Evaluation of elexacaftor–tezacaftor–ivacaftor treatment in individuals with cystic fibrosis and *CFTR*^{N1303K} in the USA: a prospective, multicentre, open-label, single-arm trial

George M Solomon, Rachel W Linnemann*, Rachel Rich, Ashleigh Streby, Brian Buehler, Eric Hunter, Kadambari Vijaykumar, William R Hunt, John J Brewington, Andras Rab, Shasha P Bai, Adrianna L Westbrook, Carmel McNicholas-Bevensee, Jong Hong, Candela Manfredi, Cristina Barilla, Shingo Suzuki, Brian R Davis, Eric J Sorscher*

Summary

Background CFTR modulators are approved for approximately 90% of people with cystic fibrosis in the USA and provide substantial clinical benefit. N1303K (Asn1303Lys), one of the most common class 2 CFTR defects, has not been approved for these therapies by any regulatory agency. Preclinical investigation by our laboratories showed N1303K CFTR activation with elexacaftor–tezacaftor–ivacaftor (ETI). In this trial, we evaluate whether ETI improves CFTR function, measured by sweat chloride and other clinical outcomes, in people with cystic fibrosis and *CFTR*^{N1303K}.

Methods In this prospective, open-label, single-arm trial, participants aged 12 years or older with cystic fibrosis encoding at least one N1303K variant and at least one CFTR^{N1303K} allele who were ineligible for modulator therapy by US Food and Drug Administration labelling were given ETI for 28 days followed by a 28-day washout period at two cystic fibrosis centres in the USA. Participants received two orally administered pills of 100 mg elexacaftor, 50 mg tezacaftor, and 75 mg ivacaftor once daily in the morning, and 150 mg ivacaftor once daily in the evening. The primary endpoint was mean change in sweat chloride from baseline up to day 28 compared with mixed-effects models. Secondary endpoints were changes in percentage of predicted FEV_1 ($ppFEV_1$), Cystic Fibrosis Questionnaire-Revised (CFQ-R) respiratory domain, BMI, and weight after ETI therapy. Safety was assessed in all participants who received at least one dose of the study drug and primary and secondary analyses were performed in all participants who took the study drug per protocol. The trial was registered at ClinicalTrials.gov (NCT03506061) and remains open for reporting purposes.

Findings Between June 7, 2022, and Oct 20, 2023, 20 participants (ten male and ten female) were enrolled and received ETI treatment. One participant was lost to follow-up but was included in intention-to-treat analyses. At 28 days, the mean sweat chloride reduction was –1·1 mmol/L (95% CI –5·3 to 3·1; p=0·61) with only one participant showing a sweat chloride decrease greater than 15 mmol/L. There was a mean increase in $ppFEV_1$ from baseline at day 28 of 9·5 percentage points (6·7–12·3; p<0·0001) with 15 (75%) participants showing at least a 5% increase in $ppFEV_1$. Improvements were also identified in mean CFQ-R respiratory domain score (20·8 increase [95% CI 11·9–29·8]; p<0·0001), BMI (0·4 kg/m² increase [0·2–0·7]; p=0·0017), and weight (1·0 kg increase [0·4–1·7]; p=0·0020) after 28 days of ETI treatment. 14 (70%) of 20 participants had adverse events (12 [60%] mild, one [5%] moderate), with one (5%) serious adverse event of hospitalisation attributed to pneumonia. No deaths were recorded in the study.

Interpretation Individuals with *CFTR*^{N1303K} showed no change in sweat chloride after 28 days of treatment with ETI. However, there were improvements in secondary clinical endpoints, which suggest clinical efficacy. Our approach provides support for the use of in vitro model systems to inform clinical trials for rare *CFTR* variants.

Funding The Cystic Fibrosis Foundation and the US National Institutes of Health.

Lancet Respir Med 2024; 12: 947–57

Published Online August 26, 2024 https://doi.org/10.1016/S2213-2600(24)00205-4

See Comment page 934

*Joint first authors

University of Alabama at Birmingham, Birmingham, AL, USA (G M Solomon MD, R Rich BS, K Vijaykumar MD, Prof C McNicholas-Bevensee PhD, C Barilla PhD, S Suzuki PhD, Prof B R Davis PhD); **Emory University, Atlanta, GA, USA** (R W Linnemann MD, A Streby MPH, B Buehler BS, E Hunter BS, W R Hunt MD, A Rab PhD, S P Bai PhD, A L Westbrook MPH, J Hong PhD, C Manfredi BS, Prof E J Sorscher MD); **Children's Healthcare of Atlanta, Atlanta, GA, USA** (R W Linnemann, Prof E J Sorscher); **Cincinnati Children's Hospital, Cincinnati, OH, USA** (J J Brewington MD); **University of Texas Health Science Center, Houston, TX, USA** (C Barilla, S Suzuki, Prof B R Davis)

Correspondence to: Dr George M Solomon, University of Alabama at Birmingham, Birmingham, AL 35294-0006, USA **gsolomon@uabmc.edu**

Introduction

Cystic fibrosis is a life-threatening genetic disorder resulting from variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.[1] Defects in CFTR prevent adequate chloride and bicarbonate transport in and out of cells.[1–3] CFTR modulator therapies have markedly improved the lives of many people with cystic fibrosis, particularly after approval by the US Food and Drug Administration (FDA) of elexacaftor–tezacaftor–ivacaftor (ETI; Trikafta, Vertex Pharmaceuticals, Boston, MA, USA) for adults and adolescents with one or more F508del (also known as Phe508del) alleles in 2019,[4–9] and subsequently for children aged at least 2 years in 2023. A large group of less common variants have also received regulatory approval in the USA for treatment with ETI.[10] With the expansion of modulators to many people with cystic fibrosis across the world, a substantial gap in access to

Research in context

Evidence before this study

Treatment with elexacaftor–tezacaftor–ivacaftor (ETI) provides substantial clinical benefit to many people with cystic fibrosis. N1303K (also known as Asn1303Lys), one of the most common *CFTR* variants, has not been approved for this therapy by regulatory agencies, and previous results examining in vitro responses to ETI have been variable. We searched PubMed for published studies assessing clinical outcomes of ETI treatment among individuals with cystic fibrosis and *CFTR*^{N1303K} from database inception to grant submission on July 21, 2021, and again on Feb 15, 2024. The search terms were "elexacaftor" and "N1303K". No language restrictions were applied. Published case reports and case series provided preliminary data suggesting improvement in some clinical endpoints. In addition, a preclinical investigation by our laboratories showed activation of N1303K CTFR^{N1303K} with ETI.

Added value of this study

This prospective, multicentre, open-label, single-arm trial evaluated treatment responses to ETI among 20 adolescents and adults with cystic fibrosis aged 12 years and older who had *CFTR*^{N1303K} and a second minimal function *CFTR* variant. We observed no significant reduction in sweat chloride after 28 days of ETI treatment. By contrast, we identified clinically meaningful improvements in secondary endpoints, including lung function, respiratory symptom scores, and nutritional outcomes, with return to pre-ETI baseline after the 28-day washout period. This trial design blends rigour with feasibility for testing ETI in rare *CFTR* variants—a setting in which new approaches are needed to expand access.

Implications of all the available evidence

Results from this prospective, open-label trial complement and expand on previous reports, all describing clinical benefits of ETI treatment for people with cystic fibrosis encoding N1303K, despite minimal to no reduction in sweat chloride. These data support further evaluation of ETI treatment for *CFTR*^{N1303K}. Future study is needed to understand the relationship between lung function and sweat chloride in individuals with *CFTR*^{N1303K}.

