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Real-world experience with CFTR modulator therapy

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Real-world experience with CFTR modulator therapy

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Editorial: Real-world experience with CFTR modulator therapy

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Editorial on the Research Topic
Real-world experience with CFTR modulator therapy

Cystic fibrosis (CF) is a severe autosomal recessive trait that is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene. The basic defect impairs the transport of chloride and bicarbonate across the apical membrane of epithelial cells leading to mucus plugging of the ducts of exocrine glands. Mucus obstruction of the small conducting airways predisposes to a vicious cycle of infection, inflammation and airway remodeling, which determine the course and prognosis of most people with CF (pwCF). Being a fatal disease with death in early childhood in the 1950s, the symptom-oriented treatment programs have gradually improved the median life expectancy in countries with well-established CF care to about 50 years in 2020.

The *CFTR* gene was identified in 1989. By the mid-1990s high-throughput screening programs were initiated to identify small molecules that improve the function of mutant CFTR. The first molecule approved for use in humans is the CFTR potentiator ivacaftor (IVA), which facilitates the opening of the CFTR ion channel. In 2010 IVA monotherapy became available for the small group of pwCF who are harboring a gating mutation in the *CFTR* gene. Of the more than 2,000 known disease-causing mutations, the 3-base pair deletion of codon 508, named p.Phe508del, is the most common mutation present in 50%–90% of CF alleles in European populations. p.Phe508del CFTR is primarily defective in folding and processing of the nascent protein at the endoplasmic reticulum. CFTR correctors stabilize critical sites during folding. The first approved correctors, lumacaftor (LUM) and tezacaftor (TEZ), in combination with IVA showed only modest improvements of lung function in phase 3 clinical trials, but they reduced the frequency of pulmonary exacerbations and have been predicted to increase the median survival by 7 years compared to symptom-oriented care (Lopez et al., 2023).

By November 2019 the outcome of phase 3 studies of the triple therapy with elexacaftor (ELX), TEZ and IVA in CF patients with one or two p.Phe508del alleles were published (Heijerman et al., 2019; Middleton et al., 2019). Study participants substantially improved in lung function, body weight and quality of life measures classified as a game changer of CF disease. Hence, the projected survival for pwCF treated with ELX/TEZ/IVA has been predicted to show large improvements in future years (Lopez et al., 2023). Consistent with this estimate, the reconstituted ELX/TEZ/IVA - Phe508del CFTR complex showed wild type conformation in cryoelectron micrographs (Fiedorczuk and Chen, 2022).

The 15 contributions of this Research Topic report on the "*Real-world experience with CFTR modulator therapy*." One of us (Tümmler) summarized the take-home message of all post-approval studies on ELX/TEZ/IVA that were published until February 2023.

Stanke et al. compared the intestinal Phe508del CFTR glycoforms at baseline and during ELX/TEZ/IVA therapy. By applying a novel protocol to separate CFTR protein isoforms with high resolution, complex glycosylated Phe508del CFTR was found to be less glycosylated and less polydisperse than mature wild type CFTR both in absence and presence of ELX/TEZ/IVA indicating that triple therapy rescues Phe508del CFTR at the endoplasmic reticulum, but does not normalize post-translational processing at the Golgi apparatus.

The FDA approved ELX/TEZ/IVA for treatment of pwCF who are carrying *CFTR* mutations that are responsive to modulator *in vitro* or *in vivo*. Carriage of two loss-of-function mutations does not fulfill the label. However, as shown by Pallenberg et al. for two *prima facie* loss-of-function splice mutations, ELX/TEZ/IVA conferred residual CFTR activity to some, but not all examined organs. This showcase tells us that the individual response of rare CFTR mutations to highly-effective CFTR modulation cannot be predicted from assays in standard cell cultures, but requires the personalized multi-organ assessment by CFTR biomarkers.

ELX/TEZ/IVA improved Phe508del CFTR function in intestinal epithelia to a level of about 40% of normal in pwCF aged 12 years and older (Graeber et al., 2022a). Berges et al. now shows that the clinically less effective combination therapy with LUM/IVA improved Phe508del CFTR activity in the intestine to about 30% of normal in children aged 2–11 years exceeding the 18% of normal found previously in pwCF aged 12 years and older. Consistent with registry reports on ivacaftor, CFTR modulator therapy is more efficient in the young age group to attenuate the basic defect than in the elderly suggesting that with early initiation of treatment the long-term outcome will be better.

The first phase 3 clinical trials on triple therapy assessed pwCF aged 12 years and older and a lung function of ppFEV1 of 40%–90% predicted of normal. The real-world studies now tell us that pwCF with severe airway obstruction ($ppFEV_1$ <40) and pwCF with well-preserved lung function ($ppFEV_1$ >90) showed less improvement in ppFEV1 than pwCF with ppFEV1 40–90 (Fila et al.). The clinical response towards ELX/TEZ/IVA depends on age and lung disease severity. Adolescents and children showed benefit in nutritional status and pulmonary function (Olivier et al.), especially the individuals with more severe lung disease prior to ELX/TEZ/IVA (within the lowest 25% of age specific ppFEV1) showed higher improvements in spirometry compared to adults in this severity group (Schütz et al.). Improvements in FEV1 upon initiation of ELX/TEZ/IVA therapy were demonstrated to go hand in hand with a lower bacterial load and less frequent detection of *Aspergillus fumigatus* in respiratory secretions (Eschenhagen et al.), a decrease of ventilation inhomogeneity and a reduction of structural lung damage (Graeber et al., 2022b; Appelt et al.). Likewise, paranasal sinus abnormalities, particularly mucopyoceles, stably decreased during treatment with LUM/IVA in p.Phe508del homozygous children with CF (Wucherpfennig et al.).

Highly effective CFTR modulation does not only substantially improve lung function, but typically also immediately improves pH and fluid homeostasis in the intestine. Nutrients are more efficiently absorbed leading to an increase of the body mass index. When ELX/TEZ/IVA therapy is initiated, abdominal symptoms may transiently emerge. PwCF often report more flatulence and abdominal pain during the first 10 days (Mainz et al.), but these abdominal symptoms improve or even disappear within the following 2 weeks.

An increase of the body mass index has been interpreted in the phase 3 CFTR modulator trials as an improvement of anthropometry. The CF clinic in Toronto now shares their experience with us that the gain of body weight is mainly due to an increase of fat mass whereas the lean body mass does not improve (Mouzaki et al.). If we want to avoid to see many obese pwCF in the future, pwCF should rapidly switch from a calorie-rich diet recommended in the pre-modulator era to the balanced mixed diet of the healthy population.

The phase 3 trials and the open-extension study did not find any neurologic or psychiatric side effects of ELX/TEZ/IVA therapy other than headache. Post approval, however, substantial mental health side effects have been reported, which impacted on day-to-day activity and quality of life (Spoletini et al., 2022). Ibrahim et al. from the CF clinic in Cork now share their experience with a dose reduction strategy in CF adults who developed anxiety, irritability, sleep disturbance and/or mental slowness after initiation of full dose treatment with ELX/TEZ/IVA. Initial discontinuation or reduction of medication and subsequent dose escalation every 4–6 weeks resulted in resolution of mental/psychological adverse events, without loss of clinical effectiveness. To put the numerous case reports on mental status changes into perspective, the CF clinic at the Charité in Berlin performed the worldwide first prospective study on the relationship between initiation of ELX/TEZ/IVA therapy and changes in mental health in CF adults (Piehler et al.). Symptoms of depression decreased, but symptoms of anxiety did not change after 8–16 weeks of treatment with ELX/TEZ/IVA. Hence, the rapid attenuation of the symptoms of CF disease upon initiation of ELX/TEZ/IVA will improve the quality of life and depressive symptoms in most pwCF, but 5%–10% of CF adults experience adverse events of mental health that deserve particular attention in the future.

CFTR modulator drugs are expensive. ELX/TEZ/IVA is not available in all countries around the globe, particularly pwCF living in low-middle income countries have currently no access to ELX/TEZ/IVA. Zampoli et al. discuss in their minireview the global inequality in the access to the life-altering CFTR modulator drugs. The CF community is asked to deal with this real-world disparity.

Finally, CFTR modulators are variant-selective therapies. Although ELX/TEZ/IVA has been primarily developed for patients with at least one p.Phe508del mutation, it has become clear that many other mutations respond to ELX/TEZ/IVA (Burgel et al., 2023). Determining which mutations respond to ELX/TEZ/IVA has the potential of improving health status and survival in responders. For those who do not respond, newer therapeutic options should be developed.

Author contributions

BT: Writing–original draft, Writing–review and editing. P-RB: Writing–original draft, Writing–review and editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Changes in cystic fibrosis transmembrane conductance regulator protein expression prior to and during elexacaftor-tezacaftor-ivacaftor therapy

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Background: Defects in expression, maturation or function of the epithelial membrane glycoprotein CFTR are causative for the progressive disease cystic fibrosis. Recently, molecular therapeutics that improve CFTR maturation and functional defects have been approved. We aimed to verify whether we could detect an improvement of CFTR protein expression and maturation by triple therapy with elexacaftor-tezacaftor-ivacaftor (ELX/TEZ/IVA).

Methods: Rectal suction biopsies of 21 p.Phe508del homozygous or compound heterozygous CF patients obtained pre- and during treatment with ELX/TEZ/IVA were analyzed by CFTR Western blot that was optimized to distinguish CFTR glycoisoforms.

Findings: CFTR western immunoblot analysis revealed that—compared to baseline—the levels of CFTR protein increased by at least twofold in eight out of 12 patients upon treatment with ELX/TEZ/IVA compared to baseline ($p < 0.02$). However, polydispersity of the mutant CFTR protein was lower than that of the fully glycosylated wild type CFTR Golgi isoform, indicating an incompletely glycosylated p.Phe508el CFTR protein isoform C* in patients with CF which persists after ELX/TEZ/IVA treatment.

Interpretation: Treatment with ELX/TEZ/IVA increased protein expression by facilitating the posttranslational processing of mutant CFTR but apparently did not succeed in generating the polydisperse spectrum of N-linked oligosaccharides that is characteristic for the wild type CFTR band C glycoisoform. Our results caution that the lower amounts or immature glycosylation of the C* glycoisoform observed in patients' biomaterial might not translate to fully restored function of mutant CFTR necessary for long-term provision of clinical benefit.

KEYWORDS

elexcaftor-tezacaftor-ivacaftor, TRIKAFTA, CFTR protein expression, CFTR glycosylation, CFTR small molecule therapeutics

1 Introduction

Cystic fibrosis (CF) is a life-shortening autosomal recessive trait of exocrine glands and CFTR-expressing epithelia that is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene (Elborn, 2016). The basic defect manifests in impaired chloride and bicarbonate transport across apical epithelial membranes of several organ systems (Elborn, 2016). The most frequent *CFTR* mutation is a 3-bp in-frame deletion, p.Phe508del, that affects the post-translational processing and trafficking and the half-life and function of the ion channel in the plasma membrane (Ameen et al., 2007).

Wild-type CFTR is synthesized in the endoplasmic reticulum (ER) as a mannose-rich glycoisoform (band B) that is converted in the Golgi-apparatus predominantly to the mature complex-glycosylated isoform (band C) (McClure et al., 2016). This maturation process is uneconomical as about 75% of wild-type CFTR is degraded by the ER-associated degradation pathway ERAD (Ward and Kopito, 1994). Maturation of mutant p.Phe508del CFTR is even more ineffective as the newly synthesized p.Phe508del CFTR fails to adopt a wild-type fold in the ER, is targeted to ER-associated degradation (Ward and Kopito, 1994) and is removed faster from the apical membrane by endocytosis (Lukacs et al., 1993). Consequently, p.Phe508del homozygous subjects express only low amounts of complex-glycosylated p.Phe508del CFTR and low or no residual p.Phe508del CFTR-mediated chloride secretory activity in the rectal mucosa, albeit studies of patient's tissue have confirmed that p.Phe508del-CFTR can be processed to reach the apical membrane, (Dray-Charier et al., 1999; Kälin et al., 1999; Kreda et al., 2005; van Meegen et al., 2013), can be complex glycosylated (Dray-Charier et al., 1999; van Barneveld et al., 2010) and can transport chloride (Bronsveld et al., 2001; Sermet-Gaudelus et al., 2002).

CFTR, like other membrane glycoproteins, has to undergo glycosylation by one or more out of 14 pathways that together rely on a total of 173 glycosyltransferases (Schjoldager et al., 2020). The non-glycosylated polypeptide chain of 1,480 amino acids can only be visualized by western-blotting from biomaterial when deglycosylation enzymes are used (Sarkadi et al., 1992; Kälin et al., 1999). The immature core-glycosylated glycoisoform B (CFTR-B) and the mature complex glycosylated glycoisoform C (CFTR-C) were observed in epithelial model cell lines that express CFTR endogenously (Varga et al., 2004), in various heterologous expression systems (Sarkadi et al., 1992; Ward and Kopito, 1994; Varga et al., 2004; Rowe et al., 2010) and human tissues such as gallbladder (Dray-Charier et al., 1999), colon (van Barneveld et al., 2010), ileum, jejunum and duodenum (Kälin et al., 1999). These mature fully glycosylated CFTR glycoisoforms C differ in size when comparing different sources (Ernst et al., 1994; Kälin et al., 1999; Varga et al., 2004). Moreover, even from one source, a complex glycosylated membrane protein like CFTR-C is not a single entity but a set of molecules that differ in structure and composition of terminal glycosylation residues (McClure et al., 2016), molecular shape and thus, its signal displays a slightly diffuse distribution when analyzed by polyacrylamide gel electrophoresis (Sarkadi et al., 1992; Ernst et al., 1994; Ward and Kopito, 1994; Dray-Charier et al., 1999; Kälin et al., 1999; Varga et al., 2004; Rowe et al., 2010; van Barneveld et al., 2010).

So far, conventional treatment of CF has been supportive, targeting downstream clinical manifestations that result from the loss of CFTR activity. These therapeutic measures improved morbidity and survival, but confer a high burden of care. Recently however, academia and industry have thus focused on the development of small molecule CFTR modulators that restore function of mutant CFTR. These approaches have made CF the first successful example of customized drug development for mutation-specific therapy whose effects therefore have implications beyond CF. Both US and European regulatory agencies (FDA and EMA) have recently approved the combination of the type III corrector elexacaftor (ELX), the type I corrector tezacaftor (TEZ) and the gating potentiator ivacaftor (IVA) for treatment of patients with CF with at least one p.Phe508del allele (Heijerman et al., 2019; Middleton et al., 2019). Taking the surrogate parameter of the forced expiratory volume in 1 s, FEV1, treatment with ELX/TEZ/IVA improved lung function by 12 percentage points in patients who are homozygous for p.Phe508del and by 14 percentage points in patients who are compound heterozygous for p.Phe508del and a so-called "minimal-function" mutation (Heijerman et al., 2019; Middleton et al., 2019). Sweat chloride concentrations as a measure of the defective CFTR—mediated chloride reabsorption in the sweat duct showed a mean 47–50 mmol/L reduction, thus decreasing to an intermediary or even normal range, suggesting that CFTR function in the sweat duct had been reverted to wild type (Heijerman et al., 2019; Middleton et al., 2019). Prior to ELX/TEZ/IVA treatment, p.Phe508del homozygous patients have received TEZ/IVA as a reference treatment (Heijerman et al., 2019).

The effect of targeted CFTR modulator therapy on CFTR protein expression has been observed during short-term exposure to transfected cell lines where constraints of ER processing and Golgi-associated post-translational glycosylation may differ compared to the effects achieved in patients' epithelial tissues. Such *in vitro* results suggest that incubation with ELX/TEZ/IVA leads to an increase of mature CFTR (Capurro et al., 2021; Becq et al., 2022) and facilitates clustering of CFTR into lipid rafts and ceramide-rich platforms (Abu-Arish et al., 2022). Closer to the *in vivo* situation are primary epithelia derived from healthy controls or p.Phe508del-CFTR homozygous CF patients in which CFTR function has been assessed with electrophysiology. These studies show that lumacaftor (LUM) (Gentzsch et al., 2017; Pranke et al., 2017) or ELX (Shaughnessy et al., 2021) can increase p.Phe508del-CFTR function in primary epithelia and that ELX/TEZ/IVA corrects p.Phe508del function to about two thirds of wild-type CFTR function (Veit et al., 2020). Western blot analysis of primary airway epithelia shows that p.Phe508del maturation can be partially corrected by LUM (Cholon et al., 2014; Veit et al., 2016) and TEZ (Gentzsch et al., 2021) and interestingly, that IVA can reduce CFTR maturation efficacy in comparison to LUM single molecule treatment (Cholon et al., 2014) or in heterologous cell lines in ELX/TEZ/IVA triple therapy (Becq et al., 2022).

However, data on the degree of *in vivo* correction of CFTR protein expression by ELX/TEZ/IVA—as a highly potent modulator

treatment which permits treatment of the majority of affected patients with CF, achieving impressive clinical effects (Heijerman et al., 2019; Middleton et al., 2019)—have remained elusive. Such data could clarify the potential sources of the heterogeneity of the individual clinical response and anticipate long-term efficacy of ELX/TEZ/IVA.

Based on the results in clinical trials, we hypothesized that treatment with ELX/TEZ/IVA promotes maturation and surface expression of p.Phe508del CFTR protein in epithelium. To test this hypothesis, we examined rectal suction biopsies of p.Phe508del homozygous and compound heterozygous CF patients with high-resolution immunoblot analysis prior and during treatment with ELX/TEZ/IVA.

2 Patients and methods

2.1 Ethics and participants

Results presented are part of a larger, multi-center trial at four study centers of the German Center for Lung Research designed to analyze effects of ELX/TEZ/IVA treatment on different clinical parameters and biomaterials (NCT04732910) (Graeber et al., 2022). Sampling of rectal biopsies for CFTR protein content by Western blot analyses was only performed in the subgroup of patients recruited at the Hannover Medical University site of the study, according to the ethical approval # 8922_BO_S_2020 from the Hannover Ethics Committee.

We obtained written informed consent from all patients included in the study, their parents or legal guardians. Patients were eligible to participate if they were at least 12 years old, homozygous for p.Phe508del-*CFTR* or compound-heterozygous for p.Phe508del-*CFTR* and a minimal function mutation, had no prior exposure to ELX/TEZ/IVA and were willing to remain on a stable medication regimen, including ELX/TEZ/IVA according to the patient labeling and the prescribing information for the duration of study participation. Exclusion criteria were an acute respiratory infection or pulmonary exacerbation at baseline, intranasal medication changes within 14 days prior to baseline and a history of transplantation. In total, 21 patients were included in our analyses of CFTR protein content of rectal biopsies.

2.2 Outline of CFTR immunoblot

To optimize the sensitivity and specificity of the immune-chemical CFTR signal, conditions for electrophoresis were selected that preferentially resolve proteins within the range of 100–300 kDa (see Section 2.4 below) and the Western blot was then probed with a mixture of four monoclonal antibodies that are known to detect CFTR epitopes with high affinity and specificity (see Section 2.6 below).

2.3 Preparation of lysates from rectal suction biopsies

Two to four rectal suction biopsies were obtained with a rectal suction biopsy tool Model SBT-100 (Trewavis Surgical, Australia) and frozen at −80°C after measurement of intestinal current according to the SOP of the ECFS DNWG, V.2.7. Frozen biopsies were thawed for 10 min at room temperature in 50 µL of SDS-rich lysis buffer (50 mM Tris pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 100 mM dithiothreitol (DTT), 1:50 proteinase inhibitor cocktail (SRE0055-1BO; Sigma Aldrich; MO, United States), 0.2 mM phenylmethylsulfonylfluoride, 1:10,000 Omnicleave endonuclease (OC7850K; Biozym; Germany). Samples were homogenized in a 1.5 mL sample tube with a fitting pistil (pistil PS, Kisker, Steinfurt) whereby care was taken to wedge the biopsy between pistil and sidewall of the tube during homogenization until the tissue was disintegrated with several up-and-down strokes of the pistil. Care was taken to rescue all material left on the surface of the pistil with a pipette tip, next the lysate was incubated at 37°C for 30 min, followed by a second homogenization procedure with several strokes of the pistil. Lysates were next sheared by pipetting the 50 µL volume ten times with a 200 µL pipette-tip. Centrifugation for 10 min at 13,000 rpm (5424R, Eppendorf, Hamburg, Germany) yielded a supernatant of 35–45 µL that was adjusted with the same volume of glycerol. For this study, biomaterials were analyzed after storage at −80°C for 7–14 days.

2.4 Gel electrophoresis

Protein content of lysates was semi-quantified with minute aliquots of 1:5; 1:10; 1:30; 1:60 serially diluted in 150 mM NaCl. One µL volumes of the diluted samples were spotted on a Whatman 3 MM paper in comparison to a serial dilution of 5.0 µg/µL to 0.1 µg/µL bovine serum albumin in 150 mM NaCl. Spots were dried, stained in Coomassie solution (0.1% Coomassie brilliant blue in 25% isopropanol, 10% acetic acid) for 10 s, and the stained Whatman paper was thoroughly rinsed using running tap water. Protein concentration of lysates was estimated by comparing staining of spotted samples to the staining of control proteins.

Electrophoresis was carried out in a Mini-PROTEAN Tetra Cell (#165-8001; Bio-Rad Laboratories GmbH; Munich; Germany) using 6% polyacrylamide (PAA; Rotiphorese Gel 30, crosslink 37.5:1; Roth; Karlsruhe, Germany). The separation matrix of 6% PAA was casted to yield a separation distance of 6.5 cm below a very narrow 4% PAA gel. Equal amounts of either baseline and treatment lysate were loaded side-by-side in a total volume of 25 µL each. Sample volumes were adjusted with 50 mM Tris pH 6.8, 2% (w/v) SDS, 50% (w/v) glycerol whereby 100 mM DTT and 1:50 proteinase inhibitor cocktail were freshly added prior to a mild denaturation step of 30 min at 37°C. 16HBE14o-cell lysates were used as a positive control on each gel. Electrophoretic mobility of samples was judged against a prestained molecular weight marker (PageRuler Plus Prestained Protein Ladder; #26619; Thermo Fisher; Darmstadt; Germany). Electrophoresis was carried out at 12 V for approximately 20 h at 4°C whereupon the electrophoresis was continued at 60 V for approximately 3 h until the 72 kDa marker had almost reached the lower edge of the polyacrylamide gel.

2.5 Transfer of proteins to membrane

Proteins were transferred to an uncharged supported nitrocellulose membrane (Amersham Protran Supported Nitrocellulose Blotting-Membrane; 0.45 µm pore size; #10600016; VWR; Darmstadt; Germany) by tank blotting in a Mini Trans-Blot

Electrophoretic Transfer Cell (#170-3935; Bio-Rad Laboratories GmbH; Munich; Germany). Polyacrylamide gels were mounted into the gel holder cassette whereby the high molecular weight edge of the gel was placed at the cassette's hinge. Transfer was done in 125 mM Tris, 950 mM glycine, 0.02% (w/v) SDS at 44 mA for approximately 23 h whereby the tank blot apparatus was submerged in ice in a Styrofoam container. Upon completion of tank blot, the polyacrylamide gel was stained with Coomassie to visualize non-transferred high molecular weight proteins.

2.6 Serial detection of CFTR and vinculin

Membranes were vertically cut between the lane containing the molecular weight marker and the adjacent samples which were separately processed.

Positions of prestained molecular weight marker bands were marked with a ballpoint pen. Next, the marker lane membrane was incubated in 0.05% (v/v) Tween20 in 140 mM NaCl, 2.7 mM KCl, 16 mM Tris, pH 7.4 (TBS) for 1 h, next for 1 h in secondary antibody solution (1:30,000 goat anti-mouse IgG (ab97040; Abcam; Cambridge; United Kingdom) in StartingBlock (#37542; Thermo Fisher; Darmstadt; Germany) with 0.1% Tween20) whereupon signals of the molecular weight marker could be visualized with horse-radish peroxidase substrates (see below).

The membranes of biopsy lysates were incubated in StartingBlock with 0.1% Tween20 for 1 h. Incubation with primary antibodies was carried out in a float-your-blot set-up: parafilm was placed in a 12 cm square petri dish using a small volume of 0.05% Tween20 in TBS between the plastic surface and the parafilm. Next, 1,000 µL of primary antibody solution (see below) were distributed onto the parafilm in a line parallel to the parafilm's edge. Next, the membrane was positioned at an angle over the parafilm whereby the side containing the proteins was oriented towards the parafilm and the edge containing the high molecular weight proteins was aligned with the primary antibody solution. To incubate the entire membrane surface with the small volume of primary antibody solution, the membrane was slowly lowered towards the parafilm starting at the edge with the high molecular weight proteins and continuing towards the edge where lower molecular weight proteins were located. The petri dish was covered with its lid and the entire assembly was placed in a container with 0.05% Tween20 in TBS to prevent evaporation. Primary antibodies were incubated for 18 h at 4°C.

Immune-reactive signals of biopsy lysates were generated by sequential probing with antibodies: 1st detection of CFTR (1st antibody: equimolar mix of CFTR-AK 596 + 570 + 217 + 660 (Cystic Fibrosis Foundation CFTR Antibody Distribution program; Chapel Hill; NC, United States) diluted 1:400 in StartingBlock with 0.1% Tween20; 2nd antibody: goat anti-mouse IgG (ab97040; Abcam; Cambridge; United Kingdom) diluted 1:7500 in StartingBlock with 0.1% Tween20); 2nd detection of CFTR (see above); stripping three times for 10 min each with 200 mM glycine, 0.1% SDS, 2% Tween20, pH 2.2; detection of vinculin (1st antibody: anti-vinculin antibody (ab130007; Abcam; Cambridge; United Kingdom) diluted 1: 500 in StartingBlock with 0.1% Tween20; 2nd antibody: goat anti-mouse IgG (ab97040; Abcam; Cambridge; United Kingdom) diluted 1:7500 in StartingBlock with 0.1% Tween20).

2.7 Development of CFTR and vinculin signals with HRP substrates and densitometry

Membranes with biopsy lysates were incubated sequentially with SuperSignal West Pico (34078; Thermo Fisher; Darmstadt; Germany) and SuperSignal West Femto Max. Sensitivity (34096; Thermo Fisher; Darmstadt; Germany). To ensure that signals in all lanes were visualized, exposure times were varied between 3 s and 30 min for PICO and next, between 3 s and 30 min for FEMTO yielding about 15 different exposures of each primary antibody target. Scans were acquired on a DNR-MF-ChemiBIS 3.2 Bio-Imaging System (Berthold Technologies, Bad Wildbad, Germany). For densitometry, an exposure with high signal intensity was selected, avoiding saturation of corresponding baseline and treatment pairs. Densitometry of digitized scans was performed with GelAnalyzer 19.1 (www.gelanalyzer.com by Istvan Lazar Jr., PhD and Istvan Lazar Sr., PhD, CSc). Datasets of CFTR isoforms were compared from paired samples obtained from specimens prior to and after the start of therapy with ELX/TEZ/IVA and the effect of ELX/TEZ/IVA was judged by Wilcoxon-signed rank test.

2.8 Censoring criteria for semi-quantification of signals generated by Western-blot

As biopsy lysates can contain mucus proteins that are detected by protein quantification of the primary lysates but do not migrate into the polyacrylamide gel matrix, we used Coomassie staining after transfer onto the membrane to ensure that the amount of proteins that have entered the gel matrix are comparable between reference or ELX/TEZ/IVA-treated sample of one patient. Furthermore, signals for epithelial vinculin were used to judge whether paired reference and ELX/TEZ/IVA-treated biomaterials were comparable with respect to the proportion sampled of epithelial tissue. If either of these two measures showed a difference between biomaterials, paired samples were excluded from semi-quantitative analysis (see Supplementary Figures S2A–L).

In detail, we have obtained CFTR Immunoblots prior to and after the start of ELX/TEZ/IVA treatment from rectal suction biopsies of 21 patients. We have judged paired biomaterials by two controls: Firstly, based on non-specific protein staining using Coomassie of leftover material after transfer of whole-cell lysates to the membrane, we have verified that the amount of proteins capable to enter the polyacrylamide matrix is comparable between samples obtained pre-treatment and after ELX/TEZ/IVA treatment from one patient. Next, specific vinculin staining was used to judge whether the paired biopsy samples contain an equivalent amount of epithelium. Based on Coomassie and/or specific vinculin staining, we excluded eight samples from further densitometric analysis of CFTR band C* since samples prior-to-treatment and corresponding ELX/TEZ/IVA samples were incomparable for eight pairs (N°2, N°7, N°8, N°11, N°12, N°17, N°18, and N°21). Densitometry confirms that censored samples differed stronger than samples included for densitometry: Signals for vinculin and metavinculin of censored eight sample pairs differed by 1.3 fold (SD 1.6 fold) when comparing prior-to-treatment and ELX/TEZ/IVA-sample. Vinculin and metavinculin signals in 14 sample pairs accepted for densitometry differed by 0.4 fold (SD 0.2 fold) when comparing prior-to-treatment and ELX/TEZ/IVA lane.

For the remaining 14 samples, CFTR band C* was quantified by densitometry from both paired samples. If possible, we have evaluated

TABLE 1 Study participants.

Patient No.[a]	*CFTR* genotype (Legacy name)	Gender	Assessment prior to ELX/TEZ/IVA therapy				Assessment during ELX/TEZ/IVA therapy			
			age (yr)	BMI (kg/m^2)	FEV1 %pred	Sweat Cl^- (mM)	age (yr)	BMI (kg/m^2)	FEV1 %pred	Sweat Cl^- (mM)
1	F508del/2184insA	F	16.0	21.9	93	106	16.3	22.5	106	20
2	F508del/CFTRdele2,3 (21 kb)	F	21.3	19.2	53	103	21.6	19.2	82	14
3	F508del/2721del11	F	14.0	17.4	85	104	14.3	18.6	94	25
4	F508del/CFTRdele2,3 (21 kb)	F	13.7	20.0	129	104	14.1	19.6	147	54
5	F508del/N1303K	F	17.3	25.0	71	109	17.6	25.7	83	101
6	F508del/CFTRdele2,3 (21 kb)	F	13.8	19.4	110	110	14.0	22.9	126	40
7	F508del/F508del	F	12.5	15.6	53	95	12.8	17.1	89	10
8	F508del/2184delA	F	14.7	18.5	104	113	15.0	18.8	115	65
9	F508del/F508del	F	13.6	19.8	107	104	13.9	19.8	112	27
10	F508del/1078delT	F	12.7	17.9	101	88	13.0	18.6	117	41
11	F508del/G542X	F	20.7	15.8	46	92	21.2	18.1	76	38
12	F508del/394delTT	F	12.0	14.2	63	98	12.4	16.5	83	70
13	F508del/F508del	F	24.3	20.2	93	101	24.6	21.3	124	79
14	F508del/1078delT	F	15.5	17.9	113	99	15.8	17.9	126	44
15	F508del/F508del	F	14.2	15.2	74	87	14.5	16.9	103	25
16	F508del/G542X	M	17.1	20.2	84	108	17.5	20.3	115	90
17	F508del/R553X	M	44.0	28.1	82	102	44.4	29.5	87	53
18	F508del/E822X	M	12.8	21.1	80	108	13.2	21.1	88	36
19[b]	F508del/F508del	M	44.8	21.1	62	108	45.2	22.5	88	52
20	F508del/F508del	M	13.9	15.8	95	98	14.2	16.1	116	30
21	F508del/2184delA	M	12.7	15.1	89	115	13.1	16.8	118	50
Median [IQR]			14.2 [13.6–17.3]	19.1 [15.8–20.2]	85 [71–100]	104 [98–108]	14.5 [13.9–17.6]	19.2 [17.9–21.3]	106 [88–117]	41 [27–54]

[a]Results presented are part of a larger, multi-center trial at four study centers of the German Center for Lung Research designed to analyze effects of ELX/TEZ/IVA treatment on different clinical parameters and biomaterials (NCT04732910) (Graeber et al., 2022). Sampling of rectal biopsies for CFTR protein content by western blot analyses was only performed in the subgroup of patients recruited at Hannover Medical School, according to the ethical approval # 8922_BO_S_2020 from the Hannover ethics committee.
[b]Patient 19 had continuously administered tezacaftor/ivacaftor for 18 months prior to triple therapy. All other study participants were modulator–naïve at baseline.

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more than one CFTR detection as indicated in Supplementary Figures S2A–L. Minute signals for either the sample obtained pre-treatment or for the sample obtained after ELX/TEZ/IVA-treatment were observed in five sample pairs (N°4, N°6, N°9, N°10, and N°13) which were resolved as follows: The intensity of CFTR band C* was minute in four control samples (N°6, N°9, N°10, and N°13), making normalization to 100% as expression level prior to start of treatment error prone. Based on obtained primary data of samples N°19 and N°20 corresponding to an increase of CFTR C* of 400%, 274% and 405%, we cautiously used a cut-off value of >300% gain in signal for band CFTR C* to describe the increase in CFTR expression in these samples. In sample pair N°4, the low intensity band C* could not be quantified in the sample obtained after treatment with ELX/TEZ/IVA and thus decrease upon treatment was not quantified in this sample pair.

In summary, we have obtained semi-quantitative data for the change in CFTR-C* expression upon treatment with ELX/TEZ/IVA for 13 out of 21 paired biosamples (see Figure 2C, source data shown in Supplementary Figure S2).

2.9 Statistics

Differences in CFTR glycoisoform expression comparing rectal suction biopsies obtained prior to treatment and patient tissue obtained under ELX/TEZ/IVA-treatment were judged by the non-parametric Wilcoxon signed-rank test. Critical values for small number of observations were judged based on: S. Siegel, Non-parametric Statistics, McGraw Hill Book Comp., London 1956, p.254 as described in E. Weber, Grundriss der biologischen Statistik, VEB Gustav Fischer Verlag, Jena 1986.

Korrelation of CFTR protein expression and clinical parameters (Supplementary Figure S3) was judged by Spearman's rank correlation coefficient with correction for ties within the data set.

3 Results

3.1 Design of the study and clinical outcome

Fifteen female and six male patients with CF participated in the study (Table 1). We obtained anthropometry, spirometry, sweat chloride concentrations and rectal suction biopsies for CFTR immunoblot analysis at the day prior to the first administration of ELX/TEZ/IVA and after 12–18 weeks (median: 16 weeks) of continuous triple therapy. Six patients were p.Phe508del homozygous and fifteen patients were p.Phe508del heterozygous in combination with a minimal function *CFTR* mutation. Twenty participants were naïve for CFTR modulation at the start of the triple therapy, only the oldest participant had been on regular combination treatment with tezacaftor/ivacaftor until the day of first assessment.

Consistent with the outcome of the phase III trials, the study participants who had been naïve for CFTR modulation improved anthropometry and lung function (Table 1). Chloride concentration in sweat test was in the pathological range at baseline (median 104 mmol/L, range 87–115 mmol/L) and decreased during exposure to ELX/TEZ/IVA by a mean of 56 mmol/L to a median sweat chloride concentration of 41 mmol/L (range 10–101 mmol/L). Sweat chloride decreased to the normal and intermediary levels in six and ten subjects, respectively, but remained in the pathological range in five subjects (Table 1).

3.2 CFTR immunoblot analysis of rectal biopsies from CF patients and healthy controls

Figure 1 compares the gel-separated CFTR immunochemical signals of intestinal and pulmonary reference cell lines commonly used in CFTR research, primary bronchial epithelial cells (PBEC) grown under air-liquid interface (ALI) condition, a rectal suction biopsy from a non-CF control and two samples from CF rectal suction biopsies. Non-CF samples all displayed a narrow band B at 130 kDa and a much broader band C between 150 and 180 kDa. Yet, wild-type CFTR band C from CaCo2, 16HBE14o- and from PBEC ALI migrated slower than wild-type CFTR from HT29 and T84 or the non-CF biopsy, confirming size and/or glycosylation differences between wild-type CFTR of different origins (Ward and Kopito, 1994; Kälin et al., 1999; McClure et al., 2016). The rectal mucosa of the healthy control shares the position, width and intensity of the CFTR immune-reactive signals of a faint band B and a strong band C at the previously published positions of 150–180 kDa (complex glycosylated wild-type CFTR-C) (Ward and Kopito, 1994; Kälin et al., 1999; McClure et al., 2016) and 130 kDa (core-glycosylated CFTR-B) (Ward and Kopito, 1994; Kälin et al., 1999; McClure et al., 2016) with those of the intestinal cell lines T84 and HT29 (Figure 1).

By applying the same experimental conditions, CFTR immune-reactive signals of the selected CF rectal biopsies are barely detectable, even at significantly longer exposure times indicating that the intestinal epithelia of CF patients express only low amounts of mutant CFTR (Figure 1). Even more important, side-by-side detection of CFTR from the non-CF control and from CF biopsies revealed that the signal of the glycosylated isoform obtained from CF biopsies is detected at a lower molecular weight, or alternatively, exhibits a more compact three-dimensional structure to allow faster migration through the polyacrylamide matrix. Twenty out of twenty-one biopsies obtained prior to start of ELX/TEZ/IVA-treatment displayed such a mutant CFTR C* signal and the typical shape of complex glycosylated wild-type CFTR band C was absent in all analyzed CF biopsies (Figure 2A; Supplementary Figure S2). We have named this differently glycosylated form of mutant CFTR, typical for CF patients' rectal biopsies, "band C*" to denote that its electrophorectic mobility is not equivalent to complex glycosylated wild-type CFTR band C.

3.3 Changes in CFTR expression patterns after initiation of ELX/TEZ/IVA treatment

We next addressed CFTR glycoisoform patterns 8–16 weeks after initiation of treatment with ELX/TEZ/IVA. To this end, we analyzed rectal suction biopsies obtained from the study participant prior to and during ELX/TEZ/IVA-treatment side-by-side by semi-quantitative immune-detection of CFTR. In 16 out of 21 lysates obtained prior to start of ELX/TEZ/IVA-treatment, the core-glycosylated isoform CFTR B at 130 kDa was seen. In all but one sample prior to and in all 21 samples obtained after ELX/

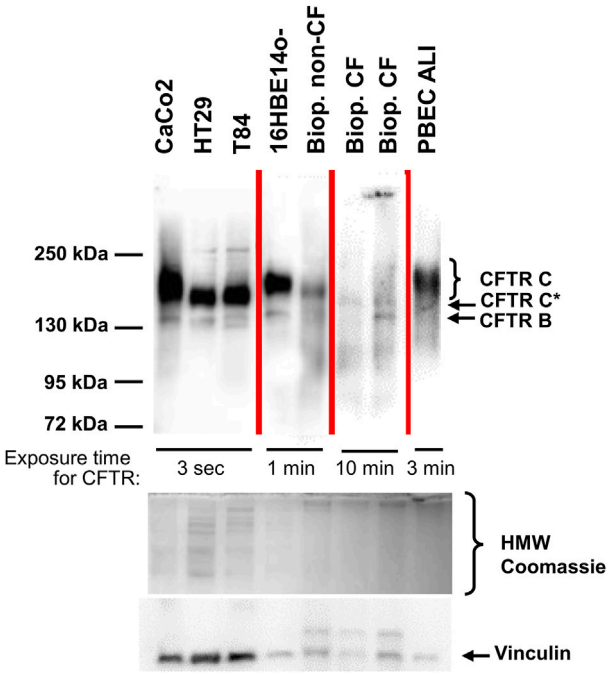

FIGURE 1
CFTR immunoblot analyses of epithelial cell lines, primary bronchial epithelium and from rectal biopsies from one healthy control and two CF samples. CFTR signals were obtained from the intestinal cancer cell lines CaCo2, HT29, T84, from the immortalized respiratory epithelial cell line 16HBE14o-, from primary peripheral bronchial epithelium (PBEC) generated by air-liquid interface (ALI) culture and compared to a rectal suction biopsy derived from a non-CF control ("Biop. non-CF") and from two CF rectal suction biopsies ("Biop. CF"). Western blots reveal a narrow band B at 130 kDa and a much broader band C between 150 and 180 kDa in all samples except for the CF patients' biopsies. Rectal biopsies from CF patients showed a band B similar to the other samples and generated a narrow signal, migrating slower than band B at approximately 140 kDa designated as CFTR C*. A poly-disperse wild-type band C visible in all other non-CF samples was absent from CF biopsies. Samples were loaded on one gel, yet this figure is a composite as visualization of signals was done using exposure times between 3 s and 10 min for CFTR as noted within the figure. Source data of all four exposures of the entire membrane is provided in the supplement. Protein amount loaded was as follows: CaCo2, HT29, T84, 16HBE14o-: 30 µg; PBEC ALI: 25 µg; rectal suction biopsies: 75 µg.

TEZ/IVA-treatment, CFTR band C* was the dominant CFTR glycoisoform in CF rectal suction biopsies (Figure 2A; Supplementary Figures S2A–L). Additionally, a low molecular weight band below 95 kDa in size could be observed in about a third and a high molecular weight band of approximately 240 kDa in size was seen in 18 out of 21 sample pairs. If present, the <95 kDa band and/or the 240 kDa band appeared in patient samples obtained at baseline and during ELX/TEZ/IVA treatment (Figure 2B, two left lanes and Supplementary Figures S2A–L).

Since the glycosylated CFTR band C* was the most prominent CFTR glycoisoform in most analyzed CF rectal suction biopsies, we aimed to quantify the change of CFTR C* induced by ELX/TEZ/IVA treatment (Figure 2C). In the majority of sample pairs an increase of CFTR C* signal intensity was seen in biopsies taken after the onset of ELX/TEZ/IVA-treatment (Figure 2B, two left lanes). In eight out of 12 sample pairs that were eligible for semi-quantification of CFTR signals (see methods for details), the signal for C* was at least twofold higher during triple therapy than at baseline (Figure 2C, $p < 0.02$, Wilcoxon signed rank test). However, in two out of 12 samples we observed a decrease in CFTR C* signal upon treatment to about half of that of the baseline sample (Figure 2B, two right lanes; Figure 2C). Of note, the strongest decline in CFTR-C* was noted in patient N°5 who is compound heterozygous for p.Phe508del and the missense variant N1303K. All other participants are either homozygous for p.Phe508del or compound heterozygous p.Phe508del with a stop or frameshift mutation, whereby no functional CFTR protein is expected from the latter class I mutations. In other words, biomaterial from the one compound heterozygous study participant who carries two, not one, class II alleles that could be improved by ELX/TEZ/IVA displayed most loss of CFTR-C* protein expression. Furthermore, absence or presence of CFTR-B and change in CFTR-C* expression was CFTR genotype-dependent among 12 cases eligible for densitometry as judged by comparable vinculin signals in samples taken prior to and after start of ELX/TEZ/IVA therapy allowed quantification of CFTR signals. Signals for CFTR-B were absent or too low for comparative quantification in biosamples from five p.Phe508del homozygotes while among seven compound heterozygotes, CFTR-B could be quantified but did not change upon ELX/TEZ/IVA therapy (Figure 2C). In contrast, increase of CFTR-C* was higher in p.Phe508del homozygotes compared to compound heterozygotes who carried only one allele: four out of five p.Phe508del homozygotes showed an increase in CFTR-C* of at least 300% while for five out of seven compound heterozygotes—carrying one p.Phe508del allele only—less than 300% increase in CFTR-C* was observed. A decrease in CFTR-C* was only seen in samples from three compound heterozygous patients.

We carefully interrogated the immunoblots for a CFTR immune-reactive signal with migratory properties intermediate between the mutant glycosylated CFTR band C* and the mature, wild-type CFTR band C seen in the 16HBE14o-control samples. We observed such a band CFTR C** in samples from three patients (Supplementary Figure S2M). However, it was a faint signal and distinguishable from wild-type CFTR band C by two criteria, i.e., a higher electrophoretic mobility and lower width of the immune-reactive signal indicating an altered shape and/or lower polydispersity of the N-glycans. This CFTR C** was visible in two samples at baseline and in one sample during ELX/TEZ/IVA therapy (Supplementary Figure S2M).

4 Discussion

Treatment of CF with small molecules to increase cell-surface CFTR expression and thereby protein function is a first successful example of mutation-specific therapy for genetic diseases. Similar approaches to restore functionality of mutated proteins can be envisioned for a large range of genetic diseases where mutations also affect synthesis, post-translational processing and trafficking and thereby protein functions. Insight into the effects of such an approach therefore has the potential to reveal broad implications for the development of, not only CFTR modulators, but also for small-molecule approaches for other genetic diseases.

In that line, our data provide initial evidence that ELX/TEZ/IVA indeed increases CFTR protein expression in rectal suction

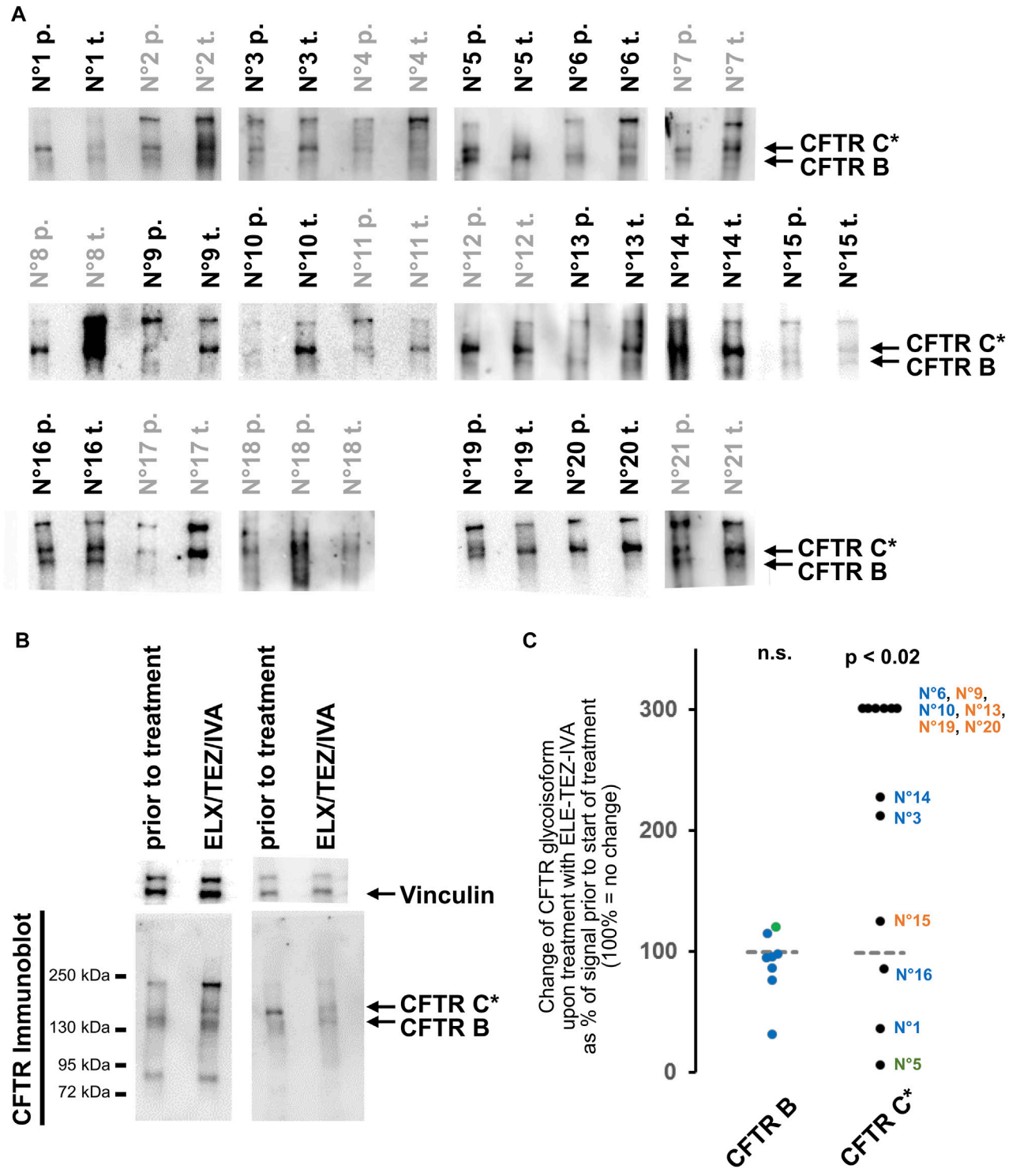

FIGURE 2
Changes in CFTR glycoisoforms from rectal suction biopsies prior to and after initiation of treatment with ELX/TEZ/IVA. **(A)** CFTR immunoblot signals of rectal biopsy lysate pairs from 21 patients. Samples prior-to-treatment (labelled "p.") and samples obtained after ELX/TEZ/IVA treatment (labelled "t.") were incomparable as judged by Coomassie staining and by vinculin detection for these eight pairs: N°2, N°7, N°8, N°11, N°12, N°17, N°18, N°21 (labelled in grey; see methods section for details on censoring). Exposures are selected to visualize band C* and B in CF samples. Calibration of C* vs. CFTR-C from 16HBE14o- is shown as source data of the entire patient cohort including Coomassie staining of high molecular weight proteins and vinculin signals in Supplementary Figure S2. **(B)** Western blot of two representative examples. Left sample pair shows an increase of CFTR C* upon treatment with ELX/TEZ/IVA (patient N°6) while right sample pair shows a decrease of CFTR* upon treatment with TEZ-ELE-IVA (patient N°1). Additionally, the left sample pair shows the <95 kDa and 240 kDa immune-reactive signals visible in about a third (<95 kDa) and all but one (240 kDa) samples. **(C)** Densitometry was carried out on side-by-side loaded lanes of pre- and post-treatment samples for 12 out of 21 biopsy lysate pairs that yielded comparable signals for whole protein content and vinculin [see Panel **(A)** and Section 2 for details on censoring]. An increase of CFTR C* is observed ($p < 0.02$, Wilcoxon signed rank test) while the signal intensity of the core glycosylated band B is unaltered by ELX/TEZ/IVA. The patient's CFTR mutation genotype is color-coded as follows: p.Phe508del homozygotes—terracotta (N°15, N°19, N°20, N°13, N°9); p.Phe508del compound heterozygotes with a class I mutation—blue (N°1, N°16, N°3, N°14, N°10, N°6); p.Phe508del/N1303K compound heterozygote—green (N°5).

biopsies in the majority of patients. Interestingly, our results also show that in rectal epithelium of CF patients the molecular weight of expressed CFTR differs between CF patients and non-CF controls suggesting persistent alterations in post-translational glycosylation, which are not affected by ELZ/TEZ/IVA. Thus, our data provide two novel aspects as explanations for the patient-to-patient variability as a possible clue to individual responses (Heijerman et al., 2019; Middleton et al., 2019).

Our data on human rectal suction biopsies collected prior to and after the start of therapy with ELX/TEZ/IVA identified a partially mature complex-glycosylated isoform CFTR C* prior to ELX/TEZ/IVA of varying intensity in all patients. Migratory properties of this band C* differed from fully glycosylated wild-type CFTR band C which we could observe in healthy controls and in intestinal epithelial cell lines and airway epithelial cells lines and primary airway epithelial cells (Figure 1). To our knowledge, our data are the first to identify this CF-typical migratory pattern of the CFTR protein in CFTR modulator naïve or treated patients. Interestingly, Sette et al. (2021) could recently visualize CFTR from patient-derived nasal epithelial conditionally reprogrammed stem cells whereby three p.Phe508del-CFTR homozygotes displayed a CFTR glycoisoform phenotype in ALI culture similar to the C*/B—combination observed in patient's intestinal epithelium *ex vivo* in our study. Moreover, Dekkers et al. (2016) showed western blot data from intestinal organoids whereby one p.Phe508del homozygote and three p.Phe508del compound heterozygotes display a faint glycosylated CFTR isoform with migratory properties at the lower rim of the wild-type signal, thus in consistency with the band CFTR-C* described in this work. Taken together, these data (Dekkers et al., 2016; Sette et al., 2021, this work), suggest that CFTR-C* represents the *status quo* of p.Phe508del-CFTR expression in CF patients *in vivo*.

Our data upon treatment with ELX/TEZ/IVA suggest that ELX/TEZ/IVA facilitated the posttranslational processing of some mutant CFTR, but apparently did not succeed in generating the poly-disperse spectrum of N-linked oligosaccharides that is characteristic for wild type CFTR band C. Migratory properties observed by polyacrylamide gel electrophoresis of the mutant complex-glycosylated isoform CFTR C* are in between those of the core-glycosylated isoform B and the mature glycoisoform C of wild-type CFTR (Figure 1). This suggests that the repertoire of glycosylation enzymes resident in the mid-to-trans-Golgi compartment (Schjoldager et al., 2020) has not been fully utilized to generate the branched and elongated N-linked oligosaccharides typical of mature wild-type CFTR (McClure et al., 2016).

Previously, western-blot data have confirmed that an increase of mature CFTR is observed upon therapy with ELX/TEZ/IVA (Capurro et al., 2021; Becq et al., 2022). We detected the mannose-rich ER isoform band B in similar amounts in epithelia of non-CF origin and in CF specimens collected at baseline or during triple therapy. In that, our results propose that the biosynthesis and turnover of the p.Phe508del-CFTR ER glycoisoform does not significantly differ from that of the wild-type protein and is not affected by triple combination CFTR modulation. Conversely, the mutant CFTR band C* glycoisoform was enhanced by ELX/TEZ/IVA in 60% of samples by at least twofold, suggesting that an improvement in CFTR processing and maturation in the ER and cis-Golgi beyond the core-glycosylation that shapes the CFTR glycoisoform B is achieved by ELX/TEZ/IVA (Figure 2). Since non-conventional trafficking has been noticed for CFTR (Yoo et al., 2002; Gee et al., 2011), it is conceivable that mutant CFTR C* can reach the plasma membrane of epithelia and function as a chloride- and bicarbonate channel which is corroborated by ELX/TEZ/IVA's effect on sweat chloride levels and other biomarkers of CFTR function (Heijerman et al., 2019; Middleton et al., 2019; Graeber et al., 2022). Notably however, the change in mutant CFTR C* induced by ELX/TEZ/IVA observed *ex vivo* was lower than the small molecule mediated correction of class II CFTR mutations *in vitro* in transfected cell lines (Han et al., 2018).

Even though only four major bands are observed in the CFTR immunoblots (B, C*, <95 kDa, 240 kDa), we noticed that the band pattern detected with antibodies raised against CFTR is more similar between two paired samples (samples prior to and after ELX/TEZ/IVA treatment) from one patient while patterns obtained from different individuals have a distinguishable signature (Figure 2C; Supplementary Figure S2). We interpret the low molecular weight bands of <95 kDa as degradation products of CFTR (Ward and Kopito, 1994; Gentzsch et al., 2004; Swiatecka-Urban et al., 2005). The high molecular weight signals at approximately 240 kDa may represent multimeric protein complexes such as ubiquitinated CFTR (Ameen et al., 2007; McClure et al., 2016) or CFTR non-covalently or covalently linked with members of the p.Phe508del CFTR interactome (Pankow et al., 2015; Vinhoven et al., 2021). Alternatively, the 240 kDa band might correspond to rootletin which can also be detected by mAb596 raised against CFTR (Sato et al., 2021). The similarity between samples taken from one patient suggests that the repertoire of glycosylation enzymes, degradation enzymes and CFTR interacting partners is likely specific for an individual and thus leads to a unique set of CFTR glycoisoforms and CFTR multiprotein species in each patient.

Studies with recombinant CFTR in transfected cell lines have revealed that neither core nor complex N-glycans are required for the correct folding of CFTR at the ER and the subsequent trafficking to the cell surface (Chang et al., 2008). However, the N-glycans enhance the productive folding and conformational stability of CFTR (Glozman et al., 2009). Defective N-glycosylation reduces the stability of CFTR, induces ubiquitination and causes more rapid turnover in post-ER compartments (McClure et al., 2016). In our study, the pharmacologically rescued band C* p.Phe508del CFTR from patients' rectal mucosa differed from the complex-glycosylated isoform C of wild-type CFTR by higher mean mobility and lower bandwidth of the immune-reactive signal on the Western blot (Figures 1, 2; Supplementary Figure S2). In the Golgi apparatus, complex glycan attachment by one of the more than 200 glycosyltransferases fine-tunes protein biogenesis (Lairson et al., 2008). The broad poly-disperse distribution of the wild type Golgi maximally tetra-antennary glycoform C of CFTR is most likely caused by repeating units of N-acetyllactosamine (O'Riordan et al., 2000). Hence, under the assumption that CFTR-C* is a partially glycosylated isoform of CFTR, it is conceivable that in the rectal biopsies we examined CFTR triple combination therapy promoted the exit of p.Phe508del CFTR from the ER (Kleizen et al., 2021), but failed to restore Golgi-resident glycosylation steps including the addition of N-acetyllactosamine repeats. Alternatively, as wild-type and mutant CFTR are modified differently post-translationally (Gong et al., 2019; Wu et al., 2022), the size difference between CFTR-B and CFTR-C* might be the result of other posttranslational modifications such as mono- or poly-ubiquinylation or sumoylation.

Any factors that influence the synthesis, processing, trafficking, half-life of mutant CFTR such as the members of the CFTR interactome (Pankow et al., 2015; Vinhoven et al., 2021) may be considered as the primary modifiers of cellular CFTR protein content. Alternative ion channels (Pinto et al., 2021) and the growth,

differentiation, ageing and remodeling of tissue (Brezillon et al., 1995; Castillon et al., 2002) constitute the secondary modifiers of ion channel function; and lifestyle, living conditions, socioeconomic status, biological age, gender, therapeutic measures and co-morbidities represent the tertiary modifiers of CFTR homeostasis.

It has been noted in ELX/TEZ/IVA treated primary cultures of nasal and pulmonary epithelia that the functional correction exceeds the biochemical correction of CFTR (Veit et al., 2020), indicating that the potentiator function of ELX in these respiratory primary epithelia (Veit et al., 2020; Shaughnessy et al., 2021) can partially compensate for mistrafficking and inadequate processing of mutant CFTR. Since the degree of clinical benefit changes within a small range of functional CFTR protein, it remains to be seen whether the strong clinical benefit of the treatment with ELX/TEZ/IVA seen in our study and the clinical trials (Heijerman et al., 2019; Middleton et al., 2019) will persist or attenuate over the years in an ageing CF population. Our results might provide a basis to understand different degrees in response and different long-term outcomes of ELX/TEZ/IVA treatment. They caution that the lower amounts or immature glycosylation of the C* glycoisoform might prevent long-term, sustained benefit of ELX/TEZ/IVA. The development of further CFTR modulators that promote the production of more and mature CFTR may increase the robustness of the functional rescue and may reduce the strong patient-to-patient variation of the clinical response. Further analyses of CFTR glycoisoforms by high-resolution western blot from patient's biosamples may thus assist to verify and monitor the individual's CFTR-C glycosylation status achieved by different CFTR small molecule therapeutics as a potential biomarker for full functional CFTR correction. Given the clinical success of ELX/TEZ/IVA triple therapy, we hope that our results can provide leverage to achieve improved functional rescue of CFTR and provide guidance for the development of approaches to rescue protein function for other diseases affected by aberrations of protein synthesis, post-translational processing and trafficking.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Hannover Medical University, according to the ethical approval # 8922_BO_S_2020 from the Hannover Ethics Committee. Written informed consent to participate in this study was provided by the participant or their parent or legal guardian.

Author contributions

BT and A-MD conceived the study. FS and BT designed the experimental approach for biosample analysis by Western blot. SP enrolled patients and provided specimens. SP, AS-H, FR, SJ, BT, and A-MD consented patients. RM and ST processed biosamples. SH and EE performed experiments. FS supervised and interpreted the Western blot analysis and provided display items for the manuscript. A-MD and BT provided and interpreted clinical data. FS and BT drafted the manuscript. A-MD discussed all data and revised the draft manuscript. All authors discussed the results and commented on the manuscript, revising it critically for content. All authors read and approved the final manuscript.

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Conflict of interest

AS-H, FR, SJ, A-MD and BT have received funding by Vertex Inc. to conduct clinical approval studies of ELX/TEZ/IVA and other CFTR modulators. RM and SJ receive personal renumeration as part of their salaries to conduct Vertex Inc. approval studies below €10.000/annum. Recruitment of patients and clinical analyses were partially funded by an independent medical grant from Vertex Pharmaceuticals Incorporated to A-MD, which did not include analyses of rectal biopsy material for western blot analyses. This funding was only granted after conceiving the study design and ethical approval of the study protocol at Hannover Medical School.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1114584/full#supplementary-material

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Early and sustained improvements of lung clearance index from two to sixteen weeks of elexacaftor/tezacaftor/ivacaftor therapy in patients with cystic fibrosis—a real world study

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Since the introduction of CFTR modulator therapies, longitudinal real-life data of lung clearance index (LCI) during treatment is scarce. In this single-centre, post-approval setting, we report data of 51 patients with different stages of lung disease, age 2–52 years with repeated measurements of forced expiratory volume as a percentage of the predicted value ($ppFEV_1$) and LCI after 2, 4, and 16 weeks of CFTR modulator treatment and at baseline. In 25 patients during elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA) treatment, significant improvements of LCI (median −1.4) and $ppFEV_1$ (median +8.3%) were observed after only 2 weeks, and were maintained after 4 and 16 weeks of treatment (LCI: -2.0, −2.2; $ppFEV_1$: +7.2%, +11.8%). We observed a significant correlation between LCI improvement at week 16 and lower baseline LCI. In 26 younger and healthier patients receiving lumacaftor/ivacaftor (LUM/IVA) treatment, no significant changes of LCI and $ppFEV_1$ occured. With ELX/TEZ/IVA, our data shows rapid, significant improvements of LCI and $ppFEV_1$ already after 2 weeks. Early LCI measurements can help to assess the patient's response to this high-cost therapy.

KEYWORDS

cystic fibrosis, modulator therapy, lung clearance index (LCI), ELX/TEZ/IVA, LUM/IVA, ventilation inhomogeneity, real-life, ppFEV1

Abbreviations: CF, cystic fibrosis; CFTR, Cystic fibrosis transmembrane conductance regulator; LCI, lung clearance index; MBW, multiple breath washout; MDCT, multi-detector computed tomography; ELX/TEZ/IVA, Elexacaftor/Tezacaftor/Ivacaftor; LUM/IVA, Lumacaftor/Ivacaftor; ppFEV:, per cent predicted forced expiratory volume in one second.

1 Introduction

Cystic fibrosis transmembrane conductance regulator (CFTR) modulator combinations, such as lumacaftor/ivacaftor (LUM/IVA) and elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA), lead to a partial restoration of CFTR function, and clinical studies have shown improvements in several clinical outcomes (Heijerman et al., 2019; Middleton et al., 2019; Nichols et al., 2022). Real-life data regarding longitudinal improvements of ventilation inhomogeneity measured by lung clearance index (LCI) is rare, with one study in CF patients with severe lung disease <40% ppFEV$_1$ (per cent predicted forced expiratory volume in one second) with LCI measurements after 2 and 4 weeks and at baseline (Stylemans et al., 2022).

Graeber and colleagues recently published improvements of both ventilation inhomogeneity and structural lung damage measured after 8–16 weeks of ELX/TEX/IVA treatment (Graeber et al., 2022). That article prompted us to evaluate our own results of LCI measurements in patients treated with triple therapy. In contrast to other groups who reported single follow-up measurements or measurements only in children age 6–11 years (Zemanick et al., 2021; Graeber et al., 2022; Mall et al., 2022), we had assessed pediatric and adult patients longitudinally over 4 months, i.e. at baseline and after 2, 4, and 16 weeks of ELX/TEX/IVA treatment, as part of a routine monitoring programme of modulator efficacy. Our aim was to show the early changes in ventilation inhomogeneity in CF patients due to modulator treatment, measured by improvement of LCI. In addition, we analysed data from patients who had been treated with LUM/IVA before 2020. Here we report on repeated measurements of LCI and ppFEV$_1$ in patients of different age groups and at various stages of lung disease.

2 Materials and methods

In this single-centre real-world evaluation, we analysed data from all patients receiving ELX/TEZ/IVA (from 2020 to July 2022) or LUM/IVA (from 2013 to 2020) treatments with LCI measurements before and after beginning modulator treatment at the CF Centre Innsbruck. Patients were selected to start modulator therapy based on their genetics and deterioration over the last 3 years. We excluded patients receiving CFTR modulators in the context of clinical trials.

All measurements were part of a routine monitoring programme for newly prescribed CFTR modulator therapy using LCI, since 2006, as a surrogate marker for monitoring progression of CF lung disease (Ellemunter et al., 2010). At baseline and at weeks 2, 4, and 16 after commencing treatment, spirometry and multiple breath washout measurements were performed to determine the patient's response to treatment. For the present evaluation, we collected data from our patient database which contains all results obtained during outpatient visits.

LCI was measured using multiple-breath washout (MBW), using nitrogen as the tracer gas. We used two different devices in our patient cohort: the EasyOne Pro® (NDD, Zurich, Switzerland) up to 2019, while the EXHALYZER D® device (Eco Medics, Duernten, Switzerland) has been used since 2017. Thus, all patients on ELX/TEZ/IVA and half of the patients from the LUM/IVA group were measured using the EXHALYZER D® device. All EXHALYZER D® measurements were reanalysed using the updated version of Spiroware® 3.3.1 (Eco Medics, Duernten, Switzerland) (Wyler et al., 2021). There is no validated correction function to address the signal correction error of the EasyOne Pro® device (Oestreich et al., 2022). The upper limit of normal for the two devices differ somewhat, with 7.0 for the EasyOne Pro® and 7.1 for the EXHALYZER D® (Fuchs et al., 2009; Wyler et al., 2021). Although two different MBW devices were used, all measurements throughout the 16 weeks of follow-up were performed with the same device in each patient.

Spirometry was measured in patients aged 6 years and older according to international standards (Quanjer et al., 2012). Since no extra multi-detector computed tomography (MDCT) scans of the chest were performed to assess modulator treatment response, the Bhalla scores (best possible score: 25) of the most recent examination before commencing therapy are displayed.

Ethics approval was obtained from the ethics committee at Medical University of Innsbruck (AN 2015-0227 353/2.5). Written informed consent was obtained from all patients and their legal representatives.

Results are expressed as medians and interquartile range. Absolute changes in ppFEV$_1$ and LCI from baseline were analysed using Repeated Measures ANOVA with *post hoc* tests (pairwise t-tests) (R Version 4.2.0, 2022). To evaluate the effect of severity of lung disease at baseline on subsequent treatment response, we divided each treatment cohort into two subgroups, with LCI and ppFEV$_1$ baseline values above or below the median, respectively. For a comparison of the two modulator groups with the whole patient cohort, the median ppFEV$_1$ was calculated from all patients aged 6 years and older (n = 153) treated at our centre in 2020.

3 Results

3.1 Patient characteristics

The patient characteristics of both treatment groups at baseline are summarised in Table 1. CFTR genetics differed between groups, since 13 of the 25 subjects on ELX/TEZ/IVA, but none on LUM/IVA, were heterozygous for the F508del CFTR mutation. Before receiving ELX/TEZ/IVA, six subjects had been treated with LUM/IVA and two subjects had been treated with tezacaftor/ivacaftor. Due to age restrictions in the licensing of ELX/TEZ/IVA, patients on triple therapy were older than LUM/IVA subjects and consequently had lower lung function, expressed as ppFEV$_1$ (median 53.8% vs. 76.6%) and higher LCI (13.1 vs. 9.6), respectively. Chest MDCT revealed more structural lung disease in the triple therapy group, i.e., median Bhalla scores of 12.5 vs. 18.0.

All patients completed the 16 weeks of follow-up, except one patient, who died after week four of modulator therapy; the cause of death remained unknown despite an autopsy. Compared to the whole cohort at our centre (patients >6 years in 2020, without lung transplantation, median ppFEV$_1$: 84.3% n = 153), the two groups had more severe lung disease.

TABLE 1 Patient characteristics at baseline before CFTR modulator therapy with either Elexacaftor/Tezacaftor/Ivacaftor (ELX/TEZ/IVA) or Lumacaftor/Ivacaftor (LUM/IVA).

