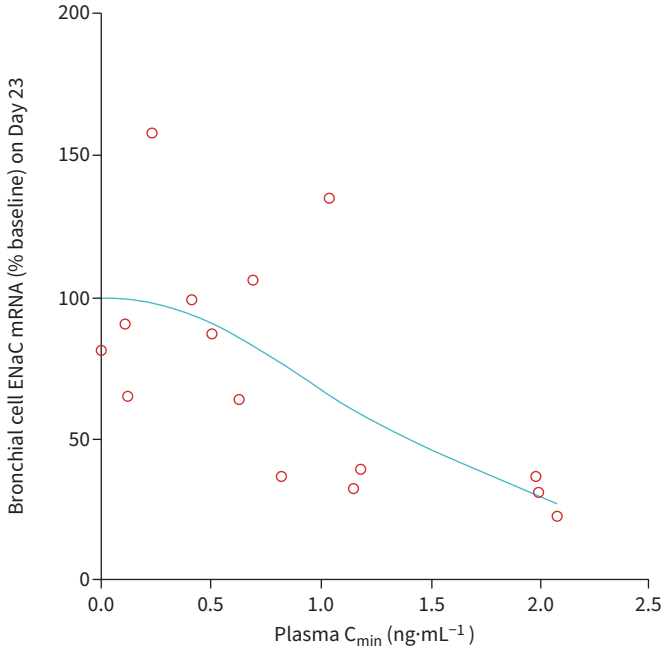

FIGURE 3 Relationship between the trough plasma concentration of ION-827359 (C_{min}) and epithelial sodium channel (ENaC) mRNA expression in cells from bronchial brushings.

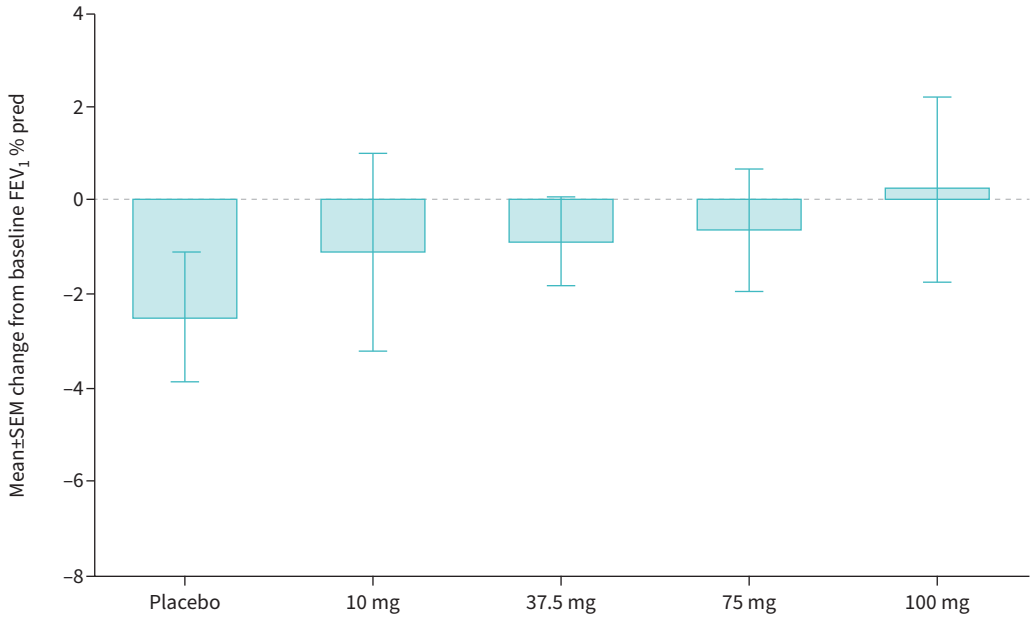

FIGURE 4 Change from baseline in % predicted forced expiratory volume in 1 s (FEV_1) in the multiple-dose cystic fibrosis cohort (safety population; n=33). Note: doses refer to those of ION-827359.

the pwCF in the study population, the data presented here support the use of a weekly ION-827359 dosage of 100 mg to ensure adequate drug levels to provide therapeutic activity.

The small fractional urinary excretion of ION-827359 seen after single and multiple doses in this study is consistent with low systemic absorption and therefore limited excretion of this compound. Low renal clearance over the first 24 h supports the hypothesis that initial plasma clearance is the result of rapid tissue uptake (primarily into the lungs) rather than excretion. Although not determined in this study, previous studies suggested that the primary route of elimination of ASOs is nuclease-mediated metabolism in tissues and excretion of shortened metabolites in urine as demonstrated with other ASOs in this chemical class [24, 25]. Once produced, these chain-shortened metabolites are rapidly eliminated in urine because they are expected to be less bound to plasma proteins. Therefore, the ultimate elimination of ION-827359 (albeit a prolonged process) is likely to consist of a combination of slow nuclease metabolism within tissues and subsequent excretion of shortened metabolites (as well as any remaining full-length oligonucleotide) into urine.

This is the first clinical study to demonstrate a reduction in target mRNA with an inhaled ASO. The PD results showed a mean 55.6% (median 65.3%) reduction in ENaC mRNA at Day 23 after treatment with the 75 mg weekly dosage of ION-827359. This is also likely to be clinically relevant, since mouse models of CF have demonstrated a significant improvement in mucus plugging and lung function with a 40% reduction in ENaC mRNA [19]. Based on the mechanism of action, ENaC inhibition is expected to increase the ASL layer. Human bronchial epithelial cells from CF patients treated with ENaC ASOs resulted in a significant increase in ASL volume (data not shown). Rehydrating airway surfaces with hypertonic saline has been shown to improve lung function and decrease the rate of pulmonary exacerbations by 50% [26]. Interestingly, ENaC mRNA reduction with a once-weekly dose was greater than that with a similar dose split into three administrations over 1 week. This further supports use of the 100 mg once weekly dose of ION-827359 in future studies.

18 of 33 pwCF were receiving treatment with CFTR modifiers (*i.e.*, ivacaftor, ivacaftor–tezacaftor, or ivacaftor–lumacaftor) at study enrolment. Given the different mechanisms of action of CFTR modifiers and ENaC inhibition, the addition of ION-827359 to CFTR modifier treatment could be expected to further address the underlying physiology of CF.

Key limitations of this study include the small sample size and variability in medications the patients with CF were receiving at baseline. The small sample size prohibits a comprehensive understanding of the

safety profile of ION-827359, and is a consequence of the phase 1/2a study design; this limitation will be addressed in larger, phase 3 studies. Both limitations influence the ability to assess efficacy of ION-827359. Larger studies may address variability in baseline medications by stratifying analyses according to baseline medication and/or restricting enrolment according to prior or current medications.

These results provide favourable evidence for the tolerability and safety of single and multiple doses of ION-827359 in this study. In addition, the PK and PD data support further investigations of ION-827359 as a potential treatment option for pwCF, regardless of the specific CTFR mutations they carry.

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Data availability: Data that underlie the results reported in this article and the respective individual participant data will not be shared.

This study is registered at https://eudract.ema.europa.eu/ with identifier number 2018-002621-27.

Author contributions: S. Sutharsan and K.B. Newman wrote the first draft of the manuscript. S. Sutharsan, R. Fischer, W. Gleiber, A. Horsley and J.S. Elborn enrolled participants and collected the data, which were analysed by the sponsor. All authors participated in the analysis and interpretation of study data, drafting and critically revising the manuscript for important intellectual content, and gave final approval of the manuscript for publication.

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