CFTR-directed therapies that improve pulmonary and overall health has emerged for individuals who remain ineligible for these treatments or whose socioeconomic background or geographical location leads to medication inaccessibility despite known efficacy.

Hundreds of variants in *CFTR* that are known to cause disease are rare or ultra-rare, presenting a considerable challenge to broadening access to CFTR modulator therapies. In 2020, the availability of CFTR modulators was advanced by an FDA approval process that considered registration based partly on ETI-responsive *CFTR* variants using the Fischer rat thyroid (FRT) cell system developed by Vertex Pharmaceuticals. Current drug approvals allow access for 177 additional distinct *CFTR* variants.[10] Notably, several of the most prevalent *CFTR* variants (including N1303K, also known as Asn1303Lys) failed to show adequate preclinical responsiveness and have not been included among the ETI approved genotypes. A complimentary but distinctly different FRT system described in 2024 showed that more than 650 *CFTR* variants might show promise for ETI responsiveness, many of which were not included in the list for FDA label expansion for ETI.[11] However, this FRT system is not currently approved for modulator expansion evaluation by the FDA or other regulatory agencies.

For more on **genetic variants in people with cystic fibrosis** see https://cftr2.org

Optimising appropriate access to approved treatment strategies such as CFTR modulators is the cornerstone of ongoing and successful efforts to substantially improve prognosis for people with cystic fibrosis. Small molecule-type approaches are also viewed as a crucial interim measure while more definitive nucleotide-based therapies are under development. So-called theratyping, which uses in vitro systems to identify additional modulator-responsive variants and insights into the molecular mechanism of the genotype, could help to augment regulatory approvals,[12] such as the newly published FRT system discussed herein.[11] Nevertheless, many people with cystic fibrosis are unable to gain access to modulator therapy because of high drug costs and restriction of insurance reimbursement to variants formally approved by regulatory agencies. With appropriate study design, small-scale clinical trials could guide future FDA labelling studies, improve patient access to these treatments,[12,13] and translate findings, such as those published by Bihler and colleagues,[11] into a new FRT model system.

N1303K is one of the most common variants in people with cystic fibrosis in the USA and is found in more than 2100 patients worldwide. N1303K CFTR is traditionally described as a maturational or processing (class 2) defect, similar to F508del. Notably, N1303K pathogenesis is not strictly limited to poor protein maturation, but also involves a channel-gating defect reminiscent of the class 3 variant G551D (also known as Gly551Asp), along with plasma membrane instability.[14] Preclinical investigation by our laboratories (to be published as a separate investigation) showed strong activation of N1303K CFTR with ETI in our investigative group's FRT cell system—a third, unique FRT system with stable transgene expression that standardises CFTR expression—and induced pluripotent stem cells (iPSCs) differentiated into airway epithelial monolayers. Although differing from the data showing 9·5% wild-type rescue by Bihler and colleagues,[11] our findings in

this complimentary FRT system coupled with positive data from airway epithelial cells derived from a donor with *CFTR*^{N1303K} led us to conclude there was the possibility of clinically relevant CFTR activation. On the basis of these findings, we hypothesised that ETI would activate N1303K CFTR in people with cystic fibrosis, resulting in a reduction of sweat chloride and clinical improvement when at least one N1303K allele is present. To investigate these findings further, we conducted a preclinical investigation followed by a prospective, multicentre, open-label, single-arm clinical trial to evaluate the efficacy of ETI as measured by CFTR restoration in individuals with cystic fibrosis and an N1303K variant who are currently without an FDA-approved genotype for modulator therapy.

Methods

Study design and participants

We conducted a preclinical investigation followed by a single-arm clinical trial. In the preclinical phase, a skin biopsy from a single person with cystic fibrosis (with a N1303K–W1282X genotype) was collected and de-identified for further processing to assess the in vitro response of N1303K CFTR to ETI before initiating the clinical trial.

After the preclinical phase was complete, we conducted a prospective, multicentre, single-arm, open-label clinical trial of ETI for individuals with cystic fibrosis and *CFTR*^{N1303K}. The study design is presented in the appendix (pp 9, 12–16). The study was conducted at two US cystic fibrosis centres, Emory University (Atlanta, GA) and University of Alabama at Birmingham (Birmingham, AL), and people with cystic fibrosis were referred to participate by 15 cystic fibrosis centres in 13 states across the USA. The planned population size was 20 participants under the parent protocol. This study was sanctioned by the Cystic Fibrosis Foundation Therapeutic Development Network (TDN). Participants were referred via the Cystic Fibrosis Foundation TDN, regional research referral consortia, or direct contact between clinical teams and study principal investigators. Each site enrolled ten participants; eligible participants were 12 years or older and carried at least one copy of the *CFTR*^{N1303K} variant and a second minimal function *CFTR* variant on the other allele, as defined in previous CFTR modulator publications.[4,5] Furthermore, reassurance about the designation of the second *CFTR* variant as a minimal function variant was provided by published data from several laboratories in various in vitro systems[11] that indicated minimal activation of CFTR in all of the second variants of enrolled participants and the expectation of negligible CFTR protein production for loss-of-function alleles (eg, severe splice variants or nonsense mutations). Only patients with genotypes not approved by the FDA for CFTR modulator therapies were included in the study. Individuals eligible for enrolment had acceptable spirometry and were clinically stable with baseline symptoms and no recent exacerbations for at least 4 weeks before screening and before the first dose of study drug. Complete inclusion and exclusion criteria are shown in the appendix (p 3). Sex data were self-reported by participants. The study protocol was approved by institutional review boards at both sites (University of Alabama at Birmingham institutional review board 300001205; Emory University institutional review board 00108656), and all participants provided written informed consent (and assent when appropriate) before participation. The complete protocol synopsis is included in the appendix (pp 12–16). The study was registered with ClinicalTrials.gov (NCT03506061).

For the **study protocol** see https://clinicaltrials.gov/study/NCT03506061

Procedures

In the preclinical phase, tissue was shipped to the Clinical Translational Core at Baylor College of Medicine (BCM; Houston, TX, USA) for the isolation of fibroblasts, which were then used for iPSC reprogramming at the Advanced Technologies Human Stem Cell Core or the Human Stem Cell and Neuronal Differentiation Core at BCM. Non-cystic fibrosis iPSCs F20 (021iPS) and M22 (BCMi-001, 003iPS) were also obtained. The iPSCs were cultured according to standard feeder-free conditions on human embryonic stem cell-qualified Matrigel (Corning, NY, USA) in mTeSR1 medium (Stem Cell Technologies, Vancouver, BC, Canada) following manufacturers' standard protocols. The human iPSC lines used for differentiation were confirmed to be pluripotent through a Pluritest assay (ThermoFisher Scientific, Waltham, MA, USA) and verified as karyotypically normal (Karyostat assay, ThermoFisher Scientific).

See **Online** for appendix

The iPSCs from a single donor with an N1303K–W1282X *CFTR* genotype were differentiated into airway basal cells (iBCs) with previous protocols from our laboratory group.[15,16] The generated iBCs were seeded on Transwell plates (Corning) and cultured for approximately 5 days before maturation into pseudostratified airway epithelium with 2 subsequent weeks of air–liquid interface culture.

FRT clonal cells with stable expression of N1303K or wild-type CFTR have been established previously.[17] The cells were cultured in Coon's modification of Nutrient Mixture F-12 Ham (F6636; Sigma, Burlington, MA, USA) supplemented with 2·68 g/L sodium bicarbonate in the presence of 5% fetal bovine serum (Gibco, Waltham, MA, USA), and were maintained at 37°C in a humidified atmosphere, with 5% CO_2 and 95% air. To establish cell culture for functional studies, FRT cells were seeded onto Transwell permeable supports and cultured for 5 days until a well polarised monolayer was formed (with trans-epithelial resistance of at least 400 Ω×cm²).