	ELX/TEZ/IVA			LUM/IVA		
	Median	Interquartile range	No. of patients	Median	Interquartile range	No. of patients
Patient characteristics						
Age [years]	24.2	17.9 to 32.7	25	19.0	9.3 to 28.5	26
Female: male [n]	19:6		25	17:9		26
CFTR delF508 mutation: homozygeous: heterozygeous [n]	12:13		25	25:0		26
Previous CFTR modulator therapy: LUM/IVA: Tezacaftor/Ivacaftor (TEZ/IVA)	6:2		25	0:0		26
MBW device for LCI measurements ExhalyzerD: EasyOne Pro [n]	25:0		25	12:14		26
Baseline Chest MDCT Score [Bhalla Score]	12.5	11 to 17	24	18.0	13 to 22	25
Lung clearance index (LCI) baseline	13.1	8.4 to 15.1	25	9.6	7.1 to 16.7	26
Percent predicted FEV_1 ($ppFEV_1$) baseline	53.8	44.5 to 85.7	25	76.6	51.8 to 91.1	20

3.2 Response to ELX/TEZ/IVA treatment

Already after 2 weeks of ELX/TEZ/IVA treatment, significant ($p < 0.0001$) absolute improvements of both LCI (by −1.4) and $ppFEV_1$ (by +8.3%) were observed (Table 2). Median changes at weeks 4 and 16 showed consistent and clinically relevant improvements of both LCI and $ppFEV_1$. Thus, the benefits of ELX/TEZ/IVA treatment started early and were maintained up to the end of the observation period. Figure 1 depicts the LCI course of the individual subjects. Not only LCI of modulator naïve patients improved, but also those who had previously been treated with another CFTR drug, predominately LUM/IVA.

Analysis of subgroups with more or less severe lung function impairment revealed that disease severity was associated with treatment response, since patients with worse baseline LCI (above the median of 13.1) showed a larger median improvement in LCI after 16 weeks than subjects with better baseline LCI values (Table 2). Figure 2 shows that the improvement in LCI after 16 weeks of treatment was significantly correlated with baseline LCI values ($r = -0.431$, $p = 0.037$), while there was no association of change in $ppFEV_1$ at week 16 with baseline $ppFEV_1$ ($r = -0.188$, $p = 0.380$).

At week 16, the changes of the two parameters LCI and $ppFEV_1$ compared to baseline showed a trend to correlate with each other, but without statistical significance ($r = -0.375$, $p = 0.071$). Change in LCI was neither associated with baseline $ppFEV_1$ ($r = 0.088$, $p = 0.683$) nor with baseline MDCT Bhalla score ($r = 0.119$, $p = 0.587$).

3.3 Response to LUM/IVA treatment

The 26 younger and healthier patients who received LUM/IVA between 2013 and 2020 experienced no significant changes in LCI (Figure 1), although the median LCI declined by −0.8 at week 16 compared to baseline (Table 2). Absolute change of $ppFEV_1$ also showed no significant benefit from LUM/IVA treatment at weeks 2–16. The only subgroup which experienced a detectable response to LUM/IVA were patients with lower initial $ppFEV_1$ (below 76.6%), with a median $ppFEV_1$ increase of 5.4% at week 16.

4 Discussion

Our data from a real world, post-approval setting showed clinically relevant and statistically significant improvements of $ppFEV_1$ and LCI already after 2 weeks of ELX/TEZ/IVA treatment, with benefits maintained 4 and 16 weeks after the first dose. There was a correlation between baseline LCI and the improvement of LCI after 16 weeks of therapy. We evaluated patients between 8 and 52 years of age with predominantly moderate lung disease, as reflected in a median $ppFEV_1$ of 53.8%. Complementing other studies, our patients had three control visits within the 4 months after initiating CFTR modulator therapy to get a clearer picture on the response to treatment.

The recent article by Graeber et al. reported LCI improvements in 45 patients heterozygous for F508del (by −2.4) and 46 homozygous for F508del (by −1.4) aged 12 years and older with ELX/TEZ/IVA treatment (Graeber et al., 2022). Only one measurement was performed 8–16 weeks after commencement of treatment, so the situation within the first weeks of treatment remains unknown. Two other studies in children 6 through 11 years described longitudinal data for LCI before and during treatment with ELX/TEZ/IVA (Zemanick et al., 2021; Mall et al., 2022). The authors observed significant improvements in LCI of −2.3 and −1.7, respectively, and $ppFEV_1$ (+11.0 and +10.2%) after 24 weeks of treatment compared to baseline. Depicting the longitudinal course of LCI during ELX/TEZ/IVA treatment in adults, only one paper describes measurements after 2 and 4 weeks with improvements of both LCI and $ppFEV_1$ by median LCI -0.6 and −1.4, and median FEV_1 +6% and +3% in older patients with severe lung disease, i.e. an $ppFEV_1$ below 40% predicted (Stylemans et al., 2022).

Regarding LUM/IVA treatment and LCI, four clinical trials showed significant LCI changes compared to baseline, with two trials focusing children from 6 to 11 years and two trials analysing patients >12 years (Milla et al., 2017; Ratjen et al., 2017; Shaw et al., 2020; Graeber et al., 2021). In single measurements obtained at 24 or 52 weeks after start of LUM/IVA, improvements of LCI between −0.8 and −1.1 were observed, whereas no change in $ppFEV_1$ compared to baseline was detected (Milla et al., 2017; Ratjen et al., 2017; Shaw et al., 2020; Graeber et al., 2021). Our data also shows small improvements in median LCI during LUM/

TABLE 2 Response to CFTR modulator therapy. ppFEV$_1$ and lung clearance index (LCI) at baseline, and absolute and relative changes at 2, 4, and 16 weeks after initiation of elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA) or lumacaftor/ivacaftor (LUM/IVA) in patients with cystic fibrosis. Six patients were not able to perform ppFEV$_1$ measurements due to age <6 years. Repeated measures ANOVA showed significant ($p < 0.0001$) benefits during ELX/TEZ/IVA treatment at each time point compared to baseline. Subgroup analyses revealed greater improvements in LCI and ppFEV$_1$ in patients with worse lung function, i.e. LCI above and ppFEV$_1$ below median, respectively.

	ELX/TEZ/IVA (2020 to 2022)			LUM/IVA (2013 to 2020)		
	Median	Interquartile range	No. of patients	Median	Interquartile range	No. of patients
Lung clearance index (LCI)						
Baseline	13.1	8.4 to 15.1	25	9.6	7.1 to 16.7	26
Absolute change from start of therapy to...						
week 2	−1.4	−2.3 to −0.6	25	−0.5	−1.5 to 0.9	26
week 4	−2.0	−3.0 to −0.5	25	−0.4	−1.4 to 0.7	26
week 16	−2.2	−3.9 to −1.1	24	−0.8	−1.7 to 0.5	26
week 16, baseline LCI below median	−1.7	−2.6 to −0.7	12	−0.4	−0.9 to 0.9	13
week 16, baseline LCI above median	−2.3	−4.4 to −2.0	12	−1.1	−3.1 to 0.2	13
Relative change (%) from start of therapy to ...						
week 2	−12.7	−18.8 to −6.1	25	−5.0	−10.7 to 8.9	26
week 4	−15.4	−23.7 to −9.2	25	−4.2	−13.4 to 4.6	26
week 16	−16.2	−30.4 to −10.5	24	−6.7	−15.1 to 4.7	26
week 16, baseline LCI below median	−22.9	−30.9 to −7.2	12	−5.7	−12.8 to 10.5	13
week 16, baseline LCI above median	−15.4	−29.4 to −13.9	12	−9.0	−17.0 to −1.0	13
Percent predicted FEV$_1$ (ppFEV$_1$)						
Baseline	53.8	44.5 to 85.7	25	76.6	51.8 to 91.1	20
Absolute change from start of therapy to...						
week 2	8.3	4.0 to 16.1	25	0.9	−4.4 to 5.6	20
week 4	7.2	5.2 to 15.8	25	−0.3	−3.9 to 9.5	20
week 16	11.8	6.6 to 15.3	24	2.8	−2.9 to 7.2	20
week 16, baseline ppFEV$_1$ below median	12.2	9.0 to 14.29	12	5.4	1.8 to 8.2	10
week 16, baseline ppFEV$_1$ above median	10.1	5.8 to 16.3	12	0.1	−6.3 to 3.9	10
Relative change (%) from start of therapy to ...						
week 2	15.0	8.1 to 28.6	25	1.5	−5.2 to 8.3	20
week 4	15.7	6.7 to 28.7	25	−0.3	−4.8 to 11.9	20
week 16	19.9	10.0 to 28.3	24	4.2	−2.2 to 13.7	20
week 16, baseline ppFEV$_1$ below median	28.6	18.9 to 41.0	12	11.4	4.0 to 15.4	13
week 16, baseline ppFEV$_1$ above median	12.1	6.6 to 21.5	12	−0.1	−6.7 to 4.4	13

IVA treatment mainly in patients with a higher LCI at baseline, but to a much lesser degree than during triple therapy. 2 weeks after beginning LUM/IVA treatment 16 of 26 patients had an improved LCI, compared to 21 of 25 patients with ELX/TEZ/IVA treatment. The cohort treated with LUM/IVA showed a younger age with preserved lung function, which could explain the smaller but still discernible effect on LCI.

Our work does have several limitations. First, it is a single-centre, not controlled or blinded study. Second, we used two different MBW devices with non-interchangeable results. However, since each patient used the same measurement device throughout the study period, we regard the intrapatient differences as suitable to represent treatment effects. Third, Exhalyzer D software update was implemented in September 2019 (Spiroware® 3.3.1) as a reaction to technical progress. To avoid the bias of overestimating LCI improvement, we used an updated software version to reanalyse all measurements of Exhalyzer D before September 2019. Fourth, the treatment groups receiving ETI or LI therapy were not comparable, since the latter were healthier and might have less room for improvement from CFTR modulator therapy.

A strength of this work is that as no limitation concerning severity of lung disease was defined as exclusion criteria, we can show data

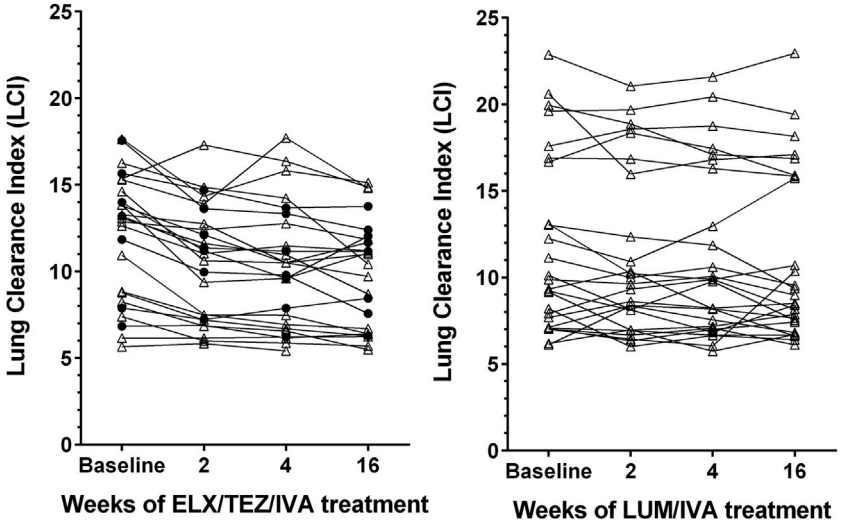

FIGURE 1
Line graphs depicting lung clearance index (LCI) in patients with cystic fibrosis after treatment with elexacaftor/tezacaftor/ivacaftor (left side, ELX/TEZ/IVA; *n* = 25) and lumacaftor/ivacaftor (right side, LUM/IVA; *n* = 26). Filled circles denote measurements in patients pretreated with another modulator, triangles denote measurements in patients who were modulator naïve before receiving ELX/TEZ/IVA.

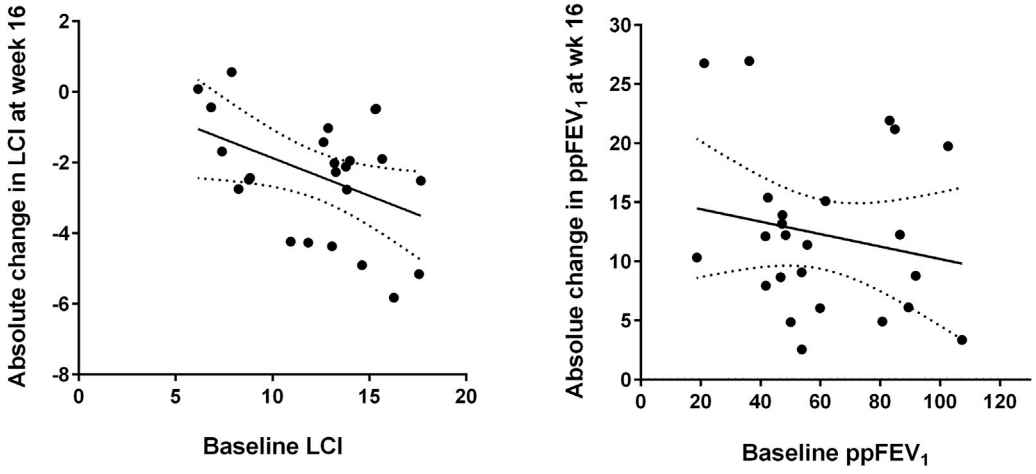

FIGURE 2
Absolute changes in lung clearance index (LCI, left) and per cent predicted forced expiratory volume in one second ($ppFEV_1$, right) after 16 weeks of elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA) treatment compared to the respective baseline values. The best-fit linear regression lines and their 95% confidence bands are displayed (see text for correlation coefficients).

from a diverse CF collective, and we also included patients with normal lung function and with baseline LCI below 7. Therefore, we could analyse the treatment response in patients with less and more severe lung disease. We found that subjects with worse baseline LCI experienced a greater benefit from ELX/TEZ/IVA at week 16 than those with less ventilation inhomogeneity. Moreover, this is the second published work after Stylemans et al. with longitudinal real-world LCI data in adults with measurements already after 2 weeks of ELX/TEZ/IVA treatment (Stylemans et al., 2022).

In conclusion, the present results from our routine monitoring programme provide further detail on the rapid treatment response during CFTR modulator therapy. If in doubt whether a patient responds to ELX/TEZ/IVA therapy, measuring LCI and $ppFEV_1$ within a few weeks after commencing modulator therapy can provide important information on the efficacy of this high-cost therapy. Our data suggest that measuring baseline LCI could help in estimating which patients might benefit the most from CFTR modulator therapy. Compared to the older drug LUM/IVA, the

improvements induced by ELX/TEZ/IVA were substantial and clinically relevant. Further studies should evaluate the long-term effects of modulator therapies on ventilation inhomogeneity, including the effectiveness after reduction of routine symptomatic CF treatments.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethikkommission der Medizinischen Universität Innsbruck, Medical University Innsbruck, Innsbruck, Austria. Written informed consent to participate in this study was provided by the participants and apos; legal guardian/next of kin.

Author contributions

DA: Validation, investigation, data curation, writing—original draft, GS: Methodology, validation, writing—review and editing, SS: Formal analysis, data curation, HE: Conceptualization, validation, resources, supervision, writing—review and editing.

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Conflict of interest

DA is a subinvestigator in clinical studies and has received sponsored travel to participate in conferences from Teva Pharmaceutical industries. GS reports fees for scientific work from CF TEAM Forschung, Innsbruck during the conduct of the study. HE is a site principal investigator in clinical studies and received honoraria for lectures from Vertex Pharmaceuticals. SS is employed by STAT-UP Statistical Consulting & Data Science GmbH, Munich, Germany. HE is a site principal investigator in clinical studies and received honoraria for lectures from Vertex Pharmaceuticals.

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Post-approval studies with the CFTR modulators Elexacaftor-Tezacaftor—Ivacaftor

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Triple combination therapy with the CFTR modulators elexacaftor (ELX), tezacaftor (TEZ) and ivacaftor (IVA) has been qualified as a game changer in cystic fibrosis (CF). We provide an overview of the body of literature on ELX/TEZ/IVA published between November 2019 and February 2023 after approval by the regulators. Recombinant ELX/TEZ/IVA-bound Phe508del CFTR exhibits a wild type conformation *in vitro*, but in patient's tissue a CFTR glyoisoform is synthesized that is distinct from the wild type and Phe508del isoforms. ELX/TEZ/IVA therapy improved the quality of life of people with CF in the real-life setting irrespective of their anthropometry and lung function at baseline. ELX/TEZ/IVA improved sinonasal and abdominal disease, lung function and morphology, airway microbiology and the basic defect of impaired epithelial chloride and bicarbonate transport. Pregnancy rates were increasing in women with CF. Side effects of mental status changes deserve particular attention in the future.

KEYWORDS

elexacaftor, tezacaftor, ivacaftor, CFTR, cystic fibrosis

Introduction

Cystic fibrosis (CF) is a severe ion channel disease of autosomal recessive inheritance that is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)* gene. Thanks to continuously improved symptomatic treatment during the last 5 decades, this lethal paediatric disease has been transformed into a chronic disorder with a median life expectancy of nowadays more than 50 years (Bell et al., 2020).

Current therapy has been symptomatic, but meanwhile CFTR modulators have arrived to the clinic that target the basic defect in CF of impaired epithelial conductance for chloride and bicarbonate (Tümmler, 2022). There are two classes of CFTR modulators: Potentiators increase the activity of CFTR at the cell surface and correctors facilitate the translation, folding, maturation and trafficking of mutant CFTR to the cell surface and/or prevent its premature degradation. Already 10 years ago the potentiator ivacaftor has been approved for the treatment of the small group of patients who carry a gating mutation in at least one of their two *CFTR* alleles. Ivacaftor is the first molecule that has been approved as a mutation-type specific medication for human use. Meanwhile the triple combination of the potentiator ivacaftor (IVA) and the two correctors elexacaftor (ELX) and tezacaftor (TEZ) has become available for the treatment of the more than 90% of people with CF (pwCF) who harbour at least one *CFTR* allele that is responsive to this medication (Middleton et al., 2019). Thanks to the strong improvements in anthropometry, lung function, reduction of pulmonary exacerbations and quality of life, triple therapy with ELX/TEZ/IVA has been qualified as a game changer in CF (Bell et al., 2020). Based on an individual person-level microsimulation

TABLE 1 Molecular action of the approved modulators on CFTR structure and function.

	Ivacaftor	Lumacaftor tezacaftor	Elexacaftor
Modulator type	Potentiator	Type I corrector	Type III corrector
Binding site in CFTR	ICL4 (photoaffinity labelling data) cleft formed by TM 4, 5, 8 (cryo-EM data)	TM 1, 2, 3, 6 (cryo-EM data)	TM 2, 10, 11, lasso motif (cryo-EM data)
Interaction with CFTR	stabilizes channel open configuration, enhances ATP-independent channel opening, stabilizes pre-hydrolytic states, reduces folding efficiency of Phe508del CFTR at the ER, destabilizes Phe508del CFTR in the plasma membrane	stabilizes early steps of CFTR biogenesis at the ER, improves co-translational folding of TMD1, facilitates binding of TMD1:NBD1 to ICL4, stabilizes the interactions NBD1: ICL4, NBD1:ICL1, TM3:TM4, TMD1:TMD2	supports assembly of the TMDs, co-potentiator

EM, electron micrograph; ICL, intracellular loop; NBD, nucleotide binding domain; TM, transmembrane helix; TMD, transmembrane domain.

model the median lifetime survival of p.Phe508del homozygous pwCF receiving ELX/TEZ/IVA plus current best supportive care has been estimated to be 71.6 years (Lopez et al., 2023). ELX/TEZ/IVA is the first CFTR modulator therapy shown to halt lung function decline over an extended time period (Lee et al., 2022). This clinical success has initiated post-approval studies on multiple preclinical and clinical aspects. Here we now provide an overview of the current body of literature on ELX/TEZ/IVA published after approval in the United States by November 2019.

CFTR modulators and their action on CFTR

Although there are more than 2,000 known sequence variants in *CFTR*, the vast majority of CF is homozygous or compound heterozygous for the most common mutation p.Phe508del. Phe508del CFTR protein is defective in posttranslational processing and trafficking. Newly synthesized Phe508del CFTR fails to adopt a wild-type fold in the endoplasmic reticulum (ER), is targeted to ER-associated degradation and is removed faster from the apical membrane by endocytosis. Consequently, p.Phe508del homozygous subjects express only low amounts of complex-glycosylated Phe508del CFTR and low or no residual Phe508del CFTR-mediated chloride and bicarbonate secretory activity. The analysis of second–site suppressor mutations revealed that a robust correction of the conformational defects of Phe508del CFTR requires the stabilization of the interfaces between the two nucleotide binding domains (NBDs) and the membrane-spanning domains (type I) and the stabilization of nucleotide binding domains 2 (NBD2) (type II) and Phe508del NBD1 (type III) (Okiyoneda et al., 2013). Combinations of type I, II and III correctors restored 50%–100% of wild-type-level Phe508del CFTR biogenesis and stability in immortalized and primary human airway epithelia (Veit et al., 2018). Concomitantly, the correctors decrease mucus concentration, relax mucus network ultrastructure, improve mucus transport and rheology of airway surface liquid, accelerate wound repair of the airway epithelium and change the plasma and cellular lipidome, in particular make the epithelial cells less susceptible to apoptosis by reducing the levels of ceramide (Gardner et al., 2020; Liessi et al., 2020; Veit et al., 2021a; Abu-Arish et al., 2022; Laselva and Conese, 2022; Ludovico et al., 2022; Morrison et al., 2022; Westhölter et al., 2022).

The yet most thoroughly characterized compound is the CFTR potentiator ivacaftor (IVA, VX-770, IUPAC name: N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide) (van Goor et al., 2009) (Table 1). The opening of the CFTR ion channel normally requires the binding and subsequent hydrolysis of ATP. In contrast, picomolar ivacaftor reversibly enhances ATP-independent opening of the channel and thereby overcomes the defective ATP-dependent opening of CF-causing gating mutations (Eckford et al., 2012; Jih and Hwang, 2013; Csanády and Töröcsik, 2019). CFTR open probability increases by stabilizing pre-hydrolytic states with respect to closed states (Kopeikin et al., 2014; Langron et al., 2018).

Lumacaftor (LUM, VX-809, IUPAC name: 3-[6-[[[1-(2,2-difluoro-1,3-benzodioxol-5-yl) cyclopropyl]carbonyl] amino]- 3-methyl-2-pyridinyl]-benzoic acid) has been the first CFTR corrector approved for use in humans (van Goor et al., 2011). This type I corrector acts early during CFTR biosynthesis (Loo and Clarke, 2017; Kleizen et al., 2021) so that Phe508del CFTR can exit the ER (Table 1). It improves the co-translational folding of transmembrane domain 1 (TMD1). The subsequent early post-translational TMD1:NBD1 packing facilitates the most critical step of Phe508del CFTR folding, i.e., the binding to cytoplasmic loop 4 (ICL4), leading to progression of domain assembly in the absence of folded Phe508del-NBD1 (Kleizen et al., 2021). Further allosteric effects of lumacaftor are the stabilization of the NBD1: ICL4 and NBD1:ICL1 interfaces, of the transmembrane helices 3 and 4 and of the TMD1:TMD2 interaction (Farinha et al., 2013; He et al., 2013; Ren et al., 2013; Hudson et al., 2017; Loo and Clarke, 2017; Laselva et al., 2018; Krainer et al., 2020).

Tezacaftor (TEZ, VX-661, IUPAC name (R)-1-(2,2-difluorobenzo [d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl) cyclopropane carboxamide) has been the second type I corrector that has been approved for the treatment of people with CF with one or two p.Phe508del alleles. Immunoblotting and *in silico* docking experiments proposed a similar composite multi-domain binding pocket for lumacaftor and tezacaftor comprised of residues within the NBD1:ICL4 interface (Molinski et al., 2018) (Table 1).

The type III corrector elexacaftor (ELX, VX-445, IUPAC name: *N*-(1,3-dimethylpyrazol-4-yl)sulfonyl-6-[3-(3,3,3-trifluoro-2,2-dimethylpropoxy)pyrazol-1-yl]-2-[(4*S*)-2,2,4-trimethylpyrrolidin-1-yl] pyridine-3-carboxamide) synergistically restores Phe508del CFTR processing in combination with type I or type II correctors (Veit et al., 2020; Becq et al., 2022) (Table 1). Moreover, elexacaftor acts as a co-potentiator of Phe508del, Gly551Asp and Met1101Lys CFTR chloride channels (Veit et al., 2021a; Laselva et al., 2021; Shaughnessy et al., 2021).

Exposure of primary p.Phe508del homozygous epithelia to a triple combination of ELX/TEZ/IVA restored Phe508del CFTR chloride channel function to about 60% of wild-type levels (Veit et al., 2020; Capurro et al., 2021). However, when p.Phe508del homozygous cells were treated with ivacaftor combined to any correctors (LUM or TEZ or ELX), the Phe508del CFTR current was unresponsive to the subsequent acute addition of ivacaftor (Cholon et al., 2014; Veit et al., 2014; 2020; Shaughnessy et al., 2022a; Becq et al., 2022). Ivacaftor diminished the folding efficiency and the metabolic stability of Phe508 CFTR at the ER and post-ER compartments and destabilized rescued Phe508del CFTR at the plasma membrane causing reduced cell surface Phe508 CFTR density and function. CFTR Western blot analysis of intestinal epithelium of people with CF with one or two p.Phe508del alleles revealed that treatment with ELX/TEZ/IVA improves posttranslational processing and trafficking of Phe508del CFTR. However, a low-complexity Phe508del CFTR glycoisoform is produced that lacks the polydisperse spectrum of N-linked oligosaccharides of mature complex glycosylated wild type CFTR (Stanke et al., 2023). Hence, triple therapy with ELX/TEZ/IVA generates and stabilizes a novel Phe508del CFTR glycoisoform that is distinct from both the wild type and mutant isoforms.

Cryo-electron microscopy of reconstituted recombinant protein identified the binding sites of elexacaftor, tezacaftor and ivacaftor in wild type and Phe508del CFTR (Fiedorczuk & Chen, 2022). Clinically most relevant, the conformations of wild type CFTR and ELX/TEZ/IVA-bound Phe508del CFTR were almost indistinguishable from each other indicating that the CFTR modulators induce the "correct" conformation in the absence of any other members of the CFTR interactome. The three drugs bind to distinct sites of the CFTR protein described by Fiedorczuk and Chen (2022) as a "triangular belt encircling the transmembrane domains". The potentiator ivacaftor binds to a cleft formed by transmembrane helices 4, 5, and 8 that stabilizes the open configuration of the ion pore in both wild type and Phe508del CFTR. Likewise, the type I corrector tezacaftor is recognized in both wild type and mutant by the same amino acid residues of transmembrane helices 1, 2, 3, and 6 and thereby probably stabilizes the early steps of CFTR biogenesis at the ER. Conversely, the type III corrector elexacaftor supports the subsequent assembly of the TMDs. ELX binds to Phe508del CFTR within the membrane mainly interacting with amino acid residues of transmembrane helices 2, 10, 11, and the N-terminal lasso motif.

Clinical pharmacology

Published data on the pharmacokinetics of ELX/TEZ/IVA in humans are scarce and needs to be extracted from the material submitted by the manufacturer Vertex to the regulators (FDA or EMA). The serum half-life is 12 h for ivacaftor and 23 h for the correctors. Thus, the label recommends a morning dose with ELX/TEZ/IVA (TRIKAFTA®, KAFTRIO®) and an evening dose with IVA (Kalydeco®). A deuterated derivative of ivacaftor, called deutivacaftor (VX-561), has a reduced rate of clearance, greater plasma concentrations at 24 h, and a longer half-life compared with ivacaftor, thereby supporting once-daily dosing (Harbeson et al., 2017). Once-daily triple therapy of deutivacaftor together with tezacaftor and the novel corrector vanzacaftor is currently being examined in clinical trials (Uluer et al., 2023). Assays for quantifying ELX, TEZ, IVA in human plasma and cell lysate have meanwhile been established by academic labs applying multiple reaction monitoring mass spectrometry (MRM/MS) (Reyes-Ortega et al., 2020) or isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) (Habler et al., 2021; Ryan et al., 2022). Pharmacokinetic modelling revealed that the transition from IVA monotherapy or dual regimens with LUM/IVA or TEZ/IVA to triple combination therapy with ELX/TEZ/IVA will approach steady state levels within 2 weeks whereby IVA and at least one corrector will remain above the half-maximal effective concentration at all times (Tsai et al., 2020). Thus, although the individual CFTR modulators are differentially metabolized by the Cyp 3A457 enzyme complex in the liver, the immediate transition from mono or dual regimens to triple therapy seems to be safe.

A challenging problem is the treatment of pwCF who are infected with non-tuberculous mycobacteria (NTM), namely, *Mycobacterium abscessus, Mycobacterium bolleti* or *Mycobacterium avium*. Chronic airway infections with NTM have become the major risk factor for quality of life and prognosis in CF (Martiniano et al., 2022). The antimycobacterial multidrug regimens that are laborious and burdened with many side effects will include macrolide antibiotics and the ansamycins rifampicin or rifabutin. Marolides inhibit and ansamycins strongly induce the Cyp-system. Correspondingly, the label lists these drugs as contraindication for CFTR modulator therapy. Based on physiologically based pharmacokinetic modeling of drug-drug interaction, Hong et al. (2022) have recently proposed a dose-adjusted ELX/TEZ/IVA therapy concomitant with NTM treatment, i.e., increased doses of ELX/TEZ/IVA 200/100/450 mg in the morning and 100/50/375 mg in the evening when ETI is co-administered with rifabutin and reduced doses of ELX/TEZ/IVA 200/100/150 mg q72 h when co-administered with clofazimine or clarithromycin, respectively.

Safety data for the CFTR modulators show that therapy is well-tolerated. However, the phase 3 clinical studies for ELX/TEZ/IVA reported an incidence of rash ranging from 4% to 10.9%. Rash appeared to be more common in female patients and in those who use hormonal contraception. Meanwhile numerous adverse skin reactions have been observed in the real-life setting ranging from skin rash, drug-induced acne, eruptive melanocytic naevi to toxic epidermal necrolysis (Goldberg et al., 2021; Leonhardt et al., 2021; Atkinson et al., 2022; Bhaskaran and Bateman, 2022; Cheng et al., 2022; Diseroad et al., 2022; Hu et al., 2022; Hudson et al., 2022; Loyd et al., 2022; Mederos-Luis et al., 2022; Muirhead et al., 2022; Okroglic et al., 2023). Desensitization protocols were often successful, but etiology has not been examined, the exception being a case report of LUM-responsive $CD4^+$ T-cell clones in non-immediate allergy (Roehmel et al., 2021).

Impact of ELX/TEZ/IVA on CFTR biomarkers

Modulation of the basic defect in chloride and bicarbonate transport by ELX/TEZ/IVA has been assessed in post-approval studies in the sweat gland, kidney and the respiratory, biliary and

intestinal epithelium of pwCF. CFTR-mediated chloride conductance of the upper respiratory epithelium improved to a mean 47% of normal with ELX/TEZ/IVA (Graeber et al., 2022a). Similarly, CFTR-mediated chloride transport of biliary and intestinal epithelium shifted into the normal range for most pwCF who carry at least one p.Phe508del allele (Graeber et al., 2022a; Bijvelds et al., 2022). In the kidney exposure to ELX/TEZ/IVA increased bicarbonate excretion to about 70% of that seen in healthy controls (Berg et al., 2022).

The responses of the sweat gland to ELX/TEZ/IVA were discordant. The sweat chloride concentration in the pilocarpine iontophoresis sweat test dropped by a mean 50 mmol/L into the intermediary or even normal range in the majority of pwCF implying that the basic defect of defective chloride reabsorption in the sweat duct had been partially or completely reversed by triple therapy (Graeber et al., 2022a). Conversely, the ß-adrenergic stimulated sweat secretion in the coil reached just about 5% of median wild type β-adrenergic sweat rate (Pallenberg et al., 2022a). Apparently β-adrenergic sweat stimulation in the coil is more stringent in its requirements for a wild type CFTR conformation whereas the reabsorption of chloride in the sweat duct tolerates residual structural and functional deficits of pharmacologically rescued mutant CFTR in the apical membrane. The limited response of the β-adrenergic sweat rate to high-efficient CFTR modulation allows the evaluation of new, potentially even more efficient CFTR modulators in the future, while the sweat chloride concentration may already have reached the limit of its sensitivity.

Exposure of ELX/TEZ/IVA to rare *CFTR* genotypes

In Europe ELX/TEZ/IVA therapy is currently approved for pwCF aged 6 years or more who carry one or two p.Phe508del alleles. The label in the US just requests the carriage of at least one CF allele that is known to be responsive to the CFTR modulator *in vitro*. Thus, about 90% of pwCF have access to ELX/TEZ/IVA. A subgroup of the remaining 10% of the population is carrying rare or even ultra-rare mutations of unknown mutant phenotype. Thus, to address this unresolved issue, researchers have characterized the association between *CFTR* genotype, phenotype and its modulation by ELX/TEZ/IVA in recombinant cells (Laselva et al., 2021; Borgo et al., 2022; Tomati et al., 2022) or patient-derived epithelial cells *in vitro* (Veit et al., 2021b; Borgo et al., 2022; Shaughnessy et al., 2022b; Furstova et al., 2022; Tomati et al., 2022). Alternatively, they combined the cell culture work with the examination of CFTR biomarkers and clinical characteristics prior and during treatment with ELX/TEZ/IVA (Anderson et al., 2021; Comegna et al., 2021; Huang et al., 2021; Terlizzi et al., 2021; Aalbers et al., 2022; Kondratyeva et al., 2022a; 2022b; Ciciriello et al., 2022; Sondo et al., 2022). A peculiar challenge are complex alleles not yet documented in the databases. Characterization of p.[Leu467Phe-Phe508del] in patient-derived organoids and primary intestinal epithelium demonstrated a more compromised CFTR function than p.Phe508del, but fortunately was susceptible to modulation by ELX/TEZ/IVA both *in vitro* and in the patient *in vivo* (Kondratyeva et al., 2022a; Kondratyeva et al., 2022b).

Numerous cases with two non-Phe508del mutations yielded outcomes of triple therapy that would not have been expected from our knowledge of the molecular pathology of CFTR. Table 2 lists the published cases that by now have been examined prior to and during ELX/TEZ/IVA therapy with CFTR biomarkers. Table 3 provides data on sweat chloride, spirometry and body weight of pwCF with advanced lung disease who participated in the French Compassionate Program of ELX/TEZ/IVA (Burgel et al., 2023).

The molecular phenotype of splice site mutations is typically predicted from the localization of the nucleotide substitution in the acceptor or donor splice sites. If an individual with CF carries a mutation in the canonical splice sites at the positions −2, −1, +1 or +2 at the intron/exon border, exon skipping will occur. The generated CFTR mRNA isoforms will typically be either rapidly degraded or translated into mutants of no or low activity. Thus, these splice mutations are assigned to the class I of minimal function. In line with expectation most class I/class I genotypes with one or two canonical splice site mutations did not respond to ELX/TEZ/IVA (Table 3). However, exceptions were noted. Triple therapy improved sweat chloride and lung function in pwCF who are homozygous for splice sites mutations affecting the inclusion of introns 18 and 26, respectively (Burgel et al., 2023). We tested two brothers who are compound heterozygous for an acceptor splice site and a donor splice site mutation flanking the same exon (Pallenberg et al., 2023). These index cases normalized CFTR function in the secretory coil of the sweat gland upon exposure to ELX/TEZ/IVA, whereas the respiratory and the intestinal epithelia were only slightly or not responsive to CFTR modulation (Table 2).

Class V splice mutations harbor the nucleotide substitution at a less conserved position of the splice site. Alternative splicing will generate both full-length and shorter CFTR mRNA isoforms. Hence, the donor splice mutation c.3717 + 5G>T has been expected to generate some wild type transcript associated with a pancreatic sufficient phenotype and a request for inclusion into the compassionate use program was denied. The index case, however, was exocrine pancreatic insufficient and the swelling assay in patient-derived intestinal organoids demonstrated a loss-of-function phenotype. The subject thus qualified for treatment with ELX/TEZ/IVA and then showed strong improvements in lung function, lung morphology and sweat chloride (Aalbers et al., 2022). On the other hand, the rather common splice mutation c.2657 + 5G>A is a class V mutation that is known to confer some residual wild-type CFTR activity (Highsmith et al., 1997; van Barneveld et al., 2008). However, ELX/TEZ/IVA therapy of pwCF with one or two c.2657 + 5G>A mutations led to only marginal or no clinical improvement (Table 3) (Burgel et al., 2023).

Asn1303Lys CFTR is post-translationally processed by other pathways than Phe508del CFTR. According to tests in recombinant cells Asn1303Lys CFTR was thought to be not responsive to CFTR modulation. However, ELX/TEZ/IVA efficiently attenuated the basic defect in numerous patients (Tables 2, 3) (Huang et al., 2021; Burgel et al., 2023). Similarly, the class II mutations p.Ala561Glu, p.Arg1066Cys and p.Met1101Lys that were non-responsive to CFTR correctors *in vitro*, were susceptible to CFTR modulation *in vivo*. ELX/TEZ/IVA significantly reduced sweat chloride and improved lung function (Table 3). Likewise, the

TABLE 2 Rare non-p.Phe508del *CFTR* genotypes assessed for ELX/TEZ/IVA—mediated CFTR modulation in pwCF by CFTR biomarkers. Sequence variants are differentiated by *CFTR* mutation class: class I, minimal function; class II, defective in protein processing and trafficking; class III: defective gating; class IV, change of ion channel conductance; class V, reduced amount of wild type CFTR.

CFTR genotype	CFTR biomarkers		References
class I—class I			
c.165-2 A>G/c.273 + 1G>A	SST:0.07/**0.23** QPIT: 84/102 NPD: 0/−3	ICM: 6/5	Pallenberg et al. (2023)
c.165-2 A>G/c.273 + 1G>A	SST:0.06/**0.23** QPIT: 110/115 NPD: 0/−1	ICM: 5/5	Pallenberg et al. (2023)
class I—class II			
p.Gly542Ter/p. [Leu467Phe-Phe508del]	ALI: 2/2		Sondo et al. (2022)
p.Glu585Ter/p. [Leu467Phe-Phe508del]	ALI: 5/5		Sondo et al. (2022)
p.Glu193Ter/p.Asn1303Lys	WPC: 0/**22** QPIT: 108/95		Huang et al. (2021)
class I—class III			
c.1585-1G>A/p.Gly1244Glu[a]	ALI: 1.7/**15**		Tomati et al. (2022)
p.Gly542Ter/p.Gly1244Glu[a]	ALI: 1.6/**16**		Tomati et al. (2022)
class III—class III			
p.Gly1244Glu*/p.Gly1244Glu[a]	ALI: 2.4/**20**		Tomati et al. (2022)

[a]FDA approved sequence variant for ELX/TEZ/IVA, therapy.
Paired values at absence and presence of ELX/TEZ/IVA. A clinically relevant improvement of CFTR activity into the normal range or in the range of CFTR-related disorders is marked in bold.
SST, sweat secretion test: ß-adrenergically stimulated sweat secretion [nL/min].
QPIT, quantitative pilocarpin iontophoresis sweat test: sweat chloride concentration [mMol/L).
NPD, nasal transepithelial potential difference: cumulative depolarization potential to chloride-free solution [mV].
ICM, intestinal current measurement: cumulative ion current of rectal biopsy upon exposure to forskolin/IBMX and carbachol [μA/cm^2].
ALI, transepithelial ion transport of primary nasal epithelial cells grown at air-liquid interface [μA/cm^2].
WPC, whole patch-clamp recording of recombinant HEK293 cells [pA/pF].

missense mutants Arg334Trp and Arg347Pro CFTR have been judged to be not accessible to modulation because of their vicinity to the ion pore. However, the carriers of these class IV mutations showed a strong clinical benefit in sweat test, lung function and anthropometry.

In summary, the test of mutations in recombinant cells *in vitro* correctly predicted the response of pwCF to ELX/TEZ/IVA for most mutations, but was erroneous for a few splice and missense mutations. This experience demonstrates that the response of pwCF with ultra-rare *CFTR* mutations to ELX/TEZ/IVA should be tested by CFTR biomarkers and clinical characteristics. These probatory trials provide proper care for the patient and improve our knowledge of the molecular pathology of CFTR.

Quality of life during ELX/TEZ/IVA therapy in patient groups not covered by phase 3 trials

The first approval of ELX/TEZ/IVA for human use was based on the outcome of phase 3 trials in pwCF aged 12 years or more with subnormal spirometry of 40%–90% FEV1 predicted. During the phase 3 trials ELX/TEZ/IVA treatment led to higher scores in all respiratory (Middleton et al., 2019) and non-respiratory domains (Fajac et al., 2022) of the Cystic Fibrosis Questionnaire-Revised, a validated measure of quality of life. Meanwhile we learnt that ELX/TEZ/IVA therapy improves the quality of life of pwCF irrespective of their anthropometry and lung function at baseline. Already after 4 months of triple therapy "patients generally reported a rapid impact on respiratory symptoms, sleep quality, general wellbeing and physical self-esteem, and a reduction in overall treatment burden. The majority of patients contrasted treatment burden, symptom severity, depression and a closed future marked by death or transplantation before ELX/TEZ/IVA, to renewed and unexpected physical strength, leading to greater self-confidence, autonomy and long-term planning, after treatment initiation" (Martin et al., 2021). Daily hospitalization and intravenous antibiotic rates were reported to decrease by 80% (Walter & Bass, 2022), which matches with the author's experience at his CF clinic. Most encouragingly, 1-month treatment with ELX/TEZ/IVA improved ppFEV1 in pwCF with advanced lung disease by 11%–13% (Carnovale et al., 2021; Martin et al., 2022) leading to a pronounced decline in CF-related transplants by 55%–83% in CF centers in the US, France and Germany (Bermingham et al., 2021; Burgel et al., 2021; Ringshausen et al., 2023). Treatment burden decreased substantially in the need for intravenous antibiotics, oxygen therapy and non-invasive ventilation (Martin et al., 2022). Therapy with ELX/TEZ/IVA was safe and efficacious post liver transplant (McKinzie et al., 2022; Ragan et al., 2022). Conversely, when ELX/TEZ/IVA was prescribed to lung transplant recipients for extrapulmonary complications of CF, triple therapy was poorly tolerated with modest perceived extrapulmonary benefit so that about 40% of patients discontinued the medication (Doligalski et al., 2022; Ramos et al., 2022). In summary, with the exception of lung transplant recipients, treatment with ELX/TEZ/IVA led to a strong improvement of the quality of life.

TABLE 3 Rare non-p.Phe508del *CFTR* genotypes assessed for ELX/TEZ/IVA - mediated CFTR modulation by sweat test, spirometry and body weight in pwCF with advanced lung disease (Burgel et al., 2023). Sequence variants are differentiated by *CFTR* mutation class: class I, minimal function; class II, defective in protein processing and trafficking; class III: defective gating; class IV, change of ion channel conductance; class V, reduced amount of wild type CFTR.

CFTR genotype	Sweat chloride	ppFEV1	Body weight
	[mMol/L]	[% predicted]	[kg]
class I—class I			
c.262-263delTT/p.Arg553Ter	*107/92*	*44/42*	*36/36*
c.357delC/c.357delC	*70/96*	*28/28*	*59/56*
c.579 + 1G>T/c.579 + 1G>T	*110/96*	*42/44*	*28/29*
c.948delT/p.Trp1282Ter	*90/138*	*30/27*	*43/47*
c.1209G>A/c.2215delG	61/47	37/47	48/50
c.1392G>T (p.Lys464Asn)/c.3528delC	*94/95*	*23/21*	*47/47*
c.1393-1G>A/c.1393-1G>A	*86/80*	*42/45*	*39/41*
c.1393-1G>A/c.1393-1G>A	*93/89*	*41/46*	*68/70*
c.1585-1G>A/c.2051_2052delAAinsG	105/95	39/60	60/61
c.1585-1G>A/c.3528delC	*96/99*	*39/50*	*38/40*
c.1585-1G>A/p.Gly542Ter	*112/111*	*27/18*	*50/50*
c.1585-1G>A/p.Arg553Ter	*124/107*	*35/39*	*50/50*
c.1679 + 1.6 kb A>G/c.1679 + 1.6 kb A>G	*92/92*	*27/27*	*47/48*
c.2051_2052delAAinsG/c.2051_2052delAAinsG	*93/104*	*24/25*	*44/43*
c.2051_2052delAAinsG/p.Gln493Ter	*102/99*	*34/35*	*53/55*
c.2051_2052delAAinsG/p.Gly542Ter	*85/86*	*46/49*	*56/56*
c.2810_2811insT/c.2989-313 A>T	*98/65*	*32/29*	*55/55*
c.2909-1 T>G/c.2909-1 T>G	68/28	32/38	95/94
c.2988 + 1G>A/c.2988 + 1G>A	*106/102*	*31/32*	*60/59*
c.2988 + 1G>A/c.2988 + 1G>A	*111/124*	*26/26*	*30/30*
c.2997_3000delAATT/p.Arg1162Ter	*100/102*	*26/26*	*43/45*
c.3469-2880_3717 + 2150del/c.3469-2880_3717 + 2150del		*30/28*	*57/57*
c.3964-3C>G/c.3964-3C>G	*95/110*	*32/42*	*47/47*
c.4139delC/p.Gly542Ter	*102/102*	*37/44*	*22/23*
c.4242 + 1G>A/c.4242 + 1G>A	88/73	31/52	25/30
c. [4242 + 1G>A; 3170delC]/p.Trp846Ter	*101/95*	*40/37*	*49/48*
p.Tyr122Ter/p.Tyr122Ter	*114/111*		*49/47*
p.Gly542Ter/p.Gly542Ter	*103/98*	*35/33*	*58/58*
p.Trp1063Ter/p.Trp1063Ter	*120/110*	*27/28*	*65/65*
p.Trp1282Ter/p.Trp1282Ter	*108/97*	*28/27*	*62/62*
p.Trp1282Ter/p.Trp1282Ter	*114/111*	*18/11*	*36/36*
p.Trp1282Ter/p.Trp1282Ter	*104/101*	*28/29*	*59/59*
class I—class II			
CFTRdele2/p.Ala561Glu	100/61	22/42	61/63
c.489 + 2 T>G/p.Iso601Phe*	79/45	51/52	62/63

(Continued on following page)

TABLE 3 (*Continued*) Rare non-p.Phe508del *CFTR* genotypes assessed for ELX/TEZ/IVA - mediated CFTR modulation by sweat test, spirometry and body weight in pwCF with advanced lung disease (Burgel et al., 2023). Sequence variants are differentiated by *CFTR* mutation class: class I, minimal function; class II, defective in protein processing and trafficking; class III: defective gating; class IV, change of ion channel conductance; class V, reduced amount of wild type CFTR.

CFTR genotype	Sweat chloride	ppFEV1	Body weight
	[mMol/L]	[% predicted]	[kg]
c.579 + 1G>T/p.Iso507del		*31/31*	*57/55*
c.2051_2052delAAinsG/p.Leu558Ser	*75/73*	*38/36*	*69/67*
c.2490 + 1G>A/p.Gly85Glu*	105/66	44/63	52/55
c.2805_2810delinsTCAGA/p.Arg1066Cys	(142)/70	23/38	62/67
c.3264delC/p.Met1101Lys*	(163)/45	37/66	36/38
p.Gln493Ter/p.Gly85Glu*	102/63	38/48	67/68
p.Arg553Ter/p.Iso507del		*25/30*	*49/49*
p.Glu585Ter/p.Arg1066Cys	100/56	25/40	33/34
p.Arg1162Ter/p.Asn1303Lys	99/90	32/61	32/33
p.Arg1162Ter/p.Asn1303Lys		23/34	51/55
p.Arg1162Ter/p.Asn1303Lys	(131)/95	46/54	51/52
class I—class IV			
c.579 + 1G>T/p. [Arg74Trp-Val201Met-Asp1270Asn]*	54/18	35/35	52/53
p.Trp1282Ter/p.Asp1152His*	38/30	43/49	69/67
class I—class V			
c.2051_2052delAAinsG/c.2657 + 5G>A	99/80	31/32	73/74
c.2988 + 1G>A/c.2657 + 5G>A		18/27	64/66
c.2988 + 1G>A/c.2657 + 5G>A	*97/87*	*28/31*	*51/50*
class II—class II			
p.Gly85Glu*/p.Gly85Glu*		24/32	75/79
p.Gly85Glu*/p.Gly85Glu*	96/76	46/60	57/60
p.Ser492Phe*/p.Arg1066Cys	73/28	34/41	54/56
p.His1085Arg*/p.Asn1303Lys	99/46	45/66	47/49
p.His1085Arg*/p.Asn1303Lys	97/23	29/62	46/49
p.Asn1303Lys/p.Asn1303Lys	109/87	19/30	50/53
p.Asn1303Lys/p.Asn1303Lys	105/96	33/92	57/62
p.Asn1303Lys/p.Asn1303Lys	93/92	44/69	41/44
p.Asn1303Lys/p.Asn1303Lys	114/76	23/32	54/56
p.Asn1303Lys/p.Asn1303Lys	96/91	20/30	61/63
class II—class IV			
p.Gly85Glu*/p.Arg334Trp	60/13	34/55	65/66
class IV—class IV			
p.Arg334Trp/p.Arg347Pro*	101/65	29/37	40/41
p.Arg347Pro*/p.Arg347Pro*	79/37	38/42	37/43
p.Arg347Pro*/p.Asn1303Lys	102/28	26/41	50/54
p.Ser364Pro*/p.Ser364Pro*	82/24	42/60	58/60

(Continued on following page)

TABLE 3 (*Continued*) Rare non-p.Phe508del *CFTR* genotypes assessed for ELX/TEZ/IVA - mediated CFTR modulation by sweat test, spirometry and body weight in pwCF with advanced lung disease (Burgel et al., 2023). Sequence variants are differentiated by *CFTR* mutation class: class I, minimal function; class II, defective in protein processing and trafficking; class III: defective gating; class IV, change of ion channel conductance; class V, reduced amount of wild type CFTR.

CFTR genotype	Sweat chloride	ppFEV1	Body weight
	[mMol/L]	[% predicted]	[kg]
class V –class V			
c.2657 + 5G>A/c.2657 + 5G>A	*107/94*	*35/33*	*33/34*

[a]FDA approved sequence variant for ELX/TEZ/IVA therapy.
Paired values at baseline and after at least 1 month of continuous treatment with ELX/TEZ/IVA; sweat chloride concentration [mMol/L] in QPIT; ppFEV1, percent of predicted Forced expiratory Volume in 1 s; body weight in kg. All individuals were modulator-naïve at baseline. Physiologically implausible sweat chloride concentrations are indicated by brackets.
Data were taken from Tables 2–4 compiled by the French Compassionate Program of the French CF reference study group (Burgel et al., 2023). The class of a mutation was allocated according to the molecular phenotype reported in the publications linked to the mutation in the CFTR1 database (http://www.genet.sickkids.on.ca/PicturePage.html). Sequence variants within canonical splice sites were assigned to class I.
PwCF, who were judged to be responders and continued therapy with ELX/TEZ/IVA, are indicated by normal font. PwCF, who were judged to be non-responders and discontinued therapy are indicated in italics.

Sinonasal function

Most pwCF have chronic rhinosinusitis resulting in nasal obstruction, nasal polyposis, sinus infections, repeated surgeries and olfactory dysfunction. Independent of age and global disease severity, ELX/TEZ/IVA therapy led within a few days to clinically meaningful and persisting improvements in sinonasal quality of life as assessed by the SinoNasal Outcome Test (SNOT-22) (DiMango et al., 2021; Douglas et al., 2021; Beswick et al., 2022a; 2022b; Shakir et al., 2022; Stapleton et al., 2022; Bode et al., 2023; Castellanos et al., 2023). Nasal polyps decreased in size or even resolved. Sinus opacification and mucosal thickening improved on CT radiographs. However, quantitative olfactory function did not significantly change according to the Smell Identification Test (Beswick et al., 2022a).

Pulmonary

The phase 3 trials reported a mean absolute increase in ppFEV1 of 14 points after 24 weeks of therapy with ELX/TEZ/IVA in pwCF with one p.Phe508del allele and a ppFEV1 of 40%–90% at baseline (Middleton et al., 2019). A similar absolute increase in the ppFEV1 of 15% was observed in French, Dutch, and Belgian CF patients with advanced pulmonary disease (ppFEV1< 40% at baseline) (Burgel et al., 2021; Kos et al., 2022; Stylemans et al., 2022). In a real-world, postapproval setting ELX/TEZ/IVA did not only significantly improve spirometry but also the lung clearance index as a measure of ventilation homogeneity (Graeber et al., 2022b; Stylemans et al., 2022). Air trapping, airway mucus plugging and bronchial wall thickening were reduced (Bec et al., 2022; Graeber et al., 2022b; FitzMaurice et al., 2022; Goralski et al., 2022; Fainardi et al., 2023). Likewise, functional MRI showed improvements in ventilation and perfusion (Streibel et al., 2023). During sleep the episodes of oxygen desaturation, apnea and hypopnea decreased in adult pwCF (Welsner et al., 2022; Giallongo et al., 2023).

Immunology and airway microbiology

In CF lung disease, mucus stasis favors chronic colonization with opportunistic pathogens, which determines the quality of life and prognosis in most pwCF. In spite of improved antimicrobial therapies, the characteristic age-dependent sequence of initial dominance of *S. aureus* followed by chronic colonization with *P. aeruginosa* has remained largely unchanged during the last 50 years. Neither monotherapy with IVA nor dual LUM/IVA changed the infection epidemiology in CF, but ELX/TEZ/IVA initiation was associated with a rapid reduction in infection-related visits and antimicrobial use among pwCF (Miller et al., 2022). After 12-month of treatment with ELX/TEZ/IVA, the detection of *Staphylococcus aureus* and *Pseudomonas aeruginosa* decreased at single CF centers by 40% or more (Pallenberg et al., 2022b; Beck et al., 2023; Sheikh et al., 2023). Sputum microbiome diversity increased (Sosinski et al., 2022). Compared to pretreatment, the total bacterial load decreased, the individual species were more evenly distributed in the community, and the individual microbial metagenomes became more similar in their composition. However, the microbial network remained vulnerable to fragmentation. The initial shift of the CF airway microbiome was attributable to the ELX/TEZ/IVA-mediated gain of CFTR activity followed by a diversification driven by a group of commensals at the 1-year time point that are typical for healthy airways (Pallenberg et al., 2022b).

CFTR is not only present in the apical epithelial membrane, but it is also intracellularly detectable in professional phagocytes where it regulates pH and chloride homeostasis of the post-Golgi network. ELX/TEZ/IVA therapy improved chloride efflux and the phagocytic and bactericidal activities of CF monocytes (Zhang et al., 2022b; Cavinato et al., 2022; Gabillard-Lefort et al., 2022), reduced neutrophilic inflammation in the lung (De Vuyst et al., 2023), reduced systemic pro-inflammatory cytokines and normalized circulating immune cell composition (Sheikh et al., 2023) even in pwCF with advanced lung disease (Dhote et al., 2023).

Intestine, pancreas, liver and nutrition

The phase 3 trials demonstrated a significant increase of BMI during ELX/TEZ/IVA therapy (Middleton et al., 2019). These improvements were confirmed in real-life settings. Parameters related to nutrient absorption such as weight, BMI, cholesterol and albumin were all significantly increased and the lipid profile improved independent of the diet composition (Carnovale et al., 2022; Petersen et al., 2022). Serum levels of fat-soluble vitamins

increased (Wright et al., 2022; Francalanci et al., 2023) even leading to singular cases of hypervitaminosis (Miller and Foroozan, 2022; Wisnieweski et al., 2022). These findings call for adjustments in vitamin supplementation. ELX/TEZ/IVA attenuated abdominal pain, gastro-oesophageal reflux, poor appetite and disorders of bowel movement (Mainz et al., 2022). Fecal markers of inflammation decreased. Pancreatic insufficiency did not improve (Schwarzenberg et al., 2022).

CFTR is not expressed by the endocrine pancreas but fibrosis and CFTR dysfunction in the ducts trigger the emergence of diabetes as the major co-morbidity in CF. Studies on the impact of ELX/TEZ/IVA on glucose homeostasis yielded conflicting outcomes. Continuous glucose monitoring (CGM) and oral glucose tolerance tests (OGTT) did not detect any difference in glucose patterns after several months of ELX/TEZ/IVA therapy in three studies (Chan et al., 2022; Crow et al., 2022; Piona et al., 2022). In contrast, glucose patterns again assessed by CGM or OGTT improved in three other studies (Korten et al., 2022; Scully et al., 2022; Steinack et al., 2023). Thus, we still do not know whether or not ELX/TEZ/IVA ameliorate glucose homeostasis and/or any of its direct determinants.

Drug-induced liver injury is known as a potential side effect of the highly lipophilic CFTR modulators (Salehi et al., 2021; Lowry et al., 2022) and the mobilization of gall stones may cause biliary colic shortly after initiation with ELX/TEZ/IVA (Safirstein et al., 2021). Upon initiation of triple CFTR modulator therapy serum levels of bilirubin and liver transaminases will mildly increase after 3 months which is sustained but does not appear to increase further in the majority of pwCF (Tewkesbury et al., 2023). A recently published observational study reported that ELX/TEZ/IVA negatively affects liver stiffness and alters bile acid metabolism in children and adolescents (Schnell et al., 2023). Bile acid profiles revealed a decrease in unconjugated and an increase in glycine-conjugated derivatives. Share wave velocity derived by Acoustic Radiation Force Impulse Imaging (ARFI) increased in the younger patients which indicates an increase of liver stiffness known to correlate with liver fibrosis. Schnell and co-workers (2023) suggest that ARFI measurements and serum levels of glycine-conjugated bile acids could serve as early markers for liver deterioration during ELX/TEZ/IVA therapy.

Reproductive tract and pregnancy

Most women with CF exhibit subfertility mainly driven by CFTR dysfunction that causes viscous cervical mucus presenting a physical barrier to sperm penetration. Thanks to the partial reversion of the basic defect and the globally improved health and prognosis, pregnancy rates are increasing in women with CF exposed to ELX/TEZ/IVA (Taylor-Cousar and Jain, 2021). According to two published case series (Kendle et al., 2021; O'Connor et al., 2021) females with CF achieved conception within a few weeks after initiating ELX/TEZ/IVA. Most women who discontinued ELX/TEZ/IVA during pregnancy out of concern for unknown fetal risk restarted because of clinical deterioration (Taylor-Cousar and Jain, 2021). Even a case of successful pregnancy and uncomplicated delivery has been reported for a woman with CF with very poor lung function (ppFEV1 23%) prior to conception (Balmpouzis et al., 2022).

ELX/TEZ/IVA pass the placental barrier (Collins et al., 2022). For example, a p.Phe508del homozygous infant was born who had been exposed to ELX/TEZ/IVA *in utero* from the p.Phe508del homozygous mother taking ELX/TEZ/IVA. The neonate presented with a false-negative neonatal CF screening test, normal pancreatic function and a borderline sweat chloride in sweat test indicating a partial reversion of the basic defect *in utero* (Fortner et al., 2021). Likewise, ELX/TEZ/IVA treatment of a p.Phe508del carrier who was pregnant with a p.Phe508del homozygous fetus, resolved a mid-gestation meconium ileus and led to the delivery of a child with normal pancreatic function and borderline sweat chloride in sweat test (Szentpetery et al., 2022).

On the other hand, recently one case of pulmonary hemorrhage and three cases of bilateral congenital cataracts were reported for infants who were exposed to ELX/TEZ/IVA *in utero* (Jain et al., 2022a; Nuytten et al., 2022).

Considering the limited data on the outcomes following CFTR modulator use during pregnancy and lactation, the MAYFLOWERS trial was initiated, which will examine the role of the continued use of modulators by comparing the pregnancy in women with CF who are modulator ineligible and in women with CF who choose to continue or discontinue CFTR modulator therapy during pregnancy and lactation (Jain et al., 2022b).

Nervous system and psychosocial issues

CFTR is ubiquitously expressed in the central and peripheral nervous system during the fetal period and remains to be predominantly expressed along the hypothalamic–hypophyseal axis postnatally. PwCF are inconspicuous in their mental activities suggesting that the dysfunction or lack of CFTR in the brain is compensated by other ion channels. The phase 3 trials and the open-extension study did not find any neurologic or psychiatric side effects of ELX/TEZ/IVA therapy other than headache. Post approval, however, adverse events related to the nervous system have been reported. Patients complained about testicular or joint pain (Rotolo et al., 2020; Prajapati et al., 2021) or–more seriously–about substantial mental status changes (Zhang et al., 2022a; Heo et al., 2022; Spoletini et al., 2022; Arslan et al., 2023). Symptoms emerged within the first 3 months after initiating ELX/TEZ/IVA therapy (Heo et al., 2022). The six patients of the first case series described their symptoms as fogginess, slurred speech, short term memory loss, word finding difficulty or other mental status changes (Heo et al., 2022). Symptoms of insomnia decreased by changing morning and evening dose. Earlier this year Arslan and colleagues (2023) reported two adolescents with CF with new-onset depression and suicide attempts shortly after starting ELX/TEZ/IVA. In line with these case series, one out of five adults with CF seen at another CF center in the US initiated or changed a psychiatric medication (Zhang et al., 2022a). Of 266 CF adults who started ELX/TEZ/IVA, nineteen individuals reported deterioration in mental health with anxiety, low mood, insomnia and "brain fog" with reduced attention and concentration span, which impacted on day-to-day activity and quality of life (Spoletini et al., 2022). Dose adjustments monitored by lung function and sweat chloride, in conjunction with psychological support and prescription of antidepressants if indicated, attenuated or

TABLE 4 Real-world response of pwCF to triple therapy with ELX/TEZ/IVA*.

Feature	Improvement	No improvement/side effect
General	quality of life (Cystic Fibrosis Questionnaire-Revised (CFQ-R) respiratory domain score)	minor or no improvement of extrapulmonary symptoms in lung transplant recipients
CFTR biomarkers	CFTR-mediated chloride reabsorption in sweat gland duct	CFTR-mediated sweat secretion of the secretory coil
	CFTR-mediated chloride conductance in respiratory epithelium	
	CFTR-mediated chloride secretion in intestinal epithelium	
	CFTR-mediated bicarbonate secretion in renal epithelium	
Sinonasal	SinoNasal Outcome Test (SNOT-22) of sinonasal quality of life	
	reduction of nasal polyposis, sinus opacification and mucosal thickening	
Lower airways	lung function improved (spirometry, multiple breath washout), reduction of mucus plugging, bronchial wall thickening, less pulmonary exacerbations, low or no sputum production	consolidations, perfusion defects invariant
Airway microbiology	reduction of bacterial load, lower detection rates of *S. aureus* and *P. aeruginosa*	microbial network remains fragile, still dysbiosis
Immunology	reduced systemic and lung inflammation	
Cardiovascular		rare: systemic arterial hypertension
Nutrition, intestine	increased absorption of nutrients and fat-soluble vitamins, weight gain	exocrine pancreatic insufficiency
CF-related diabetes	contradictory outcomes of post-approval studies	
Hepatobiliary system		bile acid metabolism; increase of liver stiffness in children and adolescents (?)
Dermatology		rare: skin rash, drurg-induced acne
Reproductive system	increase of pregnancy rate	
Mental health		mental status changes (5%—10% of adults)

*Information available from peer-reviewed original publications by 1 March 2023.

resolved the symptoms (Spoletini et al., 2022). The underlying mechanism responsible for this possible side effect of mental health remains unknown.

Change of the symptom-oriented treatment program

The highly effective triple modulator therapy reduces numerous symptoms of typical CF disease and calls for changes of the symptom-oriented treatment program. Supplementation with pancreatic enzymes and fat-soluble vitamins needs to be adapted on a case-to-case basis as it has already been individually optimized in the pre-modulator era. If the absorption of nutrients and vitamins, particularly fat absorption, improves, the nutritional recommendations can switch from a calorie-rich diet to the balanced mixed diet of the healthy population. Many pwCF already change their therapy without consulting their professional CF team.

The SIMPLIFY consortium will examine in the next years if chronic therapies can be modified or even stopped (Mayer-Hamblett et al., 2021). Already within a few days of treatment, pwCF recognize a reduction of sputum production. Lung imaging demonstrated that intraluminal mucus plugging starts to be resolved (Graeber et al., 2022b). Hence, inhalation of mucolytics may become dispensable. The first SIMPLIFY study included two parallel, multicenter, open-label, randomized, controlled, non-inferiority trials at 80 participating clinics across the USA in the Cystic Fibrosis Therapeutics Development Network (Mayer-Hamblett et al., 2022). Study participants had an almost normal spirometry. Six-week discontinuation of daily inhalation DNase or hypertonic saline did not show any significant difference in the change of ppFEV1 when compared with continuing treatment.

Open questions

ELX/TEZ/IVA has improved the quality of life and prognosis for pwCF. Table 4 summarizes our current knowledge of the response of pwCF to ELX/TEZ/IVA under real-life conditions. However, the 3 years since approval are too short to conclude whether triple modulator therapy may halt the progression of CF lung disease in the long-term. Domestic multicenter consortia like PROMISE (Nichols et al., 2021; 2022) will probably resolve this issue by stratifying the course of quality of life, anthropometry and airway disease depending on age and disease status when triple therapy was

started. Own data of the microbial airway metagenome suggest that after intermittent normalization the dysbiosis was coming back after 1 year of ELX/TEZ/IVA therapy (Pallenberg et al., 2022b). Bacterial load of the airways is reduced during ELX/TEZ/IVA but the typical CF pathogens are only rarely eradicated. Hence, for the time being we have no clue whether or not antimicrobial chemotherapy needs to be continued with the same stringency. Likewise, considering the conflicting outcome of the published studies, the impact of CFTR modulator therapy on CF-related diabetes mellitus deserves to be further clarified. ELX/TEZ/IVA normalizes salt and water metabolism. Arterial blood pressure slightly increases which may put pwCF at the same risk for cardiovascular complications as the normal population (Gramegna et al., 2022). The probably under-reported side effects of mental status changes deserve particular attention. Future studies should tell us whether these disturbances of mental health reflect an inappropriate adaptation to the medication that changes the patient's lifelong perspectives or whether–more likely—they are the inevitable consequence of the gain of CFTR function in the central nervous system that has never expressed functional CFTR before, but now has to cope with chloride channel activities that since conception had been fully compensated by other members of the neural network.

From the author's point of view the major challenge in the future will probably be the patient's adherence to treatment. The burden of the time-consuming symptom-oriented treatment programs needs to be reduced, but the improved prognosis should not get lost by man's common attitude "you ought to, but you don't." Non-adherence is linked to poor health outcomes. Annual medication adherence to IVA that is as efficacious for pwCF with gating mutations as ELX/TEZ/IVA is for pwCF with one or two p.Phe508 alleles, has been extracted for the UK patient population from data of the national specialty pharmacy database (Mehta et al., 2021). The mean proportion of days covered by medication was 0.80. Clinical efficacy of treatment is high, and the medication is extremely expensive. Thus, at each clinic the CF team should join forces to ensure high rates of adherence in pwCF in the long run.

Author contributions

BT conceived and wrote the manuscript.

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Conflict of interest

BT has received funding by Vertex Pharmaceuticals Inc. to conduct clinical approval studies of CFTR modulators, served on advisory boards of Vertex Inc. and Vertex Pharmaceuticals (Germany) and performed educational events for medical professionals on behalf of Vertex.

