SUMMARY OF RESULTS

CLINICAL TRIAL INFORMATION	
Title of Study: Randomised, Double-Blind, Placebo Controlled, Two-Part Study to Evaluate the Efficacy, Safety, Tolerability and Pharmacokinetics of a Repeat Dose of Inhaled ETD001 in People with Cystic Fibrosis	
Study Identifiers: EU clinical trial number: 2023-504092-25-00 ClinicalTrials.gov identifier (NCT number): NCT06478706 ISRCTN Registry number: 11798668	
Protocol Number: ET-ENAC-03	
Sponsor: Enterprise Therapeutics Limited Science Park Square, Falmer Brighton, BN1 9SB	
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Paediatric Regulatory Details **Is trial part of an agreed paediatric investigation plan (PIP)?** No. **Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?** No. **Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?** No.	
Results Analysis **Intermediate Data Analysis Date:** Not applicable. **Interim or Final Stage of Analysis:** This report summarizes the final stage of analysis.	
Study Centres: 21 study centres in France, Germany, Italy, and the United Kingdom of which 20 consented at least 1 participant.	
Study Period: 26 June 2024 (First Participant First Visit) – 14 November 2025 (Last Participant Last Visit)	**Phase of Development:** 2

Background and Rationale for the Study:

ETD001 is a potent and selective blocker of the epithelial sodium channel (ENaC). It was postulated that the mucus dehydration caused by the defective cystic fibrosis transmembrane conductance regulator (CFTR) airway chloride channel could be improved by blocking the reabsorption of airway liquid sodium via ENaC. ETD001 has been optimised to achieve long retention in the lung airway lumen and low/slow systemic bioavailability when delivered by the inhaled route. Thus, administration of ETD001 by inhalation was expected to enable durable target engagement in clinical studies allowing ETD001 to be the first inhaled ENaC blocker to demonstrate clinical efficacy in people with cystic fibrosis (pwCF).

This was the first study of ETD001 in pwCF. The study comprised 2 parts and was designed to evaluate the efficacy of ETD001 relative to placebo, as well as its safety, tolerability, and pharmacokinetics (PK).

Part A served as a lead-in phase to assess the safety, tolerability, and PK of twice daily (BID) ETD001 administered for 7 days before longer treatment exposure in Part B.

Part B used a crossover design to evaluate the efficacy (lung function) and safety of ETD001 BID compared with placebo after 28 days of treatment. Accordingly, the Per-protocol Set (PPS) was designated as the primary analysis population, with supportive analyses planned using the Full Analysis Set (FAS) and modified Full Analysis Set (mFAS) to inform the design of future studies. Demonstration of improved lung function at a well-tolerated dose would support further clinical development of ETD001.

Objectives and Endpoints:

Objectives	Endpoints
Part A	
Primary	
• To assess the safety and tolerability of repeat inhaled doses of ETD001 in people with cystic fibrosis (pwCF), compared to placebo.	• Incidence of adverse event (AE), serious adverse event (SAE) (including death), withdrawals due to AE. • Change from baseline in safety assessments (ECG, vital signs, spirometry, clinical laboratory results, and physical examination).
Secondary	
• To characterise the plasma and urine pharmacokinetics (PK) following repeat inhaled doses of ETD001 in pwCF.	• Derived PK parameters in plasma and urine for ETD001 after repeat inhaled dose administration.
Part B	
Primary	
• To assess the effect of repeat inhaled doses of ETD001 on percent predicted forced expiratory volume in 1 second ($ppFEV_1$) in pwCF, compared to placebo.	• Absolute change in $ppFEV_1$ from baseline to Day 28 (for either treatment period 1 or treatment period 2), in the ETD001 group compared to placebo group.
Estimand to Address the Primary Objective (Part B): In relation to study Part B, the primary estimand was the difference in mean of absolute change from baseline in $ppFEV_1$, in pwCF who completed the study with no major protocol deviations, assessed after 28 days (for each of Treatment Period 1 and Treatment Period 2), with ETD001 compared to placebo, regardless of experience of intercurrent illness or exacerbations related to viral or bacterial infections.	
Secondary	
• To assess the effect of repeat inhaled doses of ETD001 on other lung function assessments including relative change in $ppFEV_1$, forced vital capacity (FVC), FEV_1/FVC ratio and maximal mid-expiratory flow rates (FEF_{25-75}), compared to placebo.	• Relative change in $ppFEV_1$ from baseline to Day 28 (for either Treatment Period 1 or Treatment Period 2), in the ETD001 group compared to placebo group. • Absolute change in other lung function endpoints including FVC, FEV_1/FVC ratio and FEF_{25-75} from baseline to Day 28 (for either Treatment Period 1 or Treatment Period 2), in the ETD001 group compared to placebo group.
• To assess the effect of repeat inhaled doses of ETD001 on safety and tolerability in pwCF, compared to placebo.	• Incidence of AE, SAE (including death), withdrawals due to AE. • Change in safety assessments (ECG, vital signs, clinical laboratory results, and physical examination) from baseline to Day 28 (for either Treatment