48 h before CFTR functional experiments, FRT cell monolayers were treated with 5 μM VX-661 (S7059; Selleckchem, Houston, TX, USA) and 5 μM VX-445 (S8851; Selleckchem). For iPSC-derived airway epithelial bioelectric measurements, 3 μM VX-661, 5 μM VX-445, and 1 μM VX-770 (S1144; Selleckchem) were administered 48 h before analysis. In both cases, polarised cell monolayers were evaluated using an EasyMount Ussing

Chamber System (Physiologic Instruments, Reno, NV, USA). At the beginning of each recording, monolayers were transferred to Ussing chambers and bathed basolaterally in Ringer's solution containing 120 mM NaCl, 25 mM $NaHCO_3$, 3·33 mM KH_2PO_4, 0·83 mM K_2HPO_4, 1·2 mM $CaCl_2$, 1·2 mM $MgCl_2$, and 10 mM D-glucose (pH 7·4). In the apical buffer, NaCl concentration was decreased to 1·2 mM and supplemented with 140 mM sodium-gluconate to establish a chloride secretory gradient. The temperature of bathing solutions was maintained at 37°C and stirred by bubbling 5% CO_2 and 95% O_2. Once baseline readings were established and stabilised, 100 µM amiloride (A7410; MilliporeSigma, Burlington, MA, USA) was applied to both apical and basolateral monolayer surfaces to inhibit epithelial sodium channel (ENaC) activity. CFTR protein was activated by applying colforsin (commonly known as forskolin; 5 µM) for FRT and colforsin (10 µM) for iPSC-derived monolayers (F3917; MilliporeSigma). In FRT experiments, 1 µM VX-770 was also acutely added to the apical membrane surface. At the end of each recording, 10 µM $CFTR_{inh}$-172 (C2992; MilliporeSigma) was administered apically to inhibit CFTR-mediated transepithelial ion transport. Short circuit current was measured under voltage clamp conditions and change in short circuit current calculated and expressed as mean plus or minus SD compared with vehicle control (dimethyl sulfoxide).

In the clinical trial phase, participants received the study drug (two pills once daily in the morning of 100 mg elexacaftor, 50 mg tezacaftor, and 75 mg ivacaftor, and 150 mg ivacaftor once daily in the evening) by oral administration for 28 days. Dose and administration guidelines with fat-containing food were identical to what has been FDA approved for Trikafta for adults and children aged 12 years or older.[4,5]

Study participants completed study visits at screening (one visit within 28 days before commencement of the study period), baseline (day 1), day 14, day 28, and day 56 (end of the washout period, during which no trial

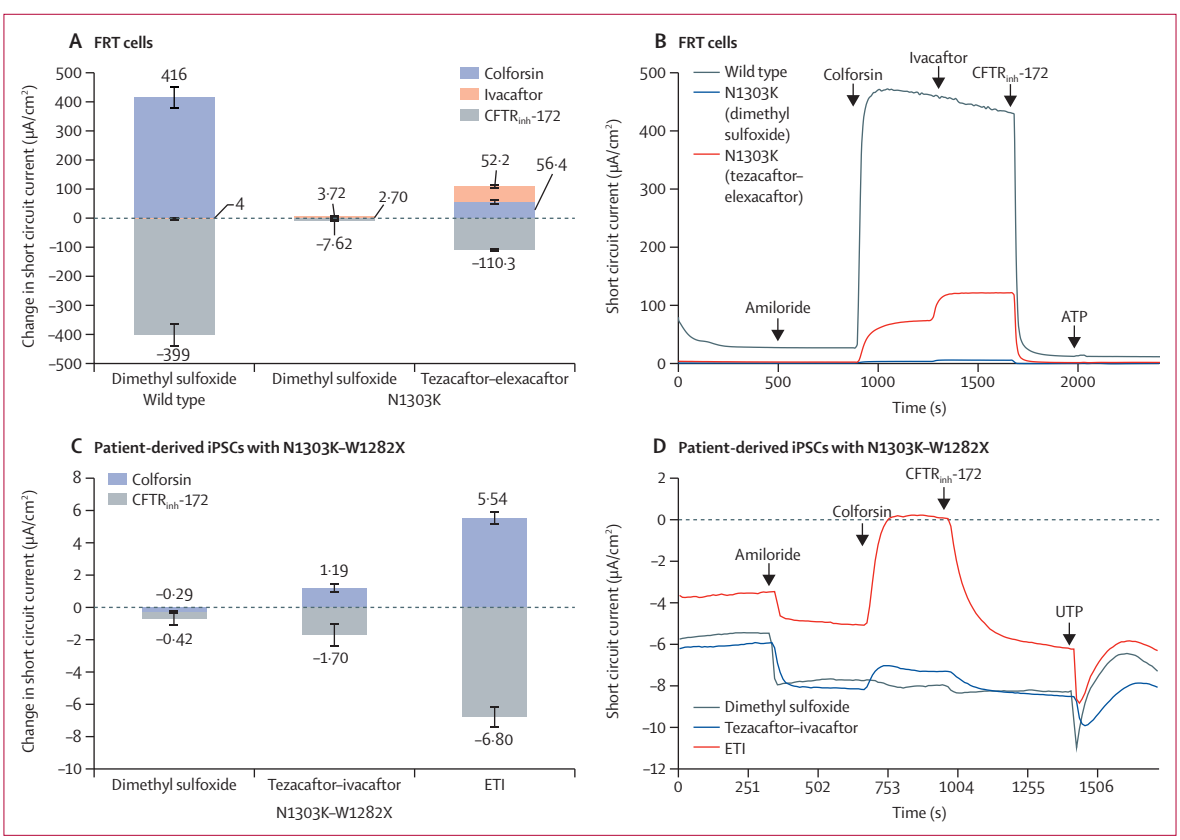

Figure 1: **N1303K CFTR correction by ETI in cell-based systems in vitro**
Data are mean (SD). (A) Efficient functional correction (26% wild-type activity) for N1303K CFTR mediated by ETI on the FRT background (n=3). (B) Representative tracings depict short circuit currents attributable to acute addition of colforsin (commonly known as forskolin; 10 µM), ivacaftor (1 µM; VX-770), and $CFTR_{inh}$-172 (10 µM) on wild-type or N1303K CFTR on the FRT cells. (C) N1303K CFTR activity in patient-derived airway epithelial monolayers differentiated from iPSCs. A minimal function (non-ETI responsive) variant (W1282X) is present on the second allele. ETI (24% of wild-type activity) displays a superior correction efficiency compared with tezacaftor–ivacaftor (5% of wild-type activity; n=11–12). (D) Representative tracings show CFTR-dependent chloride transport stimulated by colforsin (10 µM) and inhibited by $CFTR_{inh}$-172 (10 µM) in patient-derived airway epithelial monolayers differentiated from iPSCs. Nucleosides such as ATP and UTP are added to stimulate any non-CFTR channels present in these cell systems. ETI=elexacaftor–tezacaftor–ivacaftor. FRT=Fischer rat thyroid. iPSC=induced pluripotent stem cell. UTP=uridine triphosphate.

drug is administered). Baseline visits were conducted on the same day as drug administration in consistent clinical research environments. A remote telephone visit at day 7 was also done to assess adverse events. The schedule of testing for each visit is displayed in the appendix (pp 4, 9). Study procedures included routine safety and efficacy measures for monitoring ETI therapy in clinical trials, with the addition of a low-risk skin punch biopsy, peripheral blood sample, or both, to generate iPSC-derived airway epithelial monolayers. Adherence to the study drug was monitored via drug tally and accountability at each visit.