Publisher's note

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Differential effects of ELX/TEZ/IVA on organ-specific CFTR function in two patients with the rare CFTR splice mutations c.273+1G>A and c.165-2A>G

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Introduction: Evidence for the efficiency of highly-effective triple-CFTR-modulatory therapy with elexacaftor/tezacaftor/ivacaftor (ETI), either demonstrated in clinical trials or by *in vitro* testing, is lacking for about 10% of people with cystic fibrosis (pwCF) with rare mutations. Comprehensive assessment of CFTR function can provide critical information on the impact of ETI on CFTR function gains for such rare mutations, lending argument of the prescription of ETI. The mutation c.165-2A>G is a rare acceptor splice mutation that has not yet been functionally characterized. We here describe the functional changes induced by ETI in two brothers who are compound heterozygous for the splice mutations c.273+1G>C and c.165-2A>G.

Methods: We assessed the effects of ETI on CFTR function by quantitative pilocarpine iontophoresis (QPIT), nasal potential difference measurements (nPD), intestinal current measurements (ICM), β-adrenergic sweat secretion tests (SST) and multiple breath washout (MBW) prior to and 4 months after the initiation of ETI.

Results: Functional CFTR analysis prior to ETI showed no CFTR function in the respiratory and intestinal epithelia and in the sweat gland reabsorptive duct in either brother. In contrast, β-adrenergic stimulated, CFTR-mediated sweat secretion was detectable in the CF range. Under ETI, both brothers continued to exhibit high sweat chloride concentration in QPIT, evidence of low residual CFTR function in the respiratory epithelia, but normalized β-adrenergically stimulated production of primary sweat.

Abbreviations: A, adenine; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; ELX/TEZ/IVA, ETI, elexacaftor/tezacaftor/ivacaftor; G, guanine; ICM, intestinal current measurement; $LCI_{2.5}$, lung clearance index 2.5; MBW, multiple breath washout; nPD, nasal transepithelial potential difference measurement; QPIT, quantitative pilocarpine iontophoresis; PI-CF, pancreatic insufficient cystic fibrosis; pwCF, people with cystic fibrosis; SST, sweat secretion test.

Discussion: Our results are the first to demonstrate that the c.165-2A>G/c.273+1G>C mutation genotype permits mutant CFTR protein expression. We showed organ-specific differences in the expression of CFTR and consecutive responses to ETI of the c.165-2A>G/c.273+1G>C CFTR mutants that are probably accomplished by non-canonical CFTR mRNA isoforms. This showcase tells us that the individual response of rare *CFTR* mutations to highly-effective CFTR modulation cannot be predicted from assays in standard cell cultures, but requires the personalized multi-organ assessment by CFTR biomarkers.

KEYWORDS

cystic fibrosis transmembrane conductance regulator (CFTR), elexacaftor/tezacaftor/ivacaftor, c.165-2A>G, CFTR rare mutations, exon 3

1 Introduction

Cystic fibrosis (CF) is a severe ion channel disease of autosomal recessive inheritance that is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)* gene (Stoltz et al., 2015; Shteinberg et al., 2021). CFTR is a low conductance anion-selective, ATP-regulated ion channel. Its major role is to regulate chloride and bicarbonate ion movements across epithelial tissues throughout the body (Stoltz et al., 2015). More than 2,000 mutations and polymorphisms are known in the *CFTR* gene, with rare mutations remaining to be functionally characterized (Tümmler et al., 1996).

CF is the first successful example of customized drug development for mutation-specific therapy (Tümmler, 2022). CFTR correctors have been developed for improved posttranslational maturation and trafficking of mutants such as p.Phe508del that do not achieve a stable fully-folded polytopic configuration. Potentiators of CFTR activity increase chloride and bicarbonate flux across apical epithelial membranes. The potentiator Ivacaftor enhances the ATP-independent opening of the CFTR channel and thereby overcomes the defective ATP-dependent opening of CF-causing gating mutations (Van Goor et al., 2009; Eckford et al., 2012). The new triple combination therapy with the correctors elexacaftor and tezacaftor and the potentiator ivacaftor has shown to be highly efficient for the large group of patients with one or two p.Phe508del alleles regarding lung function and reduction of sweat chloride concentration (Middleton et al., 2019; Barry et al., 2021; Zemanick et al., 2021; Graeber et al., 2022; Sutharsan et al., 2022) and improvement of CFTR function in airway and intestinal epithelia (Graeber et al., 2022).

To date, approximately 90% of people with cystic fibrosis (pwCF) are eligible for highly-effective triple-CFTR-modulatory therapy with elexacaftor/tezacaftor/ivacaftor (ETI) as demonstrated in clinical trials or *in vitro* testing. However, such evidence is lacking for about 10% of pwCF with rare mutations where clinical trials are unlikely to address their response to ETI. In these cases, comprehensive assessment of CFTR function can provide critical information on the impact of ETI on CFTR function gains for such rare mutations, lending argument of the prescription of ETI.

We here describe assessment of organ-specific *in vivo* and *ex vivo* baseline CFTR function and the functional changes induced by ETI in two brothers (B1 and B2) who are compound heterozygous for the splice mutations c.273+1G>C and c.165-2A>G. c.273+1G>A is a donor splice mutation at position c.273+1 of the first nucleotide in intron 3 of the *CFTR* gene, where the obligatory conserved guanine (G) has been replaced by an adenine (A) (Tümmler, 2022). This leads to the absence of exon 3 in the CFTR mRNA messenger and a reading frame shift of the CFTR mRNA, causing different amino acids to be encoded from exon 4 onward and repeated stop signaling (Dörk et al., 1993). In other words, the c.273+1G>A mutation is a "loss of function" mutation, causing the inability to synthesize a functional CFTR protein. The mutation is assigned to *CFTR* mutation class 1.

In the acceptor splice mutation c.165-2A>G, adenine (A) is exchanged for guanine (G) at the penultimate position in intron 2. The mutation is rare and has not yet been characterized in terms of its effects on CFTR mRNA composition. However, based on CFTR acceptor splice mutations in other introns of the CFTR gene, we can again expect the skipping of exon 3 as the major consequence of the c.165-2 A>G mutation. Like c.273+1G>A, c.165-2 A>G should be a class I mutation.

The two mutations affect the canonical splice sites flanking exon 3. Exon 3 skipping from both alleles should lead to a knock-out phenotype of no functional CFTR protein provided that no other CFTR mRNA isoforms are produced. However, as shown in this report, an organ-specific rescue of CFTR function was observed by CFTR biomarkers in the two index cases who are compound heterozygous for the rare splice mutations c.165-2A>G and c.273+1G>C. The two brothers showed residual CFTR activity in sweat secretion and gained more CFTR activity during ETI triple therapy in the respiratory epithelium and the secretory coil of the sweat gland.

2 Materials and methods

In this study, we assessed the effects of ETI on CFTR function in two brothers (B1 and B2) who are compound heterozygous for the splice mutations c.273+1G>C and c.165-2A>G. For clinical evaluation, we monitored the lung clearance index 2.5 ($LCI_{2.5}$) by multiple breath washout (MBW). We quantified CFTR-function using quantitative pilocarpine iontophoresis (QPIT), nasal potential difference measurements (nPD), intestinal current measurements (ICM) and β-adrenergic sweat secretion tests (SST). All tests were performed prior to and 4 months after the initiation of ETI.

MBW testing was performed with the Exhalyzer D system (Eco Medics), and 100% oxygen was used to wash out resident nitrogen

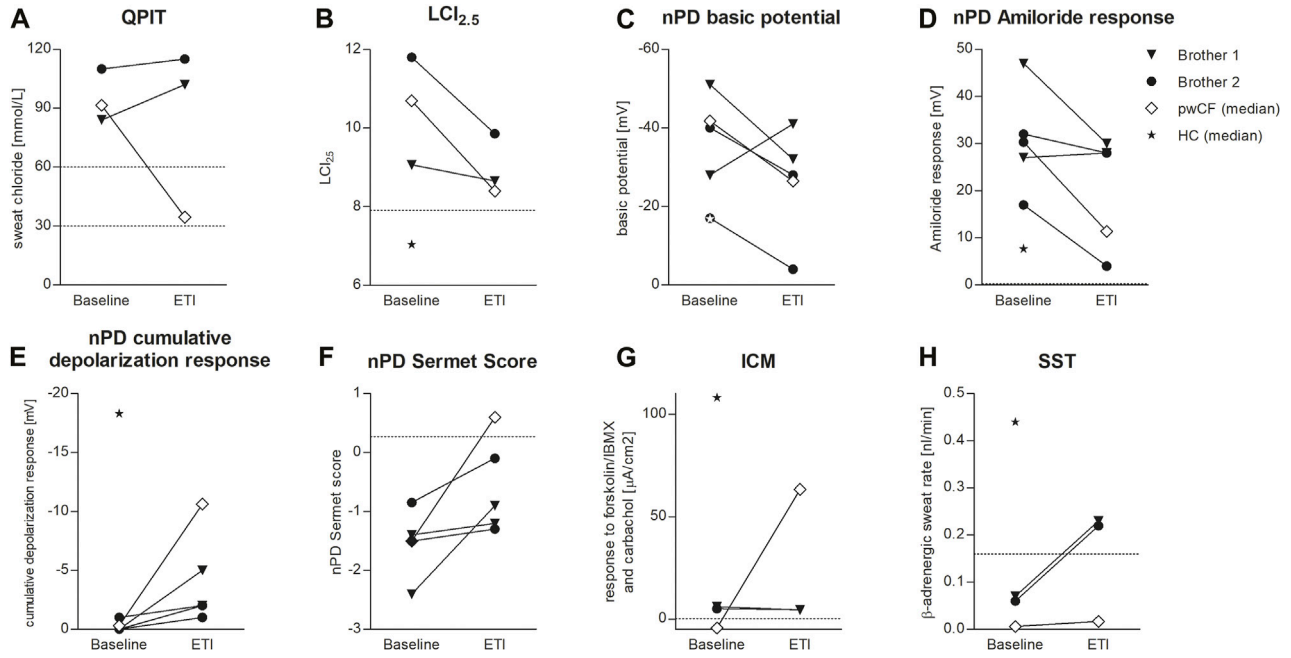

FIGURE 1
Effects of ETI on QPIT **(A)**, $LCI_{2.5}$ **(B)**, nPD basic potential **(C)**, nPD Amiloride response **(D)**, nPD cumulative depolarization response to chloride-free solution and isoproterenol **(E)**, nPD Sermet Score **(F)**, response to forskolin/IBMX and carbachol in ICM **(G)** and SST **(H)** in brother 1 (triangle) and brother 2 (circle) compared to the median effect on pwCF with one or two p.Phe508del alleles (white square) and reference values for healthy controls (HC, star). Dashed lines indicate published limits of normal for QPIT (Nährlich et al., 2013), $LCI_{2.5}$ (Wyler et al., 2021), nPD Sermet Score (Sermet-Gaudelus et al., 2010) and SST (Pallenberg et al., 2022).

from the lungs with a mouthpiece as interface (Stahl et al., 2017). All measurements were using spiroware 3.3.1 (Eco Medics) (Stahl et al., 2020; Graeber et al., 2021; Wyler et al., 2021). The upper limit of normal (ULN) was determined as 7.1 (Wyler et al., 2021).

QPIT was performed following the German national diagnostic guideline (Nährlich et al., 2013) and the guidelines of the Clinical and Laboratory Standards Institute (Wayne, 2009). Pilocarpine iontophoresis was used to stimulate the skin and sweat was collected with the Macroduct® system (Model 3700, Wescor, Logan UT, United States). Sweat chloride concentration was measured using a chloridometer (KWM 20 Chloridometer, Kreienbaum, Langenfeld, Germany) in a minimum volume of 30 µL.

NPD measurements were performed according to the Standard Operating Procedure nPD_EU001, version 1.7 (March 2013) "Nasal Potential Difference (nPD) Measurement for Diagnosis and Clinical Trials in Cystic Fibrosis" of the European Cystic Fibrosis Society (ECFS) Diagnostic Network Working Group and Clinical Trials Network and as previously described (Sermet-Gaudelus et al., 2010; Rowe et al., 2011; Graeber et al., 2018) and published recently (Graeber et al., 2022). The Sermet Score was used to discriminate between normal (>0.27) and reduced (<0.27) CFTR function in the respiratory epithelium of the nose (Sermet-Gaudelus et al., 2010).

ICM was performed according to the Standard Operating Procedure ICM_EU001, version 2.7 (October 2011) "Ion Transport in Rectal Biopsies for Diagnosis and Clinical Trials in Cystic Fibrosis" of the European Cystic Fibrosis Society (ECFS) Diagnostic Network Working Group and Clinical Trials Network modified by in-house protocol adjustments at the CF electrophysiology laboratory in Hannover (Graeber et al., 2015; Graeber et al., 2018). CFTR function in rectal tissue biopsies was quantified using the response to forskolin/IBMX and carbachol in µA/cm^2.

The β-adrenergic sweat secretion test was performed as previously published (Pallenberg et al., 2022) using the AutoBuSTeD software for automatic analysis of sweat bubble formation. Sweat rates were measured in sweat volume (nL) per time (min). A β-adrenergic sweat rate of < 0.16 nL/min was defined as impaired CFTR function.

2.1 Statistical analysis

We analyzed all data with GraphPad Prism version 9.0.1 (GraphPad Software) and R 3.6.2 (R Core Team, 2018).

3 Results

3.1 Clinical characteristics

Both patients were compound heterozygous for the rare splice mutations c.273+1G>C and c.165-2A>G. At baseline, B1 was 10.2 years old and B2 was 7.2 years, both of them presented with the phenotype of pancreatic insufficient cystic fibrosis (PI-CF). Prior to ETI, the sweat chloride concentrations were in the typical PI-CF

TABLE 1 Clinical and functional parameters of the two brothers before and under ETI therapy and reference values for pwCF with one (Δ/MF) or two (Δ/Δ) p.Phe508del alleles without CFTR-modulator therapy (baseline) and after 3 months of ETI (ETI) and healthy controls (HC).

Clinical parameters	Brother 1 (B1)				Brother 2 (B2)				References values				
	Baseline		ETI		Baseline		ETI		pwCF (baseline) median		pwCF (ETI) median		HC median
									Δ/MF	Δ/Δ	Δ/MF	Δ/Δ	
Gender	m				m				—		—		—
Phenotype	PI-CF				PI-CF				—		—		—
Age in years	10.2		10.5		7.2		7.5		—		—		—
$LCI_{2.5}$	9.06		8.66		11.8		9.86		10.3[a]	10.7[a]	7.4[a]	8.4[a]	<7.1 (ULN)[b]
Sweat chloride [mmol/L]	84		102		110		115		103[a]	91.5[a]	50[a]	34.5[a]	<30[c]
nPD	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*					
Basic potential [mV]	−51	−28	−32	−41	−17	−40	−4	−28	−42.2[d]	−41.7[d]	−28[d]	−26.4[d]	−16.8[e]
Amiloride response [mV]	47	27	30	28	17	32	4	28	24.8[d]	30.3[d]	15.1[d]	11.4[d]	7.7[e]
Cumulative depolarization response to chloride-free solution and isoproterenol [mV]	0	0	−5	−2	0	−1	−1	−2	−1.1[d]	−0.3[d]	−9.9[d]	−10.6[d]	−18.3[e]
Sermet Score	−2.4	−1.4	−0.9	−1.2	−0.85	−1.5	−0.1	−1.3	−1.1[d]	−1.5[d]	0.4[d]	0.6[d]	> 0.27[e]
ICM													
Response to forskolin/IBMX and carbachol [$\mu A/cm^2$]	6		4.4		5		4.5		−1.9[d]	−4.4[d]	59.1[d]	63.3[d]	108[f]
SST													
β-adrenergic sweat rate [nL/min]	0.07		0.23		0.06		0.22		0.006[g]		0.017[g]		0.44[g]

[a](Graeber et al., 2022).
[b](Wyler et al., 2021).
[c](De Boeck et al., 2006).
[d](Graeber et al., 2022).
[e](Sermet-Gaudelus et al., 2010).
[f](Minso et al., 2020).
[g](Pallenberg et al., 2022).

range (B1: 84 mmol/L, B2: 110 mmol/L) and $LCI_{2.5}$ values showed moderate lung ventilation inhomogeneity (B1: 9.06, B2: 11.8). After 4 months of CFTR modulator therapy with ETI, sweat chloride concentrations remained in the PI-CF range (B1: 102 mmol/L, B2: 115 mmol/L, Figure 1A) and only modest improvements in pulmonary function were seen in the $LCI_{2.5}$ values (B1: 8.66, B2: 9.86, Figure 1B; Table 1).

3.2 Effects of ETI on the CFTR function of the respiratory epithelia

CFTR function in the respiratory epithelium was determined by nasal potential difference measurement. At baseline, B1 presented with typical findings for PI-CF (for reference values see Table 1). The basic potential was in the CF range (right nostril: −51 mV, left nostril: −28 mV, Figure 1C) and we saw a high hyperpolarization response to amiloride (right: 47 mV, left: 27 mV, Figure 1D). The cumulative depolarization response to chloride-free solution and isoproterenol was absent (left and right nostril: 0 mV, Figure 1E). Under ETI, there were no relevant changes in basic potential or amiloride response. However, cumulative depolarization response to chloride-free solution and isoproterenol improved slightly as a sign of low residual CFTR function under ETI (right nostril: −5 mV, left nostril −2 mV). The Sermet score improved to some extent but remained in the CF-range (<0.27) from −2.4 to −0.9 (right nostril) and −1.4 to −1.2 (left nostril; Figure 1F; Table 1).

Prior to ETI, B2 showed a normal basic potential in the right nostril (−17 mV, Figure 1C), hyperpolarization to amiloride in the borderline CF range (17 mV, Figure 1D), and a cumulative depolarization response to chloride-free solution and isoproterenol in the CF range (0 mV, Figure 1E). In the left nostril, all values were in the typical CF range. Four months of ETI therapy led to no significant improvements in CFTR function as measured by cumulative depolarization response (Figure 1E). The basic potential increased from −17 to −4 mV (right nostril) and −40 to −28 mV (left nostril, Figure 1C). At baseline, the Sermet Score was −0.85 (right nostril) and −1.5 (left nostril) and showed mild improvements to −0.1 (right nostril) and −1.3 (left nostril) under ETI, remaining in the CF range (<0.27) (Figure 1F; Table 1).

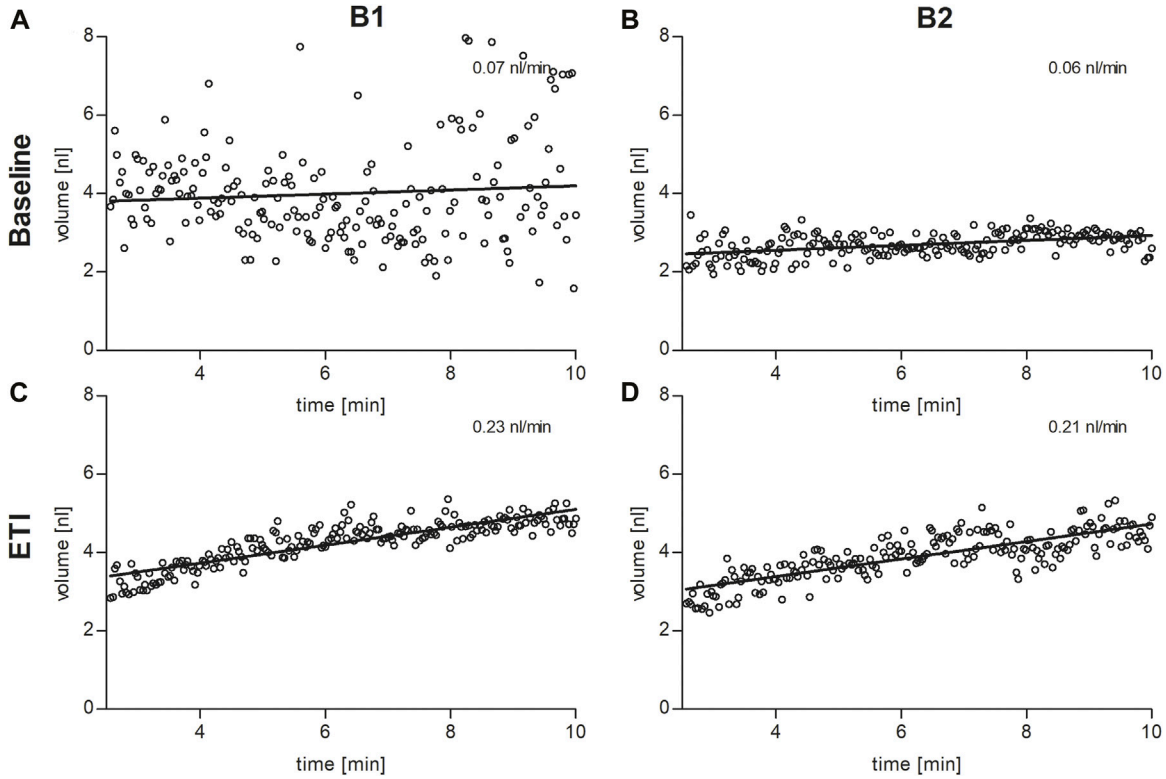

FIGURE 2
Sweat bubble formation after β-adrenergic stimulation of the skin at baseline **(A,B)** and under ETI **(C,D)** in brother 1 (B1, left column) and brother 2 (B2, right column). Each dot represents the median bubble volume per time point as calculated by the AutoBuSTeD software (Pallenberg et al., 2022). The line shows the linear correlation, the slope corresponds to the sweat rate in nL/min.

3.3 Measurement of intestinal CFTR function in rectal biopsies

In intestinal current measurement (ICM), CFTR function in rectal tissue biopsies was quantified by the response to forskolin/IBMX and carbachol in μA/cm^2. Prior to ETI, stimulation with forskolin/IBMX and carbachol induced cumulative ion currents of 6 μA/cm^2 in the biopsies of B1 and 5 μA/cm^2 in the biopsies of B2. Compared to our data from 68 healthy controls with a median response to forskolin/IBMX and carbachol of 108 μA/cm^2 (IQR: 63–198 μA/cm^2, Minso et al., 2020) and our data from pwCF with one or two p.Phe508del alleles with a median response of −1.9 and −4.4 μA/cm^2 (Graeber et al., 2022; Table 1), the brothers presented typical responses in the CF range. Thus, there was no evidence of CFTR-mediated chloride secretion in the intestinal epithelium at baseline. After 4 months of therapy with ETI, stimulation with forskolin/IBMX and carbachol induced cumulative ionic currents of 4.4 μA/cm^2 in the biopsies of B1 and 4.5 μA/cm^2 in the biopsies of B2, concluding in no improvement of CFTR-function in the intestinal epithelium (Figure 1G).

3.4 Effect of ETI on the β-adrenergic sweat rate

In the secretory epithelium of the sweat gland, following β-adrenergic stimulation with concomitant cholinergic inhibition by atropine, CFTR-mediated sweat secretion is detected as a direct indicator of CFTR function (Sato and Sato, 1984; Wine, 2022). In contrast, determination of chloride concentration in the sweat indicates the capacity for CFTR-mediated chloride reabsorption in the excretory duct of the sweat gland. We used the AutoBuSTeD software for automated processing of sweat bubble formation and our previously published protocol to analyze sweat rates in nL/min (Figure 2). Our reference values for pwCF with one or two p.Phe508del alleles (median 0.006 nL/min; IQR 0–0.027 nL/min) and healthy controls (median 0.44 nL/min; IQR 0.34–0.48 nL/min) determined the CF range (Table 1). A cut-off value as calculated by ROC analysis between these two reference cohorts of 0.16 nL/min was used to discriminate between normal and impaired CFTR function (Pallenberg et al., 2022). Prior to ETI, both brothers showed a β-adrenergic sweat rate in the upper CF range (B1: 0.07 nL/min, B2: 0.06 nL/min), indicating impaired but residual CFTR function in the sweat gland. Therapy with ETI led to a significant increase and normalization of β-adrenergic sweat rates to 0.23 nL/min (B1) and 0.22 nL/min (B2) (Figures 1H, 2).

4 Discussion

This case of the two brothers with the rare and, in part, previously uncharacterized CFTR splice mutations showed a divergence of responses in CFTR biomarkers to ETI which,

particularly in the sweat gland, was antagonistic to the findings of pwCF with one or two p.Phe508del alleles. Functional CFTR analysis prior to ETI showed no CFTR function in the respiratory (nPD) and intestinal (ICM) epithelia and in the sweat gland reabsorptive duct (QPIT) in either brother. In contrast, β-adrenergic stimulated, CFTR-mediated sweat secretion (SST) was detectable in the CF range. Under ETI, both brothers continued to exhibit high sweat chloride concentration in QPIT, a low total chloride response in ICM, evidence of low residual CFTR function in the respiratory epithelia (nPD), but normalized β-adrenergically stimulated production of primary sweat.

Our results indicate organ-specific differences in the expression of CFTR and consecutive responses to ETI of the c.165-2A>G/c.273+1G>C *CFTR* genotype. Prior to ETI, functional CFTR was detectable only in the secretory epithelium of the sweat gland by SST. ETI normalized CFTR function in the secretory apparatus of the sweat gland, whereas CFTR function in the sweat duct as assessed by QPIT and respiratory epithelium improved poorly or not at all.

The c.165-2A>G mutation affects the canonical splice acceptor site preceding exon 3 and the c.273+1 mutation affects the canonical splice donor site following exon 3. Thus, both mutations are expected to induce exon 3 skipping as the major consequence as it has been demonstrated for c.273+1G>C in the respiratory epithelium of a c.273+1G>C/p.Phe508del compound heterozygous individual with CF (Dörk et al., 1993). However, the c.165-2A>G/c.273+1G>C compound heterozygous brothers showed subtle residual CFTR function in the respiratory epithelium and substantial residual CFTR function in the secretory coil. Since β-adrenergically stimulated chloride secretion in the secretory coil of the sweat gland is exclusively executed by CFTR and is not substituted by any other ion channel (Wine, 2022), we can conclude that chloride secretion was mediated by one or more CFTR mRNA isoforms that confer residual CFTR activity. The use of cryptic splice sites may lead to a non-canonical CFTR mRNA isoform. A cryptic exon is known in intron 3 flanked by almost perfect acceptor and donor splice sites, but it encodes two termination codons so that no functional CFTR activity can be expected from this CFTR mRNA isoform (Will et al., 1994). Alternatively, minute amounts of full-length CFTR mRNA transcript may be produced if the splice mutations are somewhat leaky. This scenario may not sound plausible by first glance. However, exon skipping as the only consequence of canonical splice site mutations flanking exon 3 has been observed in compound heterozygous patients who carry a class II or a class IV missense mutation in trans (Dörk et al., 1993; Bienvenu et al., 1994). In contrast, since conception the two brothers are compound heterozygous for two class I splice site mutations that target the same exon. Thus, the spontaneous rescue of some functional CFTR activity from two class I mutations is not unlikely: we have investigated CFTR biomarkers in a singular case of a compound heterozygous patient for two non-sense mutations who demonstrated CFTR activity in the ICM (Tümmler, 2019). This index case taught us that not all carriers of two class I mutations lack CFTR activity.

This showcase illustrates that although high-throughput screening of *CFTR* mutations in recombinant cell lines allows proper classification of mutation phenotypes, and standard cell culture assays can predict rare *CFTR* mutation response to highly effective CFTR modulation, the outcome is not necessarily predictive of individual patient response to CFTR modulators *in vivo*. In summary, we provided evidence that some patients with class 1 *CFTR* mutations may benefit from ETI. Hence, when it comes to the issue whether rare or ultra-rare mutations will be responsive to CFTR modulators like ETI, the individual subject should be assessed *in vivo* with CFTR biomarkers prior and during treatment with the medication.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Hannover Medical School. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

STP: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, validation, visualization, writing—original draft, writing—review and editing. IH: resources, writing—review and editing. CD: methodology, resources. RM: methodology. MMN: formal analysis, methodology, resources, validation. GH: funding acquisition, resources, supervision, writing—review and editing. BT: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing—original draft, writing—review and editing. A-MD: conceptualization, funding acquisition, investigation, project administration, resources, supervision, validation, writing—original draft, writing—review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Real-world disparities and ethical considerations with access to CFTR modulator drugs: Mind the gap!

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The third Sustainable Development Goal (SDG), to ensure healthy lives and promote well-being for all at all ages, has particular relevance and implementation challenges amongst people living with rare diseases such as cystic fibrosis (CF). Although the treatment and projected outcome of CF has significantly improved with the advent of CF transmembrane conductance regulator protein modulator (CFTRm) therapy, there remains significant global inequality with regards to access to these life-saving and life-altering drugs. Elexacaftor, tezacaftor, and ivacaftor (ETI) triple combination therapy, first licensed in the United States in 2019, has rapidly become the standard of care for children aged 6 years and older in most high-income countries for individuals with CFTR variants responsive to ETI. Negotiated agreements for access to ETI are currently in place in North America,Europe, Israel ,Australia and New Zealand. However, less priority has been given to negotiate agreements for access to CFTRm in low-middle income countries(LMIC) with significant CF populations such as Central and South America, India, the Middle East, and Southern Africa. These countries and individuals living with CF are therefore effectively being left behind, in direct conflict with the stated principle of the 2030 SDGs. In this review, we highlight the current global inequity in access to CFTRm drugs and its impact on widening disparities between high-income countries and LMIC in CF outcomes and survival. We further discuss the reasons for this inequity and explore the ethical- and human rights-based principles and dilemmas that clinicians, families, governments, and healthcare funders must consider when prioritizing fair and affordable access to expensive CFTRm drugs. Lastly, we propose possible solutions to overcoming the barriers to accessing affordable CFTRm drugs in LMIC and illustrate with examples how access to drug therapies for other conditions have been successfully negotiated in LMIC through innovative partnerships between governments and pharmaceutical industries.

KEYWORDS

cystic fibrosis, low-middle income countries, disparity, CFTR modulator drugs, ethics

Introduction

The advent of cystic fibrosis transmembrane conductance regulator modulator (CFTRm) drugs has revolutionized the treatment of cystic fibrosis (CF) in individuals with drug-responsive CFTR variants. Elexacaftor, tezacaftor, and ivacaftor (ETI) triple combination therapy, first licensed in the United States (US) in 2019, has rapidly become standard of care in most high-

income countries (HIC) for people with CF (pwCF) aged 6 years and older with CFTR variants responsive to ETI, the commonest being F508del. Beyond clinical trials, real-world data on outcomes in pwCF on ivacaftor, the first CFTRm licensed more than a decade ago, provide compelling evidence over time of improved outcomes in nutrition, pulmonary exacerbations, and lung function. Furthermore, risk for mortality and lung transplantation are also significantly reduced (Balfour-Lynn and King, 2022; Gifford et al., 2022; Regard et al., 2022). Although long-term, real-world data are lacking for ETI, similar outcomes are reported and projected for pwCF receiving ETI, including those with advanced lung disease (Benden and Schwarz, 2021; Balfour-Lynn and King, 2022; Gifford et al., 2022; Keogh et al., 2022; Regard et al., 2022; McCoy et al., 2023). The outlook and long-term prognosis, including survival, of pwCF has without doubt substantially improved for those eligible for CFTRm and have access to these drugs, especially if initiated at a younger age when end-organ disease is not established yet. Ivacaftor is currently licensed from age 6 months, and results of phase III clinical trials for ETI in children aged 2–5 years are expected to be released soon (NCT04537793).

Worldwide, 82% of people diagnosed with CF have at least one copy of F508del and are thus eligible for ETI (Lopes-Pacheco, 2020). However, the global demography of CFTR mutations is largely determined by race and ethnicity, with the proportion of F508del in at least one allele ranging between 80% and 90% in North America, Western Europe, and Australia, less than 20% in Turkey, and extremely rare in people with sub-Saharan African or Asian ancestry (Stewart and Pepper, 2017; Lopes-Pacheco, 2020; McGarry et al., 2022). In people with African ancestry, the class-I variant 3120+1G>A is the most commonly reported mutation, present in a homozygous state in 56% of black South Africans with CF (Zampoli et al., 2021). Race and ethnic diversity are thus important biological factors determining eligibility for CFTRm, strongly favoring populations where F508del is common.

The third Sustainable Development Goal (SDG), to ensure healthy lives and promote well-being for all at all ages, enshrined in the pledge to "leave no one behind," has particular relevance and implementation challenges amongst people living with rare diseases such as CF (United Nations Committee for Development Policy, 2018). There are significant global disparities and inequalities in accessing expensive life-saving and life-altering CFTRm drugs, with only an estimated 12% of the world's total CF population receiving this therapy (Guo et al., 2022a). This inequality is likely to accelerate a widening gap in CF care and outcomes between high income countries (HIC) and low-middle income countries (LMIC).

Worldwide disparities in CF care and access to CFTRm drugs

According to the WHO, social determinants of health (SDOH) can be more important than healthcare services or lifestyle choices. Worldwide, irrespective of income or infrastructure, health and illness follow a social gradient: *the lower the socioeconomic position, the worse the health*. Non-medical factors and SDOH potentially impact 30%–55% of health outcomes (Hosseinpoor et al., 2012; World Health Organisation, 2023). In diseases such as CF, where exceptional standards of care have been established, race, ethnicity, and SDOH are important factors affecting CF outcomes in all facets of diagnosis, management, and prognosis. In many LMIC and among marginalized populations within developed nations, there is notable ascertainment bias due to lack of awareness of CF, prioritization on endemic diseases, lack of CF registries, absent or limited newborn screening (NBS) protocols, or NBS panels analyzing mutations that are not relevant to that population (Scotet et al., 2020; da Silva Filho et al., 2021). Limited access to sweat testing and full mutation analyses impedes diagnostic confirmation and hence knowledge of CFTRm eligibility.

The estimated number of confirmed pwCF from 94 countries worldwide is 105,000, with no information available from the other 109 nations (Guo et al., 2022a). The estimated global disease burden is 144,606–186,620 based on surveys, registry data, and extrapolated data. Worldwide, 40 countries have at least one CFTRm approved, but the majority are HIC with predominantly White race populations in North America, Europe, Israel, Australia and New Zealand (Figure 1). According to the annual US CF registry report, 87.6% of 25,497 pwCF eligible for CFTRm drugs in the US were receiving CFTRm drugs in 2021 (CFF, 2022). This is in stark contrast to the global picture where only 19,516 (12%) of the estimated 162,428 world CF population, both diagnosed and undiagnosed, are receiving CFTRm drugs (Guo et al., 2022b).

Unequal availability of CFTRm will only widen existing disparities in disease burden and outcomes in LMIC which have weaker healthcare infrastructure compared to HIC. Even though the reported prevalence of CF is low in many LMIC, when extrapolated to actual population data, absolute numbers of pwCF may be very high. For example, in India with a population of 1.4 billion, actual numbers of pwCF could range between 35,000 and 140,000 based on reported prevalence of 1: 10,000–1:40,000. Similarly, in 2021, there were 23 million live births with an estimated CF incidence between 680 and 2,700 (Kapoor et al., 2006; Singh et al., 2020). Without more accurate information on CFTRm-eligible mutations, it is difficult to estimate how many patients could benefit from CFTRm worldwide.

The impact of race and ethnicity on CF care and access to CFTRm

Race and ethnicity are fundamental components of a person's identity. While race classifications are often based on physical and biogenetic characteristics, especially skin color, ethnic distinctions focus on cultural characteristics such as language, history, religion, and custom. There is no genetic basis for race, and evidence from the Human Genome Project and 1000 Genomes Project revealed only 0.1% difference among races with probable shared ancestry (Collins et al., 2003; Genomes Project et al., 2015). While the realization of racial and ethnic diversity should be embraced, race has historically been the focus of disparities ever since the beginning of civilization and significantly impacts SDOH, leading to prejudice, inequity, and discrimination.

According to the 2021 US CF registry data, about 18% are self-reported as "non-white" people (CFF, 2022). Even in a well-resourced nation with access to the best possible CF care, delayed diagnosis and delayed time to first assessment after a positive NBS were noted among infants from minority

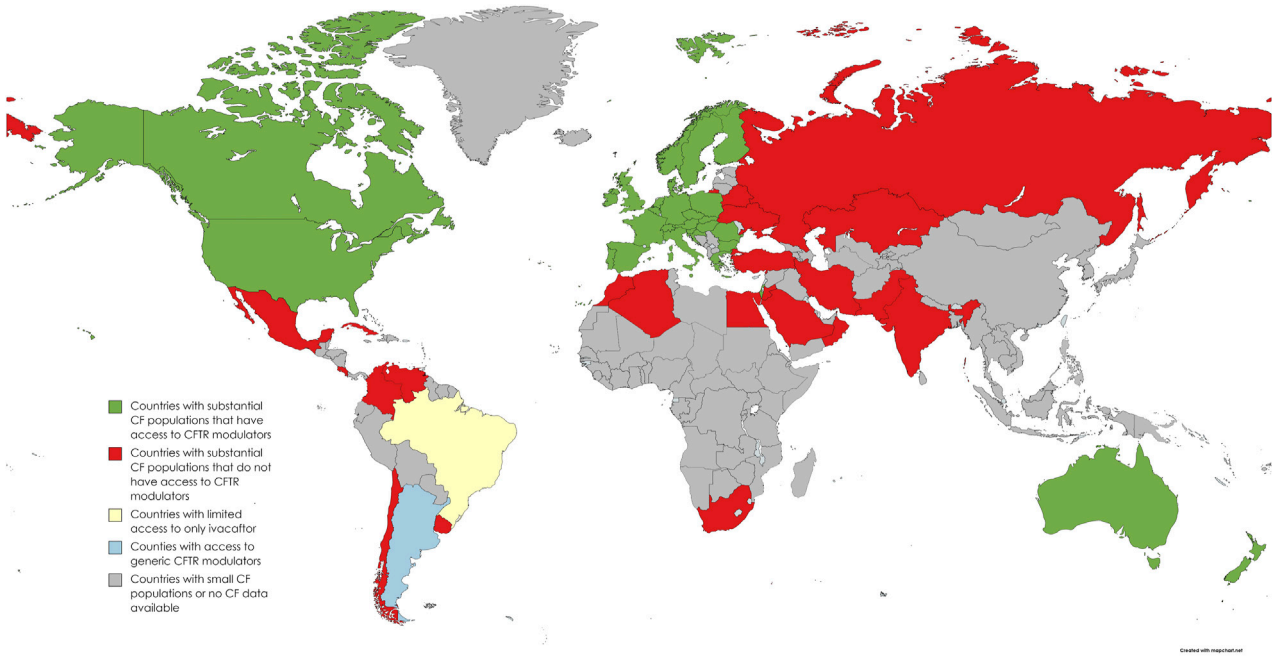

FIGURE 1
World map distribution of countries with access to CFTR modulator drugs or negotiated agreements in place (adapted from Guo et al. (2022a) and personal communications with "Vertex Save Us" campaign).

communities with worse pulmonary or nutritional morbidity. There is clear demonstration of lower lung function, higher mortality rates, and higher rates of acquiring pulmonary infections related to delayed diagnosis and limited access to care, limited health literacy, and ill effects of systemic racism. Furthermore, low-income groups experience differential exposure to tobacco smoke, access to transplantation, inclusion in clinical trials, mental health issues—all factors that impede adherence to recommended clinical care. SDOH directly and indirectly impact CF outcomes through policy, socioeconomic status, and race/ethnicity (Quittner et al., 2010; McGarry and McColley, 2021; Oates and Schechter, 2021; McGarry et al., 2022). This exciting era of CFTRm has unfortunately only exposed the existing disparities. The prevalence of F508del mutations among pwCF from minority communities in the US is lower with significant disparities in CFTRm eligibility: 92% in White people *versus* 70%–75% in African–Americans and Hispanic pwCF, respectively (McGarry and McColley, 2021). Similarly, emerging data from many LMIC reveal mutational heterogeneity, with lower F508del allele frequency (da Silva Filho et al., 2021; Singh et al., 2015; Vaidyanathan et al., 2022).

Ethical- and human rights-based framework: The case for CFTRm drugs

There are several ethical- and human rights-based principles and dilemmas that must be considered when prioritizing fair and affordable access to expensive drugs for orphan diseases such as CF (Kacetl et al., 2020). The bioethical principles most relevant to this discussion are those of justice, beneficence, and non-maleficence (Beauchamp and Childress, 2001).

Justice

Procedural justice speaks to the requirement for transparent and participative policymaking and resource allocation decisions and processes; distributive justice relates to the fair allocation of available resources; and social justice requires that every individual is treated with respect and dignity (Gericke et al., 2005). In the context of healthcare provision, justice refers to fair, equitable, and appropriate treatment in the light of what is due to individuals (Beauchamp and Childress, 2001; Gericke et al., 2005); most commonly interpreted as the need to provide basic healthcare for everyone (Kinney, 2014). The different models used to apply this principle, however, may be conflictual. It could be argued, for example, that CFTRm drugs constitute basic healthcare for pwCF.

The utilitarian approach to distributive justice maximizes public utility, often expressed as doing "the greatest good for the greatest number." This approach usually forms the ethical basis of economic evaluation for healthcare expenditure. In this context, spending substantial financial resources for a rare condition such as CF could be considered unethical because it would only benefit relatively few people rather than maximizing societal benefit, and may be deemed cost-ineffective. In the face of resource constraints, funding expensive CFTRm drugs for few people may also displace cheaper healthcare interventions for more people with common conditions (Hughes et al., 2005; Ollendorf et al., 2018). In LMIC, where there is a substantial burden of disease related to communicable diseases and SDOH, it is often argued that health

costs should be prioritized to meet the needs of the majority, with a focus on primary and preventive care. Treating relatively few pwCF with prohibitively expensive CFTRm is in conflict with utilitarian ethical principles of justice.

From a social justice perspective, a rights-based approach to resource management, in which all individuals within a society are entitled to a decent minimum healthcare standard, could require that treatment is made available for managing rare diseases such as CF. However, the basic right to healthcare is by its nature limited by resource constraints and is open to interpretation, even when enshrined in national legislation (Hughes et al., 2005).

It has been argued that health systems should not simply aim to maximize health gains across the entire population, but that the principle of fairness should also ensure that all people get a chance at meaningful health and wellbeing, or a "fair innings," regardless of whether this exceeds standard measures of cost-effectiveness. Society has been shown to value this egalitarian approach to equitable distribution of resources across patients and diseases, rather than maximizing total population health (Jena and Lakdawalla, 2017; Ollendorf et al., 2018). Furthermore, society places higher value on health improvement for individuals with poorer lifetime health prospects (Dolan et al., 2005). Applying these egalitarian values upholds the moral public and policy obligation of non-abandonment of individuals with highly specialized healthcare needs, which should be considered in policy development for rationing and resource allocation for pwCF, even in LMIC settings (Landman and Henley, 1999; Hughes et al., 2005).

Beneficence

In terms of beneficence, there is compelling real-world evidence of substantial benefits of CFTRm therapy in pwCF and responsive CFTR mutations, with reported improvements in lung function and nutrition, reduced pulmonary exacerbation rates as well as the potential to alter the trajectory of CF disease, and low mortality rates in the long term (Benden and Schwarz, 2021; Balfour-Lynn and King, 2022; Gifford et al., 2022; Keogh et al., 2022; Regard et al., 2022; Sawicki et al., 2015; Heijerman et al., 2019; Volkova et al., 2020; Carnovale et al., 2022). There is therefore clearly a conflict between the utilitarian approach to distributive justice and the principle of beneficence based on social or moral obligation (Gericke et al., 2005).

Non-maleficence

Contrary to the ethical principle of non-maleficence, lack of access to CFTRm drugs for pwCF in LMIC may cause individual and collective harms, with a widening inequality between people with and without access to CFTRm. Delayed access to CFTRm for pwCF in LMIC has likely already had a substantial impact in this widening disparity. In 2021, Stanojevic et al. (2021) estimated that introduction of ETI in Canada in the same year would reduce the number of pwCF with severe lung disease by 60% and deaths by 15% by 2030. However, the projected benefits would be halved if access were delayed to 2025 (Stanojevic et al., 2021). For LMIC, where CF-related outcomes and mortality are likely to be substantially worse, this outlook is particularly dire (Bell et al., 2020).

People without CF may, however, also incur indirect and "invisible" harms if expensive drugs are made available to pwCF at the expense of healthcare funding for more common diseases (Ollendorf et al., 2018). Reducing the financial cost of CFTRm drugs is therefore an ethical imperative, in order to reap the benefits for pwCF whilst limiting the impact on health services. The high cost of CFTRm has been questioned recently in a study reporting that actual production costs were 90% lower than the current market prices (Guo et al., 2022b). This over-inflation of market prices for a life-saving essential drug appears unethical using the principle of distributive justice and infringes the basic human right to equal access to healthcare access, thereby widening the existing inequalities in CF care globally and leaving many pwCF behind (Guo et al., 2022b).

Solutions to overcoming the barriers to accessing affordable CFTRm drugs

In LMIC, key barriers to achieving quality CF care are limited CF diagnosis and prevalence data and high costs of CF therapies, including CFTRm. Improving CF awareness, providing access to diagnostic tests, and creating national registries are needed to determine the actual disease burden of CF in these regions. As CFTR mutation analysis is expensive, research and development of targeted mutation panels would help identify regional- or population-specific mutations. Knowledge of regional CF epidemiology and specific mutation data would be valuable to ascertain the actual CFTRm eligibility of individual CF populations. Pharmaceutical companies should realize the potential value of investment in LMIC due to sheer numbers of patients who may benefit from these life-altering medications.

The major barrier to equitable and affordable global distribution and access to CFTRm is a prohibitive pricing strategy driven by pharmaceutical patenting practices that are protected by the World Trade Organization (WTO) agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS), established in 1995. Most countries are signatories to TRIPS, and under this agreement, countries are required to offer patents to pharmaceutical companies which typically are valid for 20 years, thus offering extended period market exclusivity without competition. While protecting intellectual property rights is fundamental, patent regulations and laws are open to abuse as highlighted by a recent US-based Association of Accessible Medicines report finding that the top 12 brands of innovative drugs on the US market are protected by a total of 848 patents (71 per drug), providing an average of 38 years without generic competition (AAM, 2022). Pharmaceutical companies point toward recuperation of research and development costs to justify extending patents beyond reasonable periods. However, the balance between rewarding innovation through global market monopoly and providing access to innovative life-altering drugs such as CFTRm is difficult to achieve due to competing interests of profit *versus* justice. The annual estimated minimum production cost of ETI by the originator company is $5,676 for one person, which is over 90% lower than the US listed price (Guo et al., 2022b). Such prohibitive pricing strategies are beyond the reach of

governments and health systems in LMIC and will thus leave behind pwCF in LMIC despite CFTRm eligibility. One successful strategy to achieve that balance between protecting pharmaceutical intellectual property and providing affordable access to CFTRm in LMIC is the issuing of voluntary licenses (VL) to generic drug manufacturing companies.

Voluntary licenses are contractual agreements between patent-holding pharmaceutical companies and generic manufacturing companies, which set out terms under which generic versions of the drugs can be manufactured, marketed, and distributed in specified geographical regions (Médecins Sans Frontières Technical Briefing Document, 2020). While VL practices will reduce prices of medicines and improve access, the deals often lack transparency and come with a range of restrictive conditions that undermine access to medicines through limitations on where products can be sold and by controlling the supply of active pharmaceutical ingredients (APIs) to the licensees. Additionally, VL restrictions may include varying terms through multiple licensees and overly broad scopes of the patent that include, for example, appeals to rejected applications and geographical limitations that often exclude middle-income territories (Médecins Sans Frontières Technical Briefing Document, 2020). Excluding middle-income countries is counterproductive as they are home to 75% of the world's population and 62% of the world's poor and face a double burden of communicable and non-communicable diseases (Médecins Sans Frontières Technical Briefing Document, 2020). Nonetheless, VL have been successfully negotiated for human immunodeficiency virus (HIV), tuberculosis (TB), and hepatitis C drugs to the great benefit of millions of people in LMIC suffering with these conditions. More recently, Merck and Pfizer have signed VL deals with the Medicines Patent Pool (MPP) for their oral COVID-19 anti-viral medications, such as molnupiravir and nirmatrelvir (Shadlen, 2022). The MPP, established in 2010, is a United Nations-backed public health organization working to increase access to and facilitate the development of life-saving medicines in LMIC, by helping broker VL deals between originator and generic pharmaceutical companies. The major advantage of the MPP approach is that VL deals are transparent and offer pooled licensing agreements across multiple companies and territories (Shadlen, 2022). Popularity with the MPP has gained traction since the COVID-19 pandemic. We propose that this is an appropriate and attractive pathway to facilitating rapid expansion of CFTRm in LMIC. Unlike communicable diseases, such COVID-19, HIV, TB, and hepatitis C, which affect millions of people worldwide, CF is a rare orphan disease that is not prioritized in LMIC. However, CFTRm may set the precedent as advocating for VL to access innovative medicines in LMIC for a rare disease, with potential indirect benefits for other rare diseases facing similar prohibitive patent-protected pricing strategies.

Governments and civil society may revert to local legislative options to force the issuing of compulsory licenses (CLs) to licensees if originator companies are found to abuse patent conditions or impose unreasonable restrictions on patents. A CL is a license for alternative production or importation of a generic version of a patented medicine that is granted by a government or court and does require the consent of the patent holder (Médecins Sans Frontières Technical Briefing Document, 2020). Under Article 31 of the TRIPS agreement, countries are free to determine their own grounds for issuing a CL, for example, to combat anti-competitive practices and unaffordable pricing; failure to adhere to obligations with holding patents; and when public health is at stake (as was the case with COVID-19 medications and vaccines) (Shadlen, 2022). Argentina introduced legislative reforms, permitted under the TRIPS agreement, to regulate drug patent applications and promote local manufacture of generic bioequivalent formulations through issuing of CL for the benefit of public health. Argentina is therefore the only country that currently manufactures a generic ETI formulation. However, distribution and marketing to other countries where patent agreements are in place with the originator company is not permitted, and thus global access to this cheaper generic ETI is restricted (Guo et al., 2022b). Whilst CL is more likely to be granted in circumstances of public health urgencies in LMIC, such as with COVID-19, TB, or HIV, it is reasonable to argue that similar conditions should apply for a rare condition like CF, where new CFTRm is life-saving. The issuing of CL is often a protracted and costly legal process if brought forward by civil society or interest groups. Furthermore, governments that are signatories to TRIPS are reluctant to grant CL as this may lead to retaliatory trade actions and sanctions as in the case with the ongoing patent dispute between Argentina and the United States (Elliott, 2002). Reaching a deal for issuing of VL for CFTRm in LMIC is the preferred route to avoid pwCF and families being caught up in legal challenges, which may further delay accessing treatment.

Patient-led advocacy and activism is an effective and important driver for access to CFTRm drugs. The CF Buyers Club was established in the UK to procure generic CFTRm drugs from Argentina when negotiations between the UK's National Health Service and Vertex Pharmaceuticals failed to reach an agreement over the price of Orkambi® (Cohen, 2019). Similarly, the current global "Vertex Save Us" campaign is raising awareness and generating global activism for equitable access to CFTRm drugs in LMIC. These patient-led campaigns are playing a pivotal role in supporting legal steps to challenge the status of patents of CFTRm drugs in countries such as South Africa (Robbins, 2023; VSU, 2023).

Conclusion

There are no easy solutions to correcting the global inequality and disparity in CF care, including access to CFTRm drugs. The global CF community can make a difference by raising awareness, offering clinical and diagnostic expertise, and advocating for patient rights at regional, national, and global platforms. There is strength in numbers and data, and the global CF community should unite and contribute by sharing resources and perseverant advocacy, to help initiate and direct changes in health equity, especially for pwCF who are underserved or underrepresented across the world. The prohibitive cost of CFTRm no longer affects only LMIC, but also HIC like the US, where withdrawal of the manufacturer's funding for co-payment accumulator programs has led to unaffordable increases

in out-of-pocket costs to patients and families, especially minorities. Ongoing advocacy and calls to address the unacceptably high cost of CFTRm is essential to ensure that all eligible pwCF across the world can access these life-altering drugs without discrimination (Zampoli et al., 2022; McGarry et al., 2023; Robbins, 2023).

Author contributions

MZ was the lead author and drafted, compiled, and edited the final manuscript. BM contributed to the section on ethical framework and reviewed and approved the manuscript. GP contributed to sections on world disparities and impact of race and ethnicity and reviewed and approved the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Long-term effects of lumacaftor/ivacaftor on paranasal sinus abnormalities in children with cystic fibrosis detected with magnetic resonance imaging

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Introduction: Chronic rhinosinusitis (CRS) usually presents with nasal congestion, rhinorrhea and anosmia impacts quality of life in cystic fibrosis (CF). Especially mucopyoceles pathognomonic for CRS in CF may cause complications such as spread of infection. Previous studies using magnetic resonance imaging (MRI) demonstrated early onset and progression of CRS from infancy to school age in patients with CF, and mid-term improvements of CRS in preschool and school-age children with CF treated with lumacaftor/ivacaftor for at least 2 months. However, long-term data on treatment effects on paranasal sinus abnomalities in preschool and school-age children with CF are lacking.

Methods: 39 children with CF homozygous for F508del (mean age at baseline MRI 5.9 ± 3.0 years, range 1-12 years) underwent MRI before (MRI1) and about 7 months after starting lumacaftor/ivacaftor and then annually (median 3 follow-up MRI, range 1–4) (MRI2-4). MRI were evaluated using the previously evaluated CRS-MRI score with excellent inter-reader agreement. For intraindividual analysis ANOVA mixed-effects analysis including Geisser-Greenhouse correction and Fisher's exact test, and for interindividual group analysis Mann-Whitney test were used.

Results: The CRS-MRI sum score at baseline was similar in children starting lumacaftor/ivacaftor in school age and children starting therapy at preschool age (34.6 ± 5.2 vs.32.9 ± 7.8, p = 0.847). Mucopyoceles were the dominant abnormality in both, especially in maxillary sinus (65% and 55%, respectively). In

children starting therapy in school age the CRS-MRI sum score decreased longitudinally from MRI1 to MRI2 (−2.1 ± 3.5, $p < 0.05$), MRI3 (−3.0 ± 3.7, $p < 0.01$) and MRI4 (−3.6 ± 4.7, $p < 0.01$), mainly due to a decrease in the mucopyoceles subscore (−1.0 ± 1.5, $p = 0.059$; −1.2 ± 2.0, $p < 0.05$; −1.6 ± 1.8, $p < 0.01$; and −2.6 ± 2.8, $p = 0.417$, respectively). In children starting lumacaftor/ivacaftor in preschool age, the CRS-MRI sum score remained stable under therapy over all three follow-up MRI (0.6 ± 3.3, $p = 0.520$; 2.4 ± 7.6, $p = 0.994$; 2.1 ± 10.5, $p > 0.999$ and −0.5 ± 0.5, $p = 0.740$; respectively).

Conclusion: Longitudinal paranasal sinus MRI shows improvements in paranasal sinus abnormalities in children with CF starting lumacaftor/ivacaftor therapy at school age. Further, MRI detects a prevention of an increase in paranasal sinus abnormalities in children with CF starting lumacaftor/ivacaftor therapy at preschool age. Our data support the role of MRI for comprehensive non-invasive therapy and disease monitoring of paranasal sinus abnormalities in children with CF.

KEYWORDS

cystic fibrosis, magnetic resonance imaging, airway disease, chronic rhinosinusitis, Mucus obstruction

Introduction

Chronic rhinosinusitis (CRS) in patients with cystic fibrosis (CF) is often underrecognized due to the early predominance of pulmonary symptoms, but also significantly contributes to morbidity in CF (Mainz et al., 2009; Berkhout et al., 2013). Moreover, CRS may serve as a reservoir for bacteria and leading to recurrent infections of the lower airways (Eggesbo et al., 2001; Mainz et al., 2009; Berkhout et al., 2013). Previous magnetic resonance imaging (MRI) studies employing a dedicated CRS-MRI scoring system demonstrated a high prevalence of paranasal sinus abnormalities in children with CF from infancy, and an age-dependent increase in prevalence and severity of CRS from infancy to school age (Sommerburg et al., 2020; Wucherpfennig et al., 2022a). The CRS-MRI score correlates with lung disease severity as detected by the chest MRI score in adults (Wucherpfennig et al., 2022b). Further, inflammatory markers from nasal lavage correlate with the CRS-MRI score severity (Chung et al., 2021). These results suggest a need for thorough examination of the paranasal sinuses in children with CF. The recent development of cystic fibrosis transmembrane conductance regulator modulators (CFTRm) significantly improved the therapy of CF abnormalities compared to symptomatic therapies by addressing the disease's basic defect (De Boeck and Amaral, 2016; Mall et al., 2020). Thus, sinonasal symptoms measured by 22 item Sinonasal Outcome Test (SNOT-22) improved in patients with CF older than 12 years during the first year of elexacaftor/tezacaftor/ivacaftor therapy (Stapleton et al., 2022; Bode et al., 2023). Moreover, in patients with CF 6 years and older inflammatory markers in nasal lavage decreased in the first 12 weeks after therapy initiation with ivacaftor (Mainz et al., 2021). Lumacaftor/ivacaftor was approved in the United States of America 2016 and in the EU in 2018 for the treatment of children with CF homozygous for the F508del mutation aged 6 years and older, and 2018 in the United States of America and 2019 in the EU for children with CF homozygous for the F508del mutation between two and 6 years of age. It was shown that treatment with lumacaftor/ivacaftor results in improvements in clinical parameters such as sweat chloride concentration, thriving, exocrine pancreatic function and lung function, as well as on lung abnormalities detected by chest MRI (Graeber et al., 2021; Hoppe et al., 2021). Moreover, a previous cross-sectional study showed a decrease in the CRS-MRI sum score in children (mean age 9.2 ± 4.4 years, range 3–17 years) with CF homozygous for the F508del mutation after therapy initiation with lumacaftor/ivacaftor at a mid-term follow up after at least 2 months of therapy (mean therapy duration 5.8 ± 3.7 months) (Wucherpfennig et al., 2022a). Furthermore, MRI also showed improvements of the CRS-MRI sum score in adults with at least one F508del mutation and established disease after at least 1 month of therapy with elaxacaftor/tezacaftor/ivacaftor (Wucherpfennig et al., 2022b). However, the long-term effects of treatment with CFTRm on paranasal sinus abnormalities especially when initiated early in preschool age are unknown. Thus, the hypothesis of this longitudinal real-world observational study was, that the effects of first therapy with lumacaftor/ivacaftor on the development of paranasal sinuses, and the severity of paranasal sinus abnormalities are assessable by annual paranasal sinus MRI in conjunction with the CRS-MRI scoring system in 39 preschool and school-age children with CF over a median period of 3 years.

Materials and methods

Study population

This study was approved by the institutional ethics committee (clinicaltrials.gov identifier NCT02270476, S-211/2011, S-370/2011) and written informed consent was obtained from all parents or legal guardians. The diagnosis of CF was based on newborn screening ($n = 9$), clinical symptoms ($n = 22$) or both ($n = 8$), and confirmed by increased sweat Cl^- concentrations (≥60 mmol/L), *CFTR* mutation analysis (Hirtz et al., 2004; Graeber et al., 2022). 39 children with CF homozygous for F508del (mean age at baseline MRI 5.9 ± 3.0 years, range 1–12 years) who started lumacaftor/ivacaftor therapy at our center between February 2018 and December 2020 and underwent a paranasal sinus MRI before (MRI1) and at least one subsequent annual MRI (median of 3 examinations, range one to four MRI examinations) after first prescription of lumacaftor/ivacaftor (MRI2 to MRI5), were included (Table 1, Supplementary Figure S1). 15 children first started lumacaftor/

TABLE 1 Patient baseline characteristics. Data presented as mean ± standard deviation, and absolute and relative numbers, respectively. BMI, body mass index, *CFTR, cystic fibrosis transmembrane conductance regulator*, ppFEV1, percent predicted forced expiratory volume in 1 s, SDS, standard deviation score. ***$p < 0.001$ vs. lumacaftor/ivacaftor from preschool age.

	All	Lumacaftor/ivacaftor from preschool age	Lumacaftor/ivacaftor from school age
n =	39	15	24
Age (y)	5.9 ± 3.0	3.4 ± 1.0	8.0 ± 2.0
Sex (m/f)	19/20	7/8	12/12
Height (cm)	116.4 ± 21.5	69.7 ± 16.0	128.8 ± 13.9***
Height, SDS	−0.3 ± 1.2	−0.5 ± 1.1	−0.1 ± 1.2
Weight (kg)	21.9 ± 9.5	14.3 ± 4.3	26.7 ± 8.6***
Weight, SDS	−0.3 ± 0.9	−0.4 ± 0.6	−0.2 ± 1.0
BMI (kg/m^2)	15.5 ± 1.6	15.2 ± 1.2	15.7 ± 1.7
BMI, SDS	−0.4 ± 0.7	−0.4 ± 0.7	−0.4 ± 0.7
CFTR genotype, n (%)			
F508del/F508del	39 (100)	15 (100)	24 (100)
Pancreatic insufficiency, n (%)	39 (100)	15 (100)	24 (100)
Spirometry, n (%)	32 (82)	8 (53)	24 (100)
ppFEV1	90.4 ± 15.5	88.0 ± 13.2	91.3 ± 16.3

ivacaftor in preschool age defined as an age-range of 1–5 years (mean 3.4 ± 1.0 years, range 2–5 years) and 24 children started lumacaftor/ivacaftor in school age defined as an age ≥6 years (mean 8.0 ± 2.0 years, range 6–12 years). MRI2 was performed on average 7.3 ± 4.1 months (range 1–16 months) after therapy initiation, while the interval between the consecutive annual MRI examinations was 12.5 ± 1.8 months (range 7–17 months). The mean observation time for children receiving lumacaftor/ivacaftor in preschool age was 31.6 ± 13.3 months and for children receiving lumacaftor/ivacaftor in school age 38.8 ± 12.2 months. Three examinations were aborted due to restlessness and could not be analysed. In total 143 MRI examinations were evaluated for this study. All children received additional symptomatic mucolytic therapy of the lower airways, seven received nasal saline irrigation and nine received nasal steroids. Patients who stopped lumacaftor/ivacaftor therapy due to change of CFTRm therapy regime ($n = 22$) or underwent surgery of the paranasal sinuses ($n = 1$) were excluded from this timepoint on. Cultures of upper airways were routinely obtained from nasal swabs as previously described (Supplementary Table S1) (Boutin et al., 2015; Sommerburg et al., 2020; Chung et al., 2021). Of note, some of the patients have been included with our previous reports, in which we did not assess long-term longitudinal paranasal sinus MRI abnormalities under lumacaftor/ivacaftor therapy (Stahl et al., 2017; Stahl et al., 2019; Sommerburg et al., 2020; Chung et al., 2021; Wucherpfennig et al., 2022a). 24 of them were included in our previous study on short-term effects of lumacaftor/ivacaftor therapy (Wucherpfennig et al., 2022a).

Magnetic resonance imaging

Standardized MRI of the paranasal sinuses was performed using the identical 1.5 T scanner and protocol (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany) as previously described (Oltmanns et al., 2016; Sommerburg et al., 2020; Chung et al., 2021; Wucherpfennig et al., 2022a; Wucherpfennig et al., 2022b). In brief, T2-weighted sequences, and T1-weighted sequences before and after intravenous contrast application (Dotarem, Guerbet AG, Zurich, Switzerland; or Gadovist, Bayer AG, Leverkusen, Germany) were acquired. All subjects ≤5 years were routinely sedated with oral or rectal chloral hydrate (100 mg/kg body weight, maximum dose 2 g), and monitored by MRI-compatible pulse oximetry as previously described (Wielpütz et al., 2014; Stahl et al., 2019). The previously described CRS-MRI scoring system evaluates the maxillary, frontal, sphenoid and ethmoid sinus. The score comprises items rated for each sinus such as dimensions (length, width), degree of opacification (0 = none, 1 = less than 50%, 2 = 50%–99%, and 3 = complete opacification) and the specific abnormalities mucosal swelling, mucopyoceles, polyps and effusion (0 = none, 1 = present, and 2 = present and dominant), and deformation of the semilunar hiatus for the maxillary sinus only (0 = none, 1 = mucosal prolapse, and 2 = mucosal prolapse with contact to nasal septum). The maximal CRS-MRI sum score is 68 (Sommerburg et al., 2020; Chung et al., 2021; Wucherpfennig et al., 2022a; Wucherpfennig et al., 2022b). 60 of all 143 MRI examinations were assessed by two readers achieving almost perfect inter-reader agreement (Supplementary Table S2) (Sommerburg et al., 2020; Chung et al., 2021; Wucherpfennig et al., 2022a; Wucherpfennig et al., 2022b). Results from one reader are shown in the following analyses for all examinations. Due to low prevalence and differences between age groups of the frontal sinus in our cohort the frontal sinus subscore was excluded from the CRS-MRI sum score. For more information see the online data supplement.

Statistical analyses

Data were analysed using Prism (version 9.1.0, GraphPad Software, San Diego, CA, United States) and are presented as mean ± standard deviation. For intra individual analysis for

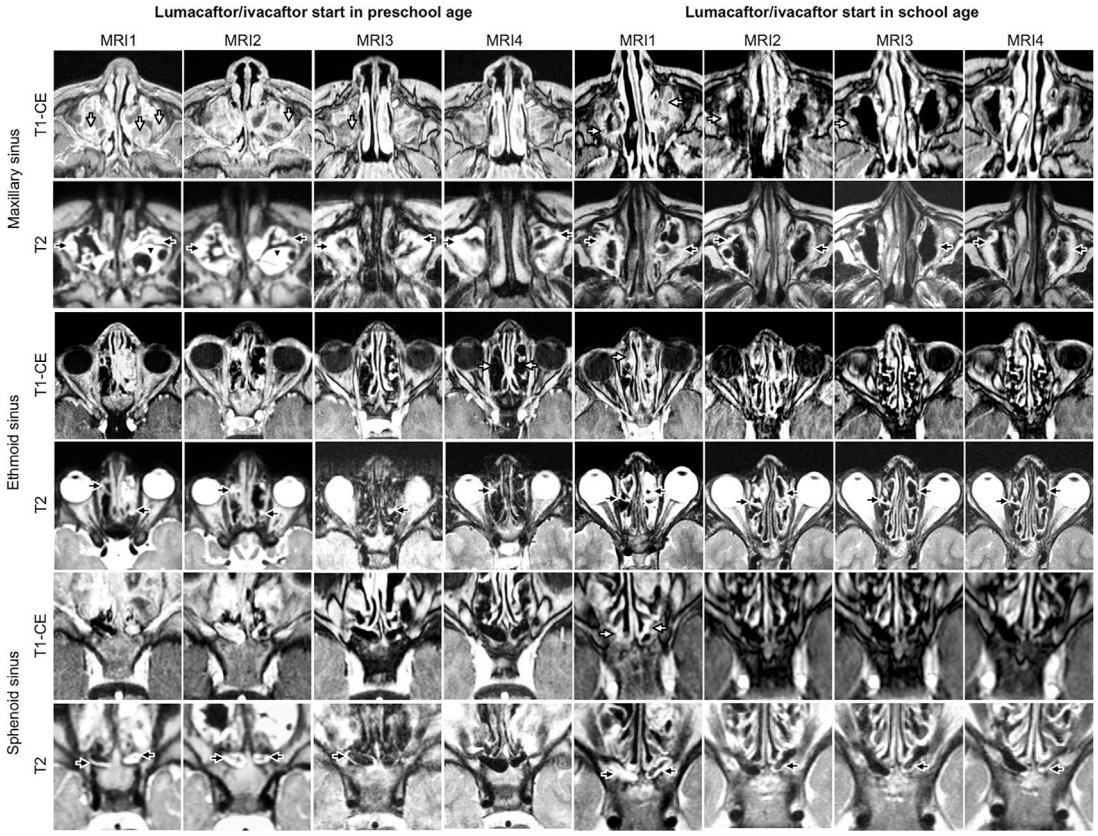

FIGURE 1
Representative examples of longitudinal magnetic resonance imaging of chronic rhinosinusitisparansal sinus abnormalities before (MRI1) and under therapy with lumacaftor/ivacaftor (MRI2-4) in children with cystic fibrosis starting lumacaftor/ivacaftor therapy in preschool and school age. Mucosal swelling is indicated by black arrows, mucopyoceles by white arrows and polyps by black arrowheads. Note the reduction of mucopyoceles in the maxillary sinus in the patient starting therapy in school age. The chronic rhinosinusitis magnetic resonance imaging (CRS-MRI) score in the patient starting therapy in preschool age (4 years at MRI1) was in MRI1 33, in MRI2 33, in MRI3 30 and in MRI4 29 and in the patient starting therapy in school age (9 years at MRI1) in MRI1 32, in MRI2 30, in MRI3 27 and in MRI4 30.

comparing two measurements Wilcoxon signed-rank test and for comparing more than two measurements ANOVA mixed-effects analysis including Geisser-Greenhouse correction, and for interindividual group analysis Mann-Whitney test and/or unpaired *t*-test were used. Prevalence and dominance were compared by Fisher's exact test. A *p*-value <0.05 was considered statistically significant.