	Period 1 or Treatment Period 2), in the ETD001 group, compared to placebo group.
• To assess the effect of repeat inhaled doses of ETD001 on Cystic Fibrosis Questionnaire–Revised (CFQ-R) (respiratory domain) in pwCF, compared to placebo.	• Change in CFQ-R (respiratory domain) from baseline to Day 28 (for either Treatment Period 1 or Treatment Period 2) in the ETD001 group, compared to placebo group.
• To characterise the plasma PK via a population PK (Pop PK) approach following repeat inhaled doses of ETD001 in pwCF.	• Population PK model generated individual PK parameters.

Study Design:

This was a randomised, double-blind, cross-over study to evaluate the effect of repeated inhaled doses of ETD001 and placebo on lung function in people aged 18 years or older with CF lung disease and FEV_1 ≥40% and ≤90% of predicted normal. The study was run in 2 parts, Part A and Part B. Participants who completed Part A were allowed to take Part in Part B; however, a minimum of 28 days was required between Visit 4 of Part A and Visit 2 of Part B.

In Part A, 1 parallel cohort of 8 participants (6 active, 2 placebo) was planned to receive ETD001 4.5 mg or placebo BID for a period of 6 days with a final morning dose on Day 7. Safety, tolerability, and PK were assessed throughout the 7-day treatment period. Blinded review of pharmacokinetic data was performed by the Sponsor. An independent Data and Safety Monitoring Board (DSMB) evaluated safety and tolerability data prior to providing recommendation to initiate Part B.

Part B was a randomised, placebo-controlled two-sequence, two-period cross-over design and enrolled a sufficient number of pwCF to ensure that 32 participants completed both 28-day treatment periods.

In Part B, participants were randomised to receive BID doses of ETD001 4.5 mg or placebo on Day 1 to 27 with a final morning dose on Day 28. All participants received both treatments; the order in which participants received the 2 treatments was randomly assigned in a 1:1 fashion across 2 sequences; neither the Investigator nor the participant knew the treatment order. The 2 treatment periods were separated by a washout period of 28 days (± 7 days) to minimise any carry over effect. Review of unblinded safety data in the study was performed as specified in the DSMB charter.

Screening procedures were expected to be completed within the 28 days prior to receiving the first dose of investigational medicinal product (IMP) in the first sequence, with follow-up assessments performed 21 days (+/- 7 days) after the final dose of IMP.

Number of Participants Planned:

Up to 60 participants were planned to be enrolled to ensure that the required number of participants completed both study parts: Part A: 8 participants and Part B: 32 participants.