All participants underwent safety assessments including the monitoring of adverse events, clinical laboratory results, vital signs, pulse oximetry, electrocardiograms, physical examinations, and ophthalmological studies (for participants aged <18 years). Adverse events or serious adverse events were reported according to local institutional review board regulations. The study was monitored by an independent data safety monitoring committee, which conducted semi-annual safety reviews of individual-level study data and any new protocol deviations. Additional (interim) reviews by the data safety monitoring committee were performed once the first four participants had completed the 28-day drug administration, again after ten participants had completed the overall trial, and then at trial completion. Except for prespecified data required at previously specified intervals by the data safety monitoring committee, the authors were masked to the per-protocol analyses until the end of trial conduct.

Outcomes

The primary endpoint of this study was absolute change in sweat chloride (mmol/L) from baseline (day 1) up to day 28, complemented by an analysis of the proportion of participants with more than 15 mmol/L change at day 28. Clinical (secondary) endpoints were the absolute change in percentage of predicted FEV_1 ($ppFEV_1$), quality-of-life symptom scores (assessed with the Cystic Fibrosis Questionnaire-Revised [CFQ-R] respiratory domain),[18] BMI (in kg/m²), and weight (in kg) from baseline (day 1) up to day 28. CFQ-R respiratory domain scores range from 0 to 100, with a minimum clinically important difference of 4 points or higher representing improved respiratory-related quality of life.[19] Safety was established by the number and severity of adverse events. Changes in all endpoints from baseline up to days 14 and 56 and day 28 up to day 56 were also assessed as post hoc analyses. In vitro responses of iPSC-derived airway epithelial monolayers will also represent a key endpoint for correlation with clinical data. The development, optimisation, and predictive values of this new assay are ongoing and additional iPSC results will be reported separately.

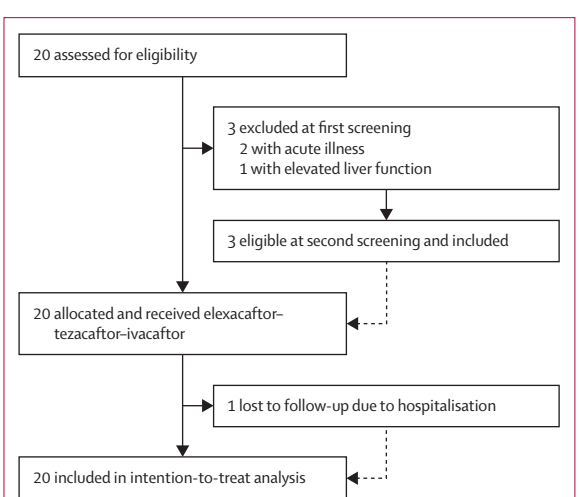

Figure 2: **Trial profile**

	Participants (N=20)
Mean age, years (SD)	25 (11)
Age distribution, years	
12 to <18	8 (40%)
≥18	12 (60%)
Sex	
Female	10 (50%)
Male	10 (50%)
Race	
Asian	0
Native American or Alaskan Native	0
Black or African American	0
Native Hawaiian or other Pacific Islander	0
Other	1 (5%)
More than one race	1 (5%)
White	18 (90%)
Ethnicity	
Hispanic or Latino	2 (10%)
Not Hispanic or Latino	18 (90%)
Cystic fibrosis medical history	
Pancreatic insufficiency	20 (100%)
Cystic fibrosis-related diabetes	4 (20%)
Chronic sinusitis	12 (60%)
Mean $ppFEV_1$ (SD)	76% (20)
$ppFEV_1$ distribution	
<50%	4 (20%)
50 to <75%	3 (15%)
≥75%	13 (65%)
Mean sweat chloride, mmol/L (SD)	109 (9)

Data are n (%) unless otherwise specified. All participants had an N1303K and minimal function *CFTR* genotype. Minimal function variants were: W1282X (n=5), G542X (n=2), I507del (n=2), E60X (n=1), 2622+1G→A (n=1), 3905insT (n=1), 1717-1G→A (n=1), Q1100P (n=1), 2184delA (n=1), 2184insA (n=1), 711+1G→T (n=1), 1154insTC (n=1), 3120+1G→A (n=1), and CFTRdele17a,17b (n=1). $ppFEV_1$=percentage of predicted FEV_1.

Table 1: **Demographic and clinical characteristics of participants at baseline**

Statistical analysis

With a sample size of 20 participants, we estimated 99% power to detect a mean sweat chloride decrease of 10 mmol/L assuming an SD of 9·7 mmol/L[20,21] under a one-sided 0·025 α-level *t* test. Categorical data are provided as counts and percentages whereas continuous data are shown as median (with IQR) or mean (with SD) and range. The absolute change from baseline to day 28 in sweat chloride, a primary outcome, was analysed through a mixed-effects model in which visit, baseline sweat chloride, age at screening, and sex were treated as fixed parameters because they are known to affect lung function variability. All analyses were conducted in the intention-to-treat population, which was all participants who received at least one dose of the study drug. The model used a compound symmetry covariance structure and random intercept to account for repeated measures within participants. Secondary outcomes such as $ppFEV_1$, CFQ-R respiratory domain, BMI, and weight were analysed with similar mixed-effects models. Least-squares means were shown for days 1 and 28, which correspond to baseline and treatment completion, and least-squares mean differences between timepoints of interest were calculated. The p values associated with the least-squares mean differences indicate whether differences were significantly different from zero. A p value less than 0·05 was considered to indicate a statistically significant difference, and tests were two-sided.

$ppFEV_1$ was calculated with the Global Lung Function Initiative 2012 equation based on age, height, race, sex, and FEV_1.[22] The R package rspiro was used for calculations. The respiratory domain score was calculated based on the CFQ-R respiratory domain. Adults and adolescents older than 14 years were scored according to the adult version of CFQ-R, whereas children 13 years and younger were scored with the child-directed version. Data management and figure generation were performed with R version 4.1.3 and mixed-effects models evaluated in SAS version 9.4. The complete statistical analysis plan is available in the appendix (pp 17–35).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or decision to submit the manuscript.

Results

In the preclinical investigation, FRT cells stably expressing the *CFTR*^{N1303K} genotype showed a response to ETI equal to 26% of that resulting from wild-type CFTR