Results

Growth of paranasal sinuses in school-age and preschool children with CF is not changed under lumacaftor/ivacaftor therapy

In all children with CF, all maxillary, sphenoid and ethmoid sinuses were present (Supplementary Table S3). The frontal sinus was undetectable in all children who started lumacaftor/ivacaftor therapy in preschool age and was detectable in only 6% of children with CF starting therapy with lumacaftor/ivacaftor in school age (p = 0.085 vs children starting therapy in preschool age) (Supplementary Table S3).

On average, on MRI1 the sinus dimensions in children starting lumacaftor/ivacaftor in school age were larger than in the group starting lumacaftor/ivacaftor in preschool age ($p < 0.05$), except for width of ethmoid sinus ($p = 0.181$). In preschool age, the growth of paranasal sinuses was more pronounced than in school age (Figures 1, 2). Overall, the growth curves of sinus dimensions of children starting therapy in preschool age harmoniously blend into the sinus dimensions at baseline MRI1 from children starting therapy at school age (Figure 2), which together with our previous longitudinal report without CFTRm therapy indicates that lumacaftor/ivacaftor did not affect growth rates (Wucherpfennig et al., 2022a).

Lumacaftor/ivacaftor therapy decreases chronic rhinosinusitis severity in school-age children with CF

In children starting lumacaftor/ivacaftor therapy in school age, the prevalence of opacified sphenoid sinuses decreased in MRI3 and MRI4 compared to MRI1 (84% and 85% vs. 100%, respectively; $p <$ 0.01 vs. MRI1). In the maxillary sinus, the prevalence of mucopyoceles decreased in MRI4 and MRI5 compared to MR1

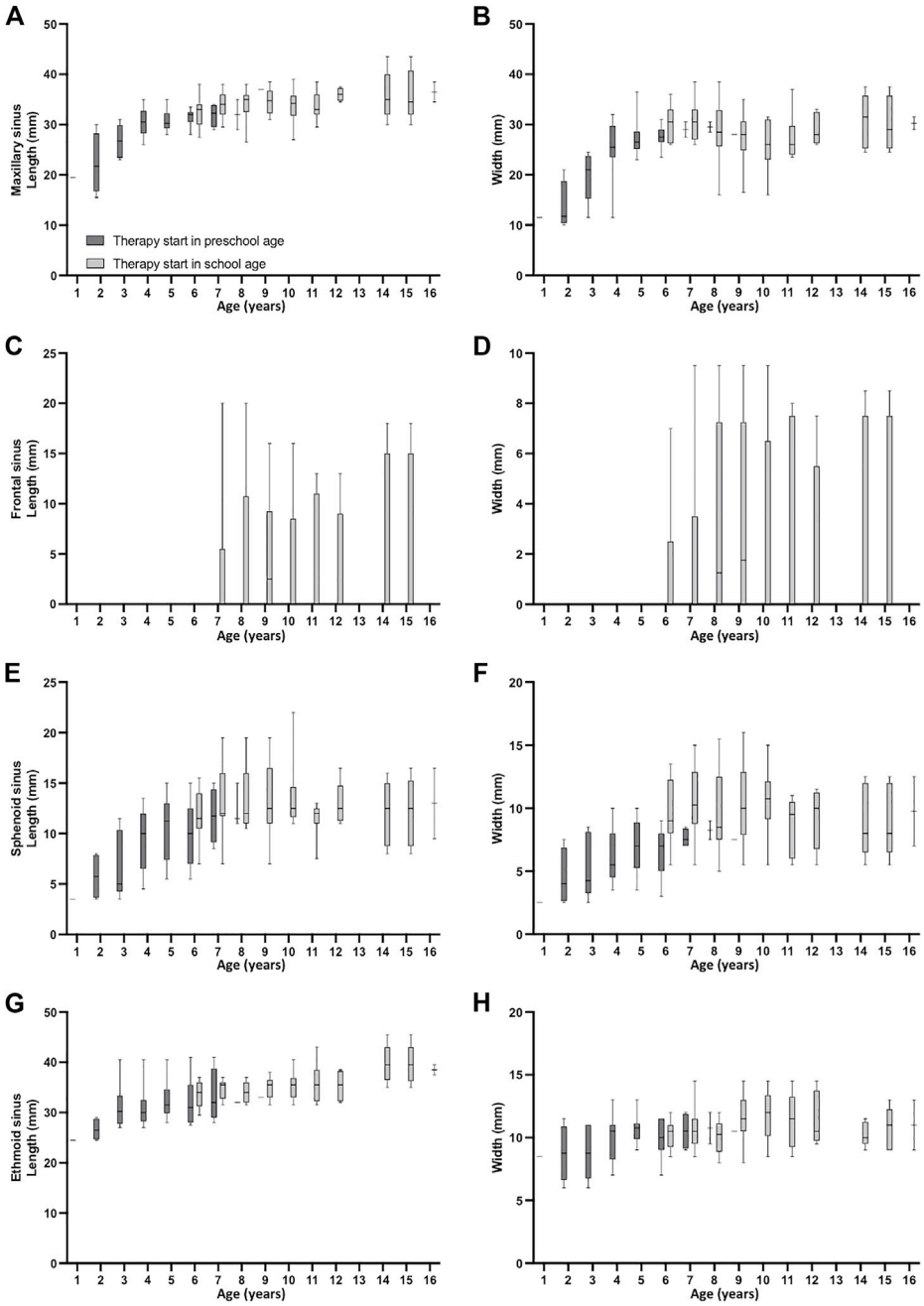

FIGURE 2
Development of paranasal sinus dimensions before (MRI1) and under therapy with lumacaftor/ivacaftor (MRI2-5) in children with cystic fibrosis starting lumacaftor/ivacaftor therapy in preschool and school age. Measurements of the dimensions of the maxillary **(A, B)**, frontal **(C, D)**, sphenoid **(E, F)** and ethmoid **(G, H)** sinus. Both sides were averaged per patient. Boxes represent 25th to 75th percentile, the median is indicated by a horizontal line, and whiskers mark 5th and 95th percentiles. Spearman correlation coefficients r with *p*-values for dimensions vs. age are indicated in each panel.

(75% and 72% vs. 92%, respectively; $p < 0.05$ vs. MRI1). Moreover, the dominance of mucopyoceles decreased especially in MRI4 and MRI5 compared to MRI1 in the maxillary sinus (25% and 17% vs. 55%, respectively; $p < 0.01$). Also, in ethmoid sinus the dominance of mucopyoceles decreased in MRI3 and MRI4 (16% and 20% vs. 33%, $p < 0.05$) (Figure 1; Table 2; Supplementary Table S4–S6). This leads to a decrease in the mucopyocele subscore in MRI3 and MRI4 compared to MRI1 (−1.2 ± 2.0 and −1.6 ± 1.8, respectively; $p < 0.05$), and conversely in an increase in mucosal swelling subscore in MRI2, MRI3, and MRI4 compared to MRI1 ($p < 0.05$). Moreover, the maxillary sinus subscore decreased from MRI1 to MRI2 and sphenoid subscore from MRI1 to MRI4 ($p < 0.05$). The CRS-MRI sum score decreased longitudinally from MRI1 to MRI2, MRI3, and MRI4 (−2.1 ± 3.5, −3.0 ± 3.7 and −3.6 ± 4.7, respectively; $p < 0.05$–0.01) (Figure 3, Supplementary Figure S2).

TABLE 2 Chronic rhinosinusitis magnetic resonance imaging (CRS-MRI) scores for the maxillary sinus in children with cystic fibrosis before (MRI1) and after start of lumacaftor/ivacaftor therapy (MRI2-5) in preschool age or school age. Prevalence n (%) and dominance n (%) of sinus abnormalities are presented on a per-sinus basis, and sinus subscore as mean ± standard deviation. *$p < 0.05$ vs. MRI1, **$p < 0.01$ vs. MRI1. For the frontal, sphenoid and ethmoid sinus please refer to Supplementary Table S4–S6.

			MRI1	MRI2	MRI3	MRI4	MRI5
Lumacaftor/ivacaftor from preschool age	**Maxillary sinus,** n		30	24	22	20	4
	Opacification	Prevalence, n (%)	26 (87)	23 (96)	20 (91)	20 (100)	4 (100)
	Mucosal swelling	Prevalence, n (%)	26 (87)	23 (96)	20 (91)	20 (100)	4 (100)
		Dominance, n (%)	2 (8)	4 (17)	3 (15)	7 (35)*	1 (25)
	Mucopyoceles	Prevalence, n (%)	25 (83)	19 (79)	20 (91)	17 (85)	4 (100)
		Dominance, n (%)	17 (65)	12 (52)	9 (45)	9 (45)	1 (25)
	Polyps	Prevalence, n (%)	20 (67)	18 (75)	16 (73)	14 (70)	4 (100)
		Dominance, n (%)	6 (23)	6 (26)	7 (35)	3 (15)	2 (50)
	Effusion	Prevalence, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Dominance, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Deformation of semilunar hiatus	Prevalence, n (%)	25 (83)	20 (83)	17 (77)	19 (95)	4 (100)
	Maxillary sinus subscore		**14.1 ± 5.6**	**14.1 ± 5.7**	**13.7 ± 6.4**	**15.6 ± 2.5**	**17.0 ± 1.0**
Lumacaftor/ivacaftor from school age	**Maxillary sinus,** n		48	42	38	40	18
	Opacification	Prevalence, n (%)	47 (98)	41 (98)	37 (97)	40 (100)	18 (100)
	Mucosal swelling	Prevalence, n (%)	47 (98)	41 (98)	37 (97)	40 (100)	18 (100)
		Dominance, n (%)	12 (26)	15 (37)	16 (43)	21 (53)*	11 (61)*
	Mucopyoceles	Prevalence, n (%)	44 (92)	38 (90)	30 (79)	30 (75)*	13 (72)*
		Dominance, n (%)	26 (55)	15 (37)	10 (27)*	10 (25)**	3 (17)**
	Polyps	Prevalence, n (%)	28 (58)	25 (60)	15 (66)	24 (60)	9 (50)
		Dominance, n (%)	9 (19)	11 (27)	11 (30)	9 (23)	4 (22)
	Effusion	Prevalence, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Dominance, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Deformation of semilunar hiatus	Prevalence, n (%)	46 (96)	40 (95)	32 (84)	35 (88)	18 (100)
	Maxillary sinus subscore		**15.3 ± 3.3**	**14.7 ± 3.4**	**14.2 ± 3.8****	**13.9 ± 4.0****	**14.0 ± 3.6***

The bold values are the sinus subscores.

Lumacaftor/ivacaftor therapy stabilizes disease progression of chronic rhinosinusitis severity in preschool children with CF

The CRS-MRI sum score was similar in preschool and school-age children with CF at MRI1 (32.9 ± 7.8 vs. 34.6 ± 5.2, $p = 0.847$) (Figures 1, 3, Supplementary Figure S2). Baseline CRS-MRI sum score was similar for children diagnosed by newborn screening without symptoms compared to those diagnosed symptomatic ($p = 0.152$) and for children positive or negative for staphyloccocus aureus in nasal swabs ($p = 0.561$) (Supplementary Figure S2). The prevalence of germs detected by nasal swabs did not differ between the groups ($p = 0.554$–0.999).

In children starting lumacaftor/ivacaftor therapy in preschool age, the prevalence of opacified sinuses and prevalence of the abnormalities remained stable during therapy. The dominance of mucopyoceles in the ethmoid sinus decreased from MRI1 to MRI3 (43% vs. 14%, $p < 0.05$) (Table 2, Supplementary Table S4–S6). The mucopyocele subscore (−1.0 ± 1.8, −0.9 ± 3.5, −0.9 ± 3.5 and −4.0 ± 1.0, respectively; $p = 0.411$–0.774) and the mucosal swelling subscore did not change during therapy from MRI1 to MRI2, MRI3, MRI4 and MRI5 (0.8 ± 1.2, 1.3 ± 1.3, 1.2 ± 1.0 and 1.5 ± 0.5, respectively; $p = 0.222$–0.942 vs. MRI1) (Figure 3, Supplementary Figure S2). The CRS-MRI sum score and the sinus subscores remained stable during therapy ($p = 0.520$–0.999 vs. MRI1) (Figure 3, Supplementary Figure S2).

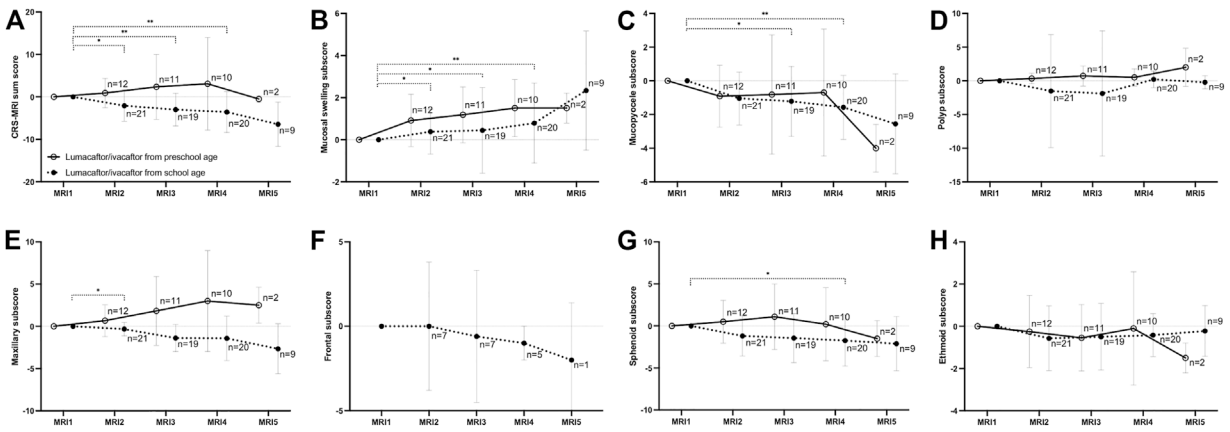

FIGURE 3
Changes of the chronic rhinosinusitis magnetic resonance imaging (CRS-MRI) sum score and subscores from baseline (MRI1) after therapy start with lumacaftor/ivacaftor (MRI2-5) in children with cystic fibrosis. The CRS-MRI sum score **(A)**, abnormality subscores **(B–D)** and sinus subscores **(E–H)** were grouped by MRI timepoints. The mean is indicated by a circle and whiskers mark the standard deviation. *$p < 0.05$ vs. MRI1, **$p < 0.01$ vs. MRI1.

Discussion

This is the first study assessing the long-term effects of treatment with lumacaftor/ivacaftor on prevalence and severity of paranasal sinus abnormalities in preschool and school-age children with CF by consecutive annual paranasal sinus MRI over a median period of 3 years. We demonstrate that in school-age children with CF homozygous for the F508del mutation severity of paranasal sinus abnormalities improves in response to therapy with lumacaftor/ivacaftor as detected by paranasal sinus MRI employing the CRS-MRI sum score. Moreover, progression of paranasal sinus abnormalities is prevented in children with CF homozygous for the F508del mutation by starting lumacaftor/ivacaftor therapy in preschool age (Figure 3; Table 2; Supplementary Table S4–S6).

In our study all maxillary, ethmoid and sphenoid sinuses were present, while the frontal sinus was present in only 6% of children with CF starting therapy with lumacaftor/ivacaftor in school age, and was undetectable in all children who started lumacaftor/ivacaftor therapy in preschool age (Supplementary Table S3). Previous CT and MRI studies similarly reported the earliest pneumatization of frontal sinus from the third year of life and a high degree of sinus aplasia in patients with CF (Kim et al., 1997; Spaeth et al., 1997; Shah et al., 2003; Sommerburg et al., 2020). Paranasal sinus dimensions in preschool and school-age children under lumacaftor/ivacaftor therapy were mostly in line with previous MRI studies assessing the development and abnormalities of paranasal sinuses in children with CF from infancy to school age without CFTRm (Sommerburg et al., 2020; Wucherpfennig et al., 2022a). Also, the growth curves of paranasal sinus dimensions under lumacaftor/ivacaftor therapy are comparable to previous longitudinal results in children without CFTRm, demonstrating a higher growth rate of paranasal sinuses in preschool than in school-age (Sommerburg et al., 2020; Wucherpfennig et al., 2022a). Further it was shown that sinus dimensions in children with CF aged between zero and 6 years are similar to sinus dimensions in a healthy control group of the same age (Sommerburg et al., 2020). This indicates in conjunction with the growth curves obtained from our previous study in children with CF without any CFTRm, that lumacaftor/ivacaftor has no effect on paranasal sinus development (Wucherpfennig et al., 2022a). Taken together, these results indicate that sinus growth does not seem to be influenced by presence and severity of paranasal sinus abnormalities nor by therapy with lumacaftor/ivacaftor.

In our cohort, the prevalence of opacified sinuses at baseline MRI1 in preschool and school-age children was in line with our previous reports (Figure 1; Table 2; Supplementary Table S4–S6) (Sommerburg et al., 2020; Wucherpfennig et al., 2022a). Moreover, baseline prevalence and dominance of the different abnormalities as well as sinus and abnormality subscores and the CRS-MRI sum score in the present study are comparable to the previous cross-sectional and the longitudinal cohort study assessing the onset and progression of paranasal sinus abnormalities in children without CFTRm therapy (Wucherpfennig et al., 2022a).

Our present study demonstrates that in children starting lumacaftor/ivacaftor therapy in school age, the prevalence of opacified sphenoid sinuses decreases longitudinally. Moreover, the prevalence and dominance of mucopyoceles decrease, especially in the maxillary sinus, leading to a decrease in mucopyoceles subscore as well as maxillary subscore (Figure 3; Table 2, Supplementary Table S4–S6). In addition, the CRS-MRI score improved in children with CF starting lumacaftor/ivacaftor therapy in school age from the first MRI examination, with subtle further improvements thereafter (Figure 3). The improvements in the CRS-MRI sum score after start of lumacaftor/ivacaftor in school-age children by an average of 2.1 score points in the first year of therapy is numerically higher than in the previous mid-term follow up MRI study in a group encompassing preschool and school-age children homozygous for F508del, which showed an improvement of the CRS-MRI sum score by 0.5 points after at least 1 month of therapy with lumacaftor/ivacaftor (Wucherpfennig et al., 2022a). Of note, the magnitude of improvements in the CRS-MRI sum score in our present study is lower than in a previous study on highly

effective CFTRm therapy with elexacaftor/tezacaftor/ivacaftor, which has shown a reduction of the CRS-MRI score in adults with CF by 6.9 points (Wucherpfennig et al., 2022b). Since it was shown, that in patients with CF aged between twelve and 60 years improvements in paranasal sinus abnormalities at CT were accompanied by improvements in SNOT-22 within the first month of therapy with elexacaftor/tezacaftor/ivacaftor, it is reasonable that improvements in paranasal sinus abnormalities detected by imaging reflect improvements in CRS (Stapleton et al., 2022). Elexacaftor/tezacaftor/ivacaftor was shown to be more effective on lung function in adults with CF compared to lumacaftor/ivacaftor therapy and also on morpho-functional lung MRI using a semiquantitative CF score (Wainwright et al., 2015; Graeber et al., 2018; Heijerman et al., 2019; Middleton et al., 2019; Mall et al., 2020; Graeber et al., 2021; Nichols et al., 2021; Wucherpfennig et al., 2022b; Graeber et al., 2022). Therefore, it is conceivable that the differences in improvements of CRS-MRI sum scores are caused by the different therapy effectiveness. Conversely, this demonstrates a high sensitivity of paranasal sinus MRI and the CRS-MRI scoring system even for less effective therapeutic strategies.

Further, our present study demonstrates that in children starting lumacaftor/ivacaftor therapy in preschool age, the prevalence of opacified sinuses as well as the prevalence of the different abnormalities remained stable during therapy, while mucopyoceles were less dominant in ethmoid sinuses after therapy start (Table 2, Supplementary Table S4–S6). The CRS-MRI score remained stable over a period of a median of three annual follow-up MRI examinations (Figure 3, Supplementary Figure S2). Of note, this result reconciles the higher improvements found in the CRS-MRI sum score in school-age children in the present study with our previous report in a cohort consisting of preschool and school-age children (Wucherpfennig et al., 2022a). The inclusion of preschool children in the previous study could explain the lower numerical improvements found as compared to the present cohort of children starting lumacaftor/ivacaftor at school age. This result also needs to be compared against our previous longitudinal study, which demonstrated an increase in the CRS-MRI sum score, in maxillary and sphenoid subscores as well as in mucopyoceles subscore especially from infancy and during the early preschool period (0–3 years) (Wucherpfennig et al., 2022a). The reduction of dominance of mucopyoceles under lumacaftor/ivacaftor therapy in preschool children thus compares beneficially against the reported worsening without CFTRm treatment (Wucherpfennig et al., 2022a). Mucopyoceles are a pathognomonic feature of paranasal sinus abnormalities in CF, and might cause complications such as headache, bone resorption with consequent spread of infection (Wagenmann and Naclerio, 1992; Zukin et al., 2017). When symptomatic, mucopyoceles are usually decompressed by endoscopic surgery (Wagenmann and Naclerio, 1992; Zukin et al., 2017). The indication for surgery is mainly based on clinical symptoms, which we could not assess in our study (Rasmussen et al., 2012). In the absence of a control group in the present study, our data may be compared against previously published results on a longitudinal cohort of children with CF, which has shown an increase in the CRS-MRI score from infancy to school-age (Wucherpfennig et al., 2022a). By comparison, lumacaftor/ivacaftor therapy starting in preschool age could be able to prevent the progression of paranasal sinus abnormalities in the sense of a preventive treatment.

Both, lumacaftor/ivacaftor and also elexacaftor/tezacaftor/ivacaftor therapy lead to a reduction in mucopyoceles especially in the maxillary sinus (Wucherpfennig et al., 2022a; Wucherpfennig et al., 2022b). The reduction of mucopyoceles was paralleled by an increase in dominance of mucosal swelling and consequently an increase in the mucosal swelling subscore. This is due to rules of the CRS-MRI scoring system, by which an opacified sinus always is assigned one dominant abnormality. Therefore, the increase in the mucosal swelling subscore is not necessarily associated with an actual increase in the severity of mucosal swelling itself but it is inverse to the reduction in prevalence and dominance of mucopyoceles.

Our study has the limitation, that we did not obtain a CRS symptom score such as SNOT-22 to systematically assess the relationship between the various structural abnormalities and inflammation or clinical disease burden. The improvements in the CRS-MRI sum score are very likely related to improvements in the sinonasal outcome test, though there has been no direct comparison yet between MRI and clinical severity (DiMango et al., 2021; Beswick et al., 2022). Further, the clinical significance of a reduction of mucopyoceles for the regional microbiome remains to be studied. Moreover, our study was performed in absence of a control group. Therefore, we compared our data may against previously published results on a longitudinal cohort of children with CF, which has shown an increase in the CRS-MRI score from infancy to school-age (Wucherpfennig et al., 2022a).

In conclusion, our study demonstrates a positive long-term effect of therapy initiation with lumacaftor/ivacaftor on paranasal sinus abnormalities in school-age and preschool children over a median period of 3 years in two ways: 1) Improvements in the CRS-MRI sum score after therapy initiation with lumacaftor/ivacaftor in school-age children with CF. 2) Prevention of an increase in the CRS-MRI sum score by therapy initiation in preschool children with CF as opposed to the previously shown worsening with natural disease progression. The observed differences in response to lumacaftor/ivacaftor therapy in school-age vs. preschool children support the concept that an early initiation of targeted therapy may be most effective in delaying or even preventing paranasal sinus abnormalities. Further, we demonstrate the potential of annual comprehensive paranasal sinus MRI as a sensitive non-invasive and radiation-free diagnostic tool for the monitoring of disease progression and therapy response of CF-related abnormalities of the upper airway tract from early childhood.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Medical Faculty of University Hospital Heidelberg. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Conception and design: LW, FW, ME, MS, S-YG, H-UK, MM, OS, and MW; Acquisition, analysis and interpretation of data: LW, FW, ME, AS, IB, JC, SZ, J-PS, AA, H-UK, MM, OS, and MW; Writing the manuscript or revising it critically for important intellectual content: LW, FW, ME, AS, IB, MS, SG, SZ, JC, J-PS, AA, H-UK, MM, OS, and MW.

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Conflict of interest

H-UK declares relationships with the following companies: Siemens, Philips, Bayer, Boehringer Ingelheim, Astra Zeneca, Merck Sharp Dohme. CPH declares advisory board membership with Boehringer Ingelheim unrelated to the present study. ME declares advisory board membership with Boehringer Ingelheim unrelated to the present study and speaker honoraria by Boehringer Ingelheim, Roche Pharma and Vertex Pharmaceuticals unrelated to the present study. MW declares advisory board membership with Boehringer Ingelheim unrelated to the present study and receives study grants from Vertex Pharmaceuticals unrelated to the present study. S-YG and MS are participants of the BIH-Charité Clinician Scientist Program funded by the Charité–Universitätsmedizin Berlin and the BIH. MM received grants from Vertex Pharmaceuticals and personal fees for participation in advisory boards or consulting from Abbvie, Antabio, Arrowhead Pharmaceuticals, Boehringer Ingelheim, Enterprise Therapeutics, Kither Biotech, Pieris Pharmaceuticals, Santhera, Sterna Biologicals, Vertex Pharmaceuticals, outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1161891/full#supplementary-material

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Effects of elexacaftor/tezacaftor/ivacaftor therapy on mental health of patients with cystic fibrosis

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Introduction: The CFTR modulator drug elexacaftor/tezacaftor/ivacaftor (ETI) was shown to improve CFTR function and clinical symptoms in patients with cystic fibrosis (CF) with at least one *F508del* allele. Recently, some case reports suggested potential side effects of ETI on mental health with an increase in depressive symptoms and even suicide attempts in patients with CF. However, the general effects of this triple combination therapy on the mental health status of patients with CF remain largely unknown.

Methods: We, therefore, performed a prospective, observational study in a real-life setting and investigated the relationship between initiation of ETI therapy and changes in mental health in adult patients with CF. We assessed Cystic Fibrosis Questionnaire-Revised (CFQ-R), Patient Health Questionnaire-9 (PHQ-9), Beck's Depression Inventory – Fast Screen (BDI-FS) and Generalized Anxiety Disorder 7-item Scale (GAD-7) at baseline and 8–16 weeks after initiation of ETI.

Results: In total, 70 adult patients with CF with at least one *F508del* allele and a median age of 27.9 years were recruited. After initiation of ETI, the CFQ-R respiratory domain score improved by 27.9 (IQR 5.6 to 47.2; $p < 0.001$). The PHQ-9 score of depressive symptoms decreased by 1.0 (IQR -3.0 to 0.3; $p < 0.05$) with an increase of 16.9% in the group with a minimal score after initiation of ETI and a decrease in the groups of mild (–11.3%) or moderate (–5.7%) scores compared to baseline. The BDI-FS score of depressive symptoms decreased from 1.0 (IQR 0.0–2.0) at baseline to 0.0 (IQR 0.0 to 2.0; $p < 0.05$) after initiation of ETI. The group with a minimal BDI-FS score increased by 8.0% after initiation of ETI, whereas the groups with mild (–4.9%), moderate (–1.6%) or severe (–1.6%) scores decreased compared to baseline. The GAD-7 score of anxiety symptoms did not change after initiation of ETI compared to baseline (0.0; IQR -2.0. to 0.0; $p = 0.112$).

Conclusion: Initiation of ETI improves symptoms of depression in adult patients with CF with at least one *F508del* allele. However, symptoms of anxiety do not change after short-term therapy with ETI.

KEYWORDS

cystic fibrosis, elexacaftor/tezacaftor/ivacaftor, mental health, depression, anxiety

1 Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder and the most common fatal monogenetic disease in Caucasian populations (Bell et al., 2020). Mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene cause impaired chloride and bicarbonate transport in epithelial organs leading to a multi-organ disease affecting mainly the lungs, gastrointestinal tract and the pancreas (Bell et al., 2020; Mall et al., 2020). In adult patients with CF, symptoms of depression are observed in ~20% and symptoms of anxiety in ~30%, which is about 2-fold higher than in the general population (~10% and ~15%, respectively) (Martin et al., 2006; Goldbeck et al., 2010; Besier and Goldbeck, 2011; Ploessl et al., 2014; Quittner et al., 2014; Graziano et al., 2020; Terlizzi and Villarroel, 2020). Symptoms of depression and anxiety are associated with reduced quality of life and adherence to airway clearance treatment (Riekert et al., 2007; Smith et al., 2010; Yohannes et al., 2012), as well as disease progression including decline in lung function, an increased rate of pulmonary exacerbations and increased mortality in patients with CF (Riekert et al., 2007; Fidika et al., 2014; Schechter et al., 2021).

Recently, it was shown that the CFTR modulator triple combination therapy with elexacaftor, tezacaftor and ivacaftor (ETI) leads to unprecedented improvements in lung function, body mass index (BMI) and self-reported respiratory symptoms in clinical trials and real-world studies in CF patients with at least one *F508del* allele (Heijerman et al., 2019; Middleton et al., 2019; Barry et al., 2021; Burgel et al., 2021; Griese et al., 2021; Graeber et al., 2022a; Nichols et al., 2022). Further, we recently showed that ETI improves F508del-CFTR function to levels of 40%–50% of normal CFTR activity in the airways and intestine, and increases lung ventilation and improves mucus plugging and other morphological changes in the lungs of patients with CF with one or two *F508del* alleles (Graeber et al., 2022a; Graeber et al., 2022b). Besides these beneficial effects, some case reports describe increased symptoms of depression and anxiety in patients with CF starting with ETI therapy (Tindell et al., 2020; Ladores and Polen, 2021; Heo et al., 2022; Arslan et al., 2023). However, the effects of ETI on depression and anxiety have not been prospectively assessed in patients with CF.

The aim of this study was, therefore, to assess the effect of ETI on depression and anxiety in adult patients with CF. To achieve this goal, we performed a prospective, observational study in 70 patients with CF and one or two *F508del* alleles and investigated quality of life with the Cystic Fibrosis Questionnaire-Revised (CFQ-R), symptoms of depression with the Patient Health Questionnaire-9 (PHQ-9) and the Beck's Depression Inventory - Fast Screen (BDI-FS) as well as symptoms of anxiety with the Generalized Anxiety Disorder 7-item Scale (GAD-7) at baseline and 8–16 weeks after initiation of ETI therapy.

2 Methods

2.1 Study population

This prospective observational post-approval study was conducted at the Christiane Herzog CF Center at Charité - Universitätsmedizin Berlin. The study was approved by the ethics committee of the Charité - Universitätsmedizin Berlin (EA2/220/18) and written informed consent was obtained from all patients included in the study. Patients were eligible to participate if they were at least 18 years old, diagnosed with CF and at least one *F508del* mutation, had no prior exposure to ETI and were willing to remain on a stable medication regimen including ETI according to the patient labeling and the prescribing information for the duration of study participation.

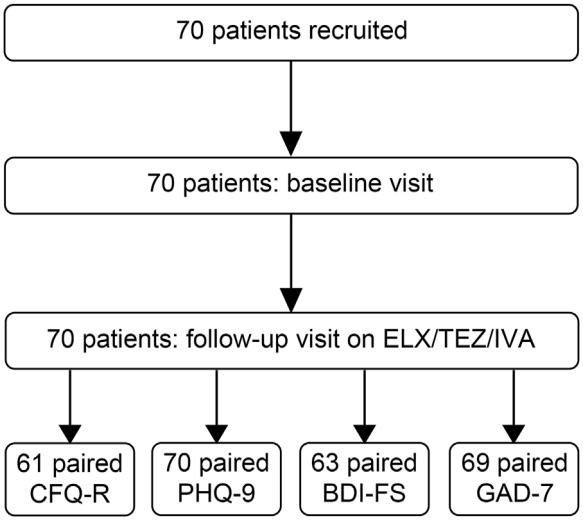

FIGURE 1
Flow chart of recruited patients with cystic fibrosis (CF) with at least one *F508del* mutation. BDI-FS = Beck's Depression Inventory-FastScreen, CFQ-R = Cystic Fibrosis Questionnaire-Revised, ETI = elexacaftor/tezacaftor/ivacaftor, GAD-7 = Generalized Anxiety Disorder Scale-7 Items, PHQ-9 = Patient Health Questionnaire-9.

CFQ-R, PHQ-9, BDI-FS and GAD-7 scores were assessed at baseline and 8–16 weeks after initiation of therapy with the approved dose of ELX 200 mg and TEZ 100 mg every 24 h in combination with IVA 150 mg every 12 h (Figure 1).

2.2 Mental health screening

To determine the effect of ETI on quality of life in patients with CF, we assessed the CFQ-R at baseline and after initiation of therapy. The CFQ-R is a questionnaire validated in CF patients to record the health-related quality of life. The questionnaire contains a total of 50 items, which in turn are divided into 12 different domains (physical functioning, emotional functioning, social functioning/school functioning, body image, eating problems, treatment burden, respiratory symptoms, digestive symptoms, vitality, health perceptions, weight, role functioning). Each domain has a range from 0 to 100, with higher scores indicating a higher patient-reported quality of life (Quittner et al., 2005; Quittner et al., 2012).

To determine the effect of ETI on symptoms of depression in patients with CF, we assessed the PHQ-9 and BDI-FS questionnaire at baseline and after initiation of therapy. The

TABLE 1 Clinical characteristics of patients with cystic fibrosis at baseline.

Patient characteristics at baseline	Median (IQR) or n (%)
Patient sample size	70
Age (years)	27.9 (22.5–34.1)
Female sex at birth	36 (51.4%)
CFTR genotype	
F508del/F508del	32 (45.7%)
F508del/minimal function mutation	22 (31.4%)
F508del/residual function mutation	15 (21.4%)
F508del/mutation not identified	1 (1.4%)
CFTR modulator therapy at baseline	
none	45 (64.3%)
Ivacaftor	2 (2.9%)
Lumacaftor/Ivacaftor	10 (14.3%)
Tezacaftor/Ivacaftor	13 (18.6%)
Pancreatic insufficiency	59 (84.3%)
CF-related diabetes	17 (24.3%)
CF-related liver disease	17 (24.3%)
CF-related arthropathy	13 (18.6%)
FEV_1% predicted	67.3 (48.0–88.4)
BMI (kg/m^2)	21.3 (19.1–23.0)

BMI, body mass index; *CFTR*, cystic fibrosis transmembrane conductance regulator, FEV1% predicted = percent predicted forced expiratory volume in one second.

PHQ-9 is a questionnaire for the detection of depressive symptoms (Kroenke et al., 2001). It identifies depressive symptoms present within the last 2 weeks. Scores ranging from 0 to 4 are considered to be minimal depressive values, scores from 5 to 9 indicate mild depression, scores from 10 to 14 moderate depression and scores ≥15 indicate severe depression. The maximum score is 27. The cut-off value for clinically relevant depressive symptoms was set at ≥10.

Since the PHQ-9 contains several items, whose variability may also be influenced by exacerbations and/or the course of CF (e.g., lack of energy, sleep disorders, loss of appetite), we used the BDI-FS as a second validated instrument to assess depression without somatic criteria. The BDI-FS is intended for use in clinical cohorts with severe underlying somatic illness (Poole et al., 2009) and measures the severity of depression by assessing non-somatic criteria for the diagnosis of major depressive disorder according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and (DSM-V) (Kliem et al., 2014). Scores ranging from 0 to 3 are considered to be minimal depressive values, scores from 4 to 6 indicate mild depression, scores from 7 to 9 moderate depression and scores ≥10 indicate severe depression. The maximum score is 21.

To determine the effect of ETI on symptoms of anxiety in patients with CF, we assessed the GAD-7 questionnaire at baseline and after initiation of therapy. The GAD-7 is a questionnaire for recording anxiety symptoms (Spitzer et al., 2006). It assesses anxiety-related complaints in the last 2 weeks. Scores ranging from 0 to 4 are minimal anxiety values, scores between 5 and 9 indicate mild generalized anxiety, scores from 10 to 14 describe moderate anxiety and scores ≥15 indicate severe generalized anxiety. The maximum score is 21. The cut-off value for clinically relevant anxiety symptoms was set at ≥10.

2.3 Statistical analysis

All data were analyzed with GraphPad Prism version 9.0.1 (GraphPad Software, San Diego, CA, USA) and R 3.6.2 (R Core Team, 2018). The data were not normally distributed and are presented as median and interquartile range (IQR). Comparisons between baseline and follow-up were tested by Wilcoxon signed-rank test. Subgroup analysis were performed in male and female patients. $p < 0.05$ was accepted to indicate statistical significance.

3 Results

3.1 Characteristics of study population

In total, 70 adult patients with CF were enrolled between September 2020 and August 2021 to assess quality of life, symptoms of depression and anxiety as well as anthropometry, spirometry, and sweat chloride concentration at baseline and 8–16 weeks after initiation of ETI therapy (Figure 1). The median age of patients at baseline was 27.9 years (IQR 22.5 – 34.1) and 51.4% were female (Table 1). 45.7% of the patients were *F508del* homozygous and the other patients were heterozygous for *F508del* and a minimal function mutation (31.4%), a residual function mutation (21.4%) or a not identified mutation (1.4%). At baseline, 64.3% of the patients had not been on previous CFTR modulator therapy, 18.6% were on treatment with tezacaftor/ivacaftor, 14.3% were on treatment with lumacaftor/ivacaftor, and 2.9% were on treatment with ivacaftor (Table 1). Patients had a median forced expiratory volume in one second % predicted (ppFEV1) of 67.3% (IQR 48.0–88.7) and BMI of 21.3 kg/m^2 (IQR 19.1–23.0) (Table 1). In our cohort, sweat chloride concentration decreased by 44.5 mmol/L (IQR -63.5 to −28.5; $p < 0.001$; Figure 2A), ppFEV1 improved by 12.1% (IQR

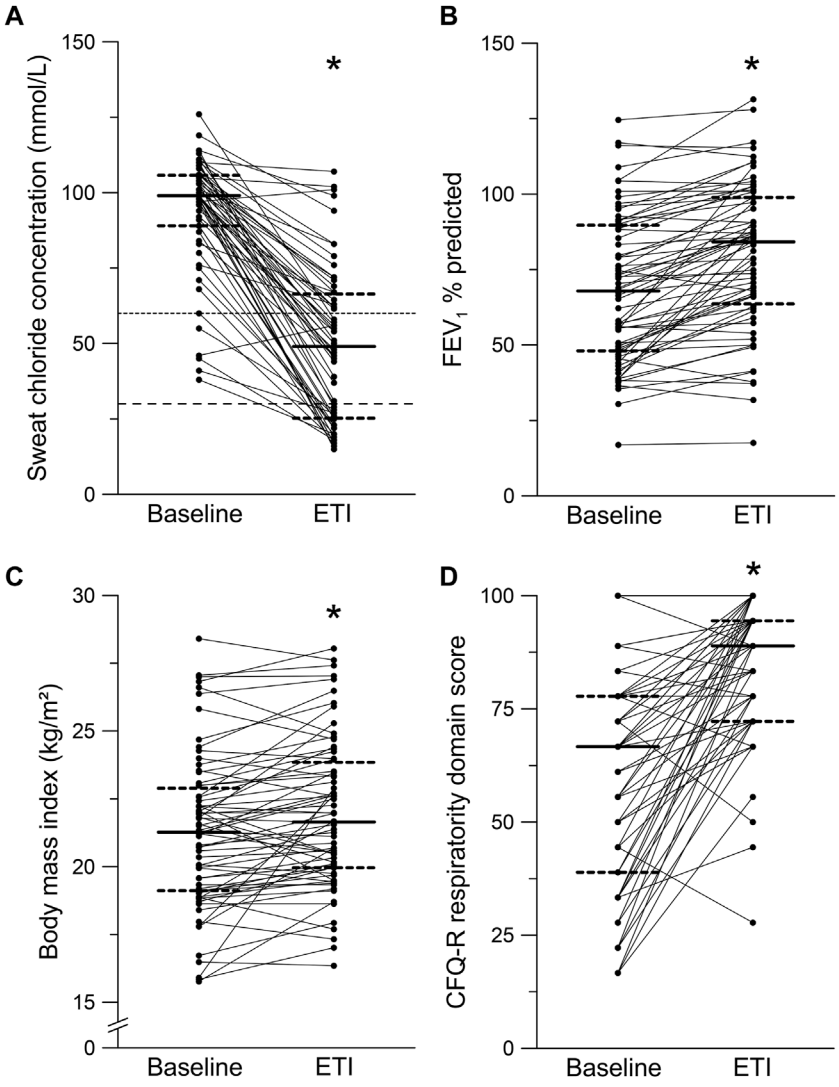

FIGURE 2
Effects of elexacaftor/tezacaftor/ivacaftor (ETI) on sweat chloride concentration, percent predicted forced expiratory volume in one second (FEV1% predicted), body mass index (BMI) and Cystic Fibrosis Questionnaire-Revised (CFQ-R) - respiratory domain. **(A–D)** Paired measurements of sweat chloride concentration **(A)**, FEV1% predicted **(B)**, BMI **(C)** and CFQ-R respiratory domain score in patients with CF and at least one *F508del* mutation at baseline and after initiation of ETI therapy. Solid lines represent the group median and dashed lines represent 25th and 75th percentile. *$p <$ 0.001 compared with baseline.

2.5 – 18.0; $p < 0.001$; Figure 2B), and BMI increased by 0.5 kg/m^2 (IQR -0.2 to 1.2; $p < 0.001$; Figure 2C) after initiation of ETI.

61 (87%) patients completed the CFQ-R, 70 patients (100%) completed the PHQ-9 questionnaire, 69 patients (99%) completed the GAD-7 questionnaire and 63 patients (90%) completed the BDI-FS questionnaire at baseline and after initiation of ETI (Figure 1).

3.2 Quality of life

The respiratory domain score of the CFQ-R, assessing the respiratory symptoms and previously used in clinical trials, increased by 27.9 (IQR 5.6 – 47.2; $p < 0.001$) (Figure 2D; Table 2). In addition, the domain scores for physical functioning ($p < 0.001$), social functioning/school functioning ($p < 0.01$), body image ($p < 0.001$), treatment burden ($p < 0.001$), vitality ($p < 0.001$), health perceptions ($p < 0.001$), and role functioning ($p < 0.001$) were improved after initiation of ETI (Table 2). On the other hand, no changes after initiation of ETI were observed for the domains emotional functioning ($p = 0.372$), eating problems ($p = 0.319$), digestive symptoms ($p = 0.860$) and weight ($p = 0.825$) (Table 2). A subgroup analysis according to gender revealed that the CFQ-R respiratory domain score improved in female 22.2 (IQR 11.1 to 38.9, $p < 0.001$) as well as male patients with CF 22.2 (IQR 6.9 to 52.8, $p < 0.001$) (Table 3).

3.3 Depression

At baseline, 81.7% reported minimal or mild and 18.3% reported moderate or severe symptoms of depression with a median score of

TABLE 2 Effects of elexacaftor/tezacaftor/ivacaftor (ETI) on quality of life determined by the Cystic Fibrosis Questionnaire-Revised (CFQ-R).

CFQ-R domain	Baseline median (IQR)	ETI median (IQR)	Change between baseline and ETI median (IQR)	*p*-Value
Physical functioning	70.8 (45.8–89.6)	89.6 (75.0–100.0)	12.5 (4.2–29.2)	<0.001
Emotional functioning	80.0 (66.7–90.0)	80.0 (66.7–93.3)	0.0 (−6.7 to 6.7)	0.372
Social functioning/school functioning	66.7 (44.4–83.3)	72.2 (50.0–83.3)	5.6 (−5.6–16.7)	<0.01
Body image	66.7 (50.0–88.9)	77.8 (55.6–100.0)	11.1 (0.0–11.1)	<0.001
Eating problems	100.0 (77.8–100.0)	100.0 (88.9–100.0)	0.0 (0.0–0.0)	0.319
Treatment burden	66.7 (55.6–77.8)	77.8 (66.7–88.9)	11.1 (0.0–16.7)	<0.001
Respiratory symptoms	66.7 (40.3–77.8)	88.9 (72.2–94.4)	22.2 (11.1–44.4)	<0.001
Digestive symptoms	77.8 (66.7–88.9)	77.8 (66.7–88.9)	0.0 (−19.4 to 11.1)	0.860
Vitality	50.0 (37.5–66.7)	66.7 (50.0–83.3)	8.3 (0.0–25.0)	<0.001
Health perceptions	55.6 (33.3–77.8)	66.7 (55.6–88.9)	11.1 (0.0–22.2)	<0.001
Weight	100.0 (66.7–100.0)	100.0 (66.7–100.0)	0.0 (0.0–33.3)	0.825
Role functioning	83.3 (66.7–91.7)	91.7 (75.0–100.0)	8.3 (0.0–16.7)	<0.001

TABLE 3 Sub group analysis of the effects of elexacaftor/tezacaftor/ivacaftor (ETI) in female and male patients with CF on quality of life determined by the Cystic Fibrosis Questionnaire-Revised (CFQ-R), symptoms of depression determined by Patient Health Questionnaire-9 (PHQ-9) and Beck's Depression Inventory-FastScreen (BDI-FS), and symptoms of anxiety with the Generalized Anxiety Disorder Scale-7 Items (GAD-7).

Questionaire	Female		Male	
	Change between baseline and ETI median (IQR)	*p*-Value	Change between baseline and ETI median (IQR)	*p*-Value
CFQ-R respiratory domain	22.2 (IQR 11.1–38.9)	<0.001	22.2 (IQR 6.9–52.8)	<0.001
PHQ-9	0.0 (IQR -2.0 to 1.0)	0.608	−1.5 (IQR -4.0 to 0.0)	<0.001
BDI-FS	0.0 (IQR -0.8 to 0.0)	0.112	0.0 (IQR -1.0 to 0.0)	<0.05
GAD-7	0.0 (IQR -1.0 to 1.0)	0.704	−1.0 (IQR -3.0 to 0.0)	<0.05

4.5 (IQR 2.0–6.8) assessed by the PHQ-9 (Figures 3A, B). In the BDI-FS questionnaire, 90.4% of patients reported minimal or mild and 9.6% reported moderate or severe symptoms of depression with a median score of 1.0 (IQR 0.0–2.0) at baseline (Figures 3C, D). After initiation of ETI, PHQ-9 scores decreased by 1.0 (IQR -3.0 to 0.3; $p < 0.05$; Figure 3A). We observed a decrease in mild (−11.3%) and moderate (−5.7%) scores, and an increase in the minimal scores (+16.9%) after initiation of ETI compared to baseline (Figure 3B). The proportion of severe scores did not change after initiation of ETI. BDI-FS scores decreased to 0.0 (IQR 0.0 to 2.0; $p < 0.05$) after initiation of ETI (Figure 3C). Mild (−4.9%), moderate (−1.6%) and severe (−1.6%) scores decreased and minimal scores increased by 8.0% after initiation of ETI (Figure 3D). Further, there was also trend towards a decrease in number of patients describing suicidal ideation. At baseline, 4 patients (5.6%) reported suicidal ideation whereas after initiation of ETI only one patient (1.4%) still reported suicidal ideation. In a gender-based subgroup analysis, both depression scores, the PHQ-9 (−1.5, IQR -4.0 to 0.0; $p < 0.001$) and the (0.0, IQR -1.0 to 0.0; $p < 0.05$) BDI-FS score improved in male patients with CF (Table 3). However, in the female subgroup, no improvement in PHQ-9 (0.0, IQR -2.0 to 1.0; $p = 0.608$) and BDI-FS (0.0, IQR -0.8 to 0.0; $p = 0.112$) were observed after initiation of ETI (Table 3).

3.4 Anxiety

At baseline, 84.3% of the patients reported minimal or mild and 15.7% reported moderate or severe symptoms of anxiety with a medium score of 2.0 (IQR 1.0 – 6.0) in the GAD-7 (Figure 4A). After initiation of ETI, GAD-7 scores did not change compared to baseline (median difference 0.0; IQR -2.0 – 0.0; $p = 0.112$; Figure 4A). We

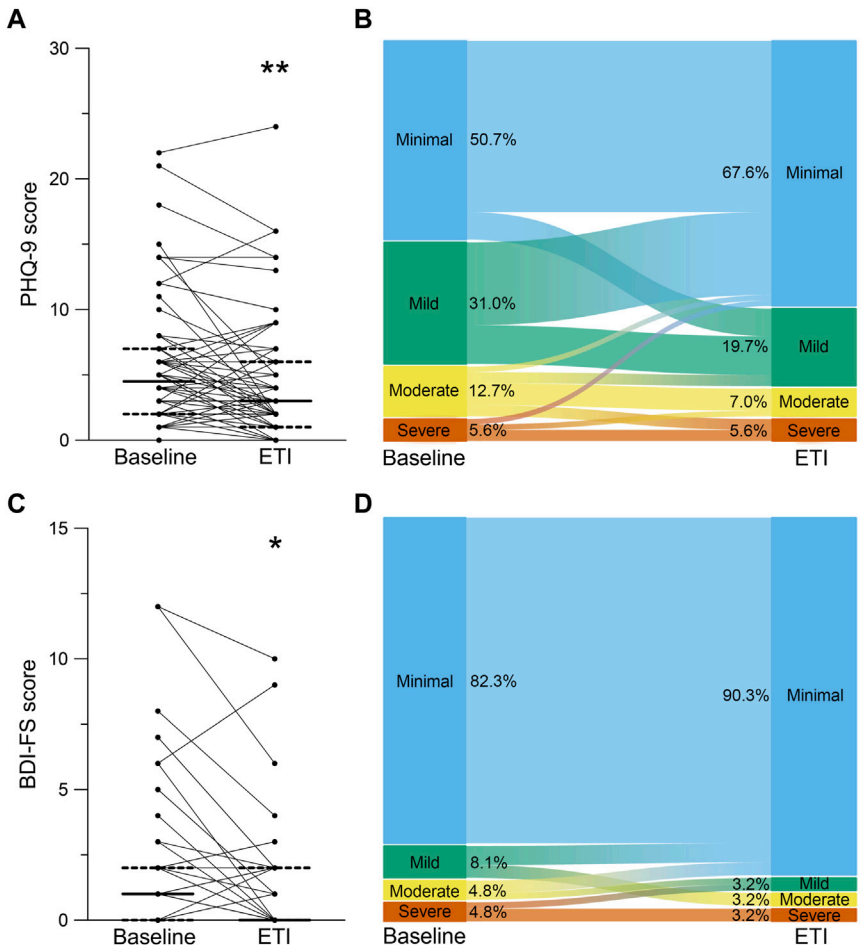

FIGURE 3
Effects of elexacaftor/tezacaftor/ivacaftor (ETI) on symptoms of depression. **(A–D)** Paired assessment of Patient Health Questionnaire-9 (PHQ-9) **(A,B)** and Beck's Depression Inventory-FastScreen (BDI-FS) **(C,D)** in patients with CF and at least one *F508del* mutation at baseline and after initiation of ETI therapy. **(B,D)** Alluvial graphic depicting the proportions of the categories minimal (blue), mild (green), moderate (yellow) and severe (red) symptoms of depression assessed by PHQ-9 **(B)** and BDI-FS **(D)** in CF patients at baseline and after initiation of ETI therapy. Solid lines represent the group median and dashed lines represent 25th and 75th percentile. *$p < 0.05$ and **$p < 0.01$ compared with baseline.

observed a trend towards a decrease in the categories of minimal (−1.5%), moderate (−2.9%) and severe (−4.2%) scores and a trend towards increase in mild (8.6%) scores (Figure 4B). In the gender-based subgroup analysis, the GAD-7 scores improved in the male subgroup (−1.0, IQR -3.0 to 0.0; $p < 0.05$), but no change was observed in the female subgroup (0.0, IQR -1.0 to 1.0; $p = 0.704$) (Table 3).

4 Discussion

This is the first prospective study assessing the impact of ETI treatment on mental health of patients with CF by using the PHQ-9, BDI-FS and GAD-7 questionnaires in a post-approval, real-world setting. In a cohort of 70 adult patients with a broad range of disease severity, the improvements in key clinical outcomes ppFEV1 and BMI, as well as sweat chloride concentration and quality of life, observed after initiation of ETI therapy were consistent with the results obtained in phase three clinical trials and previous observational studies in real-life settings (Tables 1, 2; Figure 2) (Heijerman et al., 2019; Middleton et al., 2019; Barry et al., 2021; Burgel et al., 2021; Graeber et al., 2022a; Nichols et al., 2022). We found that ETI therapy improves symptoms of depression in patients with CF with at least one *F508del* allele (Figure 3). Further, we show that ETI does not alter symptoms of anxiety in the whole cohort (Figure 4). Subgroup analysis showed that symptoms of depression and anxiety were reduced in male but not in female patients (Table 3). Collectively, our results provide novel insights into the short-term treatment with ETI on symptoms of depression and anxiety in adult patients with CF.

The quality of life assessed by the CFQ-R showed improvement in most domains after initiation of ETI in our study (Table 2). However, the emotional functioning domain and domains associated with eating and digestion, such as eating problems, digestive symptoms and weight, showed no improvement (Table 2). An analysis of the non-respiratory health-related quality of life during the previous phase 3 clinical trials showed that all, but the digestive symptoms domain were improved after

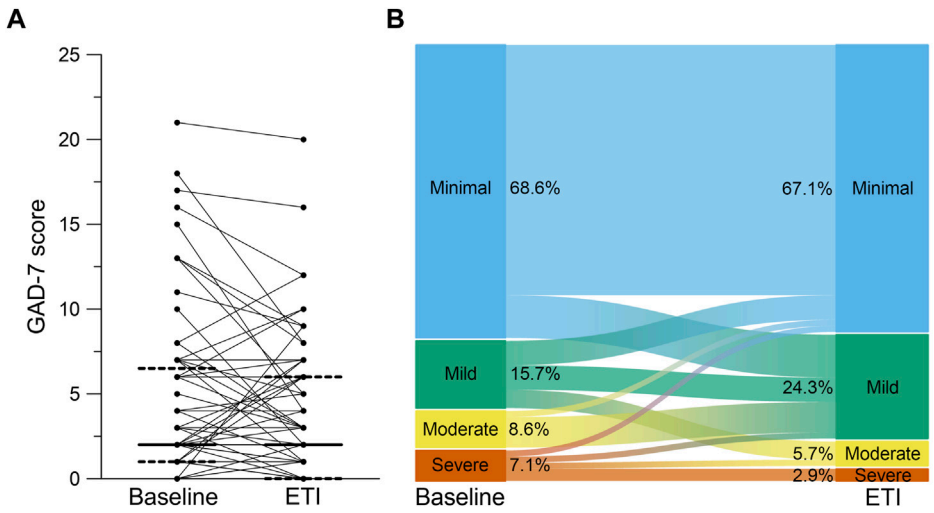

FIGURE 4
Effects of elexacaftor/tezacaftor/ivacaftor (ETI) on symptoms of anxiety. **(A)** Paired assessment of Generalized Anxiety Disorder Scale-7 Items (GAD-7) in patients with CF and at least one *F508del* mutation at baseline and after initiation of ETI therapy. **(B)** Alluvial graphic depicting the proportions of the categories minimal (blue), mild (green), moderate (yellow) and severe (red) symptoms of anxiety assessed by GAD-7 in CF patients at baseline and after initiation of ETI therapy. Solid lines represent the group median and dashed lines represent 25th and 75th percentile.

initiation of ETI (Fajac et al., 2023). However, similar to our study, another prospective real-world study observed no improvement for the emotional functioning, health perceptions, body image, and digestive symptom domains (DiMango et al., 2021). The emotional function items in the CFQ-R also partially assess symptoms of anxiety which, in line with the results of the GAD-7, could potentially explain why this domain was not improved.

In our study, we observed slightly fewer symptoms of depression and anxiety compared to previous studies on the prevalence of mental health issues in patients with CF in Europe (Figures 3, 4) (Goldbeck et al., 2010; Yohannes et al., 2012; Quittner et al., 2014; Graziano et al., 2020). This observation may be explained by the positive reports on ETI accompanying the approval as breakthrough therapy by the U.S. Food and Drug Agency in October 2019 and, therefore, the anticipation of starting with a highly effective CFTR modulator therapy at the baseline visit.

Recently, some case reports suggested potential side effects of ETI on mental health with an increase in depressive symptoms and even suicide attempts in patients with CF (Tindell et al., 2020; Ladores and Polen, 2021; Heo et al., 2022; Arslan et al., 2023). One study suggested a dose reduction in individuals with reported mental health issues after initiation of ETI, which resulted in improvement or resolution of symptoms of depression and anxiety (Spoletini et al., 2022). However, a retrospective analysis observed no significant changes in average PHQ-9 and GAD-7 scores after initiation of ETI (Zhang et al., 2022). In contrast, in our prospective study, we observed an improvement in depressive symptoms in PHQ-9 as well as BDI-FS scores after short-term treatment with ETI (Figure 3). A potential mechanism was reported in a mouse model of depression suggesting potentially beneficial effects of ivacaftor and its metabolites on the central nervous system activity profile (Schneider et al., 2018). Further, sleep quality improved in 50% of patients with CF and advanced lung disease after initiation of ETI, which could contribute to the improvement in depressive symptoms (Martin et al., 2021). Overall, two patients changed from moderate to severe depressive symptoms in the PHQ-9 in our study (Figure 3). However, both patients reported other potential causes for worsening of symptoms (problems at work and separation of partner) besides initiation of ETI underlining the multiple factors influencing mental health.

Symptoms of anxiety did not change after initiation of ETI therapy (Figure 4) which is in line with a previous retrospective study (Zhang et al., 2022). In contrast to previous case reports (Tindell et al., 2020; Spoletini et al., 2022), we did not observe an increase in anxiety symptoms on the group level. Further, only three patients changed from mild to moderate symptoms of anxiety and no patient changed to severe symptoms of anxiety after initiation of ETI (Figure 4). Another case report suggests increased symptoms of anxiety due to the life-changing effects of ETI including the fear of diminishing effectiveness over time (Ladores and Polen, 2021). However, further studies in larger patient populations will be necessary for a more comprehensive elucidation of the effects of ETI on mental health in patients with CF.

Subgroup analysis showed that although baseline values in females and males were comparable, symptoms of depression and anxiety improved in male but not in female patients with CF (Table 3). Recent case reports indicate a higher likelihood of mental health issues in females compared to males after initiation of CFTR modulators (Tindell et al., 2020; Ladores and Polen, 2021). Female sex is further associated with lower survival rates, earlier bacterial colonization, higher decrease in lung function and more frequent pulmonary exacerbations in patients with CF (Harness-Brumley et al., 2014; Montemayor et al., 2021; Montemayor and Jain, 2022; Sodhi et al., 2023). However, there was no difference in the effects on the CFQ-R respiratory domain score and lung function between female and male patients highlighting that the effects of ETI on mental health may not be directly attributable to clinical improvements. This highlights that the underlying mechanisms

of the sex differences are still unknown. Further, it is possible that the sample size in our study was not sufficient to detect more subtle effects in female patients. Therefore, larger studies powered for gender-specific differences are necessary to further elucidate this finding.

This study has some limitations. As the approval of ETI in Europe took place during the COVID-19 pandemic, this might have influenced our study results (Sakon et al., 2023). However, we observed an improvement of symptoms of depression despite the ongoing COVID-19 pandemic. The missing effects of ETI on symptoms of anxiety could be especially influenced by the pandemic as anxiety scores were observed to be elevated in the general population during the COVID-19 pandemic (Salari et al., 2020), potentially resulting in an overlap with a possible reduction of anxiety following ETI. Second, the questionnaires used for anxiety and depression are self-report measures that are useful for screening of depressive and anxiety symptoms but may lack sensitivity and might therefore not capture the full range of symptoms or severity. Novel, more CF specific questionnaires, like the recently developed Distress in Cystic Fibrosis Scale (DCFS) may provide a more comprehensive assessment of the mental health of patients with CF (Finlay et al., 2022). Third, no neuropsychiatric and neurocognitive symptoms that were recently described to be altered after initiation of ETI (Aspinall et al., 2022; Spoletini et al., 2022), nor social characteristics such as education, working and relationship status were assessed in this study. Further, this prospective real-world study with a limited sample size assessed only short-term effects of ETI. However, in a case series on patients with changes in mental health, all six patients noticed a change within the first 3 months after initiation of ETI therapy (Heo et al., 2022) that are covered in our cohort. Nevertheless, larger, multi-center, longitudinal studies over longer time periods will be necessary to identify potential long term effects of ETI on mental health.

In summary, our study demonstrates that initiation of ETI therapy leads to improvement in symptoms of depression and does not change symptoms of anxiety on a group level in adult patients with CF. However, as multiple factors influence mental health, we suggest that mental health screening including neurocognitive and neuropsychiatric symptoms should be routinely performed also after initiation of ETI in all patients with CF to identify individual patients with an increase of symptoms of depression and anxiety.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the ethics committee of the Charité - Universitätsmedizin Berlin. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conception and design of the study: LP, RT, MS, MM, and SG; acquisition, analysis and interpretation of data: all authors; first drafting of manuscript: LP, RT, MM, and SG; critical revisions and intellectual content: all authors.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Individualized approach to elexacaftor/tezacaftor/ivacaftor dosing in cystic fibrosis, in response to self-reported anxiety and neurocognitive adverse events: A case series

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The prevalence of mental health disorders is high among people with Cystic Fibrosis. The psychological symptoms in CF are associated with poor adherence, worse treatment outcomes, and greater health utilization/cost. Mental health and neurocognitive Adverse Events (AEs) have been reported with all available Cystic Fibrosis Transmembrane conductance Regulator (CFTR) modulators in small groups of patients. We report our experience with a dose reduction strategy in 10 of our patients on elexacaftor/tezacaftor/ivacaftor (7.9% of total number of patients) who self-reported developing intense anxiety, irritability, sleep disturbance and/or mental slowness after initiation of full dose treatment. Standard dose elexacaftor/tezacaftor/ivacaftor resulted in 14.3 points improvement in mean Percent Predicted Forced Expiratory Volume in 1 s ($ppFEV_1$), and a mean difference in sweat chloride of −39.3 mmol/L. We initially discontinued and/or reduced therapy according to the AEs severity, with a subsequent planned dose escalation every 4–6 weeks guided by sustainability of clinical effectiveness, absence of AEs recurrence, and patients' preferences. Clinical parameters including lung function and sweat chloride were monitored for up to 12 weeks to assess ongoing clinical response to the reduced dose regimen. Dose reduction resulted in resolution of self-reported mental/psychological AEs, without loss of clinical effectiveness ($ppFEV_1$ was 80.7% on standard dose, and 83.4% at 12 weeks on reduced dose; sweat chloride was 33.4 and 34 mmol/L on standard and reduced dose, respectively). Furthermore, in a subgroup of patients who completed 24 weeks of the reduced dose regimen, repeat low dose Computed Tomography imaging showed a significant response when compared to pre-initiation of elexacaftor/tezacaftor/ivacaftor.

KEYWORDS

case report, cystic fibrosis transmembrane conductance regulator, CFTR, anxiety, FEV-1, sweat chloride, elexacaftor/tezacaftor/ivacaftor, side-effects

1 Introduction

The prevalence of mental health disorders is high among people with Cystic Fibrosis (CF) (Quittner et al., 2014; Quittner et al., 2016). It is estimated that 5%–19% of CF adolescents and 13%–29% of CF adults have depression (Latchford and Duff, 2013; Quittner et al., 2014), along with 22% of CF adolescents and 32% of CF adults experiencing anxiety (Quittner et al., 2014). The psychological symptoms in CF have been linked to poor treatment adherence (Hilliard et al., 2015), worse clinical outcomes (Snell et al., 2014; Quittner et al., 2016), and greater healthcare utilization and costs (Snell et al., 2014).

Over the last decade, treatment with Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) modulators has resulted in significant clinical benefits. More recently, a triple combination CFTR modulator using the next-generation corrector Elexacaftor in combination with Tezacaftor/Ivacaftor (ETI), has shown high clinical efficacy in patients homozygous or heterozygous for Phe508del mutation (Heijerman et al., 2019; Middleton et al., 2019; Barry et al., 2021). Despite the positive clinical outcomes for the majority of patients, mental health and neurocognitive Adverse Events (AEs) have been reported in real-world studies with all available CFTR modulators among small groups of patients (McKinzie et al., 2017; Talwalkar et al., 2017; Dagenais et al., 2020; Heo et al., 2022). The recently published Phase 3b clinical trial of ETI showed that one participant (1/87) in the ETI group discontinued treatment due to an adverse AE of anxiety and depression, and two participants (2/88) in the tezacaftor/ivacaftor group discontinued treatment due to AEs of psychotic disorder and obsessive-compulsive disorder (Sutharsan et al., 2022). In addition, a recently published real-world study showed an incidence of self-reported mental AEs in 7.1% of patients on ETI treatment (Spoletini et al., 2022).

The mechanism behind mental health AEs reported during treatment with CFTR modulators has not been fully illuminated, but the potential pathways include: 1) Drug-drug interaction between CFTR modulators and psychotropic medications through cytochrome P450 (Jordan et al., 2016; McKinzie et al., 2017). CFTR modulators, specifically ivacaftor and lumacaftor may affect the activities of cytochrome P450 isoenzymes (CYP2C9, CYPC19, CYP3A4), therefore this may alter the level of other cytochrome P450 substrates including selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines. 2) The direct effect of CFTR modulators and their metabolites on the serotonin receptors and the CFTR receptors that are expressed ubiquitously in the nervous system (Marcorelles et al., 2014; Schneider et al., 2018).

The potential mental health AEs of CFTR modulator therapy, have focused our attention on the Phase 2 clinical trial (Keating et al., 2018). This trial demonstrated a variable but clinically significant response to different doses of the triple CFTR modulator ETI. Doses as low as 50 mg of VX-445 (elexacaftor) resulted in improvements in Percent Predicted Forced Expiratory Volume in 1 s ($ppFEV_1$) and sweat chloride of almost 11% and −38 mmol/L respectively (Keating et al., 2018). In addition, the potential clinical effectiveness of a lower dose strategy based on this Phase 2 study, allowed us to develop an adaptive strategy where we adopted a dose reduction protocol in the subgroup of patients with self-reported intense anxiety, irritability, sleep disturbance and/or mental slowness, in an attempt to minimize AEs while continuing CFTR modulator therapy. We report our experience with a dose reduction strategy.

2 Case series description

2.1 Response to full standard dose of ETI

As a standard practice in our institution, along with standard clinical measures, all adult CF patients are screened for anxiety and depression prior to commencing ETI treatment (baseline) via a series of established questionnaires, specifically the Hospital Anxiety and Depression Scale (HADS), the Patient Health Questionnaire (PHQ-9), and General Anxiety Disorder-7 (GAD-7).