Diagnosis and Main Criteria for Inclusion and Exclusion:

This study enrolled adult male and female participants with a confirmed diagnosis of CF (positive sweat chloride value ≥60 mEq/L and/or genotype with 2 identifiable mutations consistent with CF, accompanied by 1 or more clinical features consistent with the CF phenotype), with a FEV_1 ≥40% and ≤90% of predicted normal with clinically stable CF lung disease, and who were ineligible for or not receiving CFTR modulators. Participants needed to be able to reproducibly perform spirometry manoeuvres and be able to use a nebuliser.

Participants were excluded from participation for the following reasons: recent changes in medication; any abnormalities suggesting unstable pulmonary disease other than CF seen on a chest x-ray within the past year; treatment with a CFTR modulator therapy within the past 60 days; inability to withhold use of long-acting bronchodilators or anticholinergics; using oral corticosteroids within 14 days prior to Visit 1 exceeding 10 mg per day or 20 mg every other day; anticipated need for the use of diuretics, renin-angiotensin aldosterone system antihypertensive drugs, angiotensin receptor blockers, drospirenone, or trimethoprim during the study.

Test Product, Dose, and Mode of Administration:

ETD001, 4.5 mg BID, delivered by oral inhalation via nebulisation of a 3.0 mg/mL solution.

Reference Therapy, Dose, and Mode of Administration:

Placebo, BID, delivered by oral inhalation via nebulisation.

Duration of Treatment:

The duration of treatment was 7 days for Part A and 56 days for Part B (28 days on ETD001 and 28 days on placebo).

Statistical Methods:

The sample size chosen for Part A of this study for the purposes of assessing safety and tolerability was based on feasibility and considered adequate to meet the study objective.

The absolute change from baseline to Day 28 in $ppFEV_1$ was defined as the primary endpoint in Part B. The change from baseline (Visit 2 or 5) to the end of treatment visit on Day 28 (Visit 4 or 7) was the primary comparison. The planned sample size of 32 participants completing both treatment periods in Part B provided approximately 95% power at a two-sided significance level of 0.05 to detect a treatment effect of 5% (difference between groups in absolute change in $ppFEV_1$) with a pooled standard deviation (SD) of 6% with a correlation of 0.2.

The primary endpoint, Part B, was analysed using a mixed model for repeated measures (MMRM) with change from baseline $ppFEV_1$ to Day 28.

Spirometry quality grading was applied in accordance with American Thoracic Society/European Respiratory Society (ATS/ERS) acceptability and repeatability criteria. For efficacy analyses, only pre-dose $ppFEV_1$ values with an ATS/ERS quality grade of A or B were included. Post-dose ppFEV1 results, irrespective of ATS/ERS quality grade, were used for assessment of tolerability.

Number of Participants Analysed:

A total of 71 participants consented to participate (Part A: 11 participants and Part B: 60 participants), and 57 participants were randomised (Part A: 10 participants and Part B: 47 participants). A total of 57 participants were treated (Part A: 10 participants and Part B: 47 participants) and 54 participants completed the study (Part A: 9 participants and Part B: 45 participants). In Part B, a total of 35 participants were analysed for efficacy in

the per-protocol analysis set and 47 participants were evaluated in the FAS. Safety was evaluated in 57 participants in the safety analysis set (SAF) (Part A: 10 participants and Part B: 47 participants).

SUMMARY OF RESULTS

Disposition and Demography:

Part A

A total of 11 participants were screened, 10 participants (7 participants in the ETD001 group, and 3 participants in the placebo group) were enrolled, and 9 participants (7 participants in the ETD001 group and 2 participants in the placebo group) completed Part A. One participant in the placebo group permanently discontinued the study.

In the SAF, there were a total of 4 male participants (57% ETD001 group, 0% placebo group) and 6 female participants (43% ETD001 group, 100% placebo group). In Part A, the median age for participants was 40.5 years (minimum: 20 years, maximum: 51 years). All participants (100%) were White, and not Hispanic or Latino.

Part B

A total of 60 participants were screened, 47 (78%) participants (24 participants in the ETD001/placebo group, and 23 participants in the placebo/ETD001 group) were enrolled, and 45 (96%) participants (22 participants in the ETD001/placebo group and 23 participants in the placebo/ETD001 group) completed Part B.