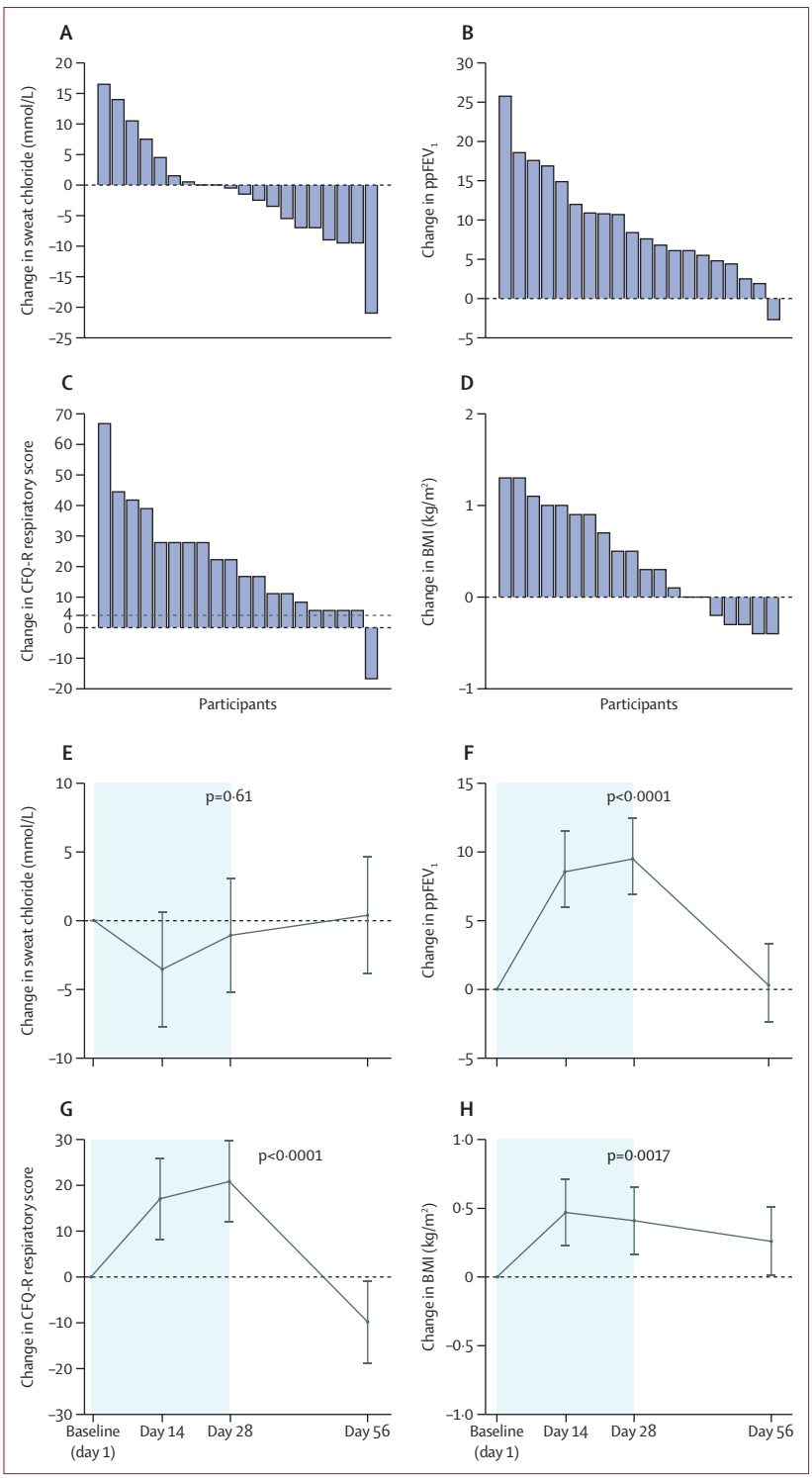

Figure 3: **Efficacy endpoints compared with baseline, per individual patient and over time**

Waterfall plots of change in sweat chloride (A), $ppFEV_1$ (B), CFQ-R respiratory domain score (C), and BMI (D) between baseline and day 28 in each participant. The dotted line in C represents the minimum clinically important difference of +4 points established for cystic fibrosis. Mean change in sweat chloride (E), $ppFEV_1$ (F), CFQ-R respiratory domain score (G), and BMI (H) from baseline to day 56. Shaded areas indicate the time participants were on ETI. Error bars show 95% CI. Significance assessed by mixed-effects adjusted paired *t* test at day 28 versus baseline. CFQ-R=Cystic Fibrosis Questionnaire-Revised. ETI=elexacaftor–tezacaftor–ivacaftor. $ppFEV_1$=percentage of predicted FEV_1.

expression (figure 1). ETI responsiveness of airway epithelium derived from cystic fibrosis iPSCs carrying the N1303K–W1282X genotype from one individual was 24% of that of non-cystic fibrosis iPSC-derived cultures (figure 1); the W1282X allele was not expected to give rise to CFTR activity in either the presence or the absence of ETI. On the basis of these data from two distinct cellular systems, we hypothesised the amount by which the N1303K variant responded to ETI.

A total of 20 participants were enrolled in the subsequent trial across the two sites between June 7, 2022, and Oct 20, 2023 (figure 2). Three participants were excluded at the initial screening due to acute illness (two [10%]) and elevated liver function (one [5%]) but were eligible upon repeat screening and therefore included. Participants had more than 99% adherence to the study drug regimen (only one participant missed two doses). Of the 20 participants, 19 (95%) completed the study up to day 56. All 20 participants were included in analyses of primary and secondary outcomes. One participant withdrew after day 28 due to illness requiring hospitalisation and did not complete the day 56 follow-up visit (figure 2). This participant was included in the analysis given that mixed-effects models use the available data from all participants through maximum likelihood estimation. There were no missing data for outcomes from baseline up to day 28. Baseline demographics and clinical characteristics of participants are summarised in table 1 and the appendix (pp 5–6). All participants enrolled had a N1303K–minimal function genotype. Minimal function variants are detailed in table 1. Of the study participants, ten (50%) were female, 12 (60%) were adults 18 years or older, 18 (90%) were White, and two (10%) were Hispanic or Latino. The population represented typical cystic fibrosis comorbidities (table 1).

Treatment with ETI did not result in a significant reduction in sweat chloride from baseline up to day 28 (mean reduction 1·1 mmol/L [95% CI –5·3 to 3·1]; p=0·61; figure 3A, table 2). However, we observed significant improvements in $ppFEV_1$ from 75·8% (73·3–78·3) at baseline to 85·3% (82·8–87·8) at day 28 of treatment, a difference of 9·5 percentage points (6·7–12·3; p<0·0001; figure 3B, table 2), which returned to baseline during the washout period. In addition, CFQ-R respiratory domain scores improved by a mean of 20·8 (11·9–29·8; p<0·0001; figure 3C, table 2), BMI increased by 0·4 kg/m² (0·2–0·7; p=0·0017; figure 3D, table 2), and weight increased by 1·0 kg (0·4–1·7; p=0·0020; table 2) at day 28 compared with baseline. Individual participants' data at day 28 are reported in the appendix (p 7).

A co-primary endpoint was the proportion of participants with a reduction in sweat chloride of more than 15 mmol/L, and waterfall plots showed that only one (5%) participant met that threshold (figure 3A). By contrast, 19 (95%) had improvements in $ppFEV_1$, with 15 (75%) having at least 5% improvement compared with baseline measurements (figure 3B). Similarly, 19 (95%) participants had positive improvements in CFQ-R respiratory domain score (figure 3C), all with benefit equal to or greater than the minimum clinically important difference of 4 points or greater.[19] Additionally, most participants (15 [75%]) had improvements in BMI (figure 3D) and ten (50%) had weight gain greater than 1 kg at day 28.

Changes in endpoints from baseline up to day 14 were similar to changes observed at day 28. At day 56 after the washout period, $ppFEV_1$, CFQ-R respiratory domain, BMI, and weight endpoints showed regression to values similar to baseline for most participants (figure 3E–H; appendix p 8). In a post-hoc analysis, no discernible correlation was observed between baseline $ppFEV_1$ and

	Baseline (day 1)	Treatment end (day 28)	Least-squares difference*	p value
Sweat chloride concentration, mmol/L	109 (105·6 to 112·4)	107·9 (104·5 to 111·3)	–1·1 (–5·3 to 3·1)	0·61
$ppFEV_1$	75·8% (73·3 to 78·3)	85·3% (82·8 to 87·8)	9·5% (6·7 to 12·3)	<0·0001
CFQ-R respiratory domain	60·6 (54·6 to 66·5)	81·4 (75·5 to 87·3)	20·8 (11·9 to 29·8)	<0·0001
BMI, kg/m²	22·1 (21·9 to 22·3)	22·5 (22·3 to 22·7)	0·4 (0·2 to 0·7)	0·0017
Weight, kg	57·5 (57·0 to 58·0)	58·5 (58·0 to 59·1)	1·0 (0·4 to 1·7)	0·0020

Data are least-squares means (95% CI) or percentage point difference (95% CI). CFQ-R=Cystic Fibrosis Questionnaire-Revised. $ppFEV_1$=percentage of predicted FEV_1. *The difference is the least-squares mean difference between day 1 and day 28 based on a mixed-effects model for repeated measures.