Between October 2020 and May 2021, we initiated ETI with 126 adult CF patients attending our service. Subsequently, a total of 10 patients (7.9% of patients on ETI) with no known psychological disorders or history of strong cytochrome P450 inducer or inhibitor use, developed self-reported anxiety, irritability, sleep disturbance and/or mental slowness within four weeks of initiation of full-dose treatment. None of these patients reported any anxiety or depression prior to ETI, and their baseline depression and anxiety screening questionnaires were within normal. The average weight and BMI of this group of patients at the start of ETI therapy were 68 kg (SD 13.9, min–max 48.7–86.4 kg) and 23.7 kg/m^2 (SD 3.02, min–max 19.8–29.5 kg), respectively. All 10 patients were on a CFTR modulator therapy prior to switching to ETI (five of these patients were on ivacaftor, and five patients were on lumacaftor/ivacaftor). At the time of self-reported mental health issues, follow-up clinical parameters in this group of patients on full standard dose of treatment (as seen in Table 1) showed significant improvement in lung function ($ppFEV_1$ was 14.3 points higher compared to baseline, $p = 0.0134$), and significant reduction in sweat chloride (mean of difference in sweat chloride was −39.3 mmol/L, $p < 0.005$), consistent with the findings of the clinical trials (Heijerman et al., 2019; Middleton et al., 2019; Barry et al., 2021). As a result, all patients wanted to stay on ETI treatment given the significant improvements they experienced with respiratory clinical parameters.

This subgroup of patients ($n = 10$) was fully assessed by our multidisciplinary team. Patients who consented for further psychological assessment were referred to the appropriate service for psychological support and/or pharmacological therapy if indicated. Of those with reported mental health AEs, four patients were referred for psychological support, and required anxiolytic medications transiently that were discontinued within 2 weeks. None of the patients required long term psychotropics given resolution of AEs shortly after ETI discontinuation or dose reduction.

2.2 Dose reduction approach

We adopted a dose reduction approach (Figure 1) in an attempt to minimize self-reported AEs but sustain adequate CFTR modulation. Our approach to dose reduction was developed in consultation with the CF multidisciplinary team and was as follows:

- In patients with severe self-reported neurocognitive and/or psychological AEs, we discontinued therapy pending AEs resolution. We subsequently re-introduced therapy at a lower dose, starting with a single tablet of ETI (100/50/75 mg) daily. We planned to reintroduce the evening dose of ivacaftor 150 mg at 4–6 weeks and considered returning to full dose therapy at 10–12 weeks. Dose escalation was guided

TABLE 1 Comparison of clinical efficacy parameters pretreatment, on full dose, and reduced dose of elexacaftor/tezacaftor/ivacaftor therapy.

	Genetic mutation	Pre- ETI treatment sweat chloride	Pre-ETI treatment FEV-1	Sweat chloride on full dose ETI	FEV-1 on full dose ETI	Sweat chloride at 4–6 weeks (on reduced dose ETI)	FEV-1 at 4–6 weeks (on reduced dose ETI)	Sweat chloride at 10–12 weeks (on reduced dose ETI)	FEV-1 at 10–12 weeks (on reduced dose ETI)	Outcome of self-reported mental AEs
Patient 1	Delta F508/ Delta F508	95 mmol/L	3.8 L/79% predicted	39 mmol/L	4.27 L/107% predicted	46 mmol/L	4.20 L/106% predicted	46 mmol/L[a]	4.23 L/106% predicted[a]	Complete resolution
Patient 2	Delta F508/ G551D	—	—	19 mmol/L	2.67 L/65% predicted	21 mmol/L	2.72 L/70% predicted	17 mmol/L[a]	2.7 L/69% predicted[a]	Complete resolution
Patient 3[b]	Delta F508/ Delta F508	62 mmol/L	3.92 L/92% predicted	30 mmol/L	4.04 L/96% predicted	28 mmol/L	4.06 L/96% predicted	20 mmol//L[c]	4.14 L/98% predicted[c]	Complete resolution
Patient 4[b]	Delta F508/ Delta F508	85 mmol/L	3.87 L/85% predicted	—	—	—	—	45 mmol/L[d]	4.49 L/98% predicted[d]	Complete resolution
Patient 5	Delta F508/ G551D	51 mmol/L	2.73 L/69% predicted	37 mmol/L	3.78 L/95% predicted	29 mmol/L	3.98 L/101 % predicted	32 mmol/L[a]	4.26 L/108% predicted[a]	Complete resolution
Patient 6	Delta F508/ Delta F508	74 mmol/L	3.36 L/84% predicted	56 mmol/L	3.32 L/83% predicted	41 mmol/L	3.40 L/85% predicted	51 mmol/L[a]	3.21 L/81% predicted[a]	Partial resolution
Patient 7	Delta F508/ Delta F508	105 mmol/L	2.06 L/53% predicted	39 mmol/L	2.47 L/64% predicted	45 mmol/L	2.34 L/61% predicted	56 mmol/L[c]	2.35 L/61% predicted[c]	Complete resolution
Patient 8	Delta F508/ Delta F508	89 mmol/L	1.3 L/48% predicted	19 mmol/L	1.83 L/68% predicted	—	—	15 mmol/L[c]	1.71 L/63% predicted[c]	Partial resolution
Patient 9	Delta F508/ G551D	47 mmol/L	1.62 L/56% predicted	28 mmol/L	1.79 L/68% predicted	19 mmol/L[a]	1.70 L/65% predicted	24 mmol/L[a]	1.77 L/67% predicted[a]	Partial resolution

ETI, Elexacaftor/Tezacaftor/Ivacaftor; FEVI, Forced Expiratory Volume in 1 s; AEs, Adverse Events.

[a]One tablet elexacaftor/tezacaftor/ivacaftor (100/50/75 mg) morning, and one tablet Ivacaftor 150 mg evening.

[b]Initial complete discontinuation of ETI prior to dose reduction strategy.

[c]Two tablets elexacaftor/tezacaftor/ivacaftor (200/100/150 mg) morning, (patients self-deviated from original plan).

[d]One Tablet elexacaftor/tezacaftor/ivacaftor (100/50/75 mg) morning (Patient opted to remain on this dose given that he developed significant mental/psychological AEs within 2 weeks of standard-dose treatment initiation that necessitated hospital admission).

Patient 3: ETI therapy discontinued for 4 weeks, sweat chloride and ppFEV-1 were 99 mmol/L and 93%, respectively at the end of the washout period.

Patient 4: Patient opted to switch CFTR modulator therapy back to lumacaftor/ivacaftor, sweat chloride and ppFEV-1 while on lumacaftor/ivacaftor were 85 mmol/L and 91%, respectively. ETI therapy recommenced on reduced dose 4 months after initial experience.

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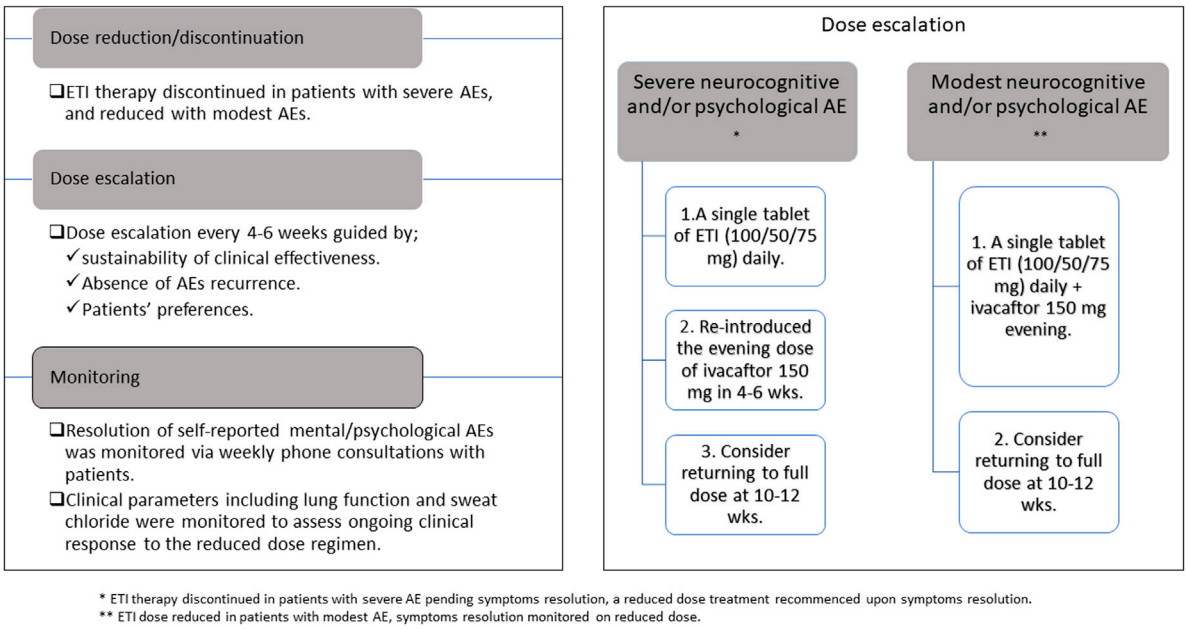

FIGURE 1
Outline of elexacaftor/tezacaftor/ivacaftor dose reduction approach.

by ongoing monitoring of clinical efficacy parameters, absence of psychological/neurocognitive AEs and patients' preferences.

- In patients with modest self-reported AEs, we reduced CFTR modulator therapy dose to a single tablet of ETI (100/50/75 mg) daily and continued the evening dose of ivacaftor 150 mg. We considered returning to full dose therapy at 10–12 weeks.

To assess ongoing clinical response to the reduced dose regimen, clinical efficacy parameters including lung function (clinical marker of sustained response), and sweat chloride (indirect measure of CFTR function restoration) (Mall et al., 2020) were recorded every 4–6 weeks for the first 12 weeks on reduced dose therapy. Members of the CF multidisciplinary team (CF clinical nurse specialist) contacted these patients weekly via phone to monitor any changes in self-reported mental/psychological AEs. The assessment of AEs resolution was subjective based on patients' perception of symptoms.

2.3 Response to reduced dose regimen

A total of nine patients commenced the dose reduction protocol and the remaining one patient opted to discontinue ETI and switched back to ivacaftor. Table 1 and Figure 2 demonstrates individual patient responses and dosing. Follow-up data showed resolution of self-reported mental/psychological AEs within 2 weeks in most patients, while their clinical efficacy parameters at 4–6 and 10–12 weeks on reduced dose were comparable to those on original full dose (mean ppFEV$_1$ was 80.7% on standard dose ETI, compared to 83.4% at 12 weeks on reduced dose; mean sweat chloride was 33.4 and 34 mmol/L on standard and at 12 weeks on reduced dose, respectively).

Whilst all nine patients adopted a sustained dosed reduction strategy, we acknowledge that two patients self-deviated from our proposed approach as highlighted in Table 1. At 12 weeks of the reduced dose regimen, six patients elected to remain on a reduced dose regimen and three patients switched back to full dose treatment. Repeat imaging in patients who completed 24 weeks on reduced dose regimen at the time of preparation of this manuscript (n = 2 of six patients) showed a significant improvement compared to imaging before initiation of modulator therapy (Figures 3, 4).

3 Discussion

Over a 12 weeks period, dose reduction in our cohort of patients who developed self-reported mental health AEs resulted in the resolution of AEs without significant change to the clinical response achieved while on full dose of therapy. In addition, two of these patients had radiological improvement at 24 weeks on this regimen. That said, the long-term outcomes of reduced dose of ETI therapy remain unclear. Continued close real-world monitoring of this group is critical and ongoing.

We believe the mental health AEs in our cohort may be related to the add on effect of the CFTR correctors elexacaftor and/or tezacaftor, as all patients in this subgroup were previously on either ivacaftor or lumacaftor/ivacaftor prior to ETI therapy. One of the hypothesized mechanisms behind the mental health AEs of ETI therapy is drug-drug interaction between ETI and psychotropic medications, but none of the patients in this group were on any psychotropic medications, this would suggest that the mental health AEs in our cohort are likely directly related to ETI therapy. Furthermore, the mental health AEs resolved

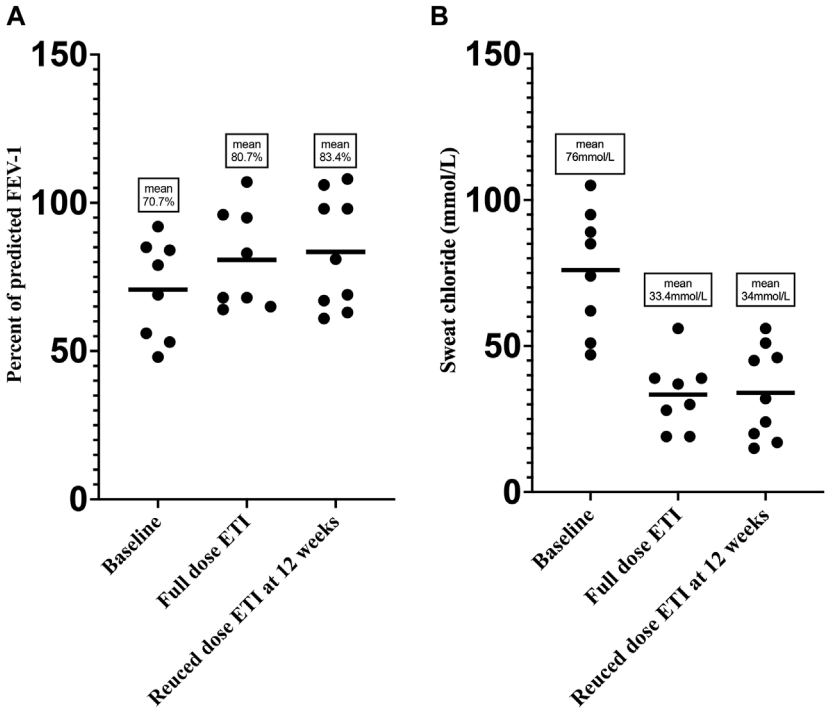

FIGURE 2
Change in **(A)** FEV_1 and **(B)** sweat chloride at baseline, on full standard dose, and reduced dose of ETI therapy.

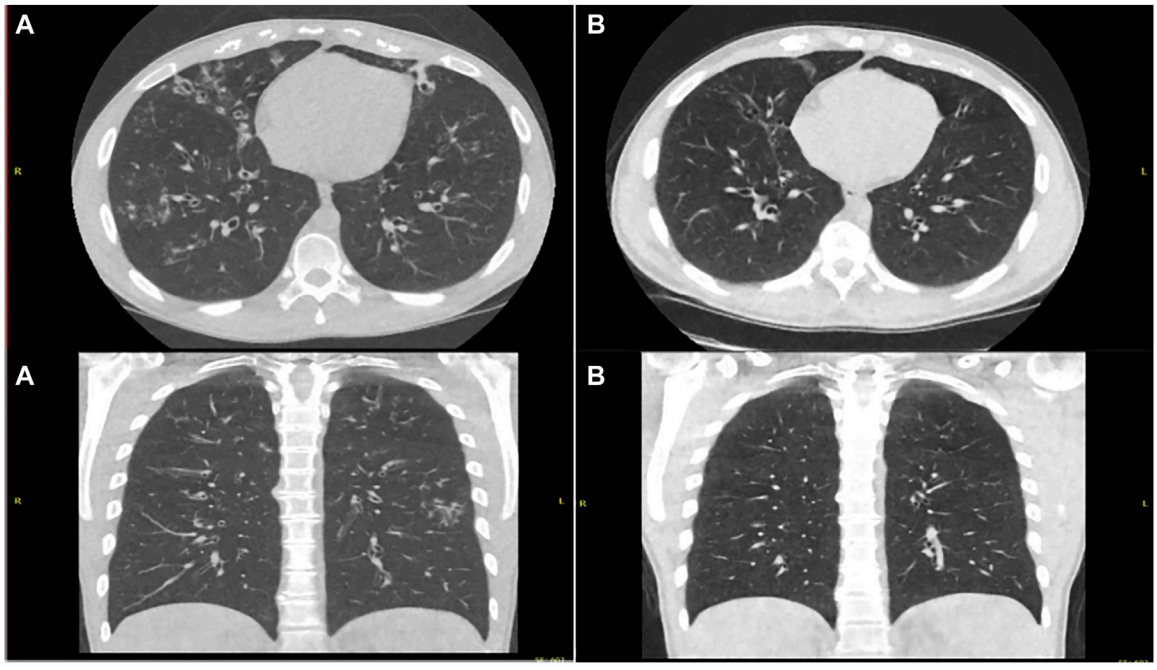

FIGURE 3
Ultra-Low Dose CT Thorax pretreatment **(A)** and post modified dose regimen **(B)** showing reduced burden of bronchiectasis, reduced caliber of bronchiectatic airways, decreased bronchial wall thickening and resolution of tree in bud opacification changes.

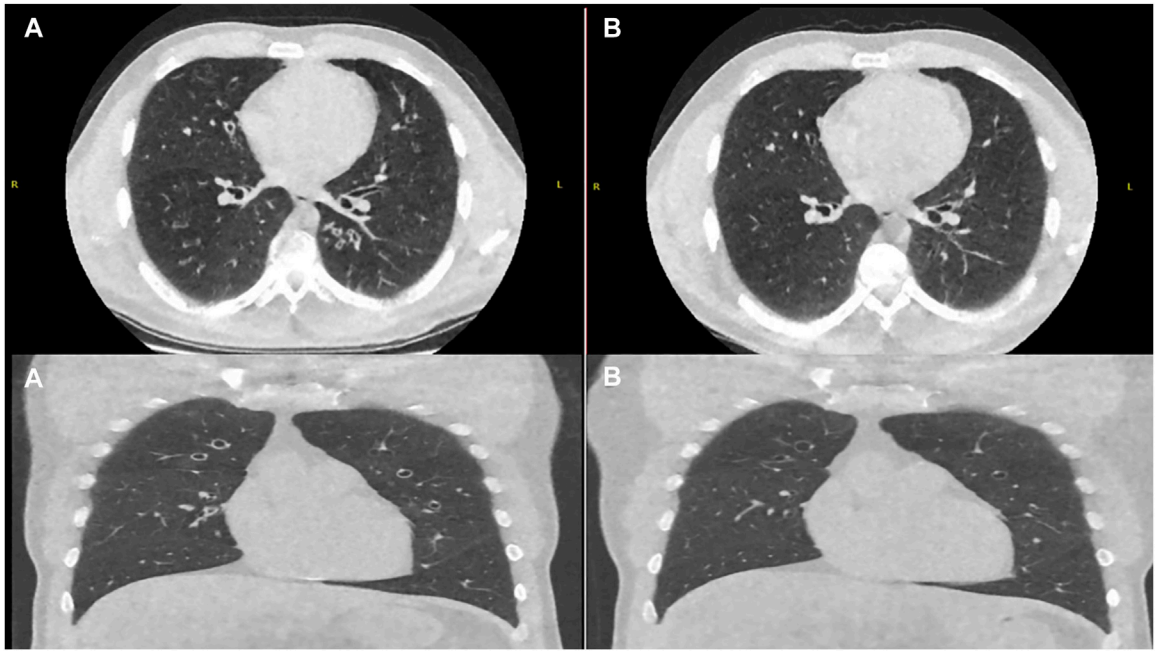

FIGURE 4
Axial and Coronal Ultra-Low Dose CT Thorax pretreatment **(A)** and post modified dose regimen **(B)** showing reduction in the degree of bronchial wall thickening and mucoid impaction.

shortly after dose reduction or discontinuation of ETI therapy. A recently published case series outlining the real-world experience of a UK CF centre not only demonstrated a similar experience with the self-reporting of mental health AEs, but also a similar incidence of 7.1% (Spoletini et al., 2022). This further supports our belief that these events may be related to ETI therapy. Moreover, the UK case series also exemplifies that dose adjustment of ETI can improve mental health AEs while sustaining clinical effectiveness, which further validates our finding and approach (Spoletini et al., 2022). Currently, there is no standardised dose reduction strategy, we attempted to achieve this but even in our case series patients deviated in their approach to dose reduction. Moving forward, as we understand more about this possible AEs a standardised strategy would be helpful to the clinical community.

Based on recent real-world analysis of serum levels of ETI in 78 adult CF patients during routine outpatient visits, 41.1% ($n = 37$) of patients had elevated serum levels of elexacaftor compared to known Pharmacokinetic (PK) values of elexacaftor (Naehrig et al., 2022). This raises the question: are we reducing the dose of ETI in our cohort of patients with self-reported mental health AEs, or are we just simply optimizing the treatment dose? The access to routine drug levels for CFTR modulators remains an issue for clinical services and access to these levels could answer this question. In addition, it could allow for further optimization of dose reduction strategies for patients with self-reported AEs.

The limitations of this work is that it reflects a single centre experience, with a modest number of cases reported. The roll out of ETI therapy was during the COVID-19 pandemic, which enforced social restrictions and isolation on vulnerable populations, such as CF patients. This in addition to concerns regarding the effect of COVID-19 on CF patients health, may have contributed to self-reported anxiety among our patients. That said, one could also hypothesise that the close monitoring of these patients after ETI dose reduction at a time where a social restriction measure was imposed on them, may have had a placebo effect in reassuring some of these patients and contributed to the improvements seen in their mental health status post ETI dose modification. However, the temporal relationship between drug initiation and AEs makes us believe that this happened independently of the pandemic. Also, the rapid and significant change in the wellbeing of CF patients post CFTR modulator therapy, may require patients to make changes to lifestyle and future planning which could potentially contribute to the self-reported anxiety.

Our real-world data and published work to date highlight that some patients do not tolerate standard full dose of CFTR modulator therapy. There is a need in small groups of patients who develop AEs, to individualize dosage so as to minimize AEs whilst continuing CFTR modulator therapy. Access to routine drug levels for ETI will compliment clinical/sweat chloride monitoring and guide dose adjustment. Routine drug levels for ETI are not made routinely available to the clinical CF community, which may potentially prohibit CF clinicians from prescribing lower dose regimens to patients who develop neuropsychiatric and/or neurocognitive AEs, for instance. Increased awareness and reporting of real-world adverse events from CFTR modulators is critical.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors on reasonable request, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals (CREC). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

The authors (HI, HD, DVM, KD, MMc, JD, CF, CH, ST, and TV) contributed to data collection and drafting the report. DMM and MM contributed to clinical and radiological monitoring of cases and aided report preparation. BP contributed to monitoring of cases and dose reduction strategy, data analysis and interpretation. All authors contributed to drafting the work and final approval.

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Conflict of interest

BP has received both speakers' fees and consulting fees from Vertex Pharmaceutical, Chiesi Pharmaceutical and Viatris Pharmaceutical, outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Real-life impact of highly effective CFTR modulator therapy in children with cystic fibrosis

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Introduction: Recently, cystic fibrosis transmembrane regulator modulator therapy with elexacaftor/tezacaftor/ivacaftor has become available for children with cystic fibrosis (CF) carrying at least one *F508del* mutation.

Objective: To assess the intermediate term effects of elexacaftor/tezacaftor/ivacaftor in children with cystic fibrosis in a real-world setting.

Methods: We performed a retrospective analysis of records of children with cystic fibrosis, who started elexacaftor/tezacaftor/ivacaftor between 8/2020 and 10/2022. Pulmonary function tests, nutritional status, sweat chloride and laboratory data were assessed before, 3 and 6 months after the start of elexacaftor/tezacaftor/ivacaftor respectively.

Results: Elexacaftor/tezacaftor/ivacaftor was started in 22 children 6–11 years and in 24 children 12–17 years. Twenty-seven (59%) patients were homozygous for *F508del* (F/F) and 23 (50%) patients were transitioned from ivacaftor/lumacaftor (IVA/LUM) or tezacaftor/ivacaftor (TEZ/IVA) to elexacaftor/tezacaftor/ivacaftor. Overall, mean sweat chloride concentration decreased by 59.3 mmol/L (95% confidence interval: −65.0 to −53.7 mmol/L, $p < 0.0001$) under elexacaftor/tezacaftor/ivacaftor. Sweat chloride concentration also decreased significantly after transition from IVA/LUM or TEZ/IVA to elexacaftor/tezacaftor/ivacaftor (−47.8 mmol/l; 95% confidence interval: −57.6 to −37.8 mmol/l, $n = 14$, $p < 0.0001$). Sweat chloride reduction was more marked in children with the F/F than in those with the F/MF genotype (69.4 vs 45.9 mmol/L, $p < 0.0001$). At 3 months follow-up, body-mass-index-z-score increased by 0.31 (95% CI, 0.2–0.42, $p < 0.0001$) with no further increase at 6 months. BMI-for-age-z-score was more markedly improved in the older group. Overall pulmonary function (percent predicted FEV_1) at 3 months follow-up increased by 11.4% (95% CI: 8.0–14.9, $p < 0.0001$) with no further significant change after 6 months. No significant differences were noted between the age groups. Children with the F/MF genotype had a greater benefit regarding nutritional

Abbreviations: BMI, body mass index; CF, cystic fibrosis; ETI, elexacaftor, tezacaftor, ivacaftor; CI, confidence interval; F/F, Patients homozygous for F508del; F/MF, Patients heterozygous for F508del and a minimal function mutation; F/U 1, Follow-up visit after 3 months of ETI treatment; F/U 2, Follow-up visit after 6 months of ETI treatment; IVA, ivacaftor; LUM, lumacaftor; ppFEV1, percent predicted FEV1; N.s, not statistically significant; SD, standard deviation; TEZ, tezacaftor; ULN, upper limit of normal.

status and pulmonary function tests than those with the F/F genotype. Adverse events led to elexacaftor/tezacaftor/ivacaftor dose reduction in three cases and a temporary interruption of therapy in four cases.

Conclusion: In a real-world setting, elexacaftor/tezacaftor/ivacaftor therapy had beneficial clinical effects and a good safety profile in eligible children with cystic fibrosis comparable to previously published data from controlled clinical trials. The positive impact on pulmonary function tests and nutritional status seen after 3 months of elexacaftor/tezacaftor/ivacaftor therapy was sustained at 6 months follow-up.

KEYWORDS

tio real-life, modulator, children, ivacaftor, tezacaftor, elexacaftor, cystic fibrosis

Introduction

Cystic fibrosis (CF) is an autosomal recessive multi-system disease, which results from mutations in the CF transmembrane conductance regulator (*CFTR*) gene (Riordan et al., 1989). While 300 CF-causing mutations and >2,000 CFTR mutations are known, the *F508del*-CFTR mutation is by far the most frequent being present in nearly 90% of people with CF (pwCF) (Riordan, 2008). Patients with *F508del-CFTR* mutations have decreased quantity and function of the CFTR protein (Ratjen et al., 2015) leading to severe disease manifestations, e.g., inborn exocrine pancreatic insufficiency, growth impairment, and progressive lung disease (Ratjen et al., 2015). Although substantial progress in the symptomatic care of pwCF was achieved over the last decades (MacKenzie et al., 2014) high disease burden and reduced life expectancy in these patients underlined the need for targeted CFTR therapies (Rang et al., 2020).

This goal was first met with the introduction of the CFTR-potentiator ivacaftor (IVA), which efficiently enhanced CFTR channel gating in the small group of CF patients with CFTR gating mutations (Ramsey et al., 2011) and achieved substantial improvements in nutritional status and pulmonary function (De Boeck et al., 2014; McKone et al., 2014). Subsequently, CFTR correctors, such as lumacaftor (LUM) and tezacaftor (TEZ) were developed, which improved CFTR processing and trafficking to epithelial surfaces. In dual combinations with ivacaftor, these substances were moderately effective in patients homozygous for *F508del* (F/F) (LUM/IVA) and in patients carrying a residual function mutation (TEZ/IVA) (Wainwright et al., 2015; Rowe et al., 2017). Since 2019, the triple-substance regimen of TEZ/IVA and the next-generation corrector elexacaftor (ELX) has been proven safe and effective (Heijerman et al., 2019; Middleton et al., 2019; Sutharsan et al., 2022) in adolescents and adults with the F/F genotype as well as in patients who were heterozygous for *F508del* and a minimal function (MF) *CFTR* mutation. ELX/TEZ/IVA (ETI) treatment resulted in so far unprecedented improvements in pulmonary function tests, respiratory symptoms and CFTR function reflected by the sweat chloride concentration, giving a new perspective to pwCF carrying at least one *F508del* mutation (Middleton et al., 2019; Sutharsan et al., 2022). However, in view of the very early onset of CF organ disease it was evident that ETI therapy should be offered to younger children and ultimately infants to tackle and prevent the sequelae of CFTR dysfunction (VanDevanter et al., 2016).

In an open-label phase 3 study, the safety, pharmacokinetics, and efficacy of ETI was examined in children aged 6 through 11 years with either F/MF or F/F genotypes (Zemanick et al., 2021). ETI therapy was safe and led to significant improvements in pulmonary function tests, sweat chloride concentration, lung clearance index (LCI) and nutritional status. In this trial, the therapeutic effects were comparable to those seen in adult patients (Zemanick et al., 2021). In a subsequent randomized, placebo-controlled trial including children 6–11 years with F/MF genotypes, the positive effects on pulmonary function, LCI, respiratory symptoms and sweat chloride were confirmed (Mall et al., 2022). Again, no safety concerns arose in the course of the trial compared to children receiving standard CF care. Consequently, in January 2022 the EMA approved the use of ETI for treatment of children with CF from the age of six. In line with most pediatric CF-centers, we intended to initiate ETI immediately in our children eligible for this treatment. In the present study, we report our experience in children and adolescents with CF during the first 6 months of ETI therapy. In contrast to the previously described controlled trials, our analysis investigates the post-approval efficacy of ETI across a heterogeneous collective of young CF-patients in a real-life setting.

Methods

We retrospectively investigated the records of all children with CF of our Cystic Fibrosis Centre for the period August 2020 to January 2023. All patients fulfilled the inclusion criteria, which were established diagnosis of CF (sweat chloride concentration ≥60 mmol/L and 2 CF-defining mutations), proof of at least one *F508del* mutation, and age ≥6 years, documenting eligibility for ETI treatment. Children participating in a clinical trial were excluded. ETI dosing was performed according to official dosage recommendations: children weighing <30 kg received ELX 100 mg once daily, TEZ 50 mg once daily, and IVA 75 mg every 12 h, whereas children weighing ≥30 kg received the full adult daily dose (ELX 200 mg once daily, TEZ 100 mg once daily, and IVA 150 mg every 12 h). Data for biometry, percentage of predicted (pp) FEV_1, alanine aminotransferase (ALT) or aspartate aminotransferase (AST), alkaline phosphatase (AP), creatinkinase (CK) and concomitant nebulized medication were collected before the start of ETI and at 3 and 6 months follow-up (F/U 1 and F/U 2) after initiation of ETI therapy. The results of hepatic sonography before start of ETI were reviewed. Sweat chloride concentrations in a modulator-naïve state were compared to results after onset of ETI

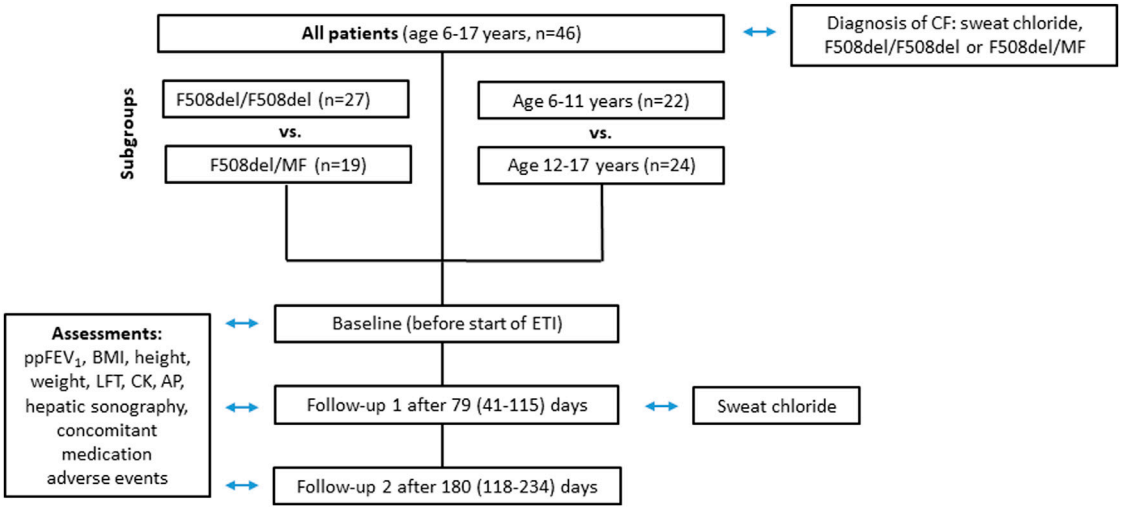

FIGURE 1
Flowchart of parameters assessed at baseline, follow-up 1 and follow-up 2 for all patients groups.

therapy. Reports of adverse effects during treatment with ETI were extracted from patient records. First, data were analyzed across the entire cohort. Subsequently, data from patients aged six through 11 years were compared to data from patients ≥12 years. Also, data of children with the F/F genotype were compared to *F508del*-heterozygous patients. Finally, the change in $ppFEV_1$ at 3 and 6 months F/U was analyzed according to baseline ppFEV1 ($ppFEV_1$ < 80% and ≥80%). This study was approved by the local ethics committee of the University of Duisburg-Essen (Study-No. 23-11141-BO).

Statistical analysis

GraphPad Prism Version 7 (GraphPad Software Inc., Boston, US) was used to analyze and visualize the data. Paired and independent *t*-tests, respectively, were used to analyze the statistical significance of the study parameters. A $p < 0.05$ was considered statistically significant. Pearson correlation coefficients were calculated using Excel 2016 1.0. All analyses were corrected for multiple testing controlling the two-sided false discovery rate (FDR) at $p < 0.05$.

Results

Entire cohort (6–17 years)

Forty-six patients (19 male, 27 female) were included in the data analysis (Figure 1). Eight patients were excluded due to current clinical trial involvement. Patient characteristics and concomitant medications are given in Table 1 and Table 2 respectively. Mean age of patients starting ETI after the approval for the age group ≥12 years in 2020 was 14.3 years (range 12.1—17.1) while mean age of patients starting ETI after the approval for children ≥6 years in 2022 was 8.6 years (range 6.0–11.9) years. Twenty-seven patients were homozygous for *F508del* (F/F). Prior modulator use was LUM/IVA in 21 patients and TEZ/IVA in two patients. The two follow-up examinations (F/U 1 and F/U 2) occurred after a mean of 79 days (range 41–115) and 180 days (range 118–234), respectively, following start of ETI therapy.

Two extreme outliers concerning the baseline sweat chloride concentration (170 mmol/l and 220 mmol/l) were excluded from further analysis. At baseline, no children had sweat chloride concentrations below the diagnostic threshold for CF of 60 mmol/L, even if pretreated with LUM/IVA or TEZ/IVA. Across the entire cohort, mean sweat chloride concentration decreased by 59.3 mmol/L (95% confidence interval [CI]: −65.0 to −53.7. mmol/L, $n = 42$) ($p < 0.0001$) compared to the modulator-naïve state (Table 3; Figure 2A). In children, who were switched from LUM/IVA or TEZ/IVA to ETI sweat chloride concentration also decreased significantly by 47.8 mmol/l (95% CI: −57.6 to −37.8 mmol/l, $n = 14$, $p < 0.0001$) (Table 3; Figure 2D). At F/U 1 and F/U 2, there was no significant correlation between the decrease in sweat chloride concentrations and the change in ppFEV1 ($r = 0.08$ and $r = 0.15$), weight ($r = 0.23$ and $r = 0.28$), weight for age z-score ($r = 0.31$ and $r = 0.27$), BMI ($r = 0.02$ and $r = 0.05$) or BMI for age-z-score ($r = -0.06$ and $r = 0.01$) when considering a correction for multiple testing. Sweat test at follow-up was borderline in 23 patients, above 60 mmol/l in 12 patients, and normal in 10 patients. In one patient, follow-up sweat sampling failed due to insufficient sweat quantity despite several attempts.

With regard to pulmonary function, ETI therapy led to a significant improvement in $ppFEV_1$ compared to baseline. At F/U 1, $ppFEV_1$ increased by a mean of 11.4 percentage points (95% CI: 8.0–14.9, $n = 45$, $p < 0.0001$). This effect was sustained at 6-month-follow-up with a mean increase in $ppFEV_1$ of 12.8%

TABLE 1 Baseline characteristics.

Characteristcs	All patients	6–11 years	12–17 years
number	46	22	24
female/male	27/19	14/8	13/11
Age (years) at baseline	11.5 (6.0–17.1)	8.6 (6.0–11.9)	14.3 (12.1–17.1)
F/U 1 (days)	79 (41–115)	88 (38–112)	80 (41–108)
F/U 2 (days)	180 (118–234)	191 (150–210)	175 (118–234)
Genotype			
F/F	27	16	11
F/MF	19	6	13
Prior modulator use			
LUM/IVA	21	15	6
TEZ/IVA	2		2

F/F, Patients homozygous for F508del; F/MF, Patients heterozygous for F508del and a minimal function mutation; LUM, Lumacaftor; IVA, ivacaftor.

TABLE 2 Concomitant medication.

Concomitant medication	—	Visit reported	Use (%)
Hypertonic saline			
6–11 years (n = 22)	Twice/once daily	Baseline	19/3 (100)
		F/U 1	20/2(100)
		F/U 2	20/2(100)
12–17 years (n = 24)	Twice/once daily	Baseline	21/3 (100)
		F/U 1	21/3(100)
		F/U 2	23/1 (100)
Dornase alfa			
6–11 years	—	Baseline	13 (59)
	—	F/U 1	13 (59)
	—	F/U 2	13 (59)
12–17 years	—	Baseline	20 (83)
	—	F/U 1	18 (83)
	—	F/U 2	18 (83)
Inhaled antibiotics			
6–11 years	—	Baseline	5(23)
	—	F/U 1	4 (18)
	—	F/U 2	5 (23)
12–17 years	—	Baseline	6 (25)
	—	F/U 1	4 (17)
	—	F/U 2	3 (13)

F/U 1, Follow-up visit after 3 months of ETI treatment; F/U 2, Follow-up visit after 6 months of ETI treatment.

(95% CI: 9.1–16.5, n = 41, $p < 0.0001$) compared to baseline. There was no significant difference between the 2 F/U visits regarding pulmonary function tests (Table 3; Figure 3A). Eighteen patients had $ppFEV_1 < 80\%$ at baseline whereas $ppFEV_1$ was ≥80% in 28 patients. Both groups experienced a significant increase in $ppFEV_1$ at both follow-up examinations after start of ETI. At F/U 1, the increase in

TABLE 3 Patient characteristics at baseline, follow-up 1 and follow–up 2.

Parameter	Patient categories	Baseline (mean, SD)	F/U 1 (mean, SD)	Change from BL at F/U 1 [mean (95% CI)]	*p*-value	F/U 2 (mean, SD)	Change from BL at F/U2 [mean (95% CI)]	*p*-value	*p*-value F/U1 vs F/U 2
ppFEV1	all patients	81.4 (15.6)	92.8 (15.5)	11.4 (8.0—14.9) (n = 45)	$p < 0.0001$	93.7 (14.46)	12.8 (9.1—16.5) (n = 41)	$p < 0.0001$	$p = 0.2629$
[%]	6–11 years	85.0 (16.2)	94.2 (16.3)	9.8 (4.6—15.1) (n = 22)	$p = 0.0009$	96.8 (14.29)	12.9 (7.1—18.7) (n = 18)	$p = 0.0002$	$p = 0.1075$
	12–17 years	77.6 (14.2)	91.3 (14.7)	13.0 (8.1—17.8) (n = 23)	$p < 0.0001$	91.1 (14.4)	12.8 (7.6—18.0) (n = 23)	$p < 0.0001$	$p = 0.8912$
	F/F	83.4 (15.5)	91.6 (15.6)	8.4 (4.8–12.1) (n = 26)	$p < 0.0001$	92.0 (14.6)	9.3 (5.1–13.5) (n = 23)	$p = 0.0001$	$p = 0.4130$
	F/MF	78.5 (15.6)	94.1 (15.7)	15.5 (9.1–22.0) (n = 19)	$p < 0.0001$	95.9 (14.5)	17.3 (11.0–23.7) (n = 18)	$p < 0.0001$	$p = 0.4634$
	ppFEV1 < 80%	65.8 (10.3)	81.3 (11.6)	15.6 (10.3–20.8) (n = 18)	$p < 0.0001$	83.9 (11.4)	18.9 (13.5-24.3 (n = 17)	$p < 0.0001$	$p = 0.0541$
	ppFEV 1 ≥ 80%	91.7 (7.7)	100.4 (13.0)	8.7 (4.1–13.2) (n = 27)	$p < 0.0006$	100.7 (12.4)	8.5 (4.0–13.1) (n = 24)	$p < 0.0007$	$p = 0.9566$
weight-for-age	all patients	−0.56 (0.98)	−0.32 (0.91)	0.24 (0.15–0.33) (n = 46)	$p < 0.0001$	−0.18 (0.95)	0.32 (0.18–0.47) (n = 42)	$p < 0.0001$	$p = 0.1461$
[z-Score]	6–11 years	−0.40 (0.90)	−0.26 (0.84)	0.14 (0.01–0.27) (n = 22)	$p = 0.0365$*	−0.07 (0.81)	0.18 (−0.01–0.37) (n = 18)	$p = 0.0657$	$p = 0.8015$
	12–17 years	−0.72 (1.05)	−0.37 (0.99)	0.34 (0.22—0.46) (n = 24)	$p < 0.0001$	−0.27 (1.06)	0.43 (0.22–0.64) (n = 24)	$p = 0.0003$	$p = 0.1243$
	F/F	−0.46 (0.93)	−0.33 (0.92)	0.13 (0.06—0.21) (n = 27)	$p = 0.0013$	−0.21 (0.89)	0.13 (−0.01–0.27) (n = 24)	$p = 0.0610$	$p = 0.7524$
	F/MF	−0.69 (1.05)	−0.29 (0.92)	0.40 (0.23–0.57) (n = 19)	$p = 0.0001$	−0.14 (1.05)	0.58 (0.33–0.83) (n = 18)	$p = 0.0002$	$p = 0.0311$*
BMI-for-age [z-score]	all patients 6–11 years	−0.15 (0.84) 0.08 (0.70)	0.16 (0.72) 0.28 (0.57)	0.31 (0.20—0.42) (n = 46) 0.20 (0.04—0.36) (n = 22)	$p < 0.0001$ $p = 0.0141$	0.18 (0.88) 0.23 (0.78)	0.38 (0.19–0.56) (n = 42) 0.19 (−0.04–0.43) (n = 18)	$p = 0.0002$ $p = 0.1035$	$p = 0.4719$ $p = 0.5677$
	12–17 years	−0.37 (0.91)	0.04 (0.82)	0.41 (0.27—0.55) (n = 24)	$p < 0.0001$	0.15 (0.97)	0.51 (0.24–0.79) (n = 24)	$p = 0.0007$	$p = 0.2202$
	F/F	−0.04 (0.82)	0.13 (0.82)	0.17 (0.09–0.24) (n = 27)	$p < 0.0001$	0.05 (0.96)	0.12 (−0.03–0.27) (n = 24)	$p = 0.1113$	$p = 0.3045$
	F/MF	−0.32 (0.85)	0.20 (0.56)	0.51 (0.30–0.72) (n = 19)	$p < 0.0001$	0.37 (0.77)	0.72 (0.38–1.06) (n = 18)	$p = 0.0003$	$p = 0.0837$
height-for-age [z-score]	all patients 6–11 years	−0.75 (1.18) −0.78 (1.11)	−0.75 (1.14) −0.82 (1.09)	0 (−0.06–0.06) (n = 46) −0.04 (−0.11-0.04) (n = 22)	$p = 0.9007$ $p = 0.3254$	−0.60 (1.03) - 0.43 (0.80)	0.01 (−0.06–0.08) (n = 42) 0.03 (−0.05–0.12) (n = 18)	$p = 0.7544$ $p = 0.3910$	$p = 0.7252$ $p = 0.0061$
	12–17 years	−0.72 (1.26)	−0.69 (1.21)	0.03 (−0.07–0.12) (n = 24)	$p = 0.5615$	- 0.72 (1.18)	−0.01 (−0.12-0.10) (n = 24)	$p = 0.8933$	$p = 0.5359$
	F/F	−0.74 (1.20)	−0.77 (1.15)	−0.03 (−0.11-0.06) (n = 27)	$p = 0.5136$	−0.49 (0.91)	0.01 (−0.10–0.12) (n = 24)	$p = 0.8611$	$p = 0.2256$
	F/MF	−0.76 (1.19)	−0.73 (1.17)	0.03 (−0.06–0.12) (n = 19)	$p = 0.5061$	−0.74 (1.19)	0.01 (−0.07–0.10) (n = 18)	$p = 0.7583$	$p = 0.7489$
Sweat chloride [mmol/l]	all patients 6–11 years	105.8 (11.2.) 105.7 (11.5)	46.5 (19.2) 47.0 (20.8)	−59.3 (−65.0 to −53.7) (n = 42) −58.7 (−67.8 to −49.5) (n = 22)	$p < 0.0001$ $p < 0.0001$	— —	— —	— —	— —
	12–17 years	106.0 (11.2)	45.9 (17.9)	−60.1 (−67.3 to −52.8) (n = 20)	$p < 0.0001$	—	—	—	—
	F/F	105.8 (11.8)	36.4 (11.7)	−69.4 (−74.3 to −64.5) (n = 24)	$p < 0.0001$	—	—	—	—
	F/MF	105.9 10.7)	59.9(19.3)	−45.9 (−54.2 to −37.7) (n = 18)	$p < 0.0001$	—	—	—	—
	Modulator switch	81.7 (14.7)	33.9 (11.9)	−47.8 (−57.6 to −37.8) (n = 14)	$p < 0.0001$	—	—	—	—

BMI, body mass index; F/F, Patients homozygous for F508del; F/MF, Patients heterozygous for F508del and a minimal function mutation; ppFEV1, percent predicted FEV1; SD, standard deviation; F/U 1, Follow-up visit after 3 months of ETI treatment; F/U 2, Follow-up visit after 6 months of ETI treatment; *, not statistically significant after correction for multiple tests.

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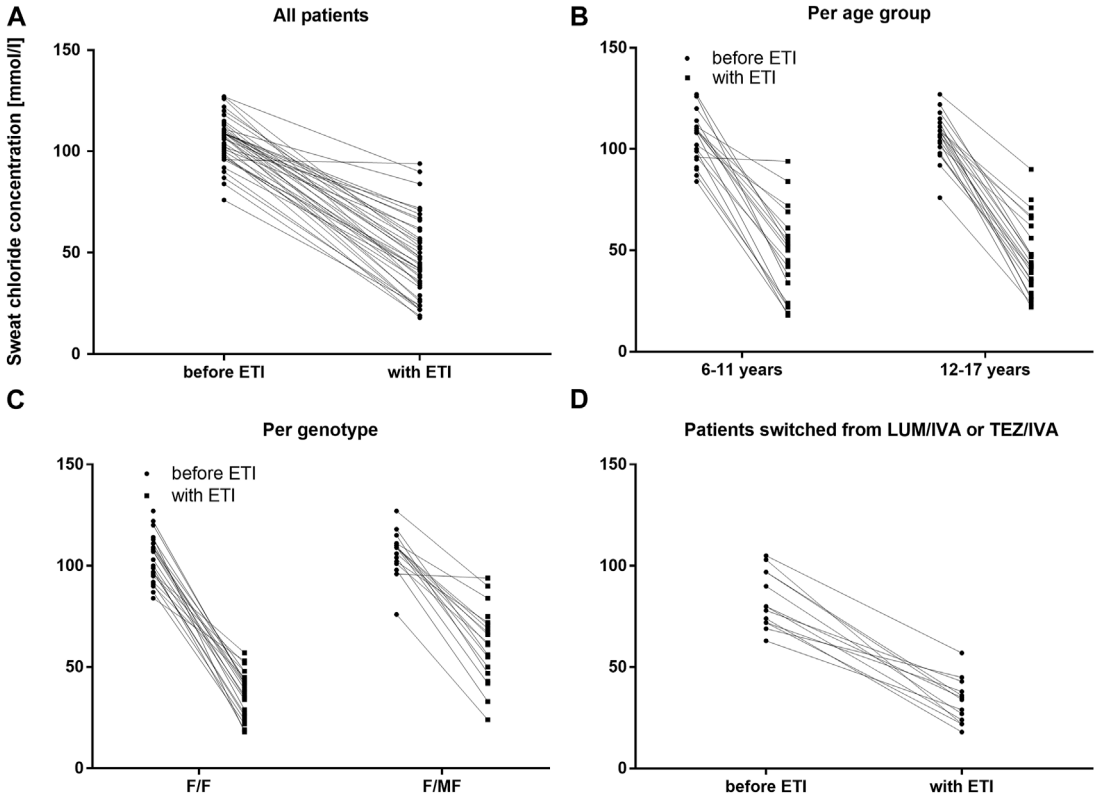

FIGURE 2
Sweat chloride concentrations before and with ETI therapy in mmol/L **(A)** Across the entire cohort before ETI (circles) and with ETI (squares) **(B)** In patients 6–11 years and in patients 12–17 years at baseline before ETI (circles) and with ETI (squares) **(C)** In patients with the F/F genotype and in patients with F/MF genotypes before ETI (circles) and with ETI (squares) **(D)** In patients switched from LUM/IVA or TEZ/IVA before ETI (circles) and with ETI (squares).

$ppFEV_1$ in patients with lower baseline pulmonary function was 6.9% higher than in the group with higher baseline $ppFEV_1$ (15.6% *versus* 8.7%, 95% CI: 0.06–13.7, $p < 0.048$, n. s after correction for multiple testing). At 6 months F/U, there was a significantly higher increase in $ppFEV_1$ in children with baseline pulmonary function <80% compared to those with higher baseline pulmonary function (18.9% *versus* 8.5%, 95% CI 3.5–17.2, $p < 0.004$). No further increase occurred in these subgroups between F/U 1 and F/U 2.

Significant improvements were seen in BMI-for-age and weight-for-age *z*-scores at F/U 1, reaching a plateau through F/U 2. Height-for-age *z*-scores remained unchanged at F/U 1 ($p = 0.901$) and F/U 2 ($p = 0.754$). Specifically, ETI resulted in a BMI *z*-score that was 0.31 higher in comparison to baseline at F/U 1 (95% CI: 0.2–0.42, $n = 46$, $p < 0.0001$). Through F/U 2, BMI-for-age *z*-score was maintained without further significant increase (mean difference to baseline 0.38, 95% CI: 0.19 to 0.56, $n = 42$, $p < 0.001$) (Table 3; Figure 4A). Similar to BMI, sustained improvement in weight-for-age *z*-score was seen through F/U 2, with a mean difference of 0.32 relative to baseline (95% CI: 0.18–0.47, $n = 42$, $p < 0.0001$). The interim analysis at F/U 1 revealed a mean increase of 0.24 compared to baseline (95% CI: 0.15 to 0.33, $n = 46$, $p < 0.0001$). Again, no marked differences between 3 and 6 months were noticed (Table 3, Figure 5A).

With regard to concomitant medication there was no relevant change in the use of hypertonic saline, dornase alfa and nebulized antibiotics after the onset of ETI therapy.

Subgroup analyses

Age groups (6–11 years versus ≥ 12 years)

There was no significant difference between the age groups regarding the effect of ETI on sweat chloride concentration at F/U 1, with a mean decrease of 58.7 mmol/l (95% CI −67.8–−49.5; $n = 22$, $p < 0.0001$) in the patients 6—11 years and a mean decrease of 60.1 mmol/l (95% CI—67.3–−52.8, $n = 20$, $p < 0.0001$) in the elder age group ($p = 0.21$). (Table 3; Figure 2B). In addition, the age groups did not differ significantly with regard to changes in pulmonary function tests as assessed by $ppFEV_1$ at F/U 1 ($p = 0.37$) and F/U 2 ($p = 0.98$). In children 6—11 years $ppFEV_1$ improved by 9.8% (95% CI 4.6—15.1; $n = 22$, $p < 0.001$) at F/U 1, whereas the elder children experienced an increase in $ppFEV_1$ of 13.0% (95% CI 8.1—17.8, $n = 20$; $p < 0,0001$ (Table 3; Figure 3B).

In the younger group of patients, there was a trend toward an increase in weight-for-age *z*-scores at F/U 1, with a mean difference of 0.14 ($p = 0.0365$, n. s after correction for multiple testing). This increase was sustained at F/U 2 although again not reaching

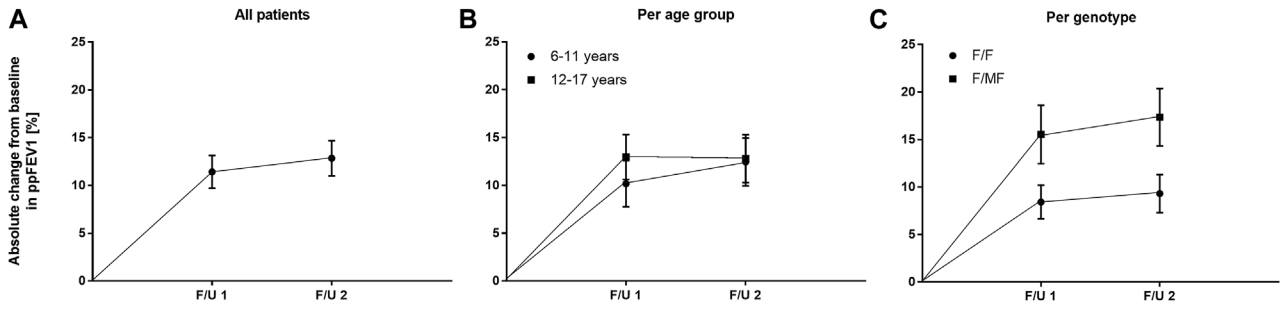

FIGURE 3
Absolute change in mean ppFEV1 before start of ETI and at F/U 1 and F/U 2 **(A)** Across the entire cohort **(B)** In patients 6–11 years (circles) and in patients 12–17 years (squares) at baseline **(C)** In patients with the F/F genotype (circles) and in patients with F/MF genotypes (squares).

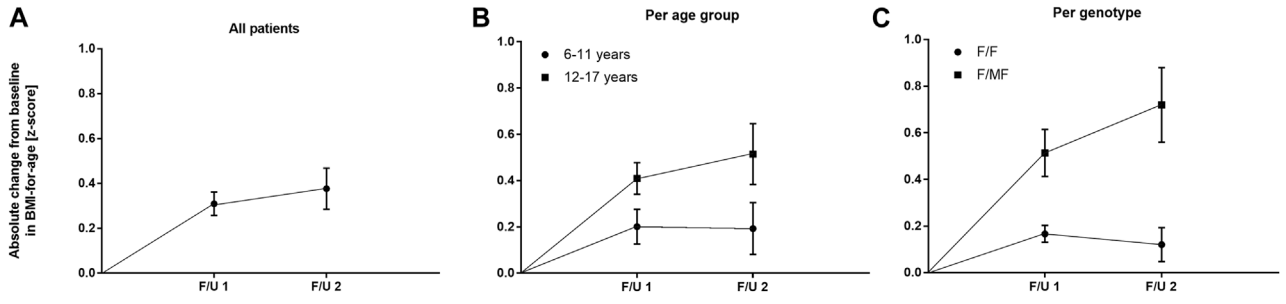

FIGURE 4
Absolute change in BMI-for-age z-score before start of ETI and at F/U 1 and F/U 2 **(A)** Across the entire cohort **(B)** In patients 6–11 years (circles) and in patients 12–17 years (squares) at baseline **(C)** In patients with the F/F genotype (circles) and in patients with F/MF genotypes (squares).

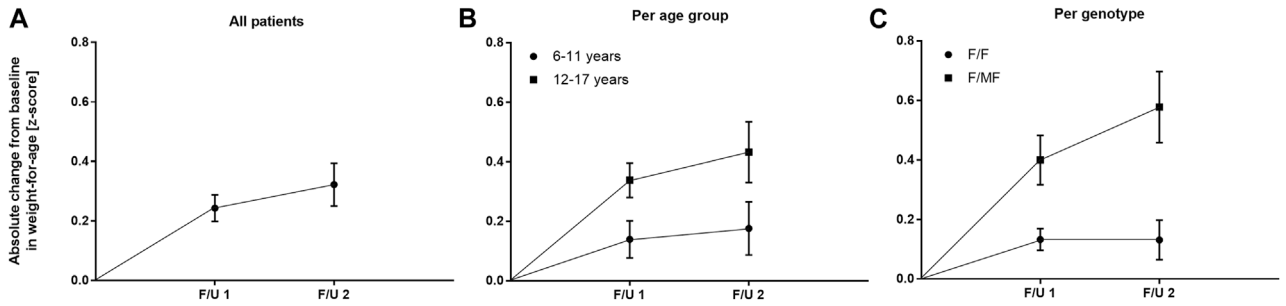

FIGURE 5
Absolute change in weight-for-age z-score before start of ETI and at F/U 1 and F/U 2 **(A)** Across the entire cohort **(B)** In patients 6–11 years (circles) and in patients 12–17 years (squares) at baseline **(C)** In patients with the F/F genotype (circles) and in patients with F/MF genotypes (squares).

statistical significance ($p = 0.0657$) (Table 3; Figure 5B). With respect to BMI-for-age *z*-score, there was a mean difference of 0.2 above the baseline (95% CI: 0.04–0.36, $n = 22$, $p = 0.0141$), whereas the absolute change from baseline at F/U 2 was not statistically significant (Table 3; Figure 4B).

In contrast, changes in BMI-for-age *z*-score in the elder group of patients revealed a statistically significant improvement at F/U 1 with a mean difference of 0.41 (95% CI: 0.27–0.55, $n = 24$, $p < 0.0001$), which was sustained at F/U 2 (mean difference 0.51, 95% CI: 0.24–0.79, $n = 24$, $p < 0.001$) (Table 3; Figure 4B). With respect to weight-for-age *z*-scores, a mean difference of 0.34 at F/U1 was observed (95% CI: 0.22–0.46, $n = 24$, $p < 0.0001$) and sustained at F/U 2 (mean difference 0.43, 95% CI: 0.22–0.64, $n = 24$, $p < 0.001$) (Table 3; Figure 5B).

The mean of individual changes between the two age groups regarding BMI-for-age z-score did not show statistically significant differences at F/U 1 ($p = 0.046$) or F/U 2 ($p = 0.082$) after correction for multiple testing. With regard to weight-for-age z-scores the elder patients experienced a significantly greater increase at F/U 1, whereas the difference between the two age-groups was insignificant at F/U 2 ($p = 0.0771$).

Height-for-age*z*-scores did not undergo a consistent significant change in either of the groups (Table 3). Solely, the children 6–11 years had an increase in height-for-age z-score between F/U 1 and F/U 2 ($p = 0.0061$).

Genotype groups (F/F *versus* F/MF)

Reductions in sweat chloride concentrations to <60 mmol/L and <30 mmol/L after ETI were found to be more prevalent among children with the F/F genotype (100.0% and 36.0%, respectively) compared to children with F/MF genotypes (42.1% and 5.3%, respectively). This observation was reflected by the significantly greater decrease in mean sweat chloride concentration in the F/F group (−69.4 mmol/L; 95% CI, −74.3–−64.9) compared to the patients with the F/MF genotype (−45.9 mmol/L; 95% CI, −54.2–−37.7) ($p < 0.0001$). (Table 3; Figure 2C).

Regarding nutritional status, absolute changes in BMI-for-age and weight-for-age z-scores showed a marked separation of the two genotype groups in favor of the F/MF group (Figure 4C; Figure 5C). Whereas weight for age z-score improvement in the F/F group was only significant at F/U 1 (0.13, 95% CI, 0.06–0.21) but not at F/U 2 ($p = 0.06$), patients with F/MF genotypes had significantly improved weight-for-age z-scores at both follow-up visits (0.40 and 0.58 relative to baseline respectively, with $p = 0.0001$ and $p = 0.0002$).

F/F patients presented a BMI-for-age z-score improvement of 0.17 at F/U 1 (95% CI, 0.09–0.24, $p < 0.0001$), which was not sustained at F/U 2 (mean difference to baseline 0.12, $p = 0.1113$) (Figure 4C). On the contrary, patients with F/MF genotype presented a significant and sustained *z*-score improvement at F/U 1 and F/U 2 (mean difference 0.51 and 0.72, respectively, with $p < 0.001$ and $p = 0.0003$).

Again, height-for-age*z*-scores did not improve significantly for either of the genotype subgroups (Table 3).

Consistent with the statistically significant improvements in weight and BMI, changes in $ppFEV_1$ compared to baseline were also greater among patients with F/MF genotype. At F/U 1 there was a trend toward a higher increase in $ppFEV_1$ in patients with the F/MF genotype compared to the F/F group (15.5% *versus* 8.4%, $p = 0.0397$, n. s after correction for multiple testing). At F/U 2, $ppFEV_1$ increased significantly by 17.3% in the F/MF group compared to 9.3% in the F/F patients ($p = 0.0274$). Both groups presented statistically relevant changes in $ppFEV_1$ related to baseline at both follow-up examinations ($p < 0.0001$) (Figure 3C).

Adverse events

ETI was generally well tolerated. A rash, considered attributable to ETI therapy, occurred in three patients, which was treated with antihistamines and did not lead to interruption of the modulator medication. At baseline, 11 patients had mild elevation of ALT or AST (<2xULN). On ultrasound before start of ETI, seven of the 11 patients with elevated hepatic enzymes had signs of CF liver disease and 2/11 patients were post liver transplantation. Of these eleven patients, only one with no morphologic signs of liver disease at baseline experienced a significant elevation of liver enzymes above 5xULN. ETI was stopped in this patient and successfully restarted with a reduced dose after normalization of liver enzymes. The other 10 patients did not have a further increase in liver enzymes. Eleven patients with normal liver enzymes at baseline experienced a mostly mild increase in ALT and/or AST. Interruption of ETI therapy was only required in two patients, both of whom were able to continue with a reduced dose. Of note, all patients with signs of liver cirrhosis on ultrasound at baseline tolerated ETI without a dose reduction.

Mild elevation of CK was a frequent finding and was less than 2-fold in all but one case. One 17-year-old boy had repetitive increases in CK up to 1026 U/L, often in association with physical exercise. Muscle MRI and CPT2-genotyping were normal and the boy continued with the regular dose.

One 16-year-old girl post liver transplantation experienced an increase in AP >6,000 U/L as well as diarrhea 3 weeks after the onset of ETI. Analysis of AP isoenzymes revealed mixed hepatic and bone origin of AP without intestinal AP. Liver enzymes, hepatic ultrasound and bone density were normal. ETI was interrupted and restarted after normalization of AP 6 weeks later without renewed elevation of AP in the further course. There were no other relevant changes in laboratory findings.

Another 16-year-old girl developed headaches and fatigue in association with the start of ETI. Ophthalmologic examination revealed bilateral papillary edema. Intracranial pressure was raised to 38 mmHg and was lowered to 20 mmHg during lumbar puncture. MRI of the brain showed no abnormalities. The vitamin A serum level was 374 µg/l (normal range 200—1,200). After 4 weeks, ETI was restarted in a reduced dose. To date, the girl has not reported a new onset of headaches or visual impairment. On follow-up fundoscopy, no papillary edema was detected. The same girl developed a psoriasis-like rash mainly on the trunk. Malassezia furfur was seen on microscopy, fungal culture was negative. Recently, skin biopsy revealed psoriasis vulgaris with no evidence of tinea corporis. The girl is scheduled for an appointment in the department of dermatology to discuss treatment options.

Discussion

Treatment with ETI has been shown to be safe and effective in adolescents and adults with at least one *F508del* mutation (Nichols et al., 2022). ETI therapy is associated with a marked clinical benefit regarding pulmonary function, growth parameters, sweat chloride concentration (Sutharsan et al., 2022), nasal potential difference (Graeber et al., 2022a) and MRI parameters (Graeber et al., 2022b) compared to previously introduced CFTR modulators. Moreover, non-respiratory health-related parameters improved in adolescents and adults taking ETI (Fajac et al., 2022). Recently, two clinical trials documented the safety and efficacy of ETI in children ≥6 years with at least one *F508del* mutation (Zemanick et al., 2021; Mall et al., 2022). More recently, the results of the phase 3 open label trial including children aged 2–5 years taking ETI therapy were

published (Goralski et al., 2023), demonstrating a significant improvement in LCI_{25} as well as reduced sweat chloride concentrations after 24 weeks of treatment. Outside clinical studies, compliance regarding the reliable drug intake is less closely monitored and as such more likely to be variable than under study conditions. Treatment efficacy may therefore differ from findings in controlled clinical trials. Real-life data in adolescents and adults (Nichols et al., 2022) have been presented including patients with severe lung disease in pre-approval compassionate use programs (Carnovale et al., 2022; Kos et al., 2022). To date, real life evidence of ETI therapy in children below 12 years of age is limited (Streibel et al., 2023).

Pulmonary function tests

In this study, we report the intermediate-term efficacy and safety of ETI therapy in 46 pediatric CF patients in a CF clinic situated in the large metropolitan Rhine-Ruhr area. Under these post-approval conditions, the positive impact of ETI was comparable to that seen in the clinical trials with children (Zemanick et al., 2021; Mall et al., 2022) and adolescents (Middleton et al., 2019). In our patients, mean $ppFEV_1$ was normal before start of ETI and improved by a mean of 11.4% across the study group. Zemanick et al. found an increase in $ppFEV_1$ of 10.2% in children carrying at least one *F508del* mutation compared to baseline (Zemanick et al., 2021). In contrast to our study, these children had undergone a wash-out period of a previously prescribed modulator. In a placebo-controlled trial, Mall et al. reported a mean in-between-group difference of 11% $ppFEV_1$ in children with a minimal function mutation compared to baseline (Mall et al., 2022). In adolescents who were homozygous for *F508del*, a mean increase in $ppFEV_1$ of 13.8% was found (Middleton et al., 2019). Real world evidence given by Streibel et al. shows a mean increase in FEV_1 z-score of 1.06 in children and adolescents after a mean of 4 months after start of ETI (Streibel et al., 2023). A significant association between pulmonary function improvements and structural MRI data in these patients was reported. Interestingly, in our study change in pulmonary function tests did not differ significantly between children 6–11 years compared to those ≥12 years. In contrast to this, children with the F/MF genotype experienced a more marked response to ETI regarding pulmonary function tests than those homozygous for *F508del* at both follow-up visits although this difference was only statistically significant at F/U 2 after correction for multiple testing. However, our results are in line with previous data (Nichols et al., 2022) and most probably reflect the modulator-naïve state prior to start of ETI in patients with the F/MF genotype. While average $ppFEV_1$ in our patients was normal at baseline, we like others (Salvatore et al., 2022) found that the subgroup of children with impaired function experienced the largest increase in $ppFEV_1$, again underlining the particular therapeutic value of ETI in these patients. We cannot reproduce the findings of Nichols et al. (Nichols et al., 2022), who described a significant correlation between the decrease in sweat chloride concentration and the increase in $ppFEV_1$ after 6 months follow-up in adolescent and adult PwCF. It remains unclear, whether in our study this effect could have been demonstrated with a larger patient group.

In patients with normal pulmonary function, ventilation inhomogeneities are detected by means of the LCI. Regrettably, we are unable to report a consistent LCI data set in our study. In the literature, treatment with ETI was associated with a significant improvement in the LCI (Zemanick et al., 2021; Graeber et al., 2022b) in excess of that previously reported for LUM/IVA in young children (Ratjen et al., 2017). Recently, a significant LCI improvement was demonstrated in children aged 2–5 years after 6 months of ETI treatment (Goralski et al., 2023). Streibel et al. reported an improvement in LCI and MRT-derived ventilation and perfusion measures after a mean follow-up of 4 months after start of ETI therapy (Streibel et al., 2023).

Growth parameters and exocrine pancreatic function

Improving and maintaining growth is of prognostic relevance regarding pulmonary function and survival in children with CF (Konstan et al., 2003; Vieni et al., 2013). In our study, mean z-scores for weight and BMI were normal at baseline, partially reflecting the effect of LUM/IVA (Hoppe et al., 2021) before start of ETI, and increased significantly after three and 6 months. In line with Zemanick et al., mean height z-scores remained unchanged after three to 6 months (Zemanick et al., 2021). The two age groups showed comparable effects of ETI on BMI and weight. Patients with the F/MF genotype experienced a greater increase in weight for age-z-score and BMI for age-z-score than those homozygous for *F508del*, which - as in the case of $ppFEV_1$ - points to the modulator-naïve state of this group. The beneficial effects on the nutritional status in our patients exceeded those seen with LUM/IVA in children with the F/F genotype (Hoppe et al., 2021).