In the PPS, there were a total of 16 male participants (46%) and 19 female participants (54%), of which 18 (95%) were of childbearing potential. The median age was 33.0 years (minimum: 18 years, maximum: 52 years). The majority of participants (91%) identified as White, and not Hispanic or Latino. In Part B, the participants in the FAS and PPS analysis sets were similar in most demographic and baseline characteristics.

Number of Participants Per Member State

Country	Screened	Randomized	Completed
Part A			
Germany	9	8	7
Italy	0	0	0
France	0	0	0
UK	2	2	2
Total	**11**	**10**	**9**
Part B			
Germany	28	22	22
Italy	14	11	11
France	8	6	6
UK	10	8	6
Total	**60**	**47**	**45**

Pharmacokinetic Results:

Following inhaled administration of ETD001 in pwCF, plasma and urine pharmacokinetic data were collected to address the prespecified secondary objectives.

In Part A, following a single 4.5 mg ETD001 dose plasma sampling to 6 hours post dose demonstrated measurable systemic exposure, with plasma concentrations showing a geometric mean (CV%) C_{max} of 18.9 ng/mL (44.8%), occurring at a median (range) T_{max} of 1.54 hours (0.50 to 2.03 hours) post-dose, followed by a decline in plasma concentrations over time. Overall ETD001 systemic exposure was characterised using non-compartmental analysis with a geometric mean (CV%) $AUC_{(0\text{-last})}$ of 85.9 ng•h/mL (34.1%), and a geometric mean (CV%) $AUC_{(0\text{-tau})}$ of 132 ng•h/mL (33.8%). Following repeated BID dosing, measurable pre-dose plasma concentrations were observed, with a geometric mean (CV%) C_{trough} of 5.17 ng/mL (76.9%) on Day 6.

Urine pharmacokinetic assessments conducted in Part A indicated the geometric mean (CV%) cumulative amount excreted in urine over the 0 to 6 hours collection interval ($A_{e(0–6)}$) was 0.189 mg (137.3%), corresponding to a geometric mean (CV%) fraction of the nominal 4.5 mg dose excreted unchanged ($F_{e(0–6)}$) of 4.20% (137.3%), while the geometric mean (CV%) renal clearance was 2.01 L/h (124.8%).

In Part B, which utilised a crossover study design, sparse plasma pharmacokinetic sampling following single and repeated BID dosing demonstrated measurable ETD001 concentrations across visits. Observed plasma concentrations were consistent with the exposure range observed in Part A. Individual PK profiles and parameter estimates were predicted by population pharmacokinetic modelling.

Efficacy Results (Part B):

Primary Analysis (MMRM): In the primary efficacy outcome of least squares mean (LSM) absolute change in $ppFEV_1$ from baseline to Day 28, a clinically meaningful and statistically significant treatment difference of 3.4 percentage points (95% confidence interval [CI]: 1.1, 5.6; p = 0.0053) was observed in the PPS population in favour of participants when receiving ETD001 compared with when receiving placebo. No period effect was observed.

Sensitivity Analysis (Mixed Effects Model [MEM]): The results of the sensitivity analysis using the MEM for the PPS population, for absolute change in $ppFEV_1$ from baseline to Day 28 was supportive of the primary analysis (using MMRM), with a least squares (LS) mean difference of 3.0 percentage points (95% CI: 0.9, 5.2) in favour of ETD001.

Supplementary Analysis (MMRM): The results of the supplementary analysis in the FAS population were consistent with the results of the primary analysis. The LS mean for absolute change in $ppFEV_1$ from baseline to Day 28 in participants when receiving ETD001 was −0.3 ± 0.7 versus −3.0 ± 0.7 when receiving placebo, with a LS mean treatment difference of 2.7 percentage points (95% CI: 0.6, 4.7; p = 0.0112). The results of the supplementary analysis in the mFAS were also consistent with the results of the primary analysis, with a treatment difference of 3.5 percentage points (95% CI: 1.3, 5.7; p = 0.0030). These data demonstrate internal consistency indicative of robustness of treatment effect.