Table 2: **Mixed-effects model means and differences of endpoints up to day 28**

	Overall (N=20)	Baseline to day 28 (active treatment; n=20)	Day 28–56 (washout period; n=19)
Any adverse event	14 (70%)	10 (50%)	8 (42%)
Maximum severity of adverse event			
Mild	12 (60%)	10 (50%)	6 (32%)
Moderate	1 (5%)	0	1 (5%)
Severe	1 (5%)	0	1 (5%)
Serious adverse event*	1 (5%)	0	1 (5%)
Most common adverse events			
Upper respiratory infection	4 (20%)	4 (20%)	0
Headache	3 (15%)	3 (15%)	0
Body aches	2 (10%)	1 (5%)	1 (5%)
Increased sputum production	2 (10%)	2 (10%)	0
Nasal congestion	2 (10%)	1 (5%)	1 (5%)
Joint pain	2 (10%)	1 (5%)	1 (5%)
Fatigue	2 (10%)	2 (10%)	0
Increased coughing	2 (10%)	2 (10%)	0

Data are n (%). *The serious adverse event was hospitalisation due to cystic fibrosis pulmonary exacerbation caused by pneumonia.

Table 3: **Adverse events**

change in $ppFEV_1$ at day 28 (r=0·19, p=0·71; appendix p 10).

14 (70%) participants had at least one adverse event (table 3). Adverse events occurring in at least 10% of participants were compatible with clinical manifestations or complications of cystic fibrosis. Most adverse events were either mild (12 [60%]) or moderate (one [5%]). All mild or moderate events resolved during the study period. During treatment, no participants had elevated alanine aminotransferase, aspartate aminotransferase, or total bilirubin that was greater than three times the upper limit of normal. A serious adverse event occurred in one (5%) patient after day 28 of treatment. This serious adverse event was hospitalisation attributed to pneumonia resulting in pulmonary exacerbation and possible modulator withdrawal syndrome. There were no deaths among study participants. No individuals in the trial discontinued the study drug due to adverse events. There were no relevant safety findings in other clinical or laboratory assessments.

Discussion

This prospective, multicentre, single-arm, open-label trial evaluated ETI treatment for individuals with cystic fibrosis encoding N1303K. Based on earlier clinical data[20,21] and our preclinical findings in relevant cell models, we projected that ETI would reduce sweat chloride by more than 15 mmol/L. In 20 participants with N1303K and a minimal function *CFTR* genotype, we found no reduction in sweat chloride despite the significant improvements in pulmonary and systemic outcomes (ie, weight and symptoms)—an unexpected finding compared with those of the previous pivotal ETI clinical studies leading to ETI regulatory agency approval.[4,5]

In sharp contrast to the findings for sweat chloride, we observed significant and clinically meaningful improvements in $ppFEV_1$, respiratory symptom scores, and nutritional outcomes. The benefit seen in $ppFEV_1$ (an increase of 9·5 percentage points) is similar to robust findings reported for patients with $CFTR^{G551D}$ who were given ivacaftor (10·6 percentage points),[5] and less than the increase noted among individuals with a F508del–minimal function genotype who were treated with ETI (13·8 percentage points).[23] Improvements in CFQ-R respiratory domain scores for patients with N1303K (by 20·8 points) were higher than in the G551D and ivacaftor study[5] (8·6 points) and similar to the F508del–minimal function trial[23] (20·2 points). Of note, patients in these pivotal studies had slightly lower baseline lung function than those in our present study (mean $ppFEV_1$ of 76% compared with 61·4–63·6% in these previous modulator pivotal trials).[4,5,23] Data in these labelling studies also showed a decline or no improvement in CFQ-R scores in the patients receiving placebo.[5,23] Furthermore, in open-label observational studies of ETI treatment, CFQ-R improved by only 12 points, indicating that our data are robust and consistent with open-label studies of ETI for individuals encoding on-label variants.[24] Taken together, these data indicate that the observed improvements in CFQ-R in our trial were larger than the boundaries of the effects of placebo-receiving participants in trials of ETI in adults conducted in the past 3–5 years, indicating that bias alone did not account for symptom improvement. Although our current results were not blinded for participants or placebo controlled, a washout period showed that relevant clinical endpoints consistently returned to pre-ETI baseline during the 28-day interval after drug treatment. Therefore, the findings that suggest clinical improvement is directly related to modulator therapy and outpaces the placebo effect from previous publications.[5,23] Our present trial controlled the endpoint methods, participant inclusion and exclusion criteria, and timing of endpoints to provide controlled data.

The present study also provided controlled trial data supporting the safety of ETI in the N1303K patient cohort. We observed the same mild adverse events reported in other people with cystic fibrosis who were given ETI, and only one serious adverse event, which occurred during the washout period. Adverse events were similar, in number and type, to other clinical trials of ETI in adolescents and adults with cystic fibrosis.[4,5]

Notably, our findings complement and expand on previous results in a study from Israel, published in 2023, that showed significant lung function and BMI improvement and diminished cystic fibrosis symptoms after ETI treatment in eight patients with N1303K (two homozygous), but an absence of sweat chloride reductions.[25] Similarly, a group of individuals with either an N1303K–N1303K genotype or an N1303K–minimal function genotype receiving compassionate-use ETI in France had a substantial benefit to lung function, showing significantly lower reductions in sweat chloride compared with patients with a F508del–minimal function genotype or G551D. Additionally, an analysis begun in 2022 by a French group analysing data from an ETI compassionate-use trial supports our findings by assembling previously published and newly reported outcomes from 11 patients homozygous for $CFTR^{N1303K}$ who were treated with ETI.[26,27] Nine of these individuals with cystic fibrosis had sweat chloride data, which revealed a median sweat chloride reduction of –10 mmol/L compared with –6·5 mmol/L in 14 patients carrying N1303K with a stop codon on the other allele in the case series, suggesting that homozygous patients might be more likely to have modest changes in sweat chloride than aer patients heterozygous for N1303K.

Although an absence of improvement in sweat chloride might be taken to suggest absent functional rescue of CFTR, our clinical findings strongly resemble those in past studies of people with cystic fibrosis showing highly effective modulator response. Potential explanations for the absence of sweat chloride reduction might include factors such as a fundamentally distinctive N1303K

mutation causing a maturation defect in the CFTR protein (managed differently in respiratory *vs* sweat glandular tissues) or ion selectivity favouring bicarbonate over chloride transport by N1303K CFTR (leading to improvement in lung epithelium but not detectable in the sweat duct). Because sweat chloride has clearly been shown to track with in vivo CFTR rescue in previous modulator studies and is regularly used as a primary or secondary outcome in clinical trials, a better understanding of our results will be important both for the basic understanding of CFTR biology and for optimising the sweat correlative endpoint for future clinical trials of rare *CFTR* variants. Our results raise the intriguing possibility that although sweat chloride reduction as a clinical trial outcome is clearly important when identified, it might not always be essential for achieving clinical improvement.