To date, we are not aware of any patient in our study reaching pancreatic sufficiency. However, fecal elastase was not systematically assessed in our patients. In the literature, there are reports of restored pancreatic function when using LUM/IVA (Vieni et al., 2013), in school-aged children taking IVA (Nichols et al., 2020) and even more so when IVA was introduced from the age of 4 months (Davies et al., 2021). Consequently, CFTR modulator therapy should be started as early as possible to maintain exocrine pancreatic function (Dave et al., 2021). So far, ETI does not seem to restore exocrine pancreatic function in children ≥12 years of age (Schwarzenberg et al., 2022). Data on fecal elastase were not reported in the pediatric trials (Mall et al., 2022; Nichols et al., 2022) including children ≥6 years of age. In children aged 2–5 years the mean increase in fecal elastase was 39.5 μg/g, with 6/75 children having a fecal elastase >200 μg/g after 6 months of ETI therapy (Goralski et al., 2023).

Sweat chloride

The sweat chloride concentration, a surrogate measure for the CFTR function (Elborn, 2016), is an important outcome parameter when assessing the efficacy of modulator therapy. With IVA, sweat chloride was reduced below the threshold for the diagnosis of CF (Ramsey et al., 2011). The modulators LUM/IVA and TEZ/IVA resulted in less impressive reductions in sweat chloride but offered a modulator option for a large group of patients carrying two *F508del* mutations or a residual function mutation. With the advent of ETI therapy, the reduction in sweat chloride was comparable to that seen with IVA (Heijerman et al., 2019; Middleton et al., 2019). In children aged 6–11 years treated with ETI, a mean change in sweat chloride of 61 mmol/L was seen with an even greater mean reduction of

70 mmol/l in *F508del* homozygous patients (Zemanick et al., 2021). In our study, mean sweat chloride concentration decreased by 59 mmol/l across all patients, with a more pronounced in effect in those carrying the F/F genotype. There was no relevant difference in sweat chloride reduction between the age groups. In four of the 12 patients with a sweat chloride concentration >60 mmol/l during ETI treatment, suboptimal compliance in several fields had been a recognized problem. Consequently, dietary advice was repeated regarding the intake of ETI and a repeat sweat test was scheduled.

Adverse events

Analysis of the adverse effects associated with ETI revealed a good safety profile as previously described for children (Zemanick et al., 2021; Goralski et al., 2023), adolescents and adults (Heijerman et al., 2019; Middleton et al., 2019). Adverse events thought to be related to ETI therapy were three transitory rash incidents in children without any other sign of acute illness. The children and their parents opted to continue the modulator medication without interruption or dose reduction. The use of an oral antihistaminic agent was considered beneficial. While rash events are known to occur frequently when initiating ETI therapy (Heijerman et al., 2019; Middleton et al., 2019) even when LUM/IVA or TEZ/IVA had been tolerated without rash, other dermatologic problems such as acne have only recently been reported in the context of ETI therapy (Hudson et al., 2022). The causal relation remains to be clarified. Regarding the new onset of psoriasis seen in our patient thus far there is no published evidence to support a connection to ETI therapy.

Elevation of liver enzymes and creatinkinase

CFTR modulator therapy containing ivacaftor is well recognized to affect hepatic enzymes. Only a small percentage of patients cannot tolerate the full ETI dose due to consistently elevated AST or ALT. All CFTR modulators are metabolized by the CYP3A4 und CYP3A5 pathway, for which extensive pharmacogenetic heterogeneity exists. At this time, there is scarce information on drug plasma levels and their potential role in explaining side effects (Choong et al., 2022). In our study, only three patients needed to reduce the ETI dose because of hepatic toxicity. Pharmacogenetic testing revealed that two of these children had a reduced CYP3A4 activity while one boy with repeated elevation of liver enzymes had increased CYP3A4 und CYP3A5 activity. A recent study observed no connection between increased modulator plasma levels and reduced CYP 3A activity (Guimbellot et al., 2022). Three of our adolescent patients received ETI post liver transplantation. In this patient group, careful monitoring is necessary in order to diagnose pharmacologic interactions with immunosuppressive agents. No guidelines exist for the use of ETI in patients post liver transplant. Recently, a case series reported good tolerability of ETI therapy in post LTX patients with varying dose regimes and good clinical benefit (Ragan et al., 2022). In our limited experience, full dose ETI was tolerated without noticeable toxicity and immunosuppressive therapy remained well controlled. Elevation of CK is another well-known side effect of CFTR modulator therapy (Heijerman et al., 2019; Middleton et al., 2019; Zemanick et al., 2021) and is often asymptomatic. In one of our patients, CK was elevated more than usually seen but no predisposing condition was identified in the boy and therapy was continued without detrimental health effects.

Pseudotumor cerebri

We report the case of a 15-year-old normal-weight girl with new onset of pseudotumor cerebri, also referred to as idiopathic intracranial hypertension (IIH) (Sheldon et al., 2017). Reports from the pre-modulator era (Obeid et al., 2011) of CF children with IIH were limited to cases with severe malnutrition and Vitamin A deficiency. To the best of our knowledge, there have been four reports of one adult and three adolescents developing intracranial hypertension while taking ETI (Miller and Foroozan, 2022; Wisniewski et al., 2022). Three patients displayed variable degrees of Vitamin A serum level elevations while one patient died due to complications of a previously unknown intracranial malformation. In our patient, the vitamin A serum levels were normal before and after onset of ETI, and no other substances linked to the development of IIH, e.g., spironolactone or tetracyclines had been prescribed (Sheldon et al., 2017). At this time, the onset of IIH in our patient remains unexplained.

Limitations

We acknowledge several limitations of the present study. First, the data presented were collected retrospectively in a single center explaining the small number of patients compared to the multicenter clinical trials. Since all eligible children with CF were advised to start ETI therapy, data from a control group are not available. Due to the retrospective nature of this work, some relevant measurements such as fecal elastase or LCI could not be included in the analysis. Moreover, the follow-op duration of 6 months was too short to draw conclusions on the long-term term effect of ETI therapy in our patients. Data presented were partly collected during the COVID-19 pandemic, and contact restrictions may have reduced pulmonary exacerbation rates in our patients as previously described (Patel et al., 2021). However, this outcome is not reported here. Since our clinical surveillance routine was not significantly modified during the pandemic we do not assume that our data were noticeably affected during this period.

Outlook

Treatment with ETI has opened a new perspective to children with CF and one *F508del* mutation. In view of the potentially life-long therapy, further prospective investigations are required to assess the long-term effects of ETI on the mental and physical health of growing children. Since safety and efficacy of ETI were recently reported in children two to 5 years of age ETI therapy should soon be offered to this age group to prevent the onset of chronic organ damage. The treatment of children with non-*F508del* mutations continues to pose a great challenge emphasizing the

importance of identifying non-*F508del* genotypes responsive to ETI or future CFTR modulators.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethik-Kommission der Universität Duisburg-Essen. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

MO, FS, MW, SvS, SuS and MS developed the design of the study. MO and AK acquired primary data. AK, MS and RH performed the statistical analysis. AK and MS produced the graphs. MO wrote the first draft of the manuscript and revised the text. AK and MS completed sections of the manuscript. All authors critically read and revised the manuscript and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effects of lumacaftor—ivacaftor therapy on cystic fibrosis transmembrane conductance regulator function in F508del homozygous patients with cystic fibrosis aged 2–11 years

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Rationale: Lumacaftor/ivacaftor was approved for the treatment of patients with cystic fibrosis who are homozygous for F508del aged 2 years and older following positive results from phase three trials. However, the improvement in CFTR function associated with lumacaftor/ivacaftor has only been studied in patients over 12 years of age, while the rescue potential in younger children is unknown.

Methods: In a prospective study, we aimed to evaluate the effect of lumacaftor/ivacaftor on the CFTR biomarkers sweat chloride concentration and intestinal current measurement as well as clinical outcome parameters in F508del homozygous CF patients 2–11 years before and 8–16 weeks after treatment initiation.

Results: A total of 13 children with CF homozygous for F508del aged 2–11 years were enrolled and 12 patients were analyzed. Lumacaftor/ivacaftor treatment reduced sweat chloride concentration by 26.8 mmol/L ($p = 0.0006$) and showed a mean improvement in CFTR activity, as assessed by intestinal current measurement in the rectal epithelium, of 30.5% compared to normal ($p = 0.0015$), exceeding previous findings of 17.7% of normal in CF patients homozygous for F508del aged 12 years and older.

Conclusion: Lumacaftor/ivacaftor partially restores F508del CFTR function in children with CF who are homozygous for F508del, aged 2–11 years, to a level of CFTR activity seen in patients with CFTR variants with residual function. These results are consistent with the partial short-term improvement in clinical parameters.

KEYWORDS

CFTR modulator therapy, cystic fibrosis (CF), CFTR function, lumacaftor/ivacaftor, intestinal current measurement (ICM), sweat chloride

Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder caused by pathogenic variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The defect affects the CFTR protein, an ion channel in the apical membrane of epithelial cells, and leads to a multisystem disease that primarily affects the lungs, pancreas and gastrointestinal tract (Elborn, 2016). F508del is the most common CFTR variant and is present in more than 60% of all CF patients worldwide on at least one allele causing a defect in the folding and trafficking of the CFTR protein, resulting in impaired epithelial chloride and bicarbonate transport (Mall and Hartl, 2014). The lung disease remains the major cause of morbidity and mortality in CF and is caused by impaired mucociliary clearance leading to mucus plugging, infection with opportunistic germs, inflammation and subsequently the development of bronchiectasis (Bell et al., 2020; Mall et al., 2020).

The CFTR corrector lumacaftor and the potentiator ivacaftor were proven in combination (LUM/IVA) as first causative treatment designed to rescue CFTR protein function in patients with CF homozygous for the F508del variant (F/F). LUM/IVA was approved in the European Union (EU) on 18 November 2015 for the treatment of patients with CF being F/F over the age of 12 years. For pediatric use, the EU marketing authorization was extended on 08 January 2018 for children aged 6–11 years and on 15 November 2018 for children aged 2 years and older. It is therefore currently the only available CFTR modulator for children with CF being F/F between 2 and 5 years.

Approval for patients over 12 years of age was based on the results of two large phase-3 trials (Wainwright et al., 2015). However, in these studies, LUM/IVA resulted in only a modest improvement in FEV1%predicted, ranging from 2.6 to 4.0 percentage points compared to placebo, with considerable heterogeneity between study participants. The subsequent 96-week open-label extension study showed that the main benefit of LUM/IVA lay in the reduction of respiratory exacerbations, suggesting a lower annual loss of lung function (Konstan et al., 2017). Authorization for the pediatric patients with CF being F/F was based on a decrease of the Lung Clearance Index (LCI) in patients aged 6 years and older of 1.09 compared to placebo after 24 weeks and a decrease in sweat chloride concentrations (SCC) of 20.8 mmol/L (Ratjen et al., 2017). In patients aged 2–5 years, a drop in SCC of −31.7 mmol/L from baseline after 24 weeks was shown, alongside a significant increase in body weight and body-mass-index (BMI) (McNamara et al., 2019). The latter phase-3 trial lacked a placebo treated control group, thus clinical findings appear less convincing, especially as the placebo-controlled study in 6–11-year-olds had precisely not been able to demonstrate a significant increase in BMI (Ratjen et al., 2017). However, the considerable decrease in SCC hinted at a substantial role of CFTR biomarker testing for the prediction of long-term benefits of LUM/IVA. Since the therapeutic concept of CFTR modulator therapy aims at mitigating the long-term loss of pulmonary function, direct evaluation of CFTR function under treatment became desirable. In addition to SCC measurement, nasal potential difference (nPD) and intestinal current measurement (ICM) are considered functional tests that can be used as even more sensitive diagnostic tools for determining CFTR activity. With these tests, our group was able to demonstrate for 53 patients with CF being F/F above the age of 12 years that LUM/IVA rescued CFTR function in nPD to a level of 10.2% and in ICM to 17.7% of normal, respectively (Graeber et al., 2018).

The first aim of this study was therefore to investigate the efficacy of LUM/IVA correction of CFTR function in sweat ducts and intestine in pediatric patients aged 2–11 years. In order to do this, we designed a sub-study as part of an ongoing prospective longitudinal observational study at our CF center and measured the CFTR biomarkers sweat chloride concentration and ICM as well as clinical outcomes like BMI, spirometry and LCI at baseline and 8–16 weeks after starting treatment with LUM/IVA. As a second aim, we wanted to determine the relationship between partial rescue of F508del-CFTR function and potential short-term clinical response to LUM/IVA.

Methods

Routine clinical data were collected using standard methods and are not described further. Additional information on the special studies briefly described below can be found in the online Supplementary Material.

Study design and participants

The prospective mono-center sub-study was conducted within a prospective longitudinal observational study in patients with CF at the CF center of the University Children's Hospital Heidelberg, Germany, belonging to the German Centre for Lung Research (DZL). Both the sub-study and the above-mentioned overarching prospective longitudinal observational study were approved by the ethics committee of the Medical Faculty of the University of Heidelberg (S-489/2015 and S-370/2011, respectively). Patients who met the inclusion criteria were eligible to participate in the sub-study. This was the case if they were at least 2 but less than 11 years old, homozygous for the CFTR variant F508del (F/F), had no previous exposure to LUM/IVA and were willing to adhere to a stable medication regimen for the duration of the study according to the regulatory approved labelling and prescribing information for the appropriate age and weight. Exclusion criteria included acute respiratory infection or pulmonary exacerbation at baseline, or concomitant disease that may pose an additional risk to LUM/IVA administration or confound the results of the study. Participation in another clinical intervention trial also led to exclusion from our study. Written informed consent was obtained from all parents or legal guardians of participating patients. In all patients participating in the sub-study, sweat chloride concentrations and ICM (Graeber et al., 2015) as well as clinical parameters including anthropometry, clinical blood

chemistry, LCI, lung function test parameters (Quanjer et al., 2012) were determined at baseline and 8–16 weeks after starting the therapy with the individually approved LUM/IVA dose. The time window for the follow-up measurements was chosen to facilitate the planning of study visits under real-life conditions and had also been chosen in our previous study with this objective when examining patients over 12 years of age (Graeber et al., 2018).

Evaluation of CFTR activity

The assessment of CFTR function in this sub-study was done exclusively by measurement of SCC and ICM using rectal biopsies.

Sweat chloride concentration (SCC)

The SCC measurement was performed according to the German guideline "Diagnose der Mukoviszidose" (Naehrlich et al., 2013) and the guidelines of the Clinical and Laboratory Standards Institute (LeGrys, 2019). Samples were collected with the Macroduct® system (Model 3700, Wescor, Logan UT, United States). Sweat chloride concentration was measured in a minimum volume of 30 µL using a chloridometer (KWM 20 Chloridometer, Kreienbaum, Langenfeld, Germany).

Intestinal current measurement (ICM)

The ICM was performed as described before (Graeber et al., 2015) at baseline and after initiation of LUM/IVA. Endoscopic forceps biopsies were performed on rectal mucosa. Tracings were evaluated by two independent readers (J.B. and S.H.) and a mean of collected scores was used as a final outcome. Quality criteria were response to test stimulation with carbachol, stability of recordings as well as baseline and final tissue resistance. Normal CFTR activity was defined as response to cAMP dependent stimulation obtained in age-matched non-CF control subjects [age: 8.7 ± 5.1 (mean ± SD)], and percentage of normal CFTR function was calculated for each patient with CF by dividing the individual cAMP-induced I_{eq} of the patient by the median cAMP induced I_{eq} of the age-matched control group as previously described (Graeber et al., 2018).

Lung clearance index (LCI)

The LCI was determined using the Exhalyzer D system (EcoMedics) with the multiple breath washout (MBW) test. 100% oxygen was used to wash residual nitrogen from the lungs using a mouthpiece as an interface (Rowe et al., 2014). All measurements were analysed using Spiroware 3.3.1 (EcoMedics) (Wyler et al., 1985; Stahl et al., 2014; Graeber et al., 2021). The upper limit of normal was set at 7.1 (Wyler et al., 1985).

Statistical data analysis

The sample size calculation was based on a previous study by our group in adult F508del/F508del CF patients (Graeber et al., 2018). We calculated the sample size for the change in IBMX/forskolin in a paired *t*-test assuming a nominal type I error of 0.05 and a power of 0.8, resulting in an estimated sample size of 10 patients. Data were analyzed with the aid of statistical software Prism version 6 (Graph Pad Software Inc., San Diego, CA, United States) and Grapher version 8 (Golden Software LLC, Golden, CO, United States). Data were presented as median with interquartile range (IQR) or mean with standard deviation (SD). Parametric data were evaluated according to paired *t*-tests. Non-parametric data were evaluated by Wilcoxon matched pairs signed rank test. A *p*-value below 0.05 was considered significant.

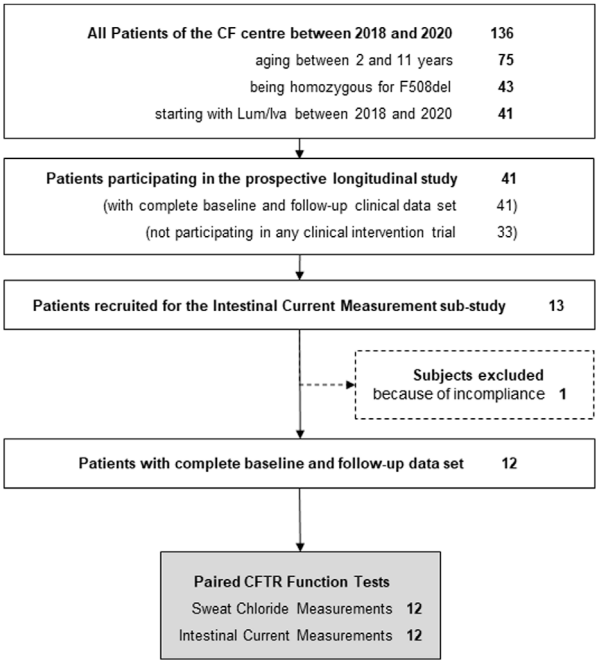

FIGURE 1
Flow chart of study design.

Results

Characteristics of study population

Between 2018 and 2020, 41 patients with CF who were F/F aged between 2 and 11 years, and who started with LUM/IVA, were treated at our CF centre. All these patients were part of an overarching prospective longitudinal study that provided the clinical data set for this sub-study. 8 of these 41 patients participated in other clinical intervention trials during this time, leaving 33 patients eligible for this sub-study (Figure 1). Of these, only 13 were finally recruited into the present sub-study, as a number of the patient's parents declined additional invasive testing as part of a trial on an approved drug. The 13 recruited patients received both the initial and follow-up ICM examination. One patient had to be excluded because he did not take LUM/IVA on the day of the second ICM, leaving 12 participants in the present ICM sub-study (Figure 1). From all 12 patients, 4 biopsies were measured for ICM at both baseline and follow-up (Figure 1). Anthropometric and clinical parameters before and during LUM/IVA treatment were also

TABLE 1 Patient characteristics at baseline and course of selected clinical parameters under LUM/IVA therapy (*n* = 12, if not stated otherwise).

Clinical characteristic	Baseline	LUM/IVA therapy	Change between baseline and LUM/IVA	*p*-value
Number of patients	12			
Age (years)	7.8 ± 2.7 (2.0–11.7)	8.2 ± 2.5 (3.5–11.9)		
Sex, female, n (%)	5 (42%)			
BMI z-score	−0.19 ± 0.80 (−1.6 to 1.2)	0.05 ± 1.02 (−1.7–2.0)	0.24 ± 0.69 (−0.3)	0.2500
Weight percentile	44.0 ± 29.7 (4.0–92.0)	50.3 ± 32.2 (5.0–97.0)	6.33 ± 10.87	**0.0195**
LCI, *n* = 10	8.1 ± 1.4 (5.9–11.2)	7.3 ± 0.8 (6.1–8.8)	−0.83 ± 0.96	**0.0332**
FEV1% predicted, *n* = 10	95.53 ± 15.72 (69.30–119.70)	91.38 ± 13.76 (68.0–113.5)	−4.15 ± 5.34	**0.0363**
VCmax % predicted, *n* = 10	94.61 ± 13.15 (75.80–109.20)	92.54 ± 13.43 (76.20–115.0)	−2.07 ± 4.61	0.1897
FEV1/VCmax % predicted, *n* = 10	99.94 ± 7.63 (87.10–111.6)	97.85 ± 9.78 (84.60–109.0)	−2.09 ± 6.15	0.3106
MEF25% predicted, *n* = 10	92.0 ± 47.44 (33.40–172.6)	82.0 ± 43.26 (27.50–160.0)	−10.0 ± 36.74	0.4117

Definition of abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 s; LCI, lung clearance index; VCmax, maximum vital capacity; MEF25, mean expiratory flow at 25% of capacity. Baseline refers to the time point of clinical evaluation before start with LUM/IVA, date of ICM, might deviate if diagnostic ICM, was performed in early childhood. Data are shown as mean ± SD (range).
Highlighted in bold are p-values below 0.05.

available for all 12 patients. Due to the young age of two patients, pulmonary function tests were only available for the remaining 10 patients. Table 1 shows in column "Baseline" the clinical characteristics at baseline of the sub-cohort of 2–11-year-old CF patients recruited for the ICM sub-study.

LUM/IVA improves CFTR function in sweat ducts and intestinal epithelia in patients with CF being F/F aged 2–11 years

To determine the effects of LUM/IVA on CFTR function *in vivo*, we measured SCC and ICM at baseline and after initiation of the therapy. SCC at baseline were 90.0 mmol/L (IQR 83.0–101.1), thus in the typical CF range. After start of LUM/IVA treatment a decrease by 26.8 mmol/L (IQR 12.9 to 46.9; *p*-value 0.0006) was observed (Figure 2A). Looking at the subjects individually, two patients had no improvement in SCC and one patient had only a very moderate improvement in SCC. Assignment of distinct symbols for each patient in this study's graphs allows for visual traceability throughout the different CFTR function tests (*cf.* below). ICM measurements showed a significant improvement in CFTR function for the investigated 2–11-year-old patients. At baseline, the absence of relevant Cl-secretion was confirmed upon cAMP-mediated stimulation (Figure 2B). In these samples, small negative currents (I_{eq} = −10.7 μA/cm^2, IQR −22.9 to −4.9) could be observed after cAMP-induced stimulation, which is consistent with previous results and most likely due to concomitant K^+ secretion (Veeze et al., 1991; Graeber et al., 2018). After initiation of LUM/IVA, cAMP-mediated stimulation increased intestinal currents to 27.3 μA/cm^2 (IQR 10.1 to 67.0; *p*-value 0.0015), corresponding to a rescue of CFTR function in the rectal mucosa to a level of 30.5% of normal (IQR 9.9–46.5). Examination of the total chloride response, which comprises cAMP-mediated stimulation augmented by cholinergic co-activation, confirmed these findings with a median of differences of 63.7 μA/cm^2 (IQR 40.7 to 109.4; *p*-value 0.0003; Figure 2C). Total chloride response under LUM/IVA corresponded to 16.0% of normal total chloride response in age matched controls (IQR 8.2–24.2). It is interesting to note that the ICM improved under LUM/IVA even in those patients who did not show any improvement in the SCC at the same time. To confirm that improved ion secretion indeed depended on CFTR function, selective inhibition with the CFTR inhibitor-172 subsequent to cAMP-mediated and cholinergic stimulation was performed. The resulting ΔI_{eq} at baseline and under therapy were compared. It could be shown that the inhibitory potential under LUM/IVA was significantly bigger than before (−28.2 μA/cm^2 vs. −9.0 μA/cm^2; *p*-value 0.024; Figure 2D), proving that cAMP-induced I_{eq} responses reflected CFTR-dependent chloride secretion. This was confirmed by a positive correlation (R2 = 0.47, *p*-value 0.014) between cAMP response and the degree of consecutive CFTR inhibition by the CFTR inhibitor-172 (Figure 3A).

Substantial concordance but no quantitative correlation among CFTR biomarkers

Next, we investigated the relationship between sweat chloride secretion and cAMP-induced chloride secretion in the ICM, because the improvements seen there after starting LUM/IVA therapy raised the question of a possible correlation between these CFTR biomarkers, such that one may predict the other. However, no correlations were observed between the change in SCC and the change in ICM response (R2 = 0.0006; *p* = 0.9440). Nonetheless, a high concordance was

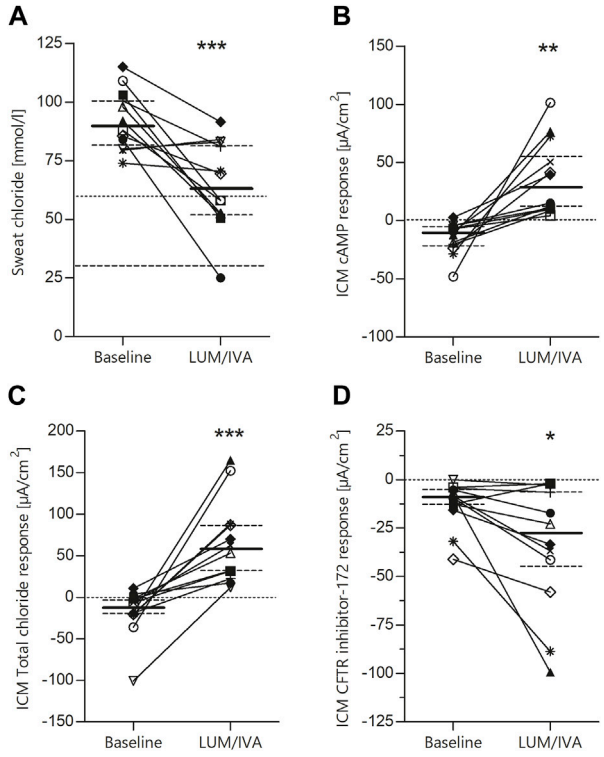

FIGURE 2
Significant improvement of CFTR function in F508del homozygous patients by LUM/IVA treatment. Paired values of individual study subjects before and after the start of LUM/IVA treatment for sweat chloride [**(A)**; median baseline 90.0, IQR 83.0–101.1; median LUM/IVA 63.8, IQR 52.0–82.0; median of difference—26.8 IQR 12.9–46.9; *p*-value 0.0006], cAMP response in rectal biopsies measured in the Ussing chamber [**(B)**; median baseline −10.7, IQR −22.9 to −4.9; median LUM/IVA 27.3, IQR 10.1–67.0; median of difference 34.0, IQR 18.9–70.8; *p*-value 0.0015], total chloride response considering intestinal current after stimulation with carbachol [**(C)**; median baseline—12.6, IQR −20.2 to −1.9; median LUM/IVA 57.8, IQR 29.5–87.3; median of difference 63.7, IQR 40.7–109.4; *p*-value 0.0003] and change of intestinal current after treatment with the CFTR inhibitor 172 [**(D)**; median baseline—9.0, IQR −13.5 to −5.1; median LUM/IVA −28.2, IQR −45.6 to −5.6; median of difference −14.5, IQR −29.2 to −2.7; *p*-value 0.0237]. Statistical evaluation by means of paired t-tests. Solid lines represent the median and dashed lines represent the 25th and 75th percentiles.

observed for the two CFTR biomarkers in 10 out of 12 patients (Figure 3B). Furthermore, all patients with CF being F/F in our sub-study showed improvements in at least one of the CFTR biomarkers.

Short-term measures of clinical outcomes show a slight benefit from LUM/IVA treatment

We investigated the response of selected clinical parameters to improved CFTR activity following LUM/IVA therapy. Conventional lung function tests showed no relevant functional improvement in a simple before and after comparison (Table 1). In the sub-cohort of 12 patients we studied, there was even a significant decrease in FEV1%predicted by −4.15 percentage points (Table 1). However, in contrast to conventional pulmonary function test, a significant improvement in LCI was observed which decreased by −0.7 with LUM/IVA treatment (median differences, *p*-value 0.0371; Table 1, Supplementary Figure S1A). Linear regression analysis revealed no quantitative correlation between the two CFTR biomarkers SCC and ICM cAMP response and LCI (sweat chloride: R2 = 0.004; $p = 0.86$, ICM: R2 = 0.06; $p = 0.50$). However, there was moderate concordance in 6 out of 10 patients for sweat chloride concentration and good concordance in 8 out of 10 patients for ICM cAMP response (Supplementary Figures S1B, C).

Regarding weight gain in patients treated with LUM/IVA, there was an increasing trend in BMI z-score, but this was not significant (*p*-value 0.25). However, it was notable that the tendency to cross the age-dependent weight percentiles upwards was significant in our cohort (median difference 4; median pre-treatment 40.5, IQR 18.3–61.8; median post-treatment 48.0, IQR 25.8–79.5; +6.3 percentiles; *p*-value 0.02; Table 1).

Discussion

This is the first study to investigate the effects of combination therapy with the CFTR modulators lumacaftor and ivacaftor (LUM/IVA) on CFTR function in organ systems such as sweat glands and the intestine using the CFTR biomarkers SCC and ICM in a cohort of 12 children with CF with two F508del less than 12 years of age and to correlate these results with short-term clinical outcome.

LUM/IVA is currently the only available CFTR modulator for children with CF who are F/F between 2 and 5 years of age and, along with tezacaftor-ivacaftor (TEZ/IVA) and the recently approved elexacaftor-tezacaftor-ivacaftor (ELX/TEZ/IVA), the only CFTR modulator therapy for children with CF who are F/F older than 6 years of age [e.g., (Ramsey and Welsh, 2017; De Boeck, 2020)]. Phase-3 studies have investigated the safety and clinical efficacy of LUM/IVA therapy in both adults and children (Wainwright et al., 2015; Konstan et al., 2017; Ratjen et al., 2017; McNamara et al., 2019) and in a previous prospective multicentre study (Graeber et al., 2018), our group was able to evaluate the effect of LUM/IVA on CFTR function in different organ systems in patients with CF who are F/F over 12 years of age using the CFTR biomarkers SCC, nPD and ICM. The present prospective monocenter observational study evaluates the *in vivo* effects of LUM/IVA on CFTR function in children aged 2–11 years by measurement of SCC and ICM, as nPD cannot yet be performed safely without sedation and with sufficient quality in infants and preschool children. Furthermore, unlike nPD, ICM can achieve standardized conditions even in very young children.

In our study in a cohort of 12 patients, the assessment of functional correction by the CFTR biomarkers SCC and ICM showed for the first time that LUM/IVA therapy results in consistent partial rescue of CFTR function in different organs in children with CF who are F/F between the ages of 2 and 11 years (Figures 2A–D). For the CFTR biomarker SCC our results show an average reduction of −26.8 mmol/L, which is comparable to the previously mentioned phase-3 studies where the reduction was −32 mmol/L in children aged 2–5 years (McNamara et al., 2019) and −20 mmol/L in children aged 6–11 years (Milla et al.,

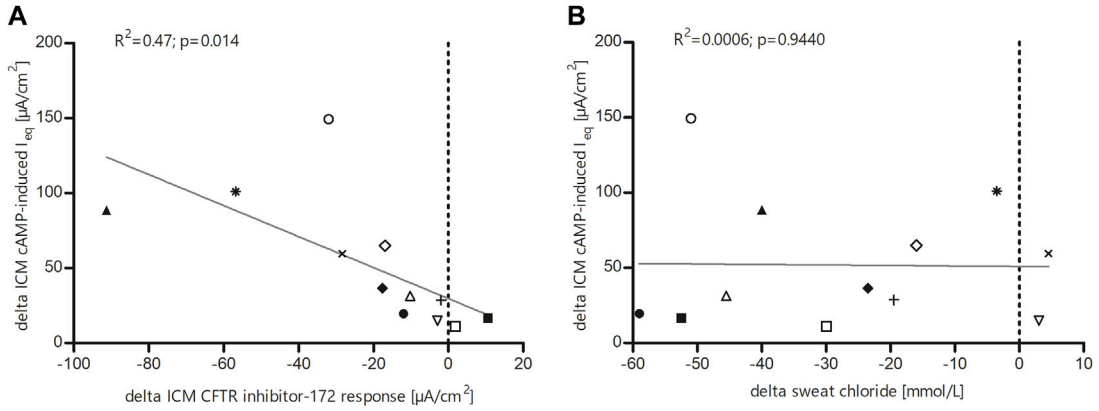

FIGURE 3
Linear regression analysis **(A)** between cAMP response and change in intestinal current after treatment with the CFTR inhibitor 172 and **(B)** between the different CFTR function surrogates sweat chloride and ICM cAMP response. **(A)** Significant correlation of individual study subjects' values within the ICM measurements of cAMP response and change in intestinal current after treatment with the CFTR inhibitor-172. **(B)** Relationship between change in ICM cAMP response and change in sweat chloride levels in 12 F508del homozygous children under LUM/IVA treatment. Change in ICM response did not correlate with change in sweat chloride levels.

2017; Ratjen et al., 2017). Compared with our previous multicenter study in patients over 12 years of age, in which we measured a reduction in SCC of −18 mmol/L (Graeber et al., 2018), and an earlier phase-2 study in adult patients, in which the decrease in SCC with LUM/IVA therapy was only −8 to −10 mmol/L (Boyle et al., 2014), this suggests that the efficacy of LUM/IVA in improving CFTR function in the sweat glands may be more pronounced in children than in adults. In this respect, the comparison with the other approved CFTR modulators TEZ/IVA and ELX/TEZ/IVA is also interesting. Recently, we were able to report results on the improvement of CFTR activity with these therapies in F/F CF patients older than 12 years. In 18 patients treated with a TEZ/IVA therapy for the first time a reduction in SCC of −13.0 mmol/L could be shown (Graeber et al., 2022a). After these patients were switched to ELX/TEZ/IVA, SCC decreased further by −50.5 mmol/L (Graeber et al., 2022a). Compared to baseline, ELX/TEZ/IVA resulted in a mean reduction in SCC of −61.0 mmol/L (Graeber et al., 2022a).

The ICM measurements performed in our sub-study cohort of children with CF being F/F from 2 to 11 years showed a rescue of cAMP-mediated stimulation of CFTR activity in the rectal epithelium to a level of about 30% of normal (Veeze et al., 1991). This is higher than demonstrated in our previous study in patients with CF who are F/F over 12 years of age, where an improvement in CFTR function to 18% was shown (Veeze et al., 1991; Graeber et al., 2018). Notably, the rescue of CFTR activity in the present study in children was also above the level of functional improvement of about 25% maximum correction shown for LUM/IVA combination therapy in in vitro studies on primary bronchial epithelial cultures from F/F CF patients (Van Goor et al., 2011). In comparison, TEZ/IVA showed slightly worse results in the age group over 12 years, where an improvement in cAMP-mediated stimulation of CFTR activity of about 14% was seen in the 18 patients included in the aforementioned study (Graeber et al., 2022a). However, after switching these patients to ELX/TEZ/IVA, this value increased to 46% of normal CFTR activity (Graeber et al., 2022a). These results are comparable to patients with CF with a Gly551Asp mutation treated with ivacaftor monotherapy, resulting in improvements of 52% of normal CFTR activity in ICM studies (Graeber et al., 2015).

Our ICM studies show that LUM/IVA combination therapy improves CFTR activity in F/F CF patients aged 2–11 years to levels comparable to the lower range of levels in patients with CF and CFTR variants with residual CFTR function (Hirtz et al., 2004). Previous nPD and ICM studies examining patients with CF with a spectrum of CFTR variants with residual function demonstrated CFTR mediated ion transport in the range of about 10%–50% of normal and showed that inherent residual function greater than about 10% of normal may be associated with long-term exocrine pancreatic sufficiency (Hirtz et al., 2004; Wilschanski et al., 2006; Stanke et al., 2008; Derichs et al., 2010). This may explain why LUM/IVA therapy even led to an improvement in faecal elastase in younger children in Phase three trials (McNamara et al., 2019). Taken together, these data suggest that the degree of functional rescue achieved with LUM/IVA may improve long-term clinical outcomes in patients with CF who are F/F. The findings on the higher efficacy of LUM/IVA therapy in childhood can therefore also be seen as an argument for starting treatment in early childhood, even if short-term clinical improvements in these patients are not always immediately observable (Milla et al., 2017; Ranganathan et al., 2017; McNamara et al., 2019; Stahl et al., 2021). However, whether pharmacological rescue of CFTR function to 30% of normal CFTR activity has an impact on the clinical course and life expectancy of patients with CF remains to be shown in longer-term prospective observational or registry studies.

Linear regression analysis revealed that the increase in cAMP response in ICM under LUM/IVA correlates well with the increasing effect of the CFTR inhibitor 172, thus providing consistent electrophysiological findings (Figure 3 A). Our analyses of the relationship between SCC and CFTR-mediated Cl-secretion determined by ICM showed no correlation but a high concordance between the two CFTR biomarkers (Figure 3B). This

was also the case in our study of LUM/IVA therapy in CF patients who are F/F over 12 years (Graeber et al., 2018). We suspect that several reasons may have contributed to this result. First, the way CFTR function is represented by the two CFTR biomarkers is different. While sweat chloride is an indirect measure of salt uptake in sweat ducts, ICM is a more direct measure of CFTR-mediated chloride flux. Furthermore, the bioavailability of LUM/IVA may vary in different tissues. Nevertheless, the analyses of the relationship between SCC, nPD and ICM in the TEZ/IVA and ELX/TEZ/IVA study mentioned above showed correlations between the three CFTR biomarkers in all treatment groups (Graeber et al., 2022a). However, this may have been due to the more pronounced improvements in CFTR activity in the ELX/TEZ/IVA-treated patients. Regardless, the results of the different studies suggest that the CFTR biomarkers are not directly interchangeable, but provide complementary information about CFTR function when tested in clinical trials (Graeber et al., 2015; Graeber et al., 2018; Graeber et al., 2022a).

The cohort studied was relatively small to evaluate short-term clinical changes with initiated LUM/IVA therapy. Nevertheless, we saw improvements in LCI and weight gain of patients under real-world conditions to levels comparable to the results of the phase three trials for LUM/IVA approval (Ratjen et al., 2017; McNamara et al., 2019). Specifically, the absolute change in LCI in our study was −0.7 (Table 1, Supplementary Figure S1A), compared with −1.0 in the cohort of children with CF aged 6–11 years (Ratjen et al., 2017) and −0.58 in the cohort of children with CF aged 2–5 years (McNamara et al., 2019). Consistent with this, a substantial concordance, but no correlation, was observed in the study sub-cohort between improvement in CFTR function, as measured by the two CFTR biomarkers SCC and ICM, and LCI as a significantly improved clinical parameter (Supplementary Figures S1B, C). These data suggest that clinical outcome is influenced by numerous factors independent of CFTR function, such as fixed airflow limitation due to irreversible structural lung damage, which may influence and mask improvement in clinical parameters, especially considering the relatively moderate degree of functional correction of CFTR function achieved with LUM/IVA. Furthermore, no improvement was seen in other lung function parameters, such as FEV1%predicted. BMI, which was also included in the phase-3 trials in children, also showed no significant improvement in our study cohort of children with CF aged 2–11 years on LUM/IVA therapy. However, the increase in body weight over the age-adjusted weight percentiles was significant in our study cohort (+6.3 percentile; *p*-value 0.02; Table 1). Thus, this study, as well as previous LUM/IVA clinical trials, shows that the heterogeneity in clinical endpoints is very high (Boyle et al., 2014; Wainwright et al., 2015; Konstan et al., 2017; Ratjen et al., 2017; Graeber et al., 2018). However, as the CFTR biomarkers SCC, nPD and ICM also showed much lower heterogeneity in our previous studies on IVA monotherapy and combination therapies with LUM/IVA, TEZ/IVA and ELX/TEZ/IVA in CF patients older than 12 years, we conclude that CFTR biomarkers are more sensitive than the established clinical endpoints FEV1%predicted and BMI to detect partial functional rescue of mutant CFTR by CFTR modulator therapy (Graeber et al., 2015; Graeber et al., 2018; Graeber et al., 2022a).

This study has several limitations. First, similar to previous observational studies on the effects of CFTR modulators, which were also conducted after the marketing authorization of the respective drug (Rowe et al., 2014; Graeber et al., 2015; Boutin et al., 2018; Ronan et al., 2018; Graeber et al., 2022b), it was not possible to include a placebo group in our study. However, the results of our CFTR biomarker measurements at baseline are consistent with previous ICM studies in independent cohorts of patients with CF who are F/F (Bronsveld et al., 2000; Bronsveld et al., 2001; Mall et al., 2004; van Barneveld et al., 2010; Graeber et al., 2018). In addition, all functional measurements were performed in a strictly paired manner to reduce background variability that may arise from unpaired comparison of CFTR biomarker measurements in different patients.

Second, in contrast to our previous study, we did not perform nPD examinations in this study, because in our experience this examination can only be performed reliably in children aged 8–9 years and older. We are aware that there are groups that perform these examinations in younger patients under sedation [e.g., (Sermet-Gaudelus et al., 2010)], but such sedation makes the procedure even more invasive, considering that the drug is already on the market. Therefore, based on the experience of good agreement between nPD and ICM measurements in the previous study, we had decided not to perform nPD in this age group.

Third, the conclusions that can be drawn from our data about the relationship between the extent of functional rescue of CFTR protein and improvement in clinical outcome are limited by 1) the small number of patients available for this study and 2) the limited duration of clinical follow-up. Therefore, evidence from longitudinal studies in larger cohorts, including patients with CF who are F/F, as well as patients with CFTR gating and residual function variants treated with different CFTR modulator therapies, is needed to further understand the relationship between the extent of functional rescue of CFTR protein and clinical response, including the long-term impact on disease progression and survival.

In summary, this study demonstrates for the first time that LUM/IVA treatment consistently induces partial rescue of CFTR function in F508del homozygous patients with CF aged 2–11 years, although clinical outcome parameters showed only partial improvement after a relatively short course of treatment. With LUM/IVA therapy, ICM in these young patients demonstrated functional rescue of F508del-CFTR in the intestinal epithelium at a level of approximately 30% of normal CFTR function, which is comparable to the CFTR activity previously observed in pancreas sufficient patients with CF who have a spectrum of CFTR variants with residual function (Hirtz et al., 2004; Stanke et al., 2008). With the approval of more effective CFTR modulators than LUM/IVA for this age group, further studies are needed to evaluate the rescue of CFTR activity. The CFTR biomarkers SCC and ICM used in this study appear to be well suited for future research to assess the relationship between therapeutic rescue in CFTR function and long-term clinical outcomes in children with CF.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Medical Faculty of University Hospital Heidelberg. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Conception and design: JB, OS, MM, and SG. Acquisition, analysis and interpretation of data: JB, SG, SH, YY, AK, MS, SH, HS, MM, and OS. Writing the manuscript or revising it critically for important intellectual content: JB, OS, MM, SG, SH, YY, AK, and MS. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MS declares concerning grants or contracts from any entity: MBW overreader Service and RIA grant from Vertex Pharmaceuticals; concerning participation on a Data Safety Monitoring Board or Advisory Board: payment to institution from Vertex Pharmaceuticals; concerning leadership or fiduciary role in other board, society, committee or advocacy group: Chairman FGM (unpaid), Secretary Group CF ERS (unpaid), Treasurer GPP (unpaid). MM received grants from Vertex Pharmaceuticals and personal fees for participation in advisory boards or consulting from Abbvie, Antabio, Arrowhead Pharmaceuticals, Boehringer Ingelheim, Enterprise Therapeutics, Kither Biotech, Pieris Pharmaceuticals, Santhera, Sterna Biologicals, Vertex Pharmaceuticals, outside the submitted work. OS received payments from Vertex Pharmaceuticals for lectures. OS, SH, AK, and JB participated in official clinical intervention trials (Vertex Pharmaceuticals) with regular payment to the institution within the last 36 months.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1188051/full#supplementary-material

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Elexacaftor-tezacaftor-ivacaftor in patients with cystic fibrosis ineligible for clinical trials: a 24-week observational study

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Introduction: Seminal clinical trials with the triple combination of elexacaftor-tezacaftor-ivacaftor (ETI) demonstrated clinical efficacy in people with cystic fibrosis (pwCF) who carry at least one F508del mutation. However, due to exclusion criteria of these clinical trials, the effect of ETI was not studied in a substantial number of pwCF. Thus, we ran a single center trial to evaluate a clinical efficacy of ETI treatment in adult pwCF who were ineligible for enrollment in registration studies.

Methods: PwCF on ETI with prior lumacaftor-ivacaftor therapy, severe airway obstruction, well-preserved lung function, or with airway infection with pathogens at risk of more rapid decline in lung function formed the study group, while all the others on ETI formed the control group. Lung function, nutritional status and sweat chloride concentration were assessed before and after initialization of ETI therapy over a 6-month period.

Results: Approximately a half of the ETI-treated pwCF at the adult Prague CF center (49 of 96) were assigned to the study group. Their mean changes in body mass index (+ 1.04 kg/m^2) and in sweat chloride concentration (–48.4 mmol/L) were similar to the control group (+ 1.02 kg/m^2; –49.7 mmol/L), while the mean change in percent predicted forced expiratory volume in 1 s (ppFEV$_1$; + 10.3 points) was significantly lower than in the control group (+ 15.8 points) (p = 0.0015). In the subgroup analysis, pwCF with severe airway obstruction (ppFEV$_1$ <40) and pwCF with well-preserved lung function (ppFEV$_1$ >90) showed a less potential for improvement in lung function during the ETI treatment than controls (median change in ppFEV$_1$ + 4.9 points and + 9.5 points, respectively).

Conclusion: PwCF not eligible for inclusion in clinical trials demonstrated improvement in lung function and nutritional status following the initiation of treatment with the ETI combination. Moderate increase in ppFEV$_1$ was observed in those with severe airway obstruction or well-preserved lung function.

KEYWORDS

cystic fibrosis, variant specific therapy, elexacaftor-tezacaftor-ivacaftor, lung function, nutritional status, sweat chloride concentration

1 Introduction

Cystic fibrosis (CF) is the most common inherited life-shortening disease among Caucasians. As indicated by a latest U.S. registry report (Cystic Fibrosis Foundation, 2022), life expectancy for people with CF (pwCF) is more than 50 years and the most frequent cause of death is progressive lung disease (Elborn, 2016).

For many decades, the cornerstone of CF care was only symptomatic approach. Mucoactive drugs along with chest physiotherapy, antibiotics, anti-inflammatory drugs, bronchodilators, oxygen therapy, noninvasive ventilation and ultimately lung transplantation have been used to treat impaired mucus clearance, airway infection and inflammation, and respiratory disorders (Girón Moreno et al., 2021).

The discovery of the cystic fibrosis transmembrane regulator (*CFTR*) gene in 1989 raised hopes for a curative therapy. Unfortunately, after more than 30 years the gene therapy is yet unavailable. However, since 2012 variant-specific therapy (VTS) has become a clinical reality. The first-in-class drug was ivacaftor (IVA), potentiating the CFTR protein function in pwCF carrying G551D or other gating mutations of the *CFTR* gene (Ramsey et al., 2011). IVA alone was followed by lumacaftor (in therapeutic combination with ivacaftor; LUM/IVA) for patients homozygous for F508del and tezacaftor (in combination with ivacaftor; TEZ/IVA) for patients homozygous for F508del or compound heterozygotes with residual function mutation (Wainwright et al., 2015; Rowe et al., 2017; Taylor-Cousar et al., 2017).

Finally, elexacaftor in combination with tezacaftor and ivacaftor (ETI) was approved for clinical use in 2019 for pwCF aged 12 years and older who carry at least one F508del mutation (Heijerman et al., 2019; Middleton et al., 2019). Subsequently, the ETI combination was shown to be superior to IVA for pwCF with the F508del along with the gating mutation, as well as to the TEZ/IVA combination for pwCF with the F508del along with the residual function mutation (Barry et al., 2021).

The above-mentioned studies demonstrated an improvement in lung function, nutritional status and quality of life together with a reduction in the pulmonary exacerbation rate and, in case of IVA and the ETI, the studies also proved a considerable decrease of sweat chloride concentration.

In general, inclusion and exclusion criteria of the respective clinical trials allowed only a clinically stable patient population to be enrolled. Thus, pwCF with severe airway obstruction (a percent predicted forced expiratory volume in one second ($ppFEV_1$) below 40) or those harboring bacteria with a high risk of a rapid decline in lung function (*Burkholderia cenocepacia*, *Burkholderia dolosa*, *Mycobacterium abscessus*) were excluded from the trials. Also, pwCF with a well-preserved lung function ($ppFEV_1$ above 90) were outside the range of 40–90, a key inclusion criterion that best sets the baseline for the change to observe statistically meaningful changes of $ppFEV_1$ during the trial.

The aim of our study was to evaluate the real-world results of ETI treatment in pwCF who do not fulfill the classical criteria for participation in the trials. We also analyzed the clinical effect of the switch to ETI from LUM/IVA, which in contrast to TEZ/IVA switch (Heijerman et al., 2019) has not been studied before.

2 Materials and methods

2.1 Study participants

Adult pwCF attending the CF Center in Prague, Czech Republic, on ETI treatment were included in the study. The diagnosis of CF was confirmed in all cases by clinical presentation, sweat test and *CFTR* gene analysis. ETI treatment was initiated between July 2021 and April 2022. First 6 months of therapy represented a time period selected for the analysis of $ppFEV_1$ and BMI on therapy (see below). Patients previously treated with LUM/IVA (n = 10), with $ppFEV_1$ below 40 (n = 15) or above 90 (n = 20), or infected with *B. cenocepacia* (n = 14) or *M. abscessus* (n = 2) were all included in the study group (n = 49; 12 of them fell into more than one category), whereas others were controls (n = 46). Patients on ETI who were first treated with IVA or TEZ/IVA were not included in the study as they were, contrary to LUM/IVA treated pwCF, already assessed in registration studies.

2.2 Assessments

Demographic data (sex and age) and information regarding airway infection and previous VST were obtained from medical records. Lung function testing (spirometry) and assessment of nutritional status (measurement of body weight and body height with calculation of body mass index; BMI) were performed according to standard procedures during routine outpatient visits. The best values of $ppFEV_1$ and BMI up to 6 months before (clinical appointments are performed every 3 months) and after initiation of ETI treatment (visits at month 1, 3, and 6) were recorded for the analysis. Sweat tests were performed using the Macroduct® Sweat Collection System and the ChloroChek® Chloridometer® Sweat Chloride Analyzer (Wescor Inc., United States) before and after the initiation of ETI treatment (between month 3 and 6).

2.3 Statistical analysis

The changes in sweat chloride concentration values and in the best $ppFEV_1$ and BMI values before and after the initiation of ETI treatment were compared between the study and control groups. Changes in best ppFEV1 values were also analyzed in subgroups of the study group. Statistical analysis was performed using TIBCO Statistica 13 (TIBCO Software Inc., USA). Normal distribution of the data was evaluated using Kolmogorov-Smirnov test and means (±SD) or medians (IQR) were used where appropriate, as well as t-tests and Mann-Whitney U-tests for comparison between groups. A p-value <0.05 was considered statistically significant.

3 Results

Demographic and clinical data of the patients on ETI are summarized in Table 1. In addition to *Pseudomonas aeruginosa*, *B. cenocepacia* and *M. abscessus*, several subjects had airway infections caused with methicillin-resistant *Staphylococcus aureus*

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TABLE 1 Demographic and clinical characteristics of the patients.

Characteristics	Study group (n = 49)	Control group (n = 47)
Sex		
Female	23 (47%)	25 (53%)
Male	26 (53%)	22 (47%)
Age at the initiation of ETI treatment		
Mean (SD), years	29.2 (7.2)	29.4 (7.2)
CFTR gene mutation		
F508del/F508del	28 (57%)	31 (66%)
F508del/other:	21 (43%)	16 (34%)
CFTRdele2,3	6	6
1898+1G>A	2	0
2134delT	1	0
2184insA	1	0
2789+5G>A	1	0
3143del9	1	1
G27R	1	0
G542X	1	1
I336K	1	1
L1335P	1	0
N1303K	1	1
Q372X	1	0
R347P	1	0
W1282X	1	0
W57G	1	0
2176delA	0	1
574delA	0	1
622-1G>C	0	1
CFTRdel1-10	0	1
L1324P	0	1
L138ins	0	1
Airway infection		
P. aeruginosa†	11 (22%)	27 (57%)
B. cenocepacia	14 (29%)	0
M. abscessus	2 (4%)	0
CFTR modulator therapy before ETI		
Lumacaftor/ivacaftor	10 (20%)	0
FEV_1		
Mean (SD), % predicted value	68.8 (30.4)	69.0 (14.1)
Distribution of FEV_1 values before ETI		

(Continued on following page)

TABLE 1 (*Continued*) Demographic and clinical characteristics of the patients.

Characteristics	Study group (n = 49)	Control group (n = 47)
>90% pred.	20 (41%)	0
≥40 to ≤90% pred.	14 (28%)	47 (100%)
<40% pred.	15 (31%)	0
Body mass index		
Mean (SD), kg/m^2	23.21 (4.30)	21.74 (2.70)
Sweat chloride concentration before ETI		
Mean (SD), mmol/L	98.0 (11.0)	100.0 (10.7)

Nominal data are n (%). CFTR, cystic fibrosis transmembrane conductance regulator. Forced expiratory volume in 1 s (FEV_1) and body mass index values were the best values in the 6 months before the initiation of ETI, treatment. †Intermittent and chronic infections with *P. aeruginosa* were both reported together.

TABLE 2 The changes in lung function, nutritional status and sweat chlorides on ETI treatment.

Parameter	Study group	Control group	*p*-value[a]
Change in FEV_1			
Mean (SD), % predicted value	+10.3 (7.8)	+15.8 (8.7)	0.0015
Females (F)	+10.9	+14.8	
Males (M)	+9.9	+17.0	
p-value[a] (F vs. M)	0.66	0.40	
Change in body mass index			
Mean (SD), kg/m^2	+1.04 (0.97)	+1.02 (0.67)	n.s.
Females (F)	+1.01	+0.92	
Males (M)	+1.05	+1.08	
p-value[a] (F vs. M)	0.59	0.42	
Change in sweat chloride concentration			
Mean (SD), mmol/L	−48.4 (18.9)	−49.7 (16.1)	n.s.

FEV_1 = forced expiratory volume in 1 s.
[a]*t*-test. n.s = not significant.

TABLE 3 Analysis of change in lung function in subgroups of the study population.

Subgroup	Change in FEV_1 median (IQR), % predicted value
Prior treatment with LUM/IVA (n = 10)	+8.6 (+7.3 to +13.0)
$ppFEV_1$ < 40%, without prior LUM/IVA treatment (n = 10)	+4.9 (+1.9 to +8.2)[a]
$ppFEV_1$ > 90%, without prior LUM/IVA treatment (n = 19)	+9.5 (+4.4 to +14.3)[b]
$ppFEV_1$ 40%–90% with pathogens at risk of more rapid FEV_1 decline, without prior LUM/IVA treatment (n = 10)	+18.7 (+9.0 to +21.6)
Control group (n = 47)	+13.3 (+9.2 to +22.3)

$ppFEV_1$ = a percent predicted forced expiratory volume in 1 s. IQR, interquartile range; LUM/IVA, lumacaftor/ivacaftor.
[a]Lower than in the control group (p = 0.0003, Mann-Whitney U test);
[b]lower than in the control group (p = 0.0067, Mann-Whitney U test).

(n = 6), *Achromobacter xylosoxidans* (n = 1), *Burkholderia stabilis* (n = 1) or *Burkholderia contaminans* (n = 1).

Changes in lung function, nutritional status and sweat chloride concentration in the study and control groups after initiation of ETI treatment are shown in Table 2. The study population reached such changes in nutritional status and sweat chloride concentration that were comparable to the control group (i.e., there were found no significant differences in changes of both parameters between the groups). The improvement of lung function in the control group was higher than in the study group. Further stratification by sex showed no statistically significant difference between males and females within each of the two groups for FEV_1 or BMI (Table 2).

Further analysis of the lung function change was based on subgrouping of the study group participants according to following parameters: prior treatment with LUM/IVA, baseline ppFEV1 values and airway infection with pathogens at risk of more rapid decline in ppFEV1. Results are summarized in Table 3.

This analysis showed that patients with severe airway obstruction or with well-preserved lung function had a lower potential for improvement with ETI treatment compared to the control group. Changes in lung function in individual subjects of studied subgroups are depicted in Figure 1.

4 Discussion

PwCF ineligible for participation in clinical trials represent a substantial part of the CF population. This is well documented in this study, where approximately a half of all patients treated with ETI (i.e., 49 of 96 patients) met one classical exclusion criterion or more. The aim of our study was to check the ETI effect on patients whose clinical conditions fall outside the inclusion criteria of clinical trials that were represented with registration studies for ETI. We believe that such postmarketing data are of paramount importance as it ensures not only a CF community, but also regulatory agencies and healthcare payers that the ETI therapy is effective in pwCF who were ineligible for classical clinical trials. For instance, the efficacy of the ETI treatment on pwCF with *B. cenocepacia* is found to be highly relevant to the Czech CF care where the prevalence of the infection is much higher (13%) compared to other European countries (4%) (Orenti et al., 2022).

Clinical data on lung function and nutritional status were collected from routine outpatient visits. We reported the best values of $ppFEV_1$ and BMI during the 6 months before and after the initiation of ETI treatment for evaluation of clinical efficacy. This approach to analyze the best $ppFEV_1$ and BMI values was chosen to minimalize the effect of intra-individual visit-to-visit variability, and for $ppFEV_1$ it was similar to the common strategy of four-week screening/run-in period in clinical trials to ensure a patient is clinically stable. This is a similar approach to the assessment of

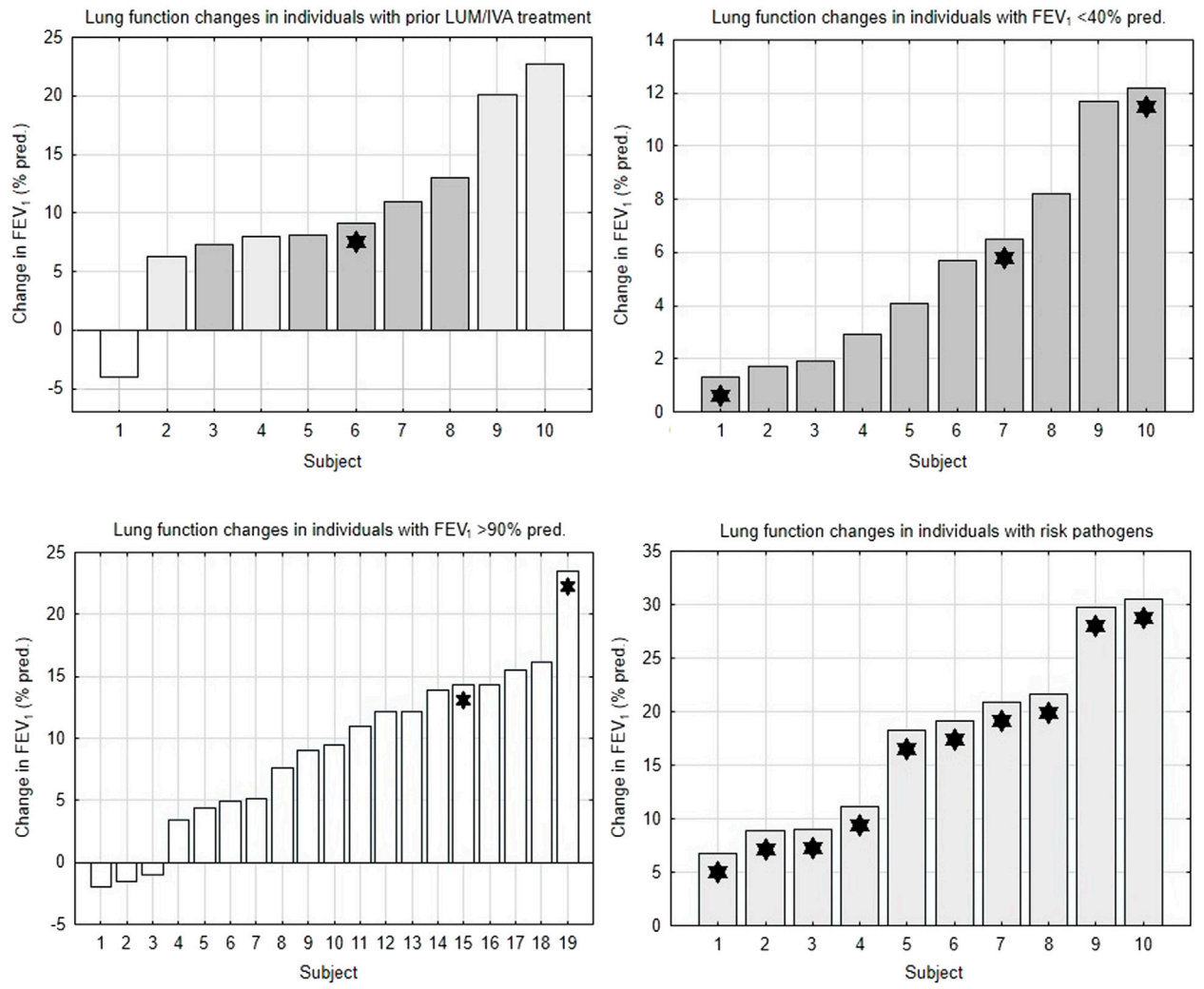

FIGURE 1
Changes in lung function in subgroups of the study population. FEV_1 = forced expiratory volume in 1 s. LUM/IVA, lumacaftor/ivacaftor. White bars = subjects with FEV_1 >90% pred. Light gray bars = subjects with FEV_1 40%–90% pred. Dark gray bars = subjects with FEV_1 <40% pred. Asterisks = subjects with pathogens at risk of more rapid FEV_1 decline.

pulmonary exacerbation in pwCF, where the current $ppFEV_1$ value is compared to the best value in last 6 months (Goss, 2019).

The control group consisted of pwCF on ETI treatment who did not meet the common exclusion criteria for clinical trials participation. Their improvement in lung function was similar to the results published in phase 3 clinical trials with the ETI treatment: $ppFEV_1$ +15.8 points in our study (over one to 6 months period) vs. +14.3 points in Week 24 (pwCF heterozygous for F508del, ETI vs. placebo) (Middleton et al., 2019) or +10.0 points in Week 4 (pwCF homozygous for F508del, ETI vs. TEZ/IVA combination) (Heijerman et al., 2019). The even better results in our cohort can be explained by the inclusion also of pwCF heterozygous for F508del with a mutation on the other allele which is regarded mild, as opposed to minimal function mutations, included as the only qualifying *CFTR* mutations in the registration study. The improvement in BMI was very comparable: +1.02 kg/m^2 (control group in our study) vs. +1.04 kg/m^2 (Middleton et al., 2019).

It is of note that our study group consisted of a heterogeneous population: subjects with severe airway obstruction as well as well-preserved lung function, subjects with pathogens at risk of more rapid FEV_1 decline and subjects with prior LUM/IVA therapy. Compared to the control group, this group as a whole showed less of improvement in lung function (i.e., +10.3 points), in contrast to a very comparable improvement in nutritional status and in a decrease in sweat chloride concentration. In the subgroup analysis, subjects with severe airway obstruction and those with well-preserved lung function showed lower potential for improvement in their lung function. Our results indicated the lung function improvement to a lesser extent than published for pwCF with advanced lung disease by Burgel et al. (Burgel et al., 2021) or O'Shea et al. (O'Shea et al., 2021). While the former publication reported an improvement in mean $ppFEV_1$ in patients without prior CFTR modulator therapy by + 12 points after 3 months (along with a reduction in the need for long-term oxygen therapy and non-invasive ventilation), and the latter showed $ppFEV_1$ +9 points after 26 days on ETI, we observed the change of median $ppFEV_1$ to be +4.9 points only. However, our observed change is similar to the work of Djavid and coworkers ($ppFEV_1$ +5.5 points after 1 month) (Djavid et al., 2021).

Data on pwCF with prior therapy with LUM/IVA combination and their switch to ETI have not been previously published. Our small group showed an improvement in lung function ($ppFEV_1$ +8.6 points) which indicates that the switch from LUM/IVA to ETI results in a very comparable outcome like the switch from TEZ/IVA to ETI (Heijerman et al., 2019).

Improvement in nutritional status in the whole study group (BMI +1.04 kg/m^2) as well as the reduction in sweat chloride concentration was similar to our control group as well as to the clinical trial study by Middleton et al. (Middleton et al., 2019).

In conclusion, pwCF not eligible for inclusion in clinical trials demonstrated improvement in lung function and in nutritional status after initiation of the treatment with the ETI combination, comparable to the population studied by respective clinical trials. Likewise, they manifested the decrease in sweat chloride concentration. Less improvement in FEV_1 was observed in a subcategory of pwCF who presented with severe airway obstruction or well-preserved lung function.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

LF and PD conception and design of the study; LF, AG, and AB acquisition of the data; LF and AB data analysis; LF and PD data interpretation; LF and PD writing of the manuscript; All authors contributed to the article and approved the submitted version.

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Conflict of interest

LF and PD recived speakers fee and advisory boards members fee from Vertex Pharmaceuticals, Inc.

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Proliferative activity of antigen-specific CD154+ T cells against bacterial and fungal respiratory pathogens in cystic fibrosis decreases after initiation of highly effective CFTR modulator therapy

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Background: Together with impaired mucociliary clearance, lung disease in cystic fibrosis (CF) is driven by dysregulation of innate and adaptive immunity caused by dysfunctional CFTR (Cystic Fibrosis Transmembrane Conductance Regulator), leading to airway infection and hyperinflamma-tion. The highly effective CFTR modulator therapy (HEMT) elexacaftor/tezacaftor/ivacaftor (ETI) generates substantial improvements in clinical outcomes of people with CF (pwCF) by restoration of CFTR activity. Aberrant immune responses of lymphocytes due to CFTR dysfunction has been described in the past, but not the effects of CFTR restoration by HEMT on these cells. We aimed to examine the effect of ETI on the proliferative activity of antigen-specific CD154 (+) T cells against bacterial and fungal species relevant in CF and on total IgG and IgE as markers of B cell adaptive immunity.

Methods: We performed *ex vivo* analyses of Ki-67 expression in antigen-specific CD154 (+) T cells against *Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus fumigatus, Scedosporium apiospermum* and *Candida albicans* from 21 pwCF by cytometric assay based on antigen-reactive T cell enrichment (ARTE), and analysis of total serum IgE and IgG before and after initiation of ETI.

Results: Mean Ki-67 expression in antigen-specific CD154 (+) T cells against *P. aeruginosa, A. fumigatus, S. apiospermum* and *C. albicans*, but not *S. aureus*, mean total serum IgG and mean total serum IgE decreased significantly after initiation of ETI. No correlation was found to change in sputum microbiology of the examined pathogens. Mean BMI and FEV1 increased significantly.

Conclusion: HEMT is associated with decreased antigen-specific CD154 (+) T cell proliferation activity in our cohort, independent of findings in sputum

microbiology of the examined pathogens. Together with the observed clinical improvement and the decrease in total IgE and IgG, this indicates effects due to CFTR restoration on CD154 (+) T cells by ETI and a reduction of B cell activation with subsequent lower immunoglobulin synthesis under HEMT therapy. These results endorse earlier evidence of CFTR dysfunction in T and B cells leading directly to aberrant immune responses with hyperinflammation.