Subgroup Analysis: For all subgroup analyses (received hypertonic saline at baseline, received dornase alpha at baseline, and received both at baseline) in the PPS population, the results demonstrate a statistically significant difference in favour of ETD001 compared with placebo.

***Exploratory Analysis*:**

For the FAS population, including all pre-dose $ppFEV_1$ grades, the LS mean treatment difference in absolute change in $ppFEV_1$ from baseline to Day 28 was 3.0 percentage points (95% CI: 1.1, 4.9; p = 0.0022) in favour of ETD001. These data indicate that any variability in $ppFEV_1$ introduced due to inclusion of all grades did not impact the efficacy analysis outcome.

Evaluation of all $ppFEV_1$ values, including pre- to post-dose $ppFEV_1$ in the PPS, showed minimal change and good recovery, with the observed LS mean treatment difference in absolute change in AUC of $ppFEV_1$ from baseline to Day 28 of 567.0 (95% CI: 125.7, 1008.3; p = 0.0136) in favour of ETD001.

Least squares mean changes in absolute change in $ppFEV_1$ from baseline through Day 56 were similar when receiving ETD001 (−1.0 ± 1.0) and when receiving placebo (−0.30 ± 1.0), indicating lack of crossover effect.

***Secondary Analysis*:**

Evaluation of the LS mean relative change in $ppFEV_1$ from baseline to Day 28 in the PPS population was 5.591 percentage points (95% confidence interval [CI], 1.575, 9.607) in favour of ETD001.

The LS mean changes in $FEF_{25-75\%}$ from baseline to Day 28 were similar when comparing participants when receiving ETD001 (−0.00 ± 0.04) with participants when receiving placebo (−0.09 ± 0.04) in the PPS.

The LS mean change in FVC from baseline to Day 28 were similar when comparing ETD001 treated participants (−0.03 ± 0.03) with placebo treated participants (−0.10 ± 0.03) in the PPS.

The LS mean treatment difference from baseline to Day 28 in absolute change in the FEV_1/FVC ratio was 0.015 (95% CI: 0.002, 0.028; p = 0.0254) in the PPS. These data are consistent with a trend in reduction in airflow obstruction, with the potential for increased benefit with prolonged dosing with this novel mucokinetic agent.

***Other Efficacy Analysis*:** In the PPS, when receiving ETD001, participants' CFQ-R (respiratory domain) mean score (SD) change from baseline to Visit 4/Visit 7 in their respiratory symptoms was −2.1 (10.64) compared with −3.7 (16.01) in participants when receiving placebo. There appears to be a smaller decline in score when receiving ETD001. It is possible that the ETD001 mechanism of action associated beneficial properties of cough and mucus clearance are a confounding variable in the lack of improvement in score in this domain.

Evaluation of other domains indicated that of the 12 domains in this instrument, 6 are showing improvement when receiving ETD001 as compared to 1 when receiving placebo. The importance of these data will be better realized upon further evaluation of ETD001 in larger and longer duration studies.

Safety Results:

Part A

In the SAF, a total of 9 participants (6 participants [86%] in the ETD001 group and 3 participants [100%] in the placebo group) experienced TEAEs and 4 participants (2 participants [29%] in the ETD001 group and 2 participants [67%] in the placebo group) experienced treatment-related TEAEs. The majority of TEAEs reported were moderate or mild. By preferred term (PT), only 1 participant in the ETD001 group reported a severe TEAE of Colitis. No participants in the ETD001 group and 1 participant (33%) in the placebo group experienced an SAE. No deaths, TEAEs leading to drug or study discontinuation or clinically meaningful changes from baseline for haematology, chemistry, or vital sign parameters were reported.