Our data also provide one example of ways in which cystic fibrosis studies directed towards a small target population can be guided by in vitro iPSC-derived airway epithelium and used to promote in vivo analysis. In that context, another clinical study based on in vitro ETI response is currently in progress to investigate patients with residual CFTR activity irrespective of genotype (entry criteria include sweat chloride <80 mmol/L or pancreatic sufficiency) and has already shown encouraging improvements of key endpoints in some individuals (NCT03506061; unpublished). Therefore, our results provide a means by which groups of people with cystic fibrosis and strong scientific rationale can facilitate focused clinical testing (if the effect size is significant) and help to facilitate modulator access in the future. However, future investigation will be necessary to define the effect size needed with this design to ensure responsiveness in ultra-rare variants.

We acknowledge some limitations in the current trial. First, an open-label format might favourably bias clinical outcome. That limitation is offset by the fact that ppFEV$_1$, CFQ-R respiratory domain scores, and BMI all returned or trended to baseline values during the washout period and that effects shown here are substantially larger than those observed in placebo groups of previous modulator investigations.[5,23,28] Our results also show a strong response in lung function similar to that associated with ivacaftor among people with cystic fibrosis encoding G551D.[23] Earlier studies of modulators in people with cystic fibrosis have not reported significant increases in FEV$_1$ or CFQ-R for control or placebo groups.[5,23] Despite the washout period and the controlled environment of a clinical trial setting, we note that observation biases might have affected patient response, particularly with regard to the CFQ-R. Second, the timeframe for ETI treatment was only 28 days. Although a longer window of observation on therapy might be advantageous, this length of treatment was chosen on the basis of the benefits observed by day 28 in pivotal ETI trials[4] while balancing travel feasibility concerns for a predominantly referral-based study. Third, although our trial enrolled a cohort with racial and ethnicity distributions similar to those of the cystic fibrosis patient population in the USA,[29] the N1303K variant has been described as frequent in Iceland, Lebanon, Tunisia, and Mediterranean regions.[30–32] Our study population might therefore not be representative of the diversity of the N1303K population globally. Furthermore, individuals homozygous for N1303K were not evaluated in the present trial. Based on clinical and preclinical data, as well as other reports involving patients with the N1303K–N1303K genotype, it appears that homozygous individuals can show pronounced modulator benefit.[26] However, this possibility was not formally tested in our study. Despite in vitro evidence and a prediction of minimal protein production by many of the second variants tested in our study, we cannot fully conclude that activation of the second variant did not contribute to the clinical effects observed. Finally, we acknowledge that the protocol including derivation of iPSCs for each participant is time intensive, which limits the ability to do rapid in vitro to in vivo correlative analyses in a theratyping approach. This limitation will be addressed with the ongoing development of methods by both our group and others in the field.

In summary, our findings shown here are meaningful for several reasons. The results establish an informative clinical trial design that might be adaptable to modulator treatment for other rare *CFTR* variants. Our results also show that reductions in sweat chloride do not universally predict the clinical effectiveness of CFTR modulators. Moreover, the data point to the importance of developing additional biomarkers and cell-based tools so that clinical benefit from ETI is not underestimated. Cell-based assays might also provide evidence for the mechanistic nature of the differences in observed sweat chloride reduction in this study compared with other CFTR modulator trials with F508del. Finally, our report provides compelling and supportive prospective clinical trial evidence that patients with the prevalent *CFTR*^{N1303K} variant have substantial improvement after ETI therapy while also showing a conventional safety profile in this patient population. The results exemplify new approaches that will be needed to expand access to patients currently ineligible for modulator treatment globally. Future research utilising this type of study design could establish whether individuals with other rare *CFTR* variants that show borderline or conflicting in vitro findings for modulator responsiveness might respond clinically to modulators.

Contributors

GMS, RWL, and EJS conceived of and designed the study. GMS, RWL, RR, AS, BB, EH, and KV recruited and enrolled participants. GMS, RWL, RR, AS, BB, EH, CM-B, and WRH conducted the study. GMS, RWL, RR, AS, CM, JH, BB, and EH monitored the study and entered data into the data registry. GMS, RWL, ALW, SPB, CB, SS, JJB, BRD, AR, CM, JH, and EJS analysed and interpreted the data. GMS, RWL, ALW, and EJS accessed and verified all data. GMS, RWL, ALW, EJS, and BRD

wrote the initial manuscript draft. All authors had access to the raw data for review and reviewed the final manuscript before submission. All authors were responsible for the decision to submit the manuscript.

Data sharing

All data will be made available with publication upon reasonable request to the corresponding author with appropriate scientific rationale.

Declaration of interest

GMS reports funding from the National Institutes of Health (NIH), the Cystic Fibrosis Foundation, the COPD Foundation, and the PCD Foundation; reports grants for clinical trials from Renovion, Vertex, Electromed, Insmed Pharmaceuticals, 4DMT, AstraZeneca, and BiomX for clinical trials; is a paid consultant for Insmed, Electromed, and Genetech; is on the steering committees for clinical trials for AstraZeneca, GlaxoSmithKline, and Genentech; is a member of the steering committee for the Bronchiectasis and NTM Research Registry; and has contracts for laboratory work from Renovion and ReCode, unrelated to the current manuscript. RWL reports grants paid to her institution from the Cystic Fibrosis Foundation and Vertex Pharmaceuticals; consulting fees from Vertex Pharmaceuticals; and support for travel to an investigator meeting from Vertex Pharmaceuticals. AS and EH report funding from the Cystic Fibrosis Foundation. WRH reports funding from NIH and the Cystic Fibrosis Foundation. JJB reports funding from NIH, the Cystic Fibrosis Foundation, and Cure Cystic Fibrosis, and has previously received honoraria for invited lectures from Vertex Pharmaceuticals. AR reports research support from NIH (HL-139876) and the Cystic Fibrosis Foundation. SPB reports grant funding from NIH and the Cystic Fibrosis Foundation. CM-B is a collaborator on the Cystic Fibrosis Foundation grant that funded this work. SS participates and has a key role in studies funded by the NIH and Cystic Fibrosis Foundation, and is an inventor on US Patent number 11401510 for the generation of airway basal stem cells from human pluripotent stem cells (issue date Aug 2, 2022). CB participates and has a key role in studies funded by NIH and the Cystic Fibrosis Foundation, and is an inventor on US Patent number 11401510. BRD has grants from NIH and the Cystic Fibrosis Foundation, and is an inventor on US Patent number 11401510. EJS reports funding from NIH (HL139876), the Cystic Fibrosis Foundation, and the Marcus Foundation; received an honorarium from the University of Iowa (Iowa City, IA, USA) for a talk regarding CFTR theratyping analysis; is working on novel compounds for treatment of cystic fibrosis that are not described or mentioned in this study but might have a future role against many CFTR mutations, including N1303K; and is a non-voting member of the Cystic Fibrosis Foundation board of directors (a not-for-profit organisation with no competing interests with this study). RR, BB, KV, ALW, JH, and CM declare no competing interests.

Acknowledgments

This study was funded by the Cystic Fibrosis Foundation (SOLOMO21A0 to GMS, LINNEM21A0 to RWL, and DAVIS21A0 to BRD) and NIH (5P30DK072482, 1K08HL138153, R01HL139876 to GMS; R01HL139876 to BRD; and R01HL139876 to EJS and BRD). We thank the participants and their families. We also thank the referring cystic fibrosis clinical teams from the clinical sites. Part of these data were presented as an abstract and workshop talk at the North American Cystic Fibrosis Conference 2023 (Phoenix, AZ, USA).

References

1 Cutting GR. Cystic fibrosis genetics: from molecular understanding to clinical application. *Nat Rev Genet* 2015; **16:** 45–56.