KEYWORDS

cystic fibrosis, antigen-specific T cells, Ki-67, *Pseudomonas aeruginosa*, Aspergillus fumigatus, total IgE, total IgG, highly effective modulator therapy

1 Introduction

Knowledge of CFTR (cystic fibrosis transmembrane regulator) dysfunction on epithelial cells and genetic mechanisms in cystic fibrosis (CF) has led to the development of potent symptomatic and mutation-specific drugs. Symptomatic treatment includes effective secretolytic substances and inhalative antibiotics. During the last decade, highly effective CFTR-modulators (HEMT) which restore CFTR activity >25% of wildtype activity have been developed and approved (Zhang et al, 2009). Today, nearly 90% of people with cystic fibrosis (pwCF) have mutations eligible for either CFTR modulator monotherapy or combination therapy (Joynt et al, 2022). Initiation of the highly efficient CFTR modulator therapy (HEMT) elexacaftor/tezacaftor/ivacaftor (ETI), consisting of two CFTR molecule correctors and one CFTR potentiator, resulted in substantial improvements in clinical outcomes of pwCF(3–5).

CF lung disease is also known to be driven by abnormal innate and adaptive immune regulation leading to hyperinflammation and ineffective immune response to respiratory pathogens. Immune dysregulation in CF is both due to genetic and acquired factors. The direct impact of CFTR dysfunction on immune cells is increasingly recognized and under investigation. In neutrophils, defective CFTR causes reduced chlorination resulting in impaired phagocytosis (Painter et al, 2006; Dickerhof et al, 2019; Meoli et al, 2022). In B cells, CFTR has been shown to play a direct role in activation, proliferation and production of inflammatory cytokines, with a subsequent increase in IgG (+)-B cells (Polverino et al, 2019). Already in the 1980ies, progression of cystic fibrosis lung disease could be linked to hypergammaglobulinemia (Wheeler et al, 1984). CFTR-dependent altered regulation of T-cell cytokine secretion leads to a shift towards a proinflammatory state and a predominant Th2 response with exaggerated IgE levels, a predisposition for allergic bronchopulmonary aspergillosis (ABPA) and simultaneously reduced defense against *Pseudomonas aeruginosa* (Moss et al, 1996; Muller et al, 2006; Mueller et al, 2011; Ratner and Mueller, 2012; Duan et al, 2021). The defective Th2 response also drives the development of multispecies cross-reactive Th2-cells as a potential risk factor for ABPA (Schwarz et al, 2023). Heterozygous asthmatic individuals with ABPA are more likely to be carriers of a CFTR mutation (Gamaletsou et al, 2018). *Candida albicans* could be identified by our study group as major inducer of cross-reactive CD154 (+) T cells contributing to *Aspergillus fumigatus*-driven inflammation in the lung in pwCF (Bacher et al, 2019).

So far, the findings have not yet been sufficient to enable the development of targeted antiinflammatory therapies for pwCF. Increasing evidence shows that there may be interindividual differences in the efficacy of HEMT and that HEMT may not be equally effective in different organs and cells in the same individual. This could apply especially to immune cells, as large interindividual differences in immune phenotypes are known to exist in humans (Okada et al, 2021). Initiation of HEMT provides the opportunity to examine in real life the relevance of CFTR-dysfunction found on immune cells *in vitro*, and of changes in host-pathogen interactions in CF. Recently, a partial restoration of macrophage phagocytosis and neutrophil efferocytosis by ETI was observed, and non-responders in the CF group were identified (Zhang et al, 2022). We therefore aimed to investigate the effects of HEMT on the proliferation activity of CD154 (+) T cells with antigenic specificity for relevant respiratory pathogens and of total IgG and IgE in pwCF. Ki-67 antigen was first described in 1983 as a marker present in proliferating cells and is widely used in tumor cell kinetics assessment, but has so far never been used to describe proliferative activity of T cells in CF (Gerdes et al, 1983; Scholzen and Gerdes, 2000).

2 Materials and methods

2.1 Patient cohort and study design

A total of 21 pwCF were included in the study. All patients gave written consent to participate. Serial samples for antigen-specific CD154 (+) T cells were taken at routine or acute visits or during hospitalization between 6.39 years and 7 days prior to and between 25 days and 2.5 years after initiation of ETI. The mean number of visits per patient was 4.83 (Wheeler et al, 1984; Painter et al, 2006; Dickerhof et al, 2019; Heijerman et al, 2019; Middleton et al, 2019; Polverino et al, 2019; Zemanick et al, 2021; Joynt et al, 2022; Meoli et al, 2022). For Ki-67 analysis, complete data were available from 21 patients for *A. fumigatus*, 14 patients for *S. apiospermum* and *C. albicans*, 8 patients for *S. aureus* and 9 patients for *P. aeruginosa*. In both the pre and post ETI group, two patients were excluded from IgE analysis due to acute ABPA, and due to incomplete data prior to initiation of ETI, 3 patients were excluded from exacerbation analysis. Baseline and demographical data are shown in Table 1.

2.2 Data collection and statistical analysis

Sample collection was conducted between May 2014 and December 2022 at Christiane Herzog Cystic Fibrosis Center,

TABLE 1 Demographic characteristics before and after ETI initiation.

Table 1 Patient characteristics	Patients (n = 21)
Gender: female/male	62%/38%
Mean age at visit (years) ± SD (range)	28.9 ± 9.4 (8–56)
Genotype	
F508del homozygous	n = 10 (47.6%)
F508del heterozygous	n = 11 (52.4%)
Exocrine Pancreatic Insufficiency	n = 19 (90.5%)
CF-related Diabetes mellitus	n = 8 (38.1%)
BMI kg/m^2 (mean, ±SD (range))	19.4 ± 2.2 (15.4–22.4)
ppFEV1 pre ETI (mean ± SD (range))	43.8 ± 16.8 (23.2–82.0)
Organ Transplantation	n = 0
Pulmonary exacerbations per year, 2 years pre ETI (n = 18[a], mean, ±SD (range))	3.3 ± 1.7 (1–5.5)
Pulmonary exacerbations per year, 2 years post ETI (n = 18[a], mean, ±SD (range))	1.5 ± 1.4 (0–3.5), $p < 0.001$
Mean number of visits per patient ±SD (range)	4.83 ± 2.24 (2–10)
Total number of visits pre ETI	n = 67
Total number of visits post ETI	n = 31
∑ pre + post ETI	n = 98
Systemic steroid therapy pre ETI, visits	n = 22 (32.8% of pre ETI)
Systemic steroid therapy post ETI, visits	n = 9 (29.0% of post ETI), ns[b]
Antifungal therapy pre ETI, visits	n = 17 (25.3% of pre ETI)
Antifungal therapy post ETI, visits	n = 2 (6.4% of post ETI), $p < 0.05$
ABPA acute + untreated pre ETI, mean total IgE (kU/L) ± SD (range)	n = 4, 1630 (1073–2,943)
ABPA acute + untreated post ETI, mean total IgE (kU/L) ± SD (range)	n = 4, 3132 (2,327–4,599)
Sputum microbiology (pre ETI, number of visits)	Positive sputum result pre ETI
Pseudomonas aeruginosa[c] (n = 43)	n = 33 (82.5%)
Staphylococcus aureus[c] (n = 38)	n = 10 (26.3%)
Aspergillus fumigatus[c] (n = 70)	n = 24 (32.9%)
Scedosporium apiospermum/spp.[c] (n = 53)	n = 7 (13.2%)
Candida albicans[c] (n = 53)	n = 19 (35.8%)
Other bacteria (n = 70)	n = 13 (13.3%)
Achromobacter xylosoxidans	n = 2 (2.8%)
Burkholderia multivorans	n = 8 (11.4%)
Serratia marcescens	n = 3 (4.3%)
Proteus mirabilis	n = 1 (1.4%)
M. intracellulare-complex	n = 2 (2.8%)
Other fungi[5] (n = 70)	n = 12 (17.1%)
Aspergillus flavus	n = 1 (1.4%)
Exophiala dermatitidis	n = 7 (10.0%)
Candida dubliniensis	n = 14 (20.0%)

(Continued on following page)

TABLE 1 (*Continued*) Demographic characteristics before and after ETI initiation.

Table 1 Patient characteristics	Patients (n = 21)
Candida guilliermondii	n = 4 (5.7%)
Candida glabrata	n = 11 (15.7%)
Penicillium spp.	n = 1 (1.4%)

[a]Patients with exacerbation data for the respective time frame.
[b]ns: not significant.
[c]Patients with Ki-67, analysis available for the respective pathogen.

Charité Universitätsmedizin Berlin, Germany, and at Cystic Fibrosis Center Westbrandenburg, Campus Potsdam, Health and Medical University Potsdam.

Ethical aspects were considered and approval for the study was gained by the Ethics Committee of Charité Universitätsmedizin Berlin, Germany (No. EA2/121/16) and by the Medical Ethics Committee Brandenburg [Landesärztekammer Brandenburg, No. AS48 (bB)/2021].

Baseline data were collected using patient records and the German patient registry software "Muko.web". For the analysis of Ki-67, subgroups with all patients with available antigen-specific CD154 (+) T cells before and after initiation of ETI were formed. Microbiology data was analyzed with respect to the subgroups.

Distribution of data was assessed with Shapiro-Wilk test for normal distribution. Associations between binary variables were assessed using Barnard`s exact test, and between quantitative variables using *t*-test. Due to unknown effect sizes, *a priori* power analyses were not performed. Data analyses were performed using Microsoft Excel Version 2301 (Build 16.0.16026.20196) and R version 4.2.1.

2.3 Antigen-specific T cells, microbiology and immunoglobulins

Antigen specific CD154 (+) T cells were derived by Antigen-reactive T cell enrichment (ARTE) which allows the detection of antigen reactive T cells against single antigens, as described elsewhere (Bacher et al, 2013). Cells were stained for multiparametric flow cytometry with Ki-67-antibodies (Miltenyi Biotec Cat# 130-119-356, RRID:AB_2857452) and flow cytometry analysis of cytokine-secreting antigen-specific T cells were performed. Antigens for T cell stimulation were chosen due to the relevance and frequency of the respective pathogens in CF, and due to their availability. Except for *C. albicans*, the chosen pathogens are considered to have a major impact on CF lung disease (Bell et al, 2020). However, *C. albicans* is isolated frequently in airways of pwCF, and its influence in CF is unclear (Chotirmall et al, 2010).

Microbiology was collected at every visit spontaneously, as induced sputum (after inhalation of 6% saline) or as physiotherapist assisted induced sputum. Additional microbiological results from other routine visits were analyzed where available if sputum could be expectorated to better reflect colonization status of the patients. Cough swab results were excluded from analysis. Serum was collected from all patients at every visit. Total serum IgG (immunoturbidimetry/nephelometry) and total serum IgE (electrochemiluminescence immunoassay) were determined in our routine laboratories (Labor Berlin/Labor Potsdam) in parallel to Ki-67 analysis.

3 Results

3.1 Baseline data/demographical data

Statistical analysis reveals a severe phenotype in our cohort, with worse lung function and nutritional status compared to the general CF population in the latest German CF registry data from 2021 (Nährlich et al, 2021). With a mean age of 28.9 years (registry: 23.0 years), the patients were older. The percentage of F508del homozygous patients was similar (47.6% in our cohort vs. 46.6% in the registry), while mean BMI (19.4 kg/m^2) and mean ppFEV1 (43.8) were lower. Detection rate of *S. aureus* was lower (19.4% vs. 50.0%) and of *P. aeruginosa* higher (64.3% vs. 33.3%) than in all birth cohorts of the German registry population. The rates of pancreatic insufficiency and diabetes were similar to the registry patients. No patients after organ transplantation were included, and the percentages of visits with systemic steroids were similar before and after ETI initiation (32.8 vs. 29.0%, ns). Significantly more pulmonary exacerbations were experienced, and more patients were under antifungal therapy before ETI initiation, mostly receiving long-term antifungal therapy against ABPA. Due to acute ABPA, four patients prior to and four patients after ETI initiation were excluded from total IgE analysis and there was a balanced relation regarding Ki-67 and analysis of *A. fumigatus* specific CD154 (+) cells, total IgG and microbiology. Patient characteristics are listed in Table 1.

3.2 General outcome data and microbiology pre and post ETI

A significant increase in mean BMI of 4.0% (19.4 vs. 20.6 kg/m^2, $p < 0.01$) and mean absolute ppFEV1 of 6.5 (43.8 vs. 50.3, $p < 0.01$) was shown after ETI initiation. We observed a significantly lower pulmonary exacerbation rate in the 2 years past vs. the 2 years prior to ETI initiation (3.3 vs. 1.5, $p < 0.001$). These data confirm the benefits of HEMT in a cohort with a relatively severe mean phenotype in real life. Nevertheless, mean BMI and ppFEV1 after initiation of ETI were still lower than in the German registry population before approval of ETI. In microbiology, we found a significant decrease in *A. fumigatus*

TABLE 2 Outcome data before and after initiation of ETI.

Table 2 Outcome data pre and post ETI	Patients (n = 21)	
BMI kg/m^2) pre ETI (mean, ±SD (range))	19.4 ± 2.2 (15.4–22.4)	$p < 0.01$**
BMI (kg/m^2) post ETI (mean ± SD (range))	20.6 ± 2.6 (14.3–24.8)	
ppFEV1 pre ETI (mean ± SD (range))	43.8 ± 16.8 (23.2–82.0)	$p < 0.01$**
ppFEV1 post ETI (mean ± SD (range))	50.3 ± 15.0 (32.0–89.0)	
Pulmonary Exacerbations pre ETI, visits	n = 52 (76.4% of pre ETI)	$p < 0.05$*
Pulmonary Exacerbations post ETI, visits	n = 16 (53.3% of post ETI)	
Total IgE (IU/mL) pre ETI (Non-ABPA, mean ± SD (range))	91.2 ± 98.6 (2.5–326.0)	$p < 0.01$**
Total IgE (IU/mL) post ETI (Non-ABPA, mean ± SD (range))	28.8 ± 22.5 (3.2–78.2)	
Total IgG (g/L) pre ETI (mean ± SD (range))	15.0 ± 3.9 (9.7–23.5)	$p < 0.001$***
Total IgG (g/L) post ETI (mean ± SD (range))	12.3 ± 3.2 (6.5–22.3)	
Sputum microbiology[a]		
Pseudomonas aeruginosa pre ETI, visits	n = 40 positive: 33 (82.5%)	ns
Pseudomonas aeruginosa post ETI, visits	n = 38 positive: 27 (71.1%)	
Staphylococcus aureus pre ETI, visits	n = 38 positive: 10 (26.3%)	ns
Staphylococcus aureus post ETI, visits	n = 36 positive: 4 (11.1%)	
Aspergillus fumigatus pre ETI, visits	n = 70 positive: 23 (32.9%)	$p < 0.001$**
Aspergillus fumigatus post ETI, visits	n = 53, positive: 1 (1.9%)	
Scedosporium apiospermum pre ETI, visits	n = 53, positive: 7 (13.2%)	ns
Scedosporium apiospermum post ETI, visits	n = 45, positive: 5 (11.1%)	
Candida albicans pre ETI, visits	n = 53, positive: 19 (35.8%)	ns
Candida albicans post ETI, visits	n = 45, positive: 21 (46.7%)	

[a]At visits with Ki-67, analysis available for the respective pathogen.

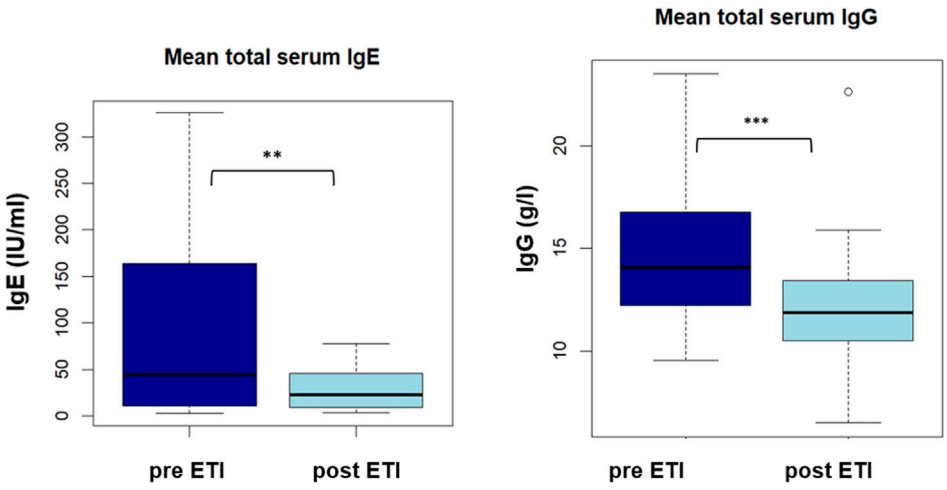

FIGURE 1
Boxplot showing mean total serum IgE and IgG (IU/ml) pre and post ETI **: $p < 0,01$.

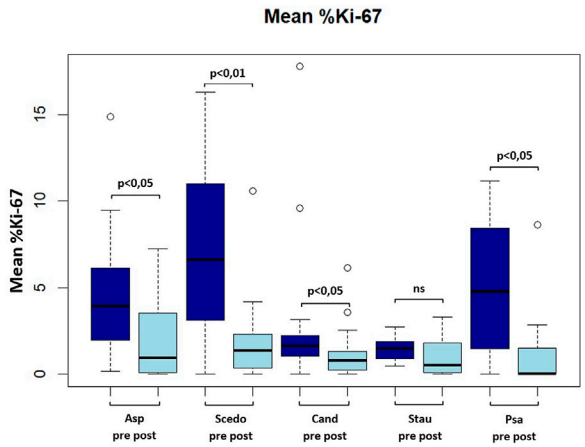

FIGURE 2
Boxplot showing mean frequency (in percent) of Ki-67(+) antigen-specific CD154(+) Tcells pre and post ETI. Asp, aspergillus fumigatus; Scedo, scedosporium apiospermum; Cand, candida albicans; Stau, staphylococcus aureus.

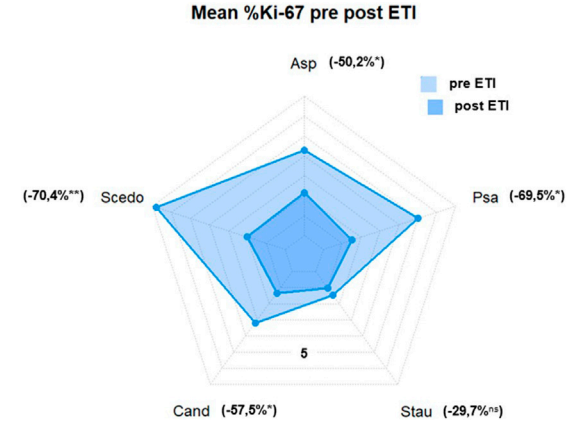

FIGURE 3
Mean %Ki-67(+) antigen-specific CD154(+) T cells. Asp, aspergillus fumigatus; Scedo, scedosporium apiospermum; Cand, candida albicans; Stau, staphylococcus aureus; Psa, pseudomonas aeruginosa (*) = $p < 0.05$, (**) = $p < 0.01$; ns, not significant.

sputum detection in our cohort (48.9 vs. 2.0%, $p < 0.001$). For *P. aeruginosa*, *Staphylococcus aureus*, *Scedosporium apiospermum* and *C. albicans* we found no significant changes in sputum detection rate for the respective groups. For general and microbiology outcome data please see Table 2.

3.3 Total IgE and total IgG

Mean total serum IgE and mean total serum IgG decreased significantly after ETI initiation (91.2 vs. 28.8 IU/mL, $p < 0.01$ and 15,0 vs. 12.3 g/L, $p < 0.001$, please see Figure 1A + B).

3.4 Ki-67 expression of antigen-specific CD154 (+)-T cells

Mean Ki-67 expression percentage in antigen-reactive CD154 (+)-T cells against *A. fumigatus* (4.28 ± 3.41 vs. 2.13 ± 2.22 (−50.2%), $p < 0.05$), *S. apiospermum* (6.79 ± 4.70 vs. 2.01 ± 2.72 (−70.4%), $p < 0.01$), *C. albicans* (3.18 ± 4.6 vs. 1.35 ± 1.63 (−57.5%), $p < 0.05$), *P. aeruginosa* (5.01 ± 4.10 vs. 1.53 ± 2.67 (−69.5%), $p < 0.05$) decreased significantly after initiation of ETI. We also found a decrease in mean Ki-67 expression percentage in antigen-reactive CD154 (+)-T cells against *S. aureus* (1.45 ± 0.69 vs. 1.02 ± 1.13 (−29.7%), ns), but this difference was not significant (please see Figure 2; Figure 3).

4 Discussion

The approval of ETI has improved morbidity and quality of life in pwCF by increasing pulmonary and overall health status (Heijerman et al, 2019; Middleton et al, 2019; Shteinberg and Taylor-Cousar, 2020; Zemanick et al, 2021). It is widely assumed that respiratory disease in pwCF results from a triad of abnormal immune cell responses, pathogen proliferation and proinflammatory respiratory environment (Keown et al, 2020). Therefore, we aimed to examine the impact of ETI *in vivo* on markers of general inflammatory response and of the T-cellular immune response to relevant airway pathogens in relation to their detection in sputum.

In our cohort, only *A. fumigatus* was found significantly less frequently after ETI initiation, which is remarkable, as significantly more patients received long-term antifungal therapy before ETI. In line with this, significantly less exacerbations were experienced, suggesting a reduction of bacterial load. There were no significant qualitative changes for cultural detection of *P. aeruginosa*, *S. aureus*, *S. apiospermum* and *C. albicans*. This partly stands in contrast to recent publications describing reduction of bacterial load and decreased detection of bacterial pathogens in CF respiratory cultures after initiation of ETI (Pallenberg et al, 2022; Beck et al, 2023). Possible causes for this divergence could be the relatively severe phenotype of our cohort and the fact that we were able to examine sputum samples even after ETI initiation. However, due to the improved mucociliary clearance, the samples examined in our study also differed in their quantity before and after ETI, as most patients under ETI were unable to expectorate more than 5 mL of sputum. This may - on the one hand—have had an influence on the bacterial load, which may at least in part explain the lower exacerbation rate. On the other hand, this may have had an influence on the cultural detection rate of *A. fumigatus* after ETI in our study. However, this does not apply to the same extent for the other pathogens we investigated, although—except for *C. albicans*, which was detected more often—the pathogens investigated in our study were also detected slightly less frequently. A link between CFTR dysfunction and defective clearing of *A. fumigatus* is assumed, so CFTR restoration by ETI with more effective immune response may be the main reason for our result (Bercusson et al, 2021). It is also in line with two recent studies, where a rapid significant decrease in *Aspergillus* spp. positive sputum cultures and a restoring of dampened *Aspergillus*-induced reactive oxygen species production

by CF phagocytes by CFTR modulators could be shown (Currie et al, 2020; Chesnay et al, 2022).

Interestingly, we found a reduction in the frequencies of Ki-67 positive antigen-specific CD154 (+)-cells for all investigated pathogens independent of their detection rate in sputum samples, which was significant for *P. aeruginosa*, *A. fumigatus*, *S. apiospermum* and *C. albicans*. Significance of Ki-67 decline could not be shown for *S. aureus*, which is most likely due to low Ki-67 frequencies already before ETI initiation in combination with only few numbers of Ki-67 examinations available for this pathogen.

Matching the reduction in Ki-67 frequencies, we found a significant reduction in total serum IgE and total serum IgG. Taken together, these results confirm previous *in vitro* findings of impaired adaptive immune regulation (Mueller et al, 2011; Polverino et al, 2019; Duan et al, 2021) in real life and suggest that CFTR restoration on B and T cells by ETI enables a more effective immune response with a decelerated disease progression (Wheeler et al, 1984). As we found heterogenous changes in microbiology after ETI initiation, reduction of antigen load by improved mucociliary clearance either with or without cross-reactivity between the fungal species examined seems to be only part of the effect of HEMT on lung disease in CF.

Limitations of this study are the relatively small number of patients and that we examined a selected patient cohort with severe phenotype. Nevertheless, these results encourage to be confirmed in a larger cohort of patients and in longitudinal studies to determine whether these effects of HEMT are maintained long-term. Analogous to total IgG, Ki-67 expression on T cells could serve as a general disease severity marker.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Charité Universitätsmedizin Berlin and Medical Ethics Committee Brandenburg (Landesärztekammer Brandenburg). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Conceptualization: PE. Project administration: CG, PE, and CS. Recruitment: PE, CS, and JM. Data acquisition: PB, PE, CG, CS, and JM. Analysis and interpretation of data: PE, PB, JM, and CS. Manuscript writing: PE, JM, PB, CG, and CS. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Spirometric and anthropometric improvements in response to elexacaftor/tezacaftor/ivacaftor depending on age and lung disease severity

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Introduction: Triple-combination cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapy with elexacaftor/tezacaftor/ivacaftor (ETI) was introduced in August 2020 in Germany for people with CF (pwCF) ≥12 years (yrs.) of age and in June 2021 for pwCF ≥6 yrs of age. In this single-center study, we analyzed longitudinal data on the percent-predicted forced expiratory volume (ppFEV1) and body-mass-index (BMI) for 12 months (mo.) after initiation of ETI by linear mixed models and regression analyses to identify age- and severity-dependent determinants of response to ETI.

Methods: We obtained data on 42 children ≥6–11 yrs, 41 adolescents ≥12–17 yrs, and 143 adults by spirometry and anthropometry prior to ETI, and 3 and 12 mo. after ETI initiation. Data were stratified by the age group and further sub-divided into age-specific ppFEV1 impairment. To achieve this, the age strata were divided into three groups, each according to their baseline ppFEV1: lowest 25%, middle 50%, and top 25% of ppFEV1.

Results: Adolescents and children with more severe lung disease prior to ETI (within the lowest 25% of age-specific ppFEV1) showed higher improvements in lung function than adults in this severity group (+18.5 vs. +7.5; p = 0.002 after 3 mo. and +13.8 vs. +7.2; p = 0.012 after 12 mo. of ETI therapy for ≥12–17 years and +19.8 vs. +7.5; p = 0.007 after 3 mo. for children ≥6–11 yrs). In all age groups, participants with more severe lung disease showed higher BMI gains than those with medium or good lung function (within the middle 50% or top 25% of age-specific ppFEV1). Regression analyses identified age as a predictive factor for FEV1 increase at 3 mo. after ETI initiation, and age and ppFEV1 at ETI initiation as predictive factors for FEV1 increase 12 mo. after ETI initiation.

Discussion: We report initial data, which suggest that clinical response toward ETI depends on age and lung disease severity prior to ETI initiation, which argue for early initiation of ETI.

KEYWORDS

cystic fibrosis, BMI, elexacaftor/tezacaftor/ivacaftor, modulator, pediatric

Introduction

The advent of highly effective cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapy has led to an unprecedented improvement in the lives of people with CF (pwCF). Licensing of triple-combination CFTR modulator therapy in the form of elexacaftor (ELX)/tezacaftor (TEZ)/ivacaftor (IVA), henceforth termed ETI, increased the availability of CFTR modulator therapy. In Germany, ETI was introduced in August 2020 for pwCF ages 12 and older (pwCF ≥12), and in June 2021 for ages 6 and older (pwCF ≥6), permitting CFTR modulator therapy for more than 85% of patients in those age strata. Until now, determinants of ETI response have not been identified. Previous studies on other CFTR modulators suggest that age at therapy initiation significantly impacts upon response to CFTR modulator therapy.

Younger age at initiation of IVA permits better preservation of lung function and a larger impact on pulmonary exacerbation (PEx) (Bui et al., 2021). In adolescents, younger age at initiation is associated with a better percent-predicted forced expiratory volume (ppFEV1) response to LUM/IVA (Nichols et al., 2022), and adolescents have shown more favorable body-mass-index (BMI) trajectories than adults in response to LUM/IVA in a large real-world setting (Muilwijk et al., 2022). Similarly, the improvements in pancreatic function observed in very young patients initiating IVA or LUM/IVA also suggest that age at initiation of CFTR modulator therapy has an impact on specific organ functions (Davies et al., 2021; Rosenfeld et al., 2018; Merlo et al., 2022; McNamara et al., 2019).

By proxy, the findings of Nichols et al., which show a significant correlation between sweat chloride reductions and ppFEV1 improvements after 6 months of therapy (Nichols et al., 2022), might also suggest that ppFEV1 response to ETI is age-dependent since improvement in sweat chloride in response to CFTR modulators other than ETI has been observed to be larger when pwCF are younger (Durmowicz et al., 2013). With regard to improvement in CFTR function in response to ETI, phase III trials showed an improvement between −45 and −49 mmol/L for the two eligible genotype combinations in adolescents ≥12 years and adults (Heijerman et al., 2019; Middleton et al., 2019). Children aged 6–11 years of both eligible genotype combinations showed an overall improvement of −61 mmol/L sweat chloride (Zemanick et al., 2021), suggesting the response to ETI also shows an age dependency with regard to the magnitude of functional CFTR improvement. However, clinical data concerning longitudinal evolvement of pulmonary function and anthropometry, supporting similar age- or severity-dependent effects, are lacking for ETI.

Our study aimed to address determinants of response to ETI through the analysis of 3-month and 1-year follow-up (FU) data from a single-center cohort of n = 226 people with CF ≥ 6 years of age. We focused on comparing age strata with the similar severity of lung disease prior to ETI initiation, hypothesizing that earlier ETI initiation confers superior benefits with regard to the key clinical endpoints ppFEV1 and BMI.

Materials and methods

Study group, data collection, and study parameters

PwCF receive standardized diagnostic procedures and treatment in our center, according to national and international guidelines, including 3-month intervals of outpatient visits. All data used for this study were recorded in an in-house electronic patient record at every visit, according to definitions predefined by the German CF Registry.

Parameters from the last outpatient visit prior to ETI initiation were obtained for baseline values, including ppFEV1, BMI, sweat chloride, prior CFTR modulator use, sex, and age at ETI initiation. Values from inpatient visits were excluded from the analysis. Sweat chloride levels were determined prior to and 3 months after ETI initiation. The changes in ppFEV1 and BMI (in kg/m^2) were collected prior to and on months 3, 6, 9, and 12 after ETI initiation. Lung function was referenced according to the Global Lung Function Initiative (GLI) (Quanjer et al., 2012). The BMI in kg/m^2 was calculated electronically by weight and height measurements at every visit.

Study design

We performed an exploratory, retrospective, single-center, post-approval cohort study. We extracted demographic data and data on ppFEV1 and BMI of all pwCF from our CF center, who received ETI therapy for at least 3 (for pwCF ≥6–11) or 12 (for pwCF ≥12) uninterrupted months. Data for pwCF ≥12 years were collected between August 2020 and January 2023, and data for pwCF ≥6, between June 2021 and January 2023. For patients ≥6–11 years of age, we only included data from FU 3 months in our analyses as the group size of further FU data was increasingly limited due to the recent approval of ETI in this age group.

Data were stratified according to age (≥6–11 yrs; ≥12–17 yrs; ≥18 yrs) and age-group-specific lung function impairment percentiles. We aimed to compare pwCF with similar degrees of lung disease across age groups. Yet, average ppFEV1 values, as a proxy for lung disease severity, are different across these age groups. We, therefore, chose to stratify pwCF according to the average ppFEV1 within their age group as reference, creating age-specific

sub-groups of lung disease severity. For this purpose, we chose the common cut-off values of the lowest and highest 25th percentile for baseline ppFEV1 of the age group in question, thereby creating three groups: "severely affected": baseline ppFEV1 ≤25th percentile (≤P25), "average": 26th–74th percentile (P50), and "less affected": ≥75th percentile (≥P75) for the age groups ≥6–11 yrs, ≥12–17 yrs, and ≥18 yrs, respectively. The age-dependent percentiles for ppFEV1 for our cohort were calculated and are also stated in Table 2.

- pwCF ≥18 years: ≤P25: 27.2 (23.4–30.4), P50: 53.1 (45.4–60.7), and
- ≥P75: 82.3 (77.0–92.9) for median (IQR)
- pwCF ≥12–17 years: ≤P25: 56.4 (50.2–61.9), P50: 83.4 (73.9–91.3), and
- ≥P75: 98.9 (95.5–110.9) for median (IQR)
- pwCF ≥6–11 years: ≤P25: 70.7 (61.4–80.7) P50: 87.7 (82.9–95.0), and
- ≥P75: 102.1 (100.6–105.4) for median (IQR)

Statistical analysis

All statistical analyses were performed with Statistical Package for Social Sciences (SPSS 28, IBM, Armonk, New York, United States). Descriptive data were calculated as the median and interquartile range (IQR). Initially, measurements were tested for normal distribution. Differences between groups were analyzed by the Mann–Whitney U test or Kruskal–Wallis test, as appropriate. Frequency differences between nominally distributed groups were calculated by a chi-squared test. Data were corrected for multiple testing by Bonferroni correction.

We used a generalized linear model to assess the effects of gender, age group, and prior CFTR modulator therapy on the outcome ppFEV1 or BMI for all pwCF at or above 12 years of age included in our analyses.

We performed regression analyses to address the effects of age, gender, and CFTR modulator therapy prior to ETI initiation, as well as baseline values for ppFEV1, BMI, and the severity of lung disease according to the previously defined percentiles at baseline. PpFEV1 or BMI gains at 3 or 12 months were considered outcomes. We included data from all pwCF at or above 6 years of age for gains at 3 months and from all pwCF above 12 years of age for gains at 12 months.

A p-value <0.05 was considered statistically significant.

Ethics

All patients or their legal guardians provided consent to the anonymized scientific use of personal clinical data for research purposes, either as written informed consent to participate at scientific studies of the German Center for Lung Research (DZL) registry (Ethics Committee Hannover Medical School, #2923-2015, Hannover Medical School) and/or the German CF registry (Ethics Committee of the Justus-Liebig-Universität FB Medizin, #AZ24/19).

Results

Clinical characteristics of the entire study population

We were able to include n = 226 pwCF from our center into our analyses. Data were obtained at a median time of 3.1 months for all n = 226 subjects included in the study and 12.4 months for n = 182 (80.5%) subjects ≥12 years after ETI initiation. Median age at ETI initiation was 22.5 yrs (IQR 13.4–30.5), of which 51.8% were female subjects, and 45.1% already underwent CFTR modulator therapy prior to ETI initiation.

The median gain of ppFEV1 at 3 months FU was +9.7 (IQR 5.1–17.8) and +9.6 (IQR 5.5–16.4) at 12 months compared to ppFEV1 at baseline. The median BMI gain at 3 months FU was +0.8 kg/m^2 (IQR 0.2–1.5) and +1.4 kg/m^2 (IQR 0.4–2.6) at 12 months compared to prior ETI initiation (Table 1, Column 1). As expected, sweat chloride decreased by 44 mmol/L after 3 months of ETI therapy with no statistical differences in sweat chloride at baseline or after 3 months of ETI therapy between the three age groups (Table 1).

Age dependency of ppFEV1 and BMI response to ETI

To query age dependency of spirometric and anthropometric responses to ETI, we first stratified our cohort into participants ≥12–17 yrs of age (n = 41) and participants ≥18 yrs of age (n = 143) (Table 1).

Participants ≤18 yrs of age included a higher proportion of female subjects and used CFTR modulator therapy significantly less prior to ETI initiation. As expected, these patients had a higher ppFEV1 but lower absolute BMI values than participants ≥18 years of age. PpFEV1 at 3 and 12 months of ETI treatment were also significantly higher (p = 0.001), and BMI absolute values were still significantly lower in the group ≥12–17 yrs of age (p = 0.001).

Assessing predictors for the outcomes of absolute ppFEV1 or BMI after 12 months of ETI therapy, we found that neither gender nor prior CFTR modulator therapy influenced absolute values of ppFEV1 or BMI. It was only age at ETI initiation, which continued to exert an impact upon these absolute values, similar to the age dependency observed between the age groups at baseline.

Regression analyses for ppFEV1 gains revealed that after 3 months of ETI therapy, age and ppFEV1 at ETI initiation were significantly associated with ppFEV1 gains (p < 0.001 and p = 0.004, respectively), while neither gender, prior CFTR modulator therapy, nor BMI or degree of lung disease severity at baseline was associated with this outcome. BMI gains at 3 months were only influenced by ppFEV1 at baseline (p = 0.008), but none of the other parameters were tested.

Our analyses with only those patients aged 12 yrs and older showed similar trends with regard to the impact of age and ppFEV1 at ETI initiation upon ppFEV1 gains at 12 months after ETI initiation (p = 0.001 and p = 0.05, respectively). For BMI gains at 12 months, age at ETI initiation did have a significant effect (p = 0.004) as did BMI at initiation (p = 0.039), but contrary to the 3-month data, ppFEV1 at initiation did not associate with BMI gains.

TABLE 1 Patients' characteristics of the total cohort and stratified into three age groups (≥18 yrs, ≥6–11 yrs, and ≥12–17 yrs). Prior ETI therapy and F508del homozygosity were distributed differently between the three age groups. PpFEV1 and BMI at ETI initiation, and after 3 and 12 months of ETI therapy showed significant age-dependent differences, as did BMI gains at 3 months but not after 12 months. PpFEV1 gains or sweat chloride changes at all time points measured did not show age-dependent differences. *p*-values refer to Kruskal–Wallis or Mann–Whitney U inter-group comparisons for continuous variables and chi-squared tests for discontinuous variables.

Characteristic	All (*n* = 226)	*p*-value	≥18 years (*n* = 143)	≥12–17 years (*n* = 41)	≥6–11 years (*n* = 42)
Age at start (yrs), median (IQR)	22.5 (13.4–30.5)	0.000	27.5 (22.9–37.1)	13.8 (12.7–15.7)	9.3 (7.0–10.5)
Sex (female, %)	51.8	0.135	49.0	65.9	47.6
CFTR_prior ETI (%)	45.1	0.001	50.3	19.5	52.4
F508del homozygous (%)	54.4	0.013	60.1	34.1	54.8
FEV1% at initiation, median (IQR)	66.3 (45.7–84.6) (*n* = 226)	0.001	53.1 (33.9–71.1) (*n* = 143)	83.4 (66.6–95.4) (*n* = 41)	87.7 (79.7–97.5) (*n* = 42)
FEV1% at FU 3 months, median (IQR)	82.6 (57.4–99.2) (*n* = 226)	0.001	64.1 (44.7–87.6) (*n* = 143)	95.8 (80.5–107.2) (*n* = 41)	99.2 (88.2–106.0) (*n* = 42)
FEV1% at FU 12 months, median (IQR)	71.8 (52.5–93.0) (*n* = 182)	0.001	62.3 (46.0–87.2) (*n* = 143)	96.0 (78.2–107.4) (*n* = 39)	------
Δ FEV1% $_{(FU\ 3\ mo.–start)}$, median (IQR)	9.7 (5.1–17.8) (*n* = 226)	0.269	10.0 (5.3–18.0) (*n* = 143)	11.0 (5.2–19.1) (*n* = 41)	7.8 (3.2–14.9) (*n* = 42)
Δ FEV1% $_{(FU\ 12\ mo.–start)}$, median (IQR)	9.6 (5.5–16.4) (*n* = 182)	0.351	9.2 (5.4–16.6) (*n* = 143)	11.1 (7.1–14.9) (*n* = 39)	------
BMI (kg/m^2) at initiation, median (IQR)	19.5 (17.3–21.5) (n = 226)	0.001	20.9 (18.9–22.4) (n = 143)	18.4 (17.1–20.3) (n = 41)	15.5 (14.7–16.4) (n = 42)
BMI (kg/m^2) at FU 3 months, median (IQR)	20.6 (18.3–22.7) (*n* = 226)	0.001	21.6 (19.8–23.8) (*n* = 143)	19.3 (17.8–21.6) (*n* = 41)	15.7 (14.8–16.8) (*n* = 42)
BMI (kg/m^2) at FU 12 months, median (IQR)	21.6 (19.4–23.9) (*n* = 182)	0.001	22.2 (19.8–24.2) (*n* = 143)	19.4 (18.1–21.7) (*n* = 39)	------
Δ BMI (kg/m^2) $_{(FU\ 3\ mo.–start)}$, median (IQR)	0.8 (0.2–1.5) (*n* = 226)	0.001	0.9 (0.4–1.7) (*n* = 143)	1.2 (0.3–1.8) (*n* = 41)	0.3 (−0.2-0.6) (*n* = 42)
Δ BMI (kg/m^2) $_{(FU\ 12\ mo.–start)}$, median (IQR)	1.4 (0.4–2.6) (*n* = 182)	0.869	1.4 (0.3–2.6) (*n* = 143)	1.3 (0.4–2.6) (*n* = 39)	-----
Chloride (mmol/L) at initiation, median (IQR)	95.0 (84.0–103.0) (n = 214)	0.495	95.0 (84.0–101.0) (n = 135)	98.0 (88.0–104.8) (n = 40)	93.0 (79.0–105.0) (n = 39)
Chloride (mmol/L) at FU 3 months, median (IQR)	44.0 (31.0–55.5) (*n* = 195)	0.890	44.0 (31.0–55.3) (n = 134)	42.5 (31.0–62.3) (*n* = 36)	45.0 (36.0–55.0) (*n* = 25)
Δ Chloride (mmol/L) $_{(FU\ 3mo–start)}$, median (IQR)	47.5 (36.0–62.0) (*n* = 188)	0.789	49.0 (36.0–61.8) (*n* = 128)	47.5 (34.5–67.3) (*n* = 36)	45.5 (39.0–59.5) (*n* = 24)

Regression analyses of only those patients in the group with the most severe lung disease (ppFEV ≤P25, age ≥6-≥18 years, *n* = 55,) provided more insights into the critical role of age at initiation of ETI. Younger age is associated with significantly higher ppFEV1 gains at 3 and 12 months after ETI initiation ($p < 0.001$ and $p < 0.002$, respectively). Neither gender, prior CFTR modulator therapy, nor BMI or degree of lung disease severity at baseline was associated with ppFEV1 gains at 3 or 12 months.

Severity of pre-existing lung disease does not impact upon ppFEV1 but on BMI response in the overall cohort

Given our results on age as a significant determinant of ppFEV1 and BMI gains, we hypothesized that pre-existing lung disease might exert different influences on ppFEV1 or BMI gains, depending on the age group analyzed. We, thus, stratified our cohort for age-specific severity of pre-existing lung disease by calculating age group-specific lung function percentiles (age-specific ppFEV1 ≤25th percentile (≤P25), 26th–74th percentile (P50), and ≥75th percentile (≥P75). This stratification did not lead to statistically significant differences with regard to age, proportion of female subjects, or prior CFTR modulator use (Supplementary Table S1). PpFEV1 gains at 3- and 12-month FU were similar in these three groups (8.1 (4.4–19.1) vs. 10.9 (5.7–19.9) vs. 9.3 (3.4–15.1) for 3-month FU ($p = 0.209$) and 9.0 (4.6–14.2) vs. 10.5 (5.6–22.0) vs. 9.2 (5.9–14.2) for 12-month FU ($p = 0.258$) for ppFEV1 ≤P25 vs. P50 vs. ≥P75, respectively.

As expected, absolute BMI values at baseline were significantly lower in the participants with the lowest lung function [19.1 (16.8–21.4) vs. 19.3 (17.0–21.2) vs. 20.2 (18.0–22.2), $p = 0.041$, for ppFEV1 ≤P25 vs. P50 vs. ≥P75, respectively]. BMI changes 3 and 12 months after initiation of ETI did show significant group differences ($p = 0.001$ at 3 months; $p = 0.022$ at 12 months), suggesting higher BMI gains in the participants with lower lung function (+1.2 (0.6–2.1) vs.+ 0.7 (0.0–1.4) vs. + 0.7 (0.0–1.3) for ppFEV1 ≤P25 vs. P50 vs. ≥P75, respectively, at 3-month FU and

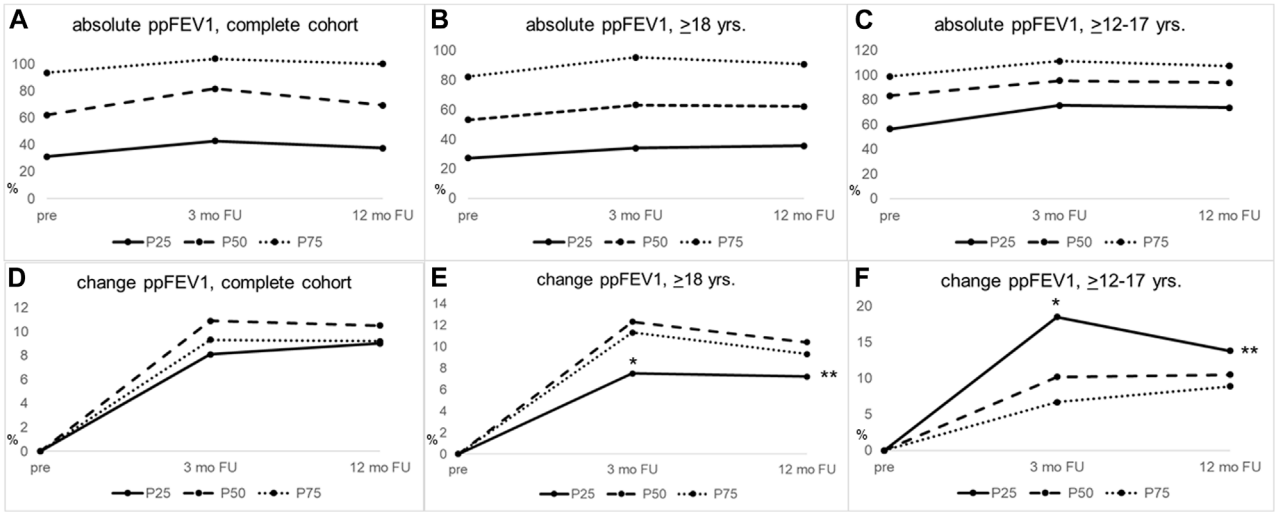

FIGURE 1
PpFEV1 gains in response to ETI therapy differ between groups of lung disease severity within the same age stratum and are significantly larger in adolescents with severe lung disease, compared to adults with severe lung disease. **(A–C)** Median absolute values of ppFEV1 for **(A)** the complete cohort, **(B)** pwCF ≥18 years, and **(C)** pwCF ≥12–17 years of age. **(D–F)** Change from baseline ppFEV for **(D)** the complete cohort, **(E)** pwCF ≥18 years of age, and **(F)** pwCF ≥12–17 years. Adults (middle column) with less severe lung disease (≥P75) showed significantly higher ppFEV1 increase than pwCF with worse lung disease (≤P25) 3 months after ETI initiation, *p*-value = 0.019. Adolescents (right column) with worse lung disease (≤P25) had significantly higher ppFEV1 gains than those with less severe lung disease (P50) 3 months after ETI initiation, *p*-value = 0.027, whereas in adults, the group with lower lung function at ETI initiation (≤P25) showed less significant ppFEV1 gains at 3 months than the group with the highest ppFEV1 percentile (≤P75; $p = 0.025$). PpFEV1 gains at 3 and 12 months of ETI treatment were significantly higher in adolescents with worse lung disease (≤P25) than adults (≤P25) at 3 (**p* = 0.002) and 12 months (***p* = 0.012). Severity of lung disease was calculated, as outlined in materials and methods. FU, follow-up; mo, months; ≤P25, baseline ppFEV1 ≤25th age-specific percentile; P50, baseline ppFEV1 26th–74th age-specific percentile; ≥P75, baseline ppFEV1 ≥75th age-specific percentile; yrs, years.

+2.2 (0.6–3.5) vs. +1.1 (0.3–2.6) vs. +1.1 (0.4–2.3) for ppFEV1 ≤P25 vs. P50 vs. ≥P75, respectively, at 12-month FU (Supplementary Table S1; Figure 1, left column).

Lung disease severity is associated with larger ppFEV1 improvements in adolescents and children but not adults

When stratifying for the severity of lung disease in the adolescents (≥12–17 years, Table 2), we observed significantly higher improvements in ppFEV1 in participants with the worst baseline lung function at 3 months post-ETI initiation (ppFEV1 +18.5 (10.6–28.2) vs. +10.2 (5.2–17.7) vs. +6.7 (2.3–14.5), $p = 0.027$ for ppFEV1 ≤P25 vs. P50 vs. ≥P75, respectively). This difference abated at 12 months post-ETI initiation (+13.8 (11.3–24.3) vs. +10.5 (5.6–24.0) vs. +8.9 (2.9–11.9), $p = 0.09$ for ppFEV1 ≤P25 vs. P50 vs. ≥P75, respectively) (Table 2; Figure 1, right column).

The more pronounced ppFEV1 gains in the adolescent participants with worse lung function were in contrast to the adult patients. Here, lower lung function at baseline was associated with significantly fewer improvements at 3 months post-ETI initiation (+7.5 (3.7–10.7) vs. +12.3 (6.2–21.6) vs. +11.3 (6.0–16.6), $p = 0.025$ for ppFEV1 ≤P25 vs. P50 vs. ≥P75, respectively), which abated at 12 months (7.2 (3.5–11.8) vs. 10.4 (5.6–22.0) vs. 9.3 (6.2–14.2), $p = 0.121$ for ppFEV1 ≤P25 vs. P50 vs. ≥P75, respectively) (Table 2; Figure 1 middle column).

When comparing adolescents (≥12–17 years) and adults with worse lung function (≤P25) for ppFEV1 gain after 3 and 12 months of ETI initiation, adolescents showed significantly higher improvements at both time points than adults ($p = 0.002$ and $p = 0.012$ for 3 and 12 months, respectively, Table 2).

Complementing our cohort with data from patients between 6 and 11 years of age supported the finding that in younger patients, those with more severe lung disease show higher gains in ppFEV1 3 months after ETI initiation (Table 2). At baseline, as expected, this cohort showed an even more preserved lung function in the three ppFEV1 percentile groups than adults and adolescents [ppFEV1 baseline: 70.7 (61.4–80.7) vs. 87.7 (82.9–95.0) vs. 102.1 (100.6–105.4) for ppFEV1 ≤P25 vs. P50 vs. ≥P75, respectively]. In these younger children, the previously described effect of a more pronounced ppFEV1 gain in adolescent patients with a lower lung function was also seen. However, this change did not reach statistical significance [+19.8 (0.4–26.0) vs. +8.4 (5.0–13.3) vs. +5.2 (1.3–7.8), $p = 0.126$ for ≤P25 vs. P50 vs. ≥P75, respectively], (Table 2; Supplementary Table S1A). Furthermore, these higher ppFEV1 gains in those children within the worst ppFEV1 stratum were not significantly different from those of the adolescents or the adult group. Yet, when comparing all children ≥6–17 yrs with a worse lung function ($n = 20$ ≤P25) than adults with ppFEV1 ≤P25, this finding attained significance with a higher gain of ppFEV1 in children (children and adolescents: +19.3 (8.0–27.3) vs. adults: +7.5 (3.7–10.7), $p = 0.007$) 3 months after ETI initiation (data not shown).

TABLE 2 Patients characteristics of the total cohort stratified for age-dependent severity of lung disease as outlined in materials and methods. PpFEV1 gains and BMI gains distribute unevenly between the age-specific ppFEV1 groups and also between the age groups when comparing groups of similar age-specific lung function severity. *p*-values refer to Kruskal-Wallis or Mann-Whitney-U inter-group comparisons for continuous variables and chi-squared tests for discontinuous variables. [f]*p*-value = 0.027 ≤P25 vs. P50 group; [g]*p*-value = 0.019 ≤P25 vs. ≥P75 groups; [h]*p*-value = 0.036 ≤P25 vs. ≥P75; [i]*p*-value = 0.006 ≤P25 vs. P50 group; [j]*p*-value = 0.026 P50 vs. ≥P75 group; [k]*p*-value = 0.066 ≤P25 vs. P50 group; [l]*p*-value = 0.046 ≤P25 vs. ≥P75; [m] *p* = 0.021; *p-value = 0.002 for ppFEV1 gains at three months of ETI therapy between ≥12–17 years age group vs. ≥18 years age group, **p-value = 0.012 for ppFEV1 gains at 12 months of ETI therapy between ≥12–17 years age group vs. ≥18 years age group. BMI, Body-mass-index; ETI, Elexacaftor/tezacaftor/ivacaftor; FU, Follow up; IQR, Inter quartile range; mo., month; yrs, years; ≤P25, baseline ppFEV1 ≤25th age-specific percentile; P50, baseline ppFEV1 26th–74th age-specific percentile; ≥P75, baseline ppFEV1 ≥75th age-specific percentile.

≥12–17 years (*n* = 41)				
Characteristics	≤P25 ppFEV1 (*n* = 10)	P50 ppFEV1 (*n* = 21)	≥P75 ppFEV1 (*n* = 10)	*p*-value
Age at start (yrs), median (IQR)	15.2 (12.7–17.0)	13.2 (12.2–15.4)	13.8 (13.7–15.8)	0.190
Sex (female, %)	70.0	61.9	70.0	0.861
CFTR_prior ETI (%)	40.0	14.3	10.0	0.164
F508del homozygous (%)	60.0	14.3	50.0	0.021
FEV1% at start, median (IQR)	56.4 (50.2–61.9) (*n* = 10)	83.4 (73.9–91.3) (*n* = 21)	98.9 (95.5–110.9) (*n* = 10)	—
FEV1% at FU 3 mo, median (IQR)	75.6 (70.6–82.2) (*n* = 10)	95.6 (82.8–104.9) (*n* = 21)	111.4 (100.2–118.4)(*n* = 10)	—
FEV1% at FU 12 mo, median (IQR)	73.6 (64.4–81.7) (*n* = 10)	94.0 (86.6–107.4) (*n* = 19)	107.6 (104.4–120.4)(*n* = 10)	—
Δ FEV1% $_{(FU\ 3\ mo.–start)}$, median (IQR)	18.5 (10.6–28.2)[f, *] (*n* = 10)	10.2 (5.2–17.7)[f] (*n* = 21)	6.7 (2.3–14.5) (*n* = 10)	0.027
Δ FEV1% $_{(FU\ 12\ mo.–start)}$, median (IQR)	13.8 (11.3–24.3)** (*n* = 10)	10.5 (5.6–24.0) (*n* = 19)	8.9 (2.9–11.9) (*n* = 10)	0.090
BMI (kg/m^2) at start, median (IQR)	17.8 (16.1–20.9) (*n* = 10)	18.1 (16.8–20.3) (*n* = 21)	19.4 (17.8–20.4) (*n* = 10)	0.495
BMI (kg/m^2) at FU 3 mo, median (IQR)	19.7 (17.8–23.7) (*n* = 10)	18.9 (17.2–21.4) (*n* = 21)	21.1 (17.7–22.5) (*n* = 10)	0.437
BMI (kg/m^2) at FU 12 mo, median (IQR)	19.5 (18.3–24.9) (*n* = 10)	19.1 (17.8–20.8) (*n* = 19)	21.1 (18.8–22.0) (*n* = 10)	0.221
Δ BMI (kg/m^2) $_{(FU\ 3\ mo.–start)}$, median(IQR)	2.0 (1.4–2.4)[g] (*n* = 10)	1.0 (0.3–1.5) (*n* = 21)	0.6 (−0.0–2.3)[g] (*n* = 10)	0.034
Δ BMI (kg/m^2) $_{(FU\ 12\ mo.–start)}$,median(IQR)	2.6 (−0.1–4.5) (*n* = 10)	1.1 (0.1–2.4) (*n* = 19)	1.1 (0.6–2.6) (*n* = 10)	0.323
≥18 years (*n* = 143)				
Characteristics	≤P25 ppFEV1 (*n* = 35)	P50 ppFEV1 (*n* = 73)	≥P75 ppFEV1 (*n* = 35)	*p*-value
Age at start (yrs), median (IQR)	35.2 (23.4–41.6)	28.0 (23.3–33.8)	24.5 (20.3–32.9)	0.092
Gender (female, %)	48.6	49.3	48.6	0.996
CFTR_prior ETI (%)	48.6	54.8	42.9	0.495
F508del homozygous (%)	65.7	58.9	57.1	0.729
FEV1% at start, median (IQR)	27.2 (23.4–30.4) (*n* = 35)	53.1 (45.4–60.7) (*n* = 73)	82.3 (77.0–92.9) (*n* = 35)	—
FEV1% at FU 3 mo, median (IQR)	34.1 (28.1–39.9) (*n* = 35)	63.3 (55.6–82.5) (*n* = 73)	95.6 (86.9–107.3) (*n* = 35)	—
FEV1% at FU 12 mo, median (IQR)	35.5 (28.4–41.3) (*n* = 35)	62.2 (55.2–78.1) (*n* = 73)	90.9 (84.4–106.3) (*n* = 35)	—
Δ FEV1% $_{(FU\ 3\ mo.–start)}$, median (IQR)	7.5 (3.7–10.7)[h, *] (*n* = 35)	12.3 (6.2–21.6) (*n* = 73)	11.3 (6.0–16.6)[h] (*n* = 35)	0.025
Δ FEV1% $_{(FU\ 12\ mo.–start)}$, median (IQR)	7.2 (3.5–11.8)** (*n* = 35)	10.4 (5.6–22.0) (*n* = 73)	9.3 (6.2–14.2) (*n* = 35)	0.121
BMI (kg/m^2) at start, median (IQR)	20.1 (17.6–21.9)[i] (*n* = 35)	20.5 (18.6–22.3)[i, j] (*n* = 73)	21.7 (20.2–24.3)[j] (*n* = 35)	0.005
BMI (kg/m^2) at FU 3 mo, median (IQR)	20.9 (19.3–24.1) (*n* = 35)	21.3 (19.8–23.5) (*n* = 73)	22.3 (21.2–25.7) (*n* = 35)	0.089
BMI (kg/m^2) at FU 12 mo, median (IQR)	22.2 (19.5–24.9) (*n* = 35)	22.0 (20.1–24.2) (*n* = 73)	22.4 (20.8–26.5) (*n* = 35)	0.438
Δ BMI (kg/m^2) $_{(FU\ 3\ mo.–start)}$, median(IQR)	1.2 (0.6–2.2)[k] (*n* = 35)	0.9 (0.3–1.6)[k] (*n* = 73)	0.7 (0.0–1.5) (*n* = 35)	0.065
Δ BMI (kg/m^2) $_{(FU\ 12\ mo.–start)}$,median(IQR)	2.0 (0.8–3.4) (*n* = 35)	1.1 (0.3–2.7) (*n* = 73)	1.1 (0.0–2.2) (*n* = 35)	0.048

(Continued on following page)

TABLE 2 (*Continued*) Patients characteristics of the total cohort stratified for age-dependent severity of lung disease as outlined in materials and methods. PpFEV1 gains and BMI gains distribute unevenly between the age-specific ppFEV1 groups and also between the age groups when comparing groups of similar age-specific lung function severity. *p*-values refer to Kruskal-Wallis or Mann-Whitney-U inter-group comparisons for continuous variables and chi-squared tests for discontinuous variables. [f]*p*-value = 0.027 ≤P25 vs. P50 group; [g]*p*-value = 0.019 ≤P25 vs. ≥P75 groups; [h]*p*-value = 0.036 ≤P25 vs. ≥P75; [i]*p*-value = 0.006 ≤P25 vs. P50 group; [j]*p*-value = 0.026 P50 vs. ≥P75 group; [k]*p*-value = 0.066 ≤P25 vs. P50 group; [l]*p*-value = 0.046 ≤P25 vs. ≥P75; [m] *p* = 0.021; [*]*p*-value = 0.002 for ppFEV1 gains at three months of ETI therapy between ≥12–17 years age group vs. ≥18 years age group, [**]*p*-value = 0.012 for ppFEV1 gains at 12 months of ETI therapy between ≥12–17 years age group vs. ≥18 years age group. BMI, Body-mass-index; ETI, Elexacaftor/tezacaftor/ivacaftor; FU, Follow up; IQR, Inter quartile range; mo., month; yrs, years; ≤P25, baseline ppFEV1 ≤25[th] age-specific percentile; P50, baseline ppFEV1 26[th]–74[th] age-specific percentile; ≥P75, baseline ppFEV1 ≥75[th] age-specific percentile.

≥6–11 years (*n* = 42)				
Characteristics	≤P25 ppFEV1 (*n* = 10)	P50 ppFEV1 (*n* = 22)	≥P75 ppFEV1 (*n* = 10)	*p*-value
Age at start (yrs), median (IQR)	10.2 (7.1–11.3)	9.0 (7.3–10.5)	8.1 (6.7–9.3)	0.203
Sex (female, %)	50.0	45.5	50.0	0.958
CFTR_prior ETI (%)	60.0	50.0	50.0	0.858
F508del homozygous (%)	60.0	50.0	60.0	0.809
FEV1% at start, median (IQR)	70.7 (61.4–80.7) (*n* = 10)	87.7 (82.9–95.0) (*n* = 22)	102.1 (100.6–105.4)(*n* = 10)	—
FEV1% at FU 3 mo, median (IQR)	83.7 (74.1–94.5) (*n* = 10)	98.8 (88.7–101.9) (*n* = 22)	107.5 (105.1–112.7)(*n* = 10)	—
Δ FEV1% $_{(FU\ 3\ mo.-start)}$, median (IQR)	19.8 (11.6–28.3) (*n* = 10)	8.4 (5.0–13.3) (*n* = 22)	5.2 (1.3–7.8) (*n* = 10)	0.126
BMI (kg/m^2) at start, median (IQR)	16.4 (15.0–18.1) (*n* = 10)	15.3 (14.4–15.8) (*n* = 22)	16.1 (15.3–16.9) (*n* = 10)	0.039
BMI (kg/m^2) at FU 3 mo, median (IQR)	17.0 (14.9–19.3)[l] (*n* = 10)	15.1 (14.3–15.8) (*n* = 22)	16.2 (15.3–17.1)[l] (*n* = 10)	0.039
Δ BMI (kg/m^2) $_{(FU\ 3\ mo.-start)}$, median(IQR)	0.8 (0.2–1.3)[m] (*n* = 10)	0.2 (−0.4–0.3) (*n* = 22)	0.3 (−0.1–0.8)[m] (*n* = 10)	0.026

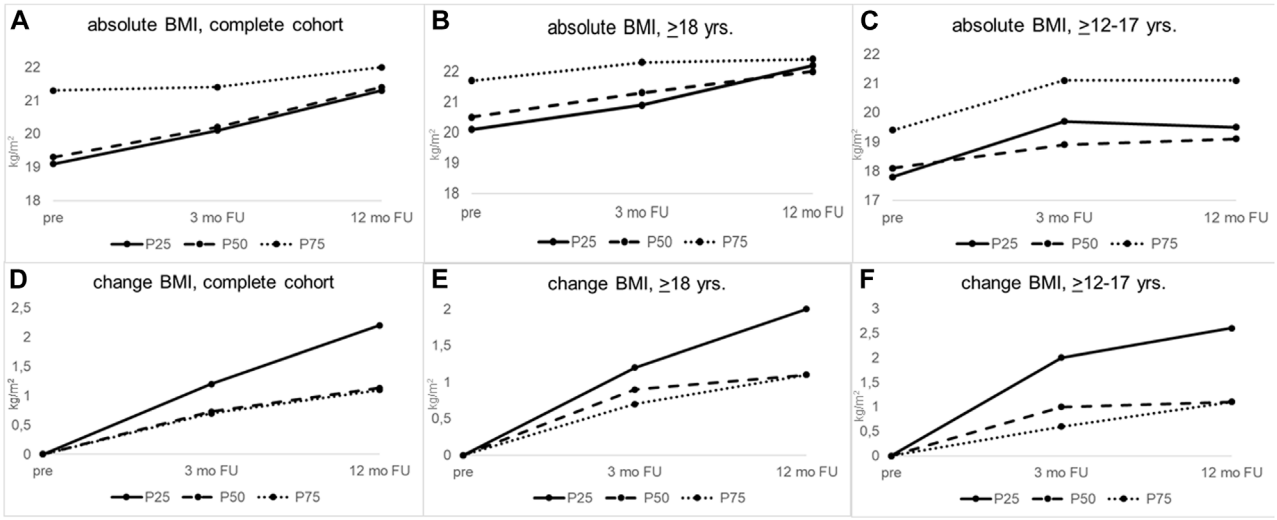

FIGURE 2
BMI gains in response to ETI therapy differ between groups of lung disease severity within the same age stratum but do not show differences in response between adolescents and adults. **(A–C)** Median absolute values of BMI for **(A)** the complete cohort, **(B)** pwCF ≥18 years, and **(C)** pwCF ≥12–17 years of age. **(D–F)** Change from baseline BMI for **(D)** the complete cohort, **(E)** pwCF ≥18 years of age, and **(F)** pwCF ≥12–17 years. In the overall cohort (left column), patients with worse lung function (≤P25) had significantly higher BMI gains at 3 (p = 0.001) and 12 months (p = 0.022) after ETI initiation than those with medium or good lung function (P50; ≥P75). The same effect was confirmed in the other age groups. Inter-age comparison did not show any statistical differences for BMI gains (middle and right column). FU, follow-up; mo, months; ≤P25, baseline ppFEV1 ≤25th age-specific percentile; P50, baseline ppFEV1 26th–74th age-specific percentile; ≥P75, baseline ppFEV1 ≥75th age-specific percentile; yrs, years.

More severe lung disease is associated with larger BMI improvement regardless of age

In the adolescent participants, there was no statistical difference in the absolute baseline BMI values when stratified according to the severity of lung disease prior to ETI initiation (Table 2; Figure 2, right column). Absolute BMI values at 3 or 12 months post-ETI initiation also did not differ statistically according to the severity of lung disease in this age group (Table 2). Participants with more severe lung disease showed higher BMI gains than those with a better lung

function at 3 (≤P25 = 2.0 vs. P50 = 1.0 vs. ≥P75 = 0.6 kg/m*2; $p = 0.034$) and 12 months (≤P25 = 2.6 vs. P50 = 1.1 vs. ≥P75 = 1.1 kg/m*2; $p = 0.323$), although these differences were statistically significant only at 3 months post-ETI initiation (Table 2; Figure 2, right column).

In contrast to the adolescent participants, adults with the most severe lung function had significantly worse absolute BMI values at baseline than patients with a better lung function (20.1 (17.6–21.9) vs. 20.5 (18.6–22.3) vs. 21.7 (20.2–24.3), $p = 0.005$ for ppFEV1 ≤P25 vs. P50 vs. ≥P75 respectively) (Table 2). These differences were lost 3 and 12 months after initiation of ETI ($p = 0.089$ and $p = 0.438$), suggesting more significant improvements in response to ETI in those participants with a worse lung function. Indeed, for adults, significantly larger improvements in BMI were observed in those pwCF with the worst lung function after both 3 (≤P25 = +1.2 vs. P50 = 0.9 vs. ≥P75 = 0.7 kg/m*2; $p = 0.065$) and 12 months (≤P25 = 2.0 vs. P50 = 1.1 vs. ≥P75 = 1.1; $p = 0.048$) (Table 2).

Interestingly, in pwCF ≥6–11 years of age (Table 2; Supplementary Table S1B), BMI improvements at 3 months were also larger in those with more severe lung function impairment (+0.8 (0.2–1.3) vs. +0.2 (−0.4-0.3) vs. +0.3 (−0.1-0.8), $p = 0.026$ for ppFEV1 ≤P25 vs. P50 vs. ≥P75, respectively).

Discussion

Our analyses provide initial data, which suggest that ETI response, as reflected in ppFEV1 and BMI levels, is dependent on age at initiation and lung disease severity. Specifically, the data show that children and adolescents with more severe lung disease (defined as age-group-specific ≤25% percentile ppFEV1) exhibited higher ppFEV1 gains at 3 and 12 months after ETI initiation than those with a better lung function at baseline in the same age group. Furthermore, inter-age comparisons of those participants affected most severely with respect to lung disease (age-specific ppFEV1 ≤P25) showed higher ppFEV1 gains in children and adolescents than adults at 3 and 12 months after ETI introduction. These findings are supported by regression analyses in the sub-group of all pwCF affected most severely (ppFEV1 ≤P25 at ETI initiation), which identified an association of younger age with higher ppFEV1 gains at 3 and at 12 months after initiation of ETI therapy.

To the best of our knowledge, our data are the first to identify larger lung function improvements toward CFTR modulator therapy in adolescents (Table 2; Figure 1), and adolescents and children (data shown in the previous section) with more severe lung function impairment than adults with more severe lung function impairment.

Previous studies also indicated a more favorable response profile of children compared to adults with regard to ppFEV1. Earlier introduction of IVA or LUM/IVA led to better preservation of lung function, a larger impact on PEx (Merlo et al., 2022), and a higher ppFEV1 response (Bui et al., 2021). More recently, Muilwijk et al., in a long-term study on data from the Dutch CF registry on real-world data on LUM/IVA responses, also found larger increases of ppFEV1 in pwCF with more advanced lung disease (Muilwijk et al., 2022). Their study incorporated longer follow-up data and more participants than most previous analyses on LUM/IVA, possibly unveiling effects not previously identifiable due to limited numbers or study duration. It seems conceivable that the larger functional improvements achieved by ETI compared to LUM/IVA (Graeber et al., 2021; Graeber et al., 2022a; Graeber et al., 2022b) provide the necessary statistical power to this study of ETI despite the lower numbers of participants. Our regression analyses support age as a central factor for ppFEV1 responses as they showed effects of age at ETI initiation on ppFEV1 gains both at 3- and 12-month follow-ups for both the entire cohort and those with most severe lung function impairment. Together, Muilwijk's and our findings suggest that younger patients with more severe lung disease experience higher gains in ppFEV1 in response to CFTR modulation.

Our analyses on improvements of ppFEV1 in those pwCF with severe lung disease are in a similar range as previous data by Burgel et al. (2021) and Carnovale et al. (2022) and do not refute their conclusions that the pwCF with the most advanced lung disease do benefit considerably from ETI. They rather extend their observation to younger age groups. Furthermore, our data indicate that in adult pwCF, compared to adolescent pwCF, those with more severe lung disease at ETI initiation take longer time to improve as the differences in ppFEV1 gains in adult pwCF, which were significant at 3 months between the three groups of lung disease severity, abated at 12 months (Table 2).

Unlike Muilwijk et al., our data failed to identify that adolescents or children show a more favorable BMI trajectory than adults (Muilwijk et al., 2022). Our data did unveil stronger BMI increases in those individuals with more severe lung function. However, these effects were seen in all age groups. These findings were supported by our regression analyses, which showed a significant impact of ppFEV1 at initiation for BMI gains at 3 months but not after 12 months of ETI therapy, whereas age only affected BMI gains after 12 months but not after 3 months, indicating a complex interplay of age and severity of lung disease at ETI initiation, for which at present, we fail to identify uni-directional effects.

Our analyses failed to identify the effects of gender on absolute values of ppFEV1 or BMI or ppFEV1 or BMI gains attained through ETI therapy, where female subjects have been shown to respond more favorably toward IVA with regard to annualized pulmonary exacerbation (PEx) rates and respond to LUM/IVA with larger BMI gains (Secunda et al., 2020; Muilwijk et al., 2021). Yet, the inclusion of multi-centric data, some of which stemming from more controlled clinical trials, makes a direct comparison difficult. These studies also addressed other CFTR modulators and thus might also suggest that ETI lacks gender-dependent response profiles seen with other CFTR modulators.

Interestingly, the models we built also failed to indicate an effect of prior CFTR modulator therapy on absolute ppFEV1 or BMI, or ppFEV1 or BMI gains. The PROMISE Study Group showed that the highest average changes in ppFEV1 were in those pwCF previously using no modulator or a two-drug combination (Nichols et al., 2022). Our findings on superior ppFEV1 gains in adolescents with severe lung function impairment compared to adults within the same range of lung function impairment might recapitulate the observations made in PROMISE since the adolescent age group contains a significantly lower proportion of modulator therapy prior to ETI initiation (19.5%) vs. the adult group (50.3%) ($p = 0.001$, Table 1). However, modulator use in children 6–11 years of age was comparable between this age group and adults, and nonetheless, the children with severe lung function impairment showed higher ppFEV1 gains than the adults with severe lung function impairment.

Our analysis has important limitations, particularly due to the heterogeneity of our subpopulations. In that line, our study cohorts differ significantly in the proportion of previous CFTR modulator therapy prior to ETI initiation (adolescents 19.5% vs. adults 50% vs.

children 6–11 years 52%). Our smaller cohort with limited follow-up data may be underpowered to produce conclusive results, particularly due to unaccounted effects of previous CFTR modulator therapy. On one hand, responses to CFTR modulator therapy may be more favorable in pwCF who had had prior therapy compared to those without because the latter group may have developed irreversible changes in the intervening time which preclude further positive responses. On the other hand, improvements in response to prior CFTR modulator therapy might eventually provide a "ceiling" effect where further improvements in either ppFEV1 or BMI cannot be observed anymore. Yet, children and adolescents, with the worst lung function (≤P25) comprised the highest proportion of previous CFTR modulator therapy (adolescents 40.0%; children 60.0%) in a comparable range to adults (48.6%). Regression analysis in this cohort with regard to the impact of previous modulator therapy did reveal significant influences of this variable.

In addition, our age sub-groups differ in the proportion of deltaF508 homozygosity, which might impact the disease severity. Interestingly, our focus sub-group (spirometry ≤P25) contains a comparable proportion of deltaF508 homozygosity across all age groups (60%–66%), which improves the comparability of these subpopulations. Along this line, other publications also failed to identify the effects of genotype–phenotype correlations, albeit also at small cohort sizes (Carnovale et al., 2022). Still, given our cohort size and its mono-centricity, we cannot exclude that previous CFTR modulator therapy or genotype influences response to ETI. Larger cohorts, preferably registry-based data and/or longer observation periods, are necessary to disentangle these aspects.

Clinical data concerning longitudinal evolvement of pulmonary function and anthropometry, supporting age-dependent effects, are yet lacking for ETI. Such data are of particular importance in view of the large number of young patients, which became and will still become eligible for ETI. Due to previous diagnostic and therapeutic improvements, this patient population will initiate CFTR modulator therapy in an unprecedented state of health. This, combined with their young age, will render the monitoring of clinical changes in response to CFTR modulator therapy, particularly challenging. Data, as we present here, which support an association of the magnitude of response toward ETI and younger age, might lend credibility to a comprehensive approach toward the initiation of ETI at the earliest age possible, as well as advocating treatment continuation even in cases, where the subjective or objective benefits might not be immediately observable due to low functional impairment at ETI initiation.

In light of the first study demonstrating long-term lung function stability in those individuals treated with ETI (Lee et al., 2022), the earliest possible initiation of ETI promises to add an unprecedented therapeutic value for pwCF. Our study is one of the few studies, which incorporates longitudinal data from pwCF aged 6+ years. Our data partially confirm our hypothesis that early initiation of ETI will prove most beneficial but limit this confirmation to those with most severe lung disease. Furthermore, our study design limits its transfer to other cohorts and clinical settings. Due to licensing timing, we included a much larger group of adults in our study than we were able to include adolescents or children, the latter of which we could only analyze after 3 months but not yet after 12 months of ETI therapy. In addition, our severity and age-specific sub-groups are of even lower numbers. These limitations may lead to statistical bias; therefore, results need to be interpreted cautiously. Our retrospective approach is by design exploratory; therefore, results need to be cautiously interpreted. We cannot exclude that follow-up data on larger groups of pwCF with longer follow-up trajectories might unveil some effects we failed to identify. In that same line, some of the identified effects might no longer be identifiable in a more heterogeneous population. Our data demonstrate a wide age range and disease severity but stem from a single CF center in Germany, albeit one of Germany's larger centers. Although this mono-centric approach permits analysis of pwCF treated according to similar standards across all ages, this approach necessarily limits generalizability, which can only be gained from larger, preferably prospective clinical trials or register-based studies. We, thus, advocate patience until larger and longer studies validate our findings by interrogating the drivers of ETI response, including, in particular, factors such as gender, age, and clinical status at initiation.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

All patients or their legal guardians provided consent to anonymized scientific use of personal clinical data for research purposes, either as written informed consent to participate in scientific studies of the German Center for Lung Research (DZL) registry (Ethics Committee Hannvovr Medical School, #2923-2015, Hannover Medical School) and/or the German CF registry (Ethics Committee of the Justus-Liebig-Universität FB Medizin, #AZ24/19). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

KS, JK, and A-MD designed the research. The patient and data recruitment was conducted by KS, SJ, FR, AS-H, CS, MW, CD, SP, RM, BT, TB, SP, and A-MD. Data analysis was performed by JK, KS, and A-MD. KS, JK, MW, FR, and A-MD wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1171544/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Gains in ppFEV1 are highest in the groups with the most severe pre-existing lung disease. **(A)** ppFEV1 gains and **(B)** BMI gains from baseline to 3 months of ETI therapy, stratified for the severity of lung disease for the age groups ≥12–17 yrs and ≥6–11 years. In both age groups, highest gains of ppFEV1 were seen in children with worst lung disease (≤P25). Highest gains in BMI were seen in adolescents within the group with the most severe lung disease (≤P25). FU, follow-up; mo, months; ≤P25, baseline ppFEV1 ≤25th age-specific percentile; P50, baseline ppFEV1 26th–74th age-specific percentile; ≥P75, baseline ppFEV1 ≥75th age-specific percentile; yrs, years.

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Weight increase in people with cystic fibrosis on CFTR modulator therapy is mainly due to increase in fat mass

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Background: Ivacaftor, the first CFTR modulator drug, leads to significant long-term improvement in lung function and weight gain. The mechanism as well as the long-term impact of ivacaftor on weight, resting energy expenditure (REE) and body composition remains to be explored.

Methods: This prospective observational study included 18 people with CF (pwCF) (age: median (range) 20 (6–58) years) carrying at least one CFTR gating mutation commencing ivacaftor. Assessments of body composition, REE and laboratory investigations were performed at baseline and 6, 12 and 24 months after treatment initiation.

Results: Treatment with ivacaftor was associated with a significantly positive change in BMI z-score at 24 months. Fat mass (mean (95% CL) of 6.5 kg (4.0; 9.0) from baseline, p = 0.0001), but not fat-free mass changed under ivacaftor treatment. There was a significant positive correlation between weight and fat mass change. Overall, there was no significant change in measured REE from baseline (mean (95% CL) of 108 kcal/d (–12; 228), p = 0.07) in our cohort. Pancreatic function and other nutritional markers did not change with treatment, with the exception of an increase in serum vitamin A levels (p = 0.006).

Conclusion: The weight gain observed in ivacaftor treated pwCF is predominantly secondary to increases in fat mass warranting early counseling of people starting on CFTR-modulating treatment with respect to healthy diet and physical exercise.

KEYWORDS

cystic fibrosis, ivacaftor treatment, nutritional status, body composition, resting energy expenditure trail registration: NCT03390985 BMI-body mass index, CFTR modulator treatment

Abbreviations: BMI, Body Mass Index; CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; CI, Confidence Interval; DXA, Dual X-ray Absorptiometry; FAO, Food and Agriculture Organization; FEVpp, Forced Expiratory Volume in one second Percent Predicted; LCI, Lung Clearance Index; PI, Pancreatic Insufficiency/Insufficient; PS, Pancreatic sufficient; REE, Resting Energy Expenditure; UNO, United Nations University; WHO, World Health Organization.

Highlights

- REE in G551D CF on ivacaftor did not change, except in 3 patients with high baseline REE
- Weight increase on ivacaftor treatment was associated with increase in fat mass
- No change in pancreas function.

Introduction

The treatment of patients with cystic fibrosis (CF) carrying mutation G551D was revolutionized with the introduction of ivacaftor into clinical care (Ramsey et al., 2011). This CFTR modulator drug increases the opening probability of CFTR channels and has been shown to improve lung function and reduce pulmonary exacerbations in patients carrying gating mutations. In addition, the use of ivacaftor has been associated with clinically significant weight gain (Ramsey et al., 2011; Stallings et al., 2016). The mechanism underlying the weight gain seen in this context, however, has not yet been fully elucidated.

PwCF typically have a lower weight, body mass index (BMI), as well as a reduced fat and lean mass compared to their peers (Newby et al., 1990; Bianchi et al., 2006; Sheikh et al., 2014). The altered body composition seen in CF may be secondary to a variety of factors, such as poor intake due to anorexia; inefficient digestion/absorption secondary to pancreatic insufficiency (PI), liver disease and/or intestinal inflammation; as well as increased energy expenditure, which occurs particularly in the context of more advanced lung disease or concurrent infections (Fried et al., 1991; Pencharz and Durie, 2000). In addition, catabolism associated with systemic inflammation can further impact patients' muscle mass (Ionescu et al., 2002). Interventions, such as nutritional rehabilitation have been shown to lead to improvements in weight (Levy et al., 1985); however, less is known about the impact of dietary modifications on the body composition of these patients. Similarly, little is known about the long term impact of CFTR modulators, such as ivacaftor on body composition (Tierney et al., 2015; Stallings et al., 2018).

The primary objective of our study was to investigate whether the weight gain seen in adults and children with CF receiving treatment with ivacaftor is associated with changes in energy expenditure. Further, we wanted to explore the change in body composition occurring in this context.

Subjects and methods

This was a prospective observational study performed at the Hospital for Sick Children and at St. Michael's Hospital in Toronto between 2013 and 2016. The Research Ethics Board of both academic institutions approved the study (SickKids Research Ethics Board (REB)# 1000036224 and SMH REB# 13–089), which was in compliance with the ethical principles outlined in the declaration of Helsinki. All participants and/or caregivers signed an informed consent prior to study enrolment. Minors signed assent forms.

Patient cohort

PwCF carrying at least one gating CFTR mutation who were commencing treatment with ivacaftor 150 mg twice daily between 2011 and 2016 were included in this observational study. Exclusion criteria were the inability to undergo assessment of body composition (via air displacement plethysmography) or energy expenditure (via indirect calorimetry).

Patients were categorized according to their pancreatic function as: 1. Insufficient (PI) if they had a fecal elastase <100 μg/g stool and/or serum trypsinogen <10 ng/ml; 2. Borderline if they had a fecal elastase ranging between 50 and 200 μg/g stool and a serum trypsinogen >10 ng/ml; and, 3. Pancreatic sufficient (PS) if they had a fecal elastase >200 μg/g stool and/or serum trypsinogen >10 ng/ml.

Monitoring

Patients were initiated on treatment when clinically stable and data were collected from their routine clinical visits prior to starting ivacaftor (pre-drug), as well as 6 months (post-6; 5.3–7.1 months), 12 months (post-12; 11.7–14.8 months) and 24 months (post-24; 23.5–28.3 months) after starting treatment with ivacaftor. Data collection included anthropometric measurements, measurements of energy expenditure and body composition, and laboratory investigations. Levels of fat-soluble vitamins were only determined annually. Lung function was assessed by spirometry (FEV1 percent predicted [FEV1pp] according to European Respiratory Society/American Thoracic Society standards using Global Lung Initiative equations (Quanjer et al., 2012)), as well as the Lung Clearance Index (LCI) using the multiple breath washout was measured (Subbarao et al., 2015).

Anthropometric measurements

Weight and height were assessed using a digital scale and a stadiometer mounted on the wall, respectively. Body mass index was calculated as kg/cm^2. BMI values were converted to z-scores using the World Health Organization references (https://www.who.int/growthref/en/).

Resting energy expenditure (REE)

Energy expenditure was measured by indirect calorimetry using VMax™ Encore 29 machine (Carefusion Medical Products, Yorba Linda, California) operated by a trained technician. Indirect calorimetry was considered successful and acceptable when the patients reached steady state for a minimum of 10 min and the respiratory quotient was within the acceptable physiologic range (0.67 and 1.35). Predicted REE was calculated using the Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) equation (Energy and protein requirements, 1985). A measured REE>110% of that predicted by the WHO equation was used to define hypermetabolism.

TABLE 1 Baseline characteristics of study cohort.

Variable		Result
≤17 years (n = 11)	mean (range)	10.6 (6.1–14.9)
>17 years (n = 7)	mean (range)	33.8 (17.8–58.3)
Gender - female	n (%)	11 (61%)
Genotype	(n)	G551D/F508del (12), G178R/F508del (1)
		G551D/2622 + 1G>A (1), G551D/E585X (1)
		G551D/3272-26A>G (2), G551D/unknown (1)
Pancreatic insufficiency	n (%)	12 (67%)
CF-related diabetes	n (%)	1 (6%)

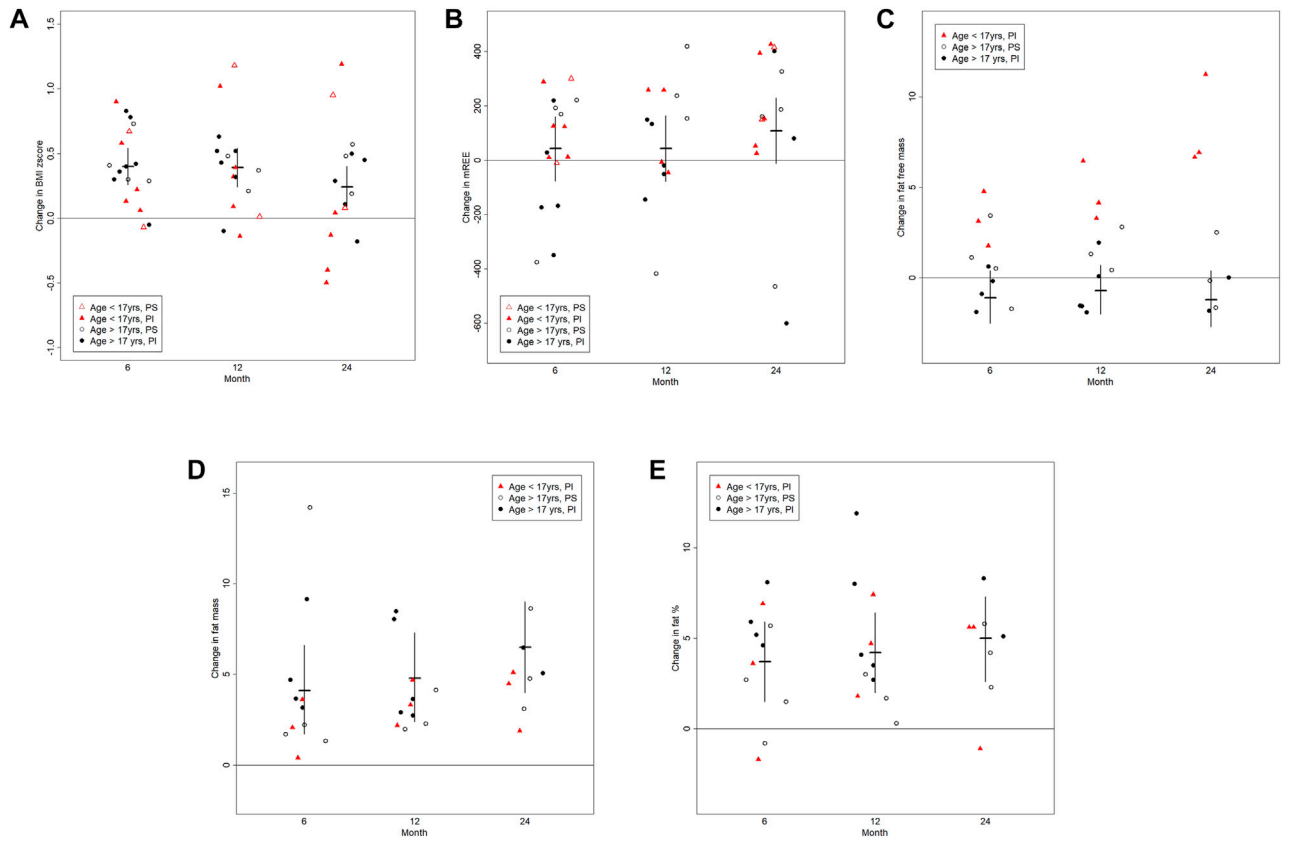

FIGURE 1
The graphs shows changes in the **(A)** body mass index (BMI, kg/cm^2), **(B)** resting energy expenditure (REE (kcal/d)), **(C)** fat free mass (kg), **(D)** fat mass (kg) and **(E)** fat percent (%) between baseline and follow-up visits at post-6, post-12 and post-24 months. Results are visualized as scattered dot plots reflecting individual results. The horizontal line represents the mean and the vertical lines represent 95% confidence limits.

Body composition

Body composition assessments were performed using air displacement plethysmography (BOD POD 2007A, Life Measurements Inc.). Patients were asked to wear either tight fitting underwear or a bathing suit and their hair was covered with a tightly fitting cap. The subjects were asked to sit in the chamber and breathe calmly without moving during the measurements. Two 40-s measurements were taken per person; measurements were considered successful when the deviation in body volume was less than 3 mL/L subject size. The average of the two measurements was used in the analyses.

Laboratory investigations

Laboratory investigations included nutritional markers (e.g., albumin, fat soluble vitamins), markers of pancreatic function

TABLE 2 Summary data of the clinical visits before and after patients started on ivacaftor.

	Repeated measures of values at each visit model controls for age at baseline				Repeated measures of change from visit 1 (baseline); model controls for baseline value and age at baseline		
	Baseline	Post-6	Post-12	Post-24	Post-6—Baseline	Post-12—Baseline	Post-24—Baseline
	Estimate (n)				*Estimate (95%CL) p-value*		
	95%CL						
Primary Outcome							
mREE/kcal/day	1466 (18)	1500 (16)	1513 (13)	1576 (14)	42 (−77; 160)	43 (−79; 164)	108 (−12; 228)
	1347; 1585	1378; 1623	1384; 1643	1449; 1703	0.5	0.5	0.076
mREE/kcal/day/kg	30.3 (18)	28.5 (16)	27.6 (12)	27.1 (13)	−1.8 (−3.7; 0.2)	−2.9 (−5.0;-0.8)	−3.1 (−5.2;-1.1)
	27.3; 33.2	25.5; 31.5	24.4; 30.7	23.9; 30.2	0.075	0.009	0.005
mREE, % predicted	111.4 (18)	110.3 (16)	102.9 (12)	104.7 (13)	−1.5 (−9.5; 6.6)	−8.3 (−17.7; 1.1)	−6.0 (−15.0; 3.1)
	102.2; 120.6	100.7; 120.0	91.9; 113.8	94.1; 115.3	0.7	0.080	0.18
Secondary Outcomes							
Anthropometric Measures							
BMI z-score	0.50 (18)	0.90 (18)	0.88 (16)	0.73 (15)	0.40 (0.26; 0.54)	0.39 (0.24; 0.54)	0.24 (0.09; 0.40)
	0.05; 0.94	0.46; 1.34	0.43; 1.33	0.29; 1.18	<.0001	<.0001	0.003
Body Composition (fat)							
Fat mass (kg)	12.3 (13)	16.5 (11)	16.8 (11)	18.3 (8)	4.1 (1.7; 6.6)	4.8 (2.4; 7.3)	6.5 (4.0; 9.0)
	5.8; 18.8	10.0; 23.1	10.3; 23.3	11.7; 24.9	0.003	0.0007	0.0001
Fat mass (kg)[a]	6.7 (10)	11.6 (8)	11.5 (8)	13.7 (5)	5.7 (−0.6; 12.1)	6.3 (−0.1; 12.6)	8.6 (2.2; 14.9)
	−2.9; 16.4	1.9; 21.4	1.8; 21.3	3.8; 23.5	0.072	0.052	0.013
Fat %	21.1 (13)	25.0 (11)	25.7 (11)	26.4 (8)	3.7 (1.5; 5.9)	4.2 (2.0; 6.4)	5.0 (2.6; 7.3)
	14.9; 27.4	18.7; 31.3	19.4; 32.0	20.1; 32.8	0.003	0.0008	0.0004
Fat %[a]	15.1 (10)	19.4 (8)	19.6 (8)	21.5 (5)	4.7 (0.3; 9.0)	4.9 (0.6; 9.1)	6.9 (2.5; 11.2)
	6.4; 23.9	10.5; 28.2	10.8; 28.5	12.6; 30.4	0.037	0.030	0.0056
Body Composition (lean)							
Fat free mass (kg)	42.5 (13)	43.2 (11)	43.9 (11)	45.0 (8)	0.6 (−1.1; 2.3)	1.2 (−0.4; 2.9)	2.3 (0.5; 4.1)
	34.9; 50.1	35.5; 50.8	36.2; 51.5	37.3; 52.7	0.5	0.12	0.01
Fat free mass (kg)[a]	50.6 (10)	50.6 (8)	50.8 (8)	50.2 (5)	0.0 (−1.3; 1.3)	0.2 (−1.1; 1.6)	−0.4 (−2.0; 1.1)
	40.1; 61.2	40.0; 61.2	40.3; 61.4	39.6; 60.8	>0.9	0.7	0.5

[a]Age at baseline >17 years, mREE-measured resting energy expenditure.

(serum trypsinogen, fecal elastase), as well as markers of inflammation, such as C-reactive protein and absolute neutrophil count.

Statistical analyses

Data across the clinic visits were modeled in two ways: 1) observed value across all visits and 2) the change from baseline at each follow-up visit. The mixed-effects model for repeated measurements (MMRM; PROC MIXED; SAS statistical software package, SAS Institute, Cary, NC) was used to control for the within subject correlation of the repeated measures across visits. Changes from baseline controlled for baseline values as a covariate in the model. We tested the effect of pancreatic status by adding it to the model of change from baseline, and we tested the effect of age and change in weight in the models of REE across visits and change from baseline. Results are provided as estimates plus 95% confidence limits (CI). The primary outcome was change in REE and the secondary outcome were change in body composition. We also analyzed the change in other variables, which in total can be grouped into 7 broad categories (including REE, body composition, anthropometric measures, lung function, inflammation markers, pancreatic function status and

TABLE 3 Summary data of pancreas status, lung function and inflammation markers before and after patients started on ivacaftor.

	Repeated measures of values at each visit				Repeated measures of change from baseline visit model controls for baseline value		
	Baseline	Post-6	Post-12	Post-24	Post-6–Baseline	Post-12–Baseline	Post-24–Baseline
	Estimate (n)				Estimate (95%CL) *p*-value		
	95%CL						
Pancreas status-Insufficient							
Trypsinogen (ng/mL)	3.54 (11)	2.79 (10)	2.59 (8)	4.80 (3)	−0.88 (−1.44;-0.32)	−0.91 (−1.51;-0.31)	1.43 (0.37; 2.50)
	2.86; 4.23	2.08; 3.49	1.79; 3.39	3.53; 6.07	0.0043	0.0054	0.011
Fecal Elastase (μg.g fat)*	15.6 (8)	16.3 (9)	14.4 (7)	19.9 (5)			
	10.9; 20.3	11.8; 20.8	9.4; 19.3	13.9; 26.0			
Pancreas status-Sufficient							
Trypsinogen (ng/mL)	34.5 (6)	16.3 (6)	31.4 (5)	30.5 (3)	−16.6 (−42.9; 9.8)	0.1 (−26.9; 27.2)	−6.7 (−35.6; 22.2)
	16.2; 52.9	−2.1; 34.6	12.0; 50.7	7.8; 53.2	0.2	>0.9	0.6
Fecal Elastase (μg.g fat)*	234 (3)	159 (3)	259 (3)	209 (3)			
	82; 386	13; 306	111; 407	58; 360			
Nutritional markers							
Vitamin A (μmol/L)	1.32 (17)		1.52 (16)	1.65 (13)		0.21 (0.01; 0.40)	0.33 (0.12; 0.54)
	1.13; 1.52		1.32; 1.72	1.42; 1.88		0.04	0.006
Vitamin E (μmol/L)	19.6 (17)		21.6 (17)	22.8 (13)		2.2 (−1.2; 5.7)	3.7 (−0.1; 7.4)
	16.0; 23.3		18.0; 25.2	18.8; 26.8		0.19	0.057
Vitamin D (μmol/L)	77 (14)		88 (17)	84 (12)		14.3 (1.7; 26.9)	2.7 (−11.2; 16.6)
	62; 93		74; 103	68; 100		0.030	0.7
Albumin (g/L)	42.6 (17)		43.0 (17)	42.8 (13)		0.2 (−1.0; 1.4)	0.3 (−1.2; 1.7)
	41.5; 43.6		41.9; 44.0	41.6; 44.1		0.7	0.7
Inflammation markers							
CRP (mg/L)	4.1 (18)		2.0 (14)	1.6 (10)		−1.5 (−3.2; 0.1)	−2.5 (−4.4;-0.7)
	2.5; 5.6		0.3; 3.8	−0.5; 3.7		0.064	0.016

(Continued on following page)

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TABLE 3 (*Continued*) Summary data of pancreas status, lung function and inflammation markers before and after patients started on ivacaftor.

	Repeated measures of values at each visit				Repeated measures of change from baseline visit model controls for baseline value		
	Baseline	Post-6	Post-12	Post-24	Post-6–Baseline	Post-12–Baseline	Post-24–Baseline
	Estimate (n)				Estimate (95%CL) *p*-value		
	95%CL						
WBC (9×10^9/L)	8.8 (18)	7.7 (15)	7.6 (17)	7.4 (15)	−1.1 (−2.2;-0.1)	−1.2 (−2.2;-0.2)	−1.4 (−2.5;-0.3)
	7.6; 10.1	6.4; 9.0	6.4; 8.9	6.1; 8.7	0.037	0.022	0.011
Neutrophil count (9×10^9/L)	5.9 (10)	4.1 (9)	4.5 (11)	3.9 (10)	−1.9 (−3.1;-0.6)	−1.8 (−3.1;-0.5)	−2.0 (−3.4;-0.7)
	4.6; 7.1	2.8; 5.5	3.3; 5.7	2.6; 5.1	0.005	0.009	0.005
Lung function							
FEV1, % predicted	72.1 (18)	85.2 (18)	86.4 (17)	84.0 (16)	13.3 (8.0; 18.6)	14.6 (9.1; 20.0)	12.2 (6.7; 17.7)
	63.5; 80.7	76.6; 93.8	77.7; 95.0	75.3; 92.7	<.0001	<.0001	<.0001
FVC, % predicted	90.6 (18)	101.3 (18)	103.1 (17)	97.9 (14)	10.8 (7.3; 14.3)	12.6 (9.1; 16.2)	7.4 (3.6; 11.1)
	84.9; 96.4	95.6; 107.1	97.3; 109.0	91.9; 103.9	<.0001	<.0001	0.0003
LC2.5	13.3 (11)	11.2 (11)	11.1 (10)	11.1 (5)	−2.4 (−3.2;-1.5)	−2.5 (−3.4;-1.5)	−2.5 (−3.8;-1.3)
	11.1; 15.6	9.0; 13.5	8.9; 13.4	8.7; 13.5	<.0001	<.0001	0.0003
Other							
Sweat Chloride (mmol/L)	83 (18)	43 (11)	36 (16)	38 (15)	−41 (−47;-34)	−46 (−53;-40)	−44 (−50;-37)
	77; 88	35; 50	30; 42	32; 45	<.0001	<.0001	<.0001

Change model does not control for baseline. Grey shaded areas indicate that the results do not meet the adjusted significant level of $p = 0.007$ (see METHOD).

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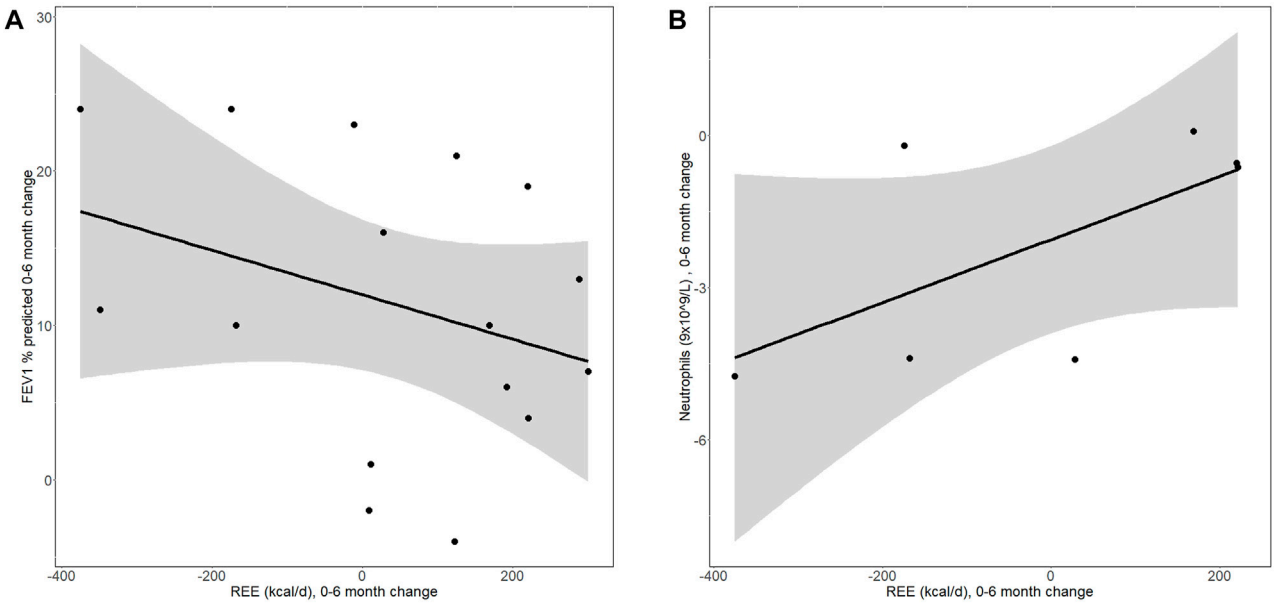

FIGURE 2
The graphs show correlation between the post-6 months change in **(A)** REE (kcal/d) and FEV1pp and **(B)** REE (kcal/d) and neutrophil count (9×10^9/L). Dots reflect individual results. Line represents Pearson correlation and the grey shaded area the 95%confidence limit. **(A)** $r^2 = -0.33$ (−0.69; 0.16), $p = 0.2$, n = 16) and **(B)** $r^2 = 0.64$ (−0.04; 0.92), $p = 0.12$, n = 7). Tough the sample was too small to reach significance, the trend/direction of these correlations are interesting.

nutritional markers). Therefore, we set the level of significance at 0.05/7 = 0.007. Lastly, we calculated the Pearson correlation between the change in REE and other variables for the change between baseline visit and the post-6 months visit, as most of the changes happened during this time frame.

Results

Eighteen pwCF were included in this study; 7 subjects were younger than 17 years of age at the time of the baseline visit (Table 1). The majority of the pwCF (n = 12) were pancreatic insufficient (PI), 3 pwCF were pancreatic sufficient (PS) and 3 had fecal elastase and serum trypsinogen measurements in the borderline range. For the purpose of the analyses, pwCF with a borderline pancreas function were grouped with the PS patients.

Anthropometrics

Over the 24-month treatment period all pwCF demonstrated an increase in BMI z-score ($p = 0.003$). This change in anthropometrics occurred in the first 6 months of treatment, with no significant changes thereafter (Figure 1A and Table 2). One pwCF with PS was removed from the post-12 and post-24 analyses because this patient underwent gastric bypass surgery. The changes in anthropometric measures were independent of the pancreas function status (pancreatic status effect on change in weight, $p > 0.9$).

Resting energy expenditure

Measurements of the REE were available for all 18 pwCF at baseline, for 16 pwCF 6 months post treatment initiation as well as for 13 and 14 pwCF at 12 and 24 months post treatment initiation, respectively. At baseline 7 pwCF were hypermetabolic. With respect to the entire cohort, there was no significant change in the ratio of measured to predicted REE or clinically significant change in the absolute REE over the treatment course of months (Figure 1B and Table 2). However, the 3 pwCF with the highest measured to predicted REE ratio of 136%, 186% and 146% at baseline showed a decrease to 103%, 108% and 100% at 24 months post drug (Supplementary Figure 1A).

Body composition (fat free mass)

Data on body composition were available for 13 pwCF at pre-drug assessment and for 11 pwCF 6 months post drug, as well as for 11 and 8 pwCF at 12 and 24 months post drug assessment. There was no significant change in fat free mass over the observation time, except for the 3 children ≤17 years of age for which we had body composition data (difference post-6 months to baseline (age)): 4.8 kg (12), 3.1 kg (15) and 1.8 kg (16)) (Figure 1C).

Body composition (fat mass)

In contrast to the fat free mass, fat mass increased by a mean (95%CL) of 6.5 kg (4.0; 9.0), $p = 0.0001$ 24 months post drug. The change in fat mass was independent of the pancreatic function status

(p = 0.8) and occurred in both children and adults (Figure 1D and Table 2).

Given the above, treatment was associated with an overall mean increase of 5 percent body fat (95%CL (2.7; 7.3), p = 0.0004) by 24 months) (Figure 1E and Table 2). Individual increase in fat mass correlated with increase in body weight (Supplementary Figure 1B).

Pancreatic function

There was no significant change in pancreatic function measured by fecal elastase and serum trypsinogen over the course of the study (Table 3). In pwCF with PI, fecal elastase remained <100 μg/g stool and in those with PS, fecal elastase remained >200 μg/g stool. The three pwCF classified as having a borderline pancreatic function remained in the borderline range over the 24-month period.

Biochemical nutritional markers

Except for one pwCF with low vitamin A, D and E levels at baseline, which normalized during the follow-up period, no other patient had fat-soluble vitamin deficiencies on regular vitamin supplementation. There was no change in vitamin D and E levels with treatment, but we observed an increase in serum vitamin A levels (p = 0.006; Table 3). Most of the pwCF (9/12) in whom vitamin A levels increased had also been on vitamin A supplementation.

Lung function and inflammation

Treatment with ivacaftor was associated with an improvement in FEV1pp (p = 0.0001) and a reduction in the lung clearance index (p = 0.0003) in 2 years. Furthermore, we observed a trend in declining serum C-reactive protein levels (p = 0.016) and a statistically significantly decrease in the absolute neutrophil count levels (p = 0.005) (Table 3).

We next wanted to see whether the change on weight or REE correlated with change in lung function or inflammation. There was no correlation between change in weight and lung function or inflammatory markers. However, while statistical significance was not reached mainly due to small numbers, we observed a negative correlation between REE and lung function (r^2 = −0.33 (−0.69; 0.16), p = 0.2) as well as positive correlation between REE and neutrophil count in sputum (r^2 = 0.64 (−0.04; 0.92), p = 0.12) (Figure 2).

Discussion

In this prospective, observational study we have shown that the weight gain seen following ivacaftor treatment in children and adults with CF resulted predominantly from increases in fat mass, which occurred largely in the first 6 months of treatment. Fat free mass did not change significantly with treatment, with the exception of those who were in the pediatric age range at the time of ivacaftor initiation. Weight and fat mass gains were not associated with a decline in REE, nor were they associated with changes in pancreatic function or other serum markers of nutritional status.

At 12 months following treatment start with ivacaftor, 46% of pwCF had excess adiposity compared to 38% at baseline. In contrast to the z-BMI, which showed an initial increase in 6 months and then no further increase, the fat mass as well as the %body fat continue to increase over the 24 months observations. Clinicians should be aware of the excess adiposity seen in these patients, particularly during treatment with CFTR modulator. More importantly, BMI is an inadequate indicator of obesity (Javed et al., 2015), as only two pwCF at baseline assessment and one pwCF at 12 months post-drug had a z-BMI >2. Considering the rates of increased body fatness in these subjects, the association between fat free mass and lung function and the importance of the latter in terms of long-term outcomes, physical activity should be recommended to all children and adults with CF. Further, all pwCF starting on CFTR-modifying treatment should receive dietary consultation aiming for a healthy balanced diet.

Previous studies have reported on weight gain induced by CFTR-modulating treatment, though with a shorter duration of follow-up (Ramsey et al., 2011; Borowitz et al., 2016). One study evaluated change in REE and body composition in pwCF on ivacaftor treatment over an observation period of 3 months (Stallings et al., 2018). In contrast to our findings, Stalling et al. (Stallings et al., 2018) observed an increase in fat free mass and fat mass in pwCF on ivacaftor using dual x-ray absorptiometry (DXA) to assess body composition, with an overall increase in %body fat by 1.7% ± 2.3%. Another study saw no difference in fat mass in a 28d-short term ivacaftor-placebo crossover trial in 20 people carrying at least on G551D mutation using bioelectric impedance analysis to evaluate for body composition changes. However, in King's cohort fat mass increased in the first 6 months on ivacaftor as did weight without any further increase between 6 and 24 months (King et al., 2021). This is different to our observation showing continuous increase in fat mass and fat% of the body composition over the 2 years.

Discrepancy in findings may be due to differences in the methodologies used to assess body composition, the differences in the follow-up time, as well as differences in the baseline body composition of the cohorts. In our study we chose to use air displacement plethysmography (BOD POD®) instead of DXA as we aimed for repeated assessments of body composition over a 2-year period. BOD POD® does not expose patients to radiation, is convenient and well accepted by research participants. Despite the fact that BOD POD® is fairly new compared to DXA, it has been used extensively in both pediatric and adult research, and has been shown to generate results comparable to DXA (Talbert et al., 2009; Gracia-Marco et al., 2012; Campisi et al., 2015; Polfuss et al., 2016).

In our study cohort, there was no significant change in REE when expressed as percent predicted REE. This is different to (Stallings et al., 2018), who showed a change in REE over an observation period of 3 months and a negative correlation between the changes in the ratio of measured to predicted REE and weight gain. As per the authors most of the subjects had measured to predicted REE <100%, which is unusual for pwCF. Our cohort was very heterogeneous with regards to their baseline measured to predict REE. Nevertheless, most of the pwCF showed a decline in their measured to predicted REE%pred following ivacaftor treatment, particularly those 3 patients with highest REE% pred at baseline. Since we did not measure lean body mass, but rather fat free mass using the BOD POD®, we cannot provide REE ratios in respect to the individual lean body mass.

There was no improvement in pancreatic function throughout the duration of the study. This may have to do with the age of the pwCF at the time of ivacaftor initiation. According to current belief there is an age threshold in CF upon which the pancreas is likely to be irreversibly damaged due to end organ destruction with fibrous-fatty tissue replacement, though case reports have shown recovery of pancreas function in adolescent patients (Gould et al., 2022). Beyond energy expenditure and pancreatic function, other factors could have contributed to the weight gain seen with treatment, such as improved nutrient absorption and/or changes in appetite. Increased absorption in the context of treatment with ivacaftor could possibly be directly related to improved CFTR function at the level of the intestinal epithelium. It has been shown that ivacafor treatment led to an improvement in intestinal pH in pwCF (Gelfond et al., 2017). Reduction in intestinal inflammation as a consequence of ivacaftor-restored CFTR function can also contribute to improved nutrient absorption (Ooi et al., 2018).

In our cohort, treatment with ivacaftor led to a decrease in markers of systemic inflammation, such as CRP and the absolute neutrophil count, which is a systemic marker of inflammation. There is supportive evidence that the reduction in systemic inflammation may be one of the explanations for the increase in body weight in patients on CFTR-modulating treatment.

Ratjen et al. have shown a correlation between a decrease in systemic inflammation markers and weight gain in Pseudomonas-negative CF patients treated with azithromycin as anti-inflammatory therapy (Ratjen et al., 2012). Association between inflammation and nutritional status, is further supported by the study of (Naon et al., 1993) who had shown that ivacaftor reduced pulmonary inflammatory markers in an observational study of 12 pwCF carrying G551D (Hisert et al., 2017). Recently, reduction in systemic inflammation was shown in 20 pwCF treated with highly efficient CFTR modulator (elexacaftor/tezacaftor/ivacaftor) who also had significant improvement in weight, BMI and nutritional parameters (Carnovale et al., 2022).

However, our sample for analysis was small and the results should be interpreted with caution. Future studies to elucidate the association between systemic inflammation and weight gain may need to focus on those pwCF with low weight z-scores and abnormal REE.

The limitation of the study is the relatively small sample size as we were recruiting only pwCF with G551D starting on the highly efficient CFTR modulator ivacaftor. Another limitation is the paucity of data on dietary intake, as well as physical activity levels, which could have affected weight and body composition changes. Lastly, this was an observational study reflecting "real-life" setting of CFTR-modulation in pwCF. This means that drug compliance was not controlled for.

In summary, we have demonstrated that long-term treatment with ivacaftor is associated with weight gain, which is predominantly driven by an increase in fat mass that occurs in the first 6 months of treatment. Changes in REE and pancreatic function are not responsible for the changes seen in our cohort. A significant proportion of pwCF following ivacaftor treatment has excess adiposity and should receive appropriate counseling regarding lifestyle changes.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by SickKids Research Ethics Board and St. Micheal's Research Ethics Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Study concept and design: MM, TG, FR, ET; Acquisition of data: TG, JA, KG, Analysis and interpretation of data: MM, TG, AD, FR, and ET; Drafting of manuscript: MM, TG; Critical revision of the manuscript: MM, AD, JA, KG, FR, ET, and TG; Study supervision: TG. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The study was funded by Vertex Pharmaceutical as Investigator initiated grant to TG. Vertex had no influence on the study design, collection, analysis or result interpretation.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1157459/full#supplementary-material

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Dynamics of abdominal symptoms during the start of a new therapy with elexacaftor/tezacaftor/ivacaftor using the novel CFAbd-day2day questionnaire

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Background: Elexacaftor–tezacaftor–ivacaftor (ETI) is a novel, highly effective CFTR modulator combination proven to enhance lung function and body weight in people with cystic fibrosis (pwCF) carrying a F508del mutation. Recently, we revealed significant reductions in abdominal symptoms (AS) in German, British, and Irish pwCF after 24–26 weeks of ETI using the CFAbd-Score, the first patient-reported outcome measure (PROM) specifically developed and validated for pwCF following FDA guidelines. Notably, many pwCF reported marked changes in their AS during the first days of the new treatment. To capture these immediate effects, we developed the CFAbd-day2day, a CF-specific GI-diary, following FDA and COSMIN guidelines.

Aim: To prospectively capture the immediate dynamics of AS using the CFAbd-day2day 14 days before and 14–28 days after ETI initiation. In addition, we aim to provide validation steps of the novel PROM concerning sensitivity to changes.

Methods: To develop the CFAbd-day2day, focus groups (community voice = pwCF and their proxies and CF specialists from different fields) were repeatedly consulted. Before and during the new ETI therapy, pwCF prospectively scored AS on a daily basis with the CFAbd-day2day.

Results: Altogether, 45 pwCF attended in five CF centers prospectively completed the CFAbd-day2day before (mean ± sd:14 ± 7 days) and after (mean ± sd: 28 ± 23 days) ETI initiation. On the one hand, cumulative scores significantly decreased during the 3–4-week time frame after ETI initiation, compared to 2 weeks prior to therapy. On the other hand, many patients who revealed a relatively stable level of

AS before ETI reported changes during the first days of treatment with the highly effective CFTR modulators. Factors like pain and flatulence increased in up to 21% of patients during the first 14 days of therapy, but they improved during days 15–27.

Conclusion: The CFAbd-day2day, specifically developed and in the process of validation to prospectively capture GI symptoms in pwCF, provides new substantial insights into the dynamics of AS in pwCF receiving a new treatment with ETI. This novel tool is also helpful in prospectively monitoring patients with specific GI problems. International implementation and further validation steps of the diary are ongoing.

KEYWORDS

gastrointestinal, patient-reported outcome measure, prospective, symptom score, diary, CFAbd-Score

1 Introduction

For long, abdominal involvement in cystic fibrosis (CF), the most common lethal inherited disease of the Caucasian population, received little attention. Since the availability of pancreatic enzyme supplementation therapy (PERT) in mostly (Ratjen and Doring, 2003) pancreatic insufficient patients, pulmonary infection and lung destruction became the major reason for premature death in approximately 90% of people with CF (pwCF) (Martin et al., 2016; Deutsches Mukoviszidose-Register Berichtsband, 2020). The causative gene defect results in abnormal production and function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, crucially affecting both the respiratory and gastrointestinal systems. Apart from the upper and lower airways, the ATP-gated anion channel is highly expressed in the pancreas, gut, and bile ducts, leading to a CF-specific pattern of gastrointestinal (GI) complaints (Ooi et al., 2016; Freedman et al., 2018). In addition to exocrine pancreatic insufficiency, present from birth in approximately 85% of pwCF, impaired intestinal passage by meconium ileus, distal intestinal obstruction syndrome (DIOS), and constipation are typical complications in pwCF (Munck et al., 2016; Ooi and Durie, 2016; Stefano et al., 2022). These are complemented by factors like intestinal dysbiosis caused by frequent antibiotic treatments, cough-associated reflux, and maldigestion, resulting in malresorption of nutrients, which then allow bacterial fermentation, gases, and diarrhea as principal symptoms of untreated exocrine pancreatic insufficiency (Caley et al., 2023a). Finally, endocrine liberation of insulin is also hampered by the destruction of pancreatic beta cells, leading to CF-related diabetes in a substantial proportion of pwCF during adulthood (Caley et al., 2023b).

Introduction of highly effective CFTR modulator therapies (HEMTs) in pwCF carrying a rare gating mutation like G551D over a decade ago revealed that correction and potentiation of the defective and/or malfunctioning CFTR channel have marked effects on the health status of pwCF beyond pulmonary function (Ramsey et al., 2011; Davies et al., 2013; Bodewes and Wilschanski, 2018; King et al., 2021). Patients substantially gained weight and thrived if therapy was introduced in childhood (Davies et al., 2016). At the same time, from personal experience with our patients (Mainz et al., 2018), we learned that HEMTs had effects on abdominal symptoms (AS). However, the lack of validated CF-specific PROMs focusing on GI involvement impeded former rigorous research to adequately assess the apparently substantial changes during early HEMT with ivacaftor in pwCF carrying a gating mutation.

To fill this gap, we developed the CFAbd-Score in different steps following FDA guidelines for the development of a PROM (U.S. Department of Health and Human Services Food and Drug Administration, 2019), including CF patients and their families (community voice), as well as professional CF specialists at several time points. Initially, the PROM had been named JenAbd-Score (Tabori et al., 2017a), and after condensing it from a 5-sided questionnaire to a one-sided PROM, which comprises 28 symptoms grouped in five domains, it was renamed "CFAbd-Score" (Tabori et al., 2017b; Jaudszus et al., 2019; Mainz et al., 2023; Jaudszus et al., 2022; Mainz et al., 2022; Caley et al., 2023b). Presently, it is available in eleven languages and implemented in more than 25 studies around the world (Boon et al., 2020; Mainz et al., 2023; Ng et al., 2021; Mainz et al., 2022; Raun et al., 2022).

Recently, we implemented the CFAbd-Score in an international study with 107 pwCF from Germany and the UK before and at a median of 24 weeks during therapy with elexacaftor–tezacaftor–ivacaftor (ETI). Therein, ETI was found to substantially improve the total CFAbd-Score and its five domains: "pain," "GERD," "disorders of bowel movement," "disorders of appetite," and "quality of life impairment" (Mainz et al., 2022). Quite a similar improvement pattern was observed during ETI in a parallel study administering the CFAbd-Score to 103 Irish and British pwCF before and after 1, 2, 6, and 12 months of ETI (Mainz et al., 2023). In both studies, the CFAbd-Score was observed to have high sensitivity to the changes induced by the new therapy with ETI, which is considered a game changer in CF. A recent multicenter study in the United States also found a significant reduction of GI symptoms in 263 pwCF receiving ETI. However, unlike results obtained with the CF-specific CFAbd-Score (Mainz et al., 2022; Mainz et al., 2023), changes assessed with questionnaires evaluated and validated for other GI pathologies, but not for pwCF [patient assessment of upper gastrointestinal disorders-symptom (PAC-SYM), patient assessment of constipation-symptom (PAC-SYM), and patient assessment of constipation-quality of life (PAC-QOL)], did not reach the level of clinical significance (Schwarzenberg et al., 2022).

Nevertheless, during the early phases after HEMT introduction, patients reported experiencing many GI symptoms, which may not be adequately represented by a PROM that addresses GI symptoms

retrospectively with a 2-week recall period. Furthermore, after HEMT initiation some symptoms could arise more frequently and markedly, but only for quite a short period of time, which may not be adequately recorded with a questionnaire focused on capturing the overall frequency of GI symptoms over the past 14 days.

Consequently, based on the CFAbd-Score, we developed the CFAbd-day2day questionnaire, a prospective diary PROM. This questionnaire is being validated following FDA and COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN) guidelines (Mokkink et al., 2010; U.S. Department of Health and Human Services Food and Drug Administration, 2019). Analogous to the development of the CFAbd-Score, pwCF and their families, as well as professional CF specialists (community voice), were included to optimize the wording and structuring of the PROM at several time points. The resulting prospective CF-specific GI-symptom diary CFAbd-day2day© is suitable for closely recording AS.

As the selection and wording of questions included in the CFAbd-Score have been found to be highly sensitive for capturing and quantifying GI symptoms in pwCF receiving a new ETI therapy (Mainz et al., 2023; Mainz et al., 2022), substantial changes concerned rewording of questions to allow prospective GI symptom inquiring on a daily basis. Furthermore, leaving room for individual comments and observations not covered by the questionnaire, such as eating habits and changes in PERT, appeared essential for the CFAbd-day2day.

The aim of this study was to prospectively assess changes in abdominal symptoms on a daily basis during a new, highly effective CFTR-modulating ETI therapy in pwCF. Specifically, we aimed to capture GI symptoms for a period of up to 14 days prior to commencing the new therapy, as well as during 14–28 days of the new therapy, using the novel CFAbd-day2day© questionnaire.

2 Methods

2.1 Participants

A total of 51 pwCF attending five German CF care centers in Brandenburg an der Havel/Potsdam (n = 22), Tübingen (n = 15), Gießen (n = 7) and Frankfurt am Main (n = 7) were prospectively considered to complete the CFAbd-day2day© before and after ETI initiation. Of note, 10 pwCF recruited at the CF centers in Brandenburg an der Havel/Potsdam for this project, which focuses on prospective short term changes in the CFAbd-day2day, were also included in the study previously published in 2022 (Mainz et al., 2022). Further inclusion criteria were: confirmed evidence of CF by two positive sweat tests and/or detection of two CFTR mutations, carrying at least one allele with F508del, as a requirement for ETI therapy initiation. Exclusion criteria were: inability to comply with the study procedures or assessments and below 6 years of age. Eligible subjects were included independent of their severity of pulmonary function (FEV1pred), airway colonization with specific pathogens, and comorbidities. A history of concomitant GI manifestations as well as food allergy or intolerance was recorded. Data acquisition was performed using *pseudonymization* and after written consent from the parents/legal guardian or from the pwCF themselves if their age was above 18 years.

2.2 Assessment of symptoms—CFAbd-day2day©

Abdominal symptoms were recorded daily using the CFAbd-day2day© questionnaire. The CFAbd-day2day© is a diary version of the CFAbd-Score. Therefore, the CFAbd-day2day© also comprises 28 items grouped into five domains. However, in addition to the modified recall period, some questions have been adapted to prospectively focus on each observational day. The questionnaire also includes an optional comments section for recording changes in dietary habits, enzyme and medication use, and menstrual symptoms. A major intrinsic advantage of the diary character is the ability to record not only symptom frequencies but also their daily intensity. Printed copies of the questionnaire were issued to the participants through the local CF care providers and/or research coordinators. Scoring and analyses of completed, pseudonymized questionnaires were centrally performed at the CF center in Brandenburg an der Havel, Germany.

2.3 Statistical analyses

Individual baseline values for each of the 28 items included in the CFAbd-day2day© questionnaire were obtained by computing medians of each item over the time frame prior to ETI therapy initiation for each pwCF. Dynamics of symptoms were assessed by computing daily proportions of subjects reporting either improvement or worsening with respect to the aforementioned baseline values for each of the 28 symptoms assessed with the CFAbd-day2day© questionnaire. Furthermore, within-subject averaged absolute daily variation measures were computed for each subject and each questionnaire item to quantify the levels of variation over three time frames: prior to ETI therapy and 2 and

TABLE 1 Distribution of responses in regard to relevant time frames.

	Time frame	Proportion of pwCF
Pre-ETI		
	14 days	38/50 (76%)
	10–13 days	4/50 (8%)
	7–9 days	5/50 (10%)
	1–6 days	3/50 (6%)
During ETI		
	27 or more days	29/50 (58%)
	21–26 days	9/50 (18%)
	14–20 days	3/50 (6%)
	10–13 days	3/50 (6%)
	7–9 days	0/50 (0%)
	1–6 days	1/50 (2%)

TABLE 2 Demographics and clinical characteristics of pwCF included in this study.

Demographics total (n) age (median, range)	45 10 (6–55) years
Age <18	37 (82.2) n (%)
Age ≥18	8 (17.8)
Female	30 (66.7)
Male	15 (33.3)
Genotype	
F508del homozygous	25 (55.5%)
F508del heterozygous	19 (42.2%)
F508del/unknown	1 (2.2%)
Previous CFTR modulator therapy	
Yes	20 (44.4%)
No	25 (55.5%)
Lumacaftor + ivacaftor	16 (35.5%)
Tezacaftor/ivacaftor + ivacaftor	3 (6.7%)
Ivacaftor	1 (2.2%)
CF-associated diseases/conditions	
Exocrine pancreatic insufficiency	Yes: 42/45 (93.3%)
	No: 3/45 (6.7%)
Meconium ileus	Yes: 8/45 (17.8%)
	No: 37/45 (82.2%)
Distal intestinal obstruction syndrome (DIOS)	Yes: 0 (0%)
	No: 45 (100%)
CF-related diabetes (CFRD)	Yes: 3/45 (6.7%)
	No: 36/45 (80%)
	Unknown: (13.3%)
CF-related liver disease (CFLD)	Yes: 9/45 (20%) Liver fibrosis: 4/45 (8.9%)
	Secondary biliary cirrhosis: 1/45 (2%)
	Hepatic steatosis grade 1: 1/45 (2%)
	Unspecified: 3/45 (6.7%)
	No: 30/45 (66.7%)
	Unknown: 6/45 (13.3%)
Report on food allergies or intolerances	Yes: 8/45 (17.8%)
	No: 37/45 (82.2%)
Lung function (FEV1%pred) (conducted in 41 patients[a])	88.3% ± 17.1%
Body mass index (BMI)	
Patients ≥18 years of age, n = 8 (17.8%) BMI (mean ± sd)	23.9 ± 4.0 kg/m^2
Patients <18 years of age, n = 37 (82.2%) BMI-for-age z-scores (mean ± sd)	−0.34 ± 1.0

[a]Baseline FEV_1%pred values from four pwCF were not available.

4 weeks after ETI therapy initiation. These measures were computed by averaging the absolute changes experienced by each patient between consecutive days in each item over the aforementioned time frames. Additionally, the maximum deviation with respect to the median in each item was identified for each patient within each time frame. In order to find out the period of time (prior to ETI therapy and 2 and 4 weeks after ETI therapy initiation) at which symptoms reported by the cohort underwent maximum changes, exploratory analyses to compare medians of these measures of variability were conducted using non-parametric Wilcoxon signed-rank tests. Effects of ETI therapy on cumulative CFAbd-day2day responses were assessed by mapping the frequency of events registered by each patient for each item in the CFAbd-day2day© with the following scale: not at all → 0, once → 1, 2–3 times → 2, 4–7 times → 3, more than 7 times → 4, and daily → 5. Afterward, items were grouped into five different domains as defined for the 2-week CFAbd-Score (Jaudszus et al., 2019). Scores for each domain and a total score were calculated for both the pre-ETI time frame and the 2-week time period involving the third and fourth weeks after ETI therapy initiation. Comparisons of domains and total scores were conducted using non-parametric Wilcoxon signed-rank tests. Prior to these tests, normality assumptions were tested using quantile–quantile plots as well as the Shapiro–Wilk test.

3 Results

A total of 51 pwCF were recruited for this study, of which 50 pwCF completed the questionnaire prior to ETI initiation (median: 15 days, IQR [14, 15] days). 45 (90%) of them completed the questionnaire after ETI initiation (median: 25 days, IQR [25, 27] days). Detailed information about the rates of daily answers is provided in Table 1. All 45 pwCF (median age: 10 [6–55] years) who completed the questionnaire during both time frames (Brandenburg an der Havel/Potsdam (40%), Gießen (16%), Tübingen (31%), and Frankfurt am Main (13%)), were included in the final analysis. In this cohort, 30 (66.7%) pwCF were female and 15 (33.3%) were male (Table 2). The mean time frame of included patients who completed the diary was (mean ± sd) −14 ± 7 days and 28 ± 23 days prior to and after commencing ETI therapy, respectively.

3.1 Dynamics of symptoms

There was a high between- and within-subject variability in the responses of patients, and the dynamics over the considered time periods followed a rather individual profile in all 28 symptoms assessed with the CFAbd-day2day© (Figure 1).

Figure 2 shows the proportions of patients reporting improvement with respect to the corresponding baseline value for each of the 28 items included in the CFAbd-day2day© over a maximum period of 4 weeks. As the question regarding "Pain intensity" depends on a positive answer for the occurrence of "Pain," these two questions were condensed into a single conditional question referred to as "Abdominal pain intensity" throughout this article.

In two items, namely, "Vomiting times" and "Stool color," no improvement with respect to baseline values was reported by the patients within this time frame. The highest proportion of patients

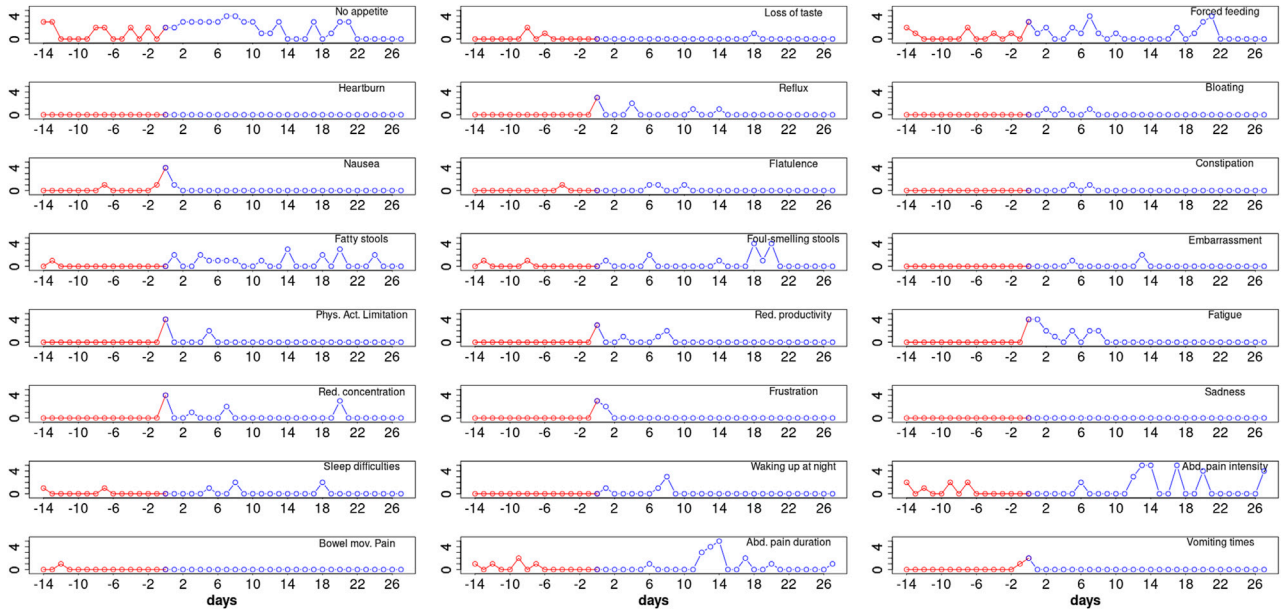

FIGURE 1
CFAbd-day2day© protocol from a single CF patient (8 years old, male, homozygous for F508del, pancreatic-insufficient, and pre-treated with Orkambi) revealing baseline symptoms prior to ETI (red) as well as evolution of the daily burden of GI-related symptoms during the first 27 days of therapy (blue) for 24 of the 28 items included in the CFAbd-day2day© (*y*-axis represent score responses on a 0–5 Likert scale, where higher scores quote a higher burden of symptoms).

(25%) reporting improvement was observed for the item "Foul-smelling stools" on day 23 after ETI therapy initiation. Other items for which the proportion of patients reporting improvement reaching almost the 20% level over this time frame were "No appetite" (19%), "Physical activity limitation" (19%), and "Fatty stools" (19%), with the former observed already on day 7 and the latter two from day 20 onward. On the other hand, symptoms for which the proportion of patients did not surpass the 10% level were "Loss of taste," "Forced feeding," "Heartburn," "Nausea," "Constipation," "Sadness," "Waking up at night," and "Pain during bowel movements."

The proportions of patients reporting worsening in symptoms within the first 4 weeks after ETI treatment initiation (Figure 2) were the highest for "Number of bowel movements," for which 27% of patients on day 18 reported having a higher burden with respect to the time frame prior to ETI therapy. The second highest proportion was observed for "Flatulence," reaching the maximum (21%) on day 7. Interestingly, after ETI therapy initiation, one patient reported an increase in the number of "Vomiting times," and two patients reported worsening in their "Stool color." Other items with relatively low worsening rates, as reported by the patients over this time frame, were "Difficulty falling asleep," "Waking up at night," "Physical activity limitation" (in the three items max: 9%), "Loss of taste," "Bloating" (in both max: 8%), "Reflux," "Embarrassment," "Sadness," "Pain during bowel movements" (in all max: 7%), and "Heartburn" (max: 3%).

Table 3 shows the averaged absolute daily changes in responses from patients reporting improvement or worsening in their GI symptoms (see Figures 2, 3) over the three considered periods of time: prior to ETI therapy and 1–14 and 15–27 days after ETI therapy initiation. According to this measure, variability levels for "Constipation" were significantly higher during the first 2 weeks after ETI therapy initiation. On the other hand, the variability in "No appetite," "Reflux," "Physical activity limitation," "Abdominal pain intensity," and "Abdominal pain duration" was significantly lower during the third and fourth weeks after ETI therapy initiation compared to the time frame prior to commencing ETI. However, compared to the 15–27-day time frame after ETI therapy initiation, the variability in "No appetite," "Nausea," "Reduced productivity," "Fatigue," "Reduced concentration," "Difficulty falling asleep," "Waking up at night," "Abdominal pain intensity," "Pain during bowel movements," and "Abdominal pain duration" was significantly higher during the first 2 weeks of ETI treatment.

Comparing maximal absolute deviations from the median over the three time frames revealed that the maximum deviations in all items occurred within the first 2-week period. This was significant for items regarding "No appetite," "Constipation," "Fatigue," "Waking up at night," and "Abdominal pain duration" (Table 4). On the other hand, maxima in the item "Foul-smelling stools" observed within the period of first 2 weeks were significantly higher only when compared to the pre-ETI therapy time frame. The maxima for the five items, namely, "Nausea," "Reduced productivity," "Difficulty falling asleep," "Abdominal pain intensity," and "Pain during bowel movements," within the first 2-week time frame were significantly higher compared to those observed within the third and fourth weeks after ETI initiation.

3.2 Response to ETI therapy initiation

Effects of ETI therapy assessed with averaged CFAbd-day2day© responses revealed a highly significant decrease in the median total score (p = 0.00001). Changes in four domains, namely, "Pain," "GERD," "Disorders of bowel movement," and "Quality of life impairment," were statistically significant (p = 0.0003, 0.01, 0.02, and 0.01, respectively), although medians for "GERD" and "Quality of life impairment" resulted equal for the two time frames (Table 5).

3.3 Correlation with CFAbd-Score©

Averaged CFAbd-day2day© responses of pwCF and retrospective CFAbd-Score© covering the 2 weeks prior to ETI initiation showed a strong correlation (Pearson r = 0.63; n = 32; $p < 0.001$) (Figure 4A). On the other hand, the correlation between both domains (Pearson r = 0.58; n = 24; $p < 0.01$) was slightly lower at 3–4 weeks (Figure 4B).

4 Discussion

With availability of highly effective CFTR modulators like ETI, a game changer in treatment of CF, it is essential to thoroughly identify the spectrum of effects of the novel medication (Bell et al., 2019; King et al., 2021). At the same time, it may be a historic opportunity to record the burden of symptoms in pwCF who