Part B

In the SAF, TEAEs were reported in a total of 42 participants (89.0%) when receiving ETD001, including 22 participants (47.0%) with treatment-related TEAEs, and 29 participants (64.0%) when receiving placebo, including 11 participants (24%) with treatment-related TEAEs. The majority of TEAEs reported were moderate or mild.

By PT, 3 participants (6%), when receiving ETD001, reported severe TEAEs of Infective pulmonary exacerbation of CF (2 participants) and Dyspnoea (1 participant). One participant (2%), when receiving placebo, reported a severe TEAE of Antibiotic therapy.

A total of 8 participants (17%) when receiving ETD001 and 2 participants (4%) when receiving placebo experienced an SAE. One participant (2%) when receiving ETD001 experienced a treatment-related SAE of Dyspnoea.

Among participants that received ETD001, a single participant (2%) experienced a TEAE of Dyspnoea leading to study drug withdrawal. By PT, 2 participants (4%) when receiving ETD001 experienced TEAEs leading to study discontinuation: Dyspnoea and Infective pulmonary exacerbation of CF (1 participant each).

One AE of hyperkalaemia (single central laboratory value of 5.58mmol/L) was reported. No stopping rules for hyperkalaemia were met. No deaths or clinically meaningful changes from baseline for haematology, chemistry, or vital sign parameters were reported. Importantly, there did not appear to be any safety concerns related to electrolyte imbalances or cardiovascular AEs.

Conclusion:

- A clinically meaningful and statistically significant difference in the primary efficacy outcome of LSM absolute change in $ppFEV_1$ from baseline to Day 28 in favour of ETD001 was observed in the PPS and FAS analysis population.
- Secondary and supplementary endpoints also demonstrated treatment benefit in favour of ETD001, further confirming robustness of treatment effect observed in favour of ETD001 over a 28-day period.
- Overall, ETD001 was well-tolerated, with good study drug compliance, and a safety profile consistent with that expected in this CF study population receiving inhaled medicines.
- Measurable systemic exposure to ETD001 was observed following inhaled administration in pwCF with T_{max} ranging between 0.5 to 2 hours post single 4.5 mg dose.
- Overall, plasma exposure to ETD001 following single dose inhaled administration was associated with moderate interindividual variability for peak and total exposure measures, while pre-dose

concentrations following multiple doses exhibited greater variability, which may in part reflect the defined pre-dose sampling window (−2.5 to 0 hours).

- The geometric mean fraction of the ETD001 total nominal dose recovered in urine over 6 hours was low ($Fe_{(0-6)}$ of 4.20%), but is an underestimate considering the achieved pulmonary deposited dose will be lower.
- Sparse plasma PK data collected in Part B were consistent with the exposure range observed in Part A and were incorporated in the population PK analysis, enabling model-based estimation of individual PK profiles and exposure metrics for each participant and study day.
- In summary, this study provides clinical proof-of-concept (PoC) for ETD001, an ENaC blocker, in pwCF not eligible for or not receiving CFTR modulator treatment. The findings support further evaluation in larger and longer-term studies, including in a broader population.

ADDITIONAL INFORMATION

Global Substantial Modifications:

Protocol/Amendment	Issue Date
Version 1.0, Original Protocol	20 March 2023
Version 2.0, Amendment 1	15 September 2023
Version 3.0, Amendment 2	15 December 2023
Version 4.0, Amendment 3	13 January 2025
Version 5.0, Amendment 4	27 January 2025
Version 6.0, Amendment 5	16 May 2025

Global Interruption and re-starts:

None.

Limitations and Caveats:

This study had some limitations. The sample size was modest, and the treatment duration was limited to 28 days, precluding direct assessment of longer-term clinical outcomes. In addition, the study population excluded individuals receiving CFTR modulators; therefore, further investigation will be required to evaluate applicability of these data in a broader population of pwCF.

Declaration of Accuracy:

Enterprise Therapeutics Limited confirms that the information provided in this summary is accurate to the best of its knowledge.