2 Spielberg DR, Clancy JP. Cystic fibrosis and its management through established and emerging therapies. *Annu Rev Genomics Hum Genet* 2016; **17:** 155–75.

3 Hwang TC, Yeh JT, Zhang J, Yu YC, Yeh HI, Destefano S. Structural mechanisms of CFTR function and dysfunction. *J Gen Physiol* 2018; **150:** 539–70.

4 Heijerman HGM, McKone EF, Downey DG, et al. Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. *Lancet* 2019; **394:** 1940–48.

5 Middleton PG, Mall MA, Dřevínek P, et al. Elexacaftor–tezacaftor–ivacaftor for cystic fibrosis with a single Phe508del allele. *N Engl J Med* 2019; **381:** 1809–19.

6 Middleton PG, Taylor-Cousar JL. Development of elexacaftor–tezacaftor–ivacaftor: highly effective CFTR modulation for the majority of people with cystic fibrosis. *Expert Rev Respir Med* 2021; **15:** 723–35.

7 Zemanick ET, Taylor-Cousar JL, Davies J, et al. A phase 3 open-label study of elexacaftor/tezacaftor/ivacaftor in children 6 through 11 years of age with cystic fibrosis and at least one F508del allele. *Am J Respir Crit Care Med* 2021; **203:** 1522–32.

8 Gramegna A, Contarini M, Bindo F, Aliberti S, Blasi F. Elexacaftor-tezacaftor-ivacaftor: the new paradigm to treat people with cystic fibrosis with at least one p.Phe508del mutation. *Curr Opin Pharmacol* 2021; **57:** 81–88.

9 Goralski JL, Hoppe JE, Mall MA, et al. Phase 3 open-label clinical trial of elexacaftor/tezacaftor/ivacaftor in children aged 2–5 years with cystic fibrosis and at least one F508del allele. *Am J Respir Crit Care Med* 2023; **208:** 59–67.

10 Vertex. FDA accepts Vertex's supplemental new drug applications for TRIKAFTA (elexacaftor/tezacaftor/ivacaftor and ivacaftor), SYMDEKO (tezacaftor/ivacaftor and ivacaftor) and KALYDECO (ivacaftor) for additional CFTR mutations. Sept 1, 2020. https://investors.vrtx.com/news-releases/news-release-details/fda-accepts-vertexs-supplemental-new-drug-applications-trikaftar (accessed Jan 25, 2024).

11 Bihler H, Sivachenko A, Millen L, et al. In vitro modulator responsiveness of 655 *CFTR* variants found in people with cystic fibrosis. *J Cyst Fibros* 2024; published online Feb 21. https://doi.org/10.1016/j.jcf.2024.02.006.

12 Clancy JP, Cotton CU, Donaldson SH, et al. CFTR modulator theratyping: current status, gaps and future directions. *J Cyst Fibros* 2019; **18:** 22–34.

13 Durmowicz AG, Lim R, Rogers H, Rosebraugh CJ, Chowdhury BA. The US Food and Drug Administration's experience with ivacaftor in cystic fibrosis: establishing efficacy using in vitro data in lieu of a clinical trial. *Ann Am Thorac Soc* 2018; **15:** 1–2.

14 Phuan PW, Son JH, Tan JA, et al. Combination potentiator ('co-potentiator') therapy for CF caused by CFTR mutants, including N1303K, that are poorly responsive to single potentiators. *J Cyst Fibros* 2018; **17:** 595–606.

15 Hawkins FJ, Suzuki S, Beermann ML, et al. Derivation of airway basal stem cells from human pluripotent stem cells. *Cell Stem Cell* 2021; **28:** 79–95.e8.

16 Suzuki S, Hawkins FJ, Barillà C, Beermann ML, Kotton DN, Davis BR. Differentiation of human pluripotent stem cells into functional airway basal stem cells. *STAR Protoc* 2021; **2:** 100683.

17 Han ST, Rab A, Pellicore MJ, et al. Residual function of cystic fibrosis mutants predicts response to small molecule CFTR modulators. *JCI Insight* 2018; **3:** e121159.

18 Quittner AL, Buu A, Messer MA, Modi AC, Watrous M. Development and validation of the Cystic Fibrosis Questionnaire in the United States: a health-related quality-of-life measure for cystic fibrosis. *Chest* 2005; **128:** 2347–54.

19 Quittner AL, Modi AC, Wainwright C, Otto K, Kirihara J, Montgomery AB. Determination of the minimal clinically important difference scores for the Cystic Fibrosis Questionnaire-Revised respiratory symptom scale in two populations of patients with cystic fibrosis and chronic *Pseudomonas aeruginosa* airway infection. *Chest* 2009; **135:** 1610–18.

20 Boyle MP, Bell SC, Konstan MW, et al. A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del *CFTR* mutation: a phase 2 randomised controlled trial. *Lancet Respir Med* 2014; **2:** 527–38.

21 Vermeulen F, Le Camus C, Davies JC, Bilton D, Milenković D, De Boeck K. Variability of sweat chloride concentration in subjects with cystic fibrosis and G551D mutations. *J Cyst Fibros* 2017; **16:** 36–40.

22 Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3–95 year age range: the global lung function 2012 equations. *Eur Respir J* 2012; **40:** 1324–43.

23 Ramsey BW, Davies J, McElvaney NG, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med* 2011; **365:** 1663–72.

24 Nichols DP, Paynter AC, Heltshe SL, et al. Clinical effectiveness of elexacaftor/tezacaftor/ivacaftor in people with cystic fibrosis: a clinical trial. *Am J Respir Crit Care Med* 2022; **205:** 529–39.

25 Sadras I, Kerem E, Livnat G, et al. Clinical and functional efficacy of elexacaftor/tezacaftor/ivacaftor in people with cystic fibrosis carrying the N1303K mutation. *J Cyst Fibros* 2023; **22:** 1062–69.

26 Burgel PR, Sermet-Gaudelus I, Girodon E, et al. Gathering real-world compassionate data to expand eligibility for elexacaftor/tezacaftor/ivacaftor in people with cystic fibrosis with N1303K or other rare *CFTR* variants: a viewpoint. *Eur Respir J* 2024; **63:** 2301959.

27 Burgel PR, Sermet-Gaudelus I, Durieu I, et al. The French Compassionate Program of elexacaftor-tezacaftor-ivacaftor in people with cystic fibrosis with advanced lung disease and no F508del *CFTR* variant. *Eur Respir J* 2023; **61:** 2202437.

28 Coton J, Le HH, Veuillet V, et al. Do patients with cystic fibrosis participating in clinical trials demonstrate placebo response? A meta-analysis. *J Cyst Fibros* 2019; **18:** 461–67.

29 Cystic Fibrosis Foundation. Annual data report 2022. September, 2023. https://www.cff.org/medical-professionals/patient-registry (accessed Jan 25, 2024).

30 Zolin A, Adamoli A, Bakkeheim E, et al. ECFSPR 2022 annual data report. 2024. https://www.ecfs.eu/sites/default/files/Annual%20Report_2022_ECFSPR_20240603.pdf (accessed Jan 31, 2024).

31 Farra C, Menassa R, Awwad J, et al. Mutational spectrum of cystic fibrosis in the Lebanese population. *J Cyst Fibros* 2010; **9:** 406–10.

32 Fredj SH, Messaoud T, Templin C, des Georges M, Fattoum S, Claustres M. Cystic fibrosis transmembrane conductance regulator mutation spectrum in patients with cystic fibrosis in Tunisia. *Genet Test Mol Biomarkers* 2009; **13:** 577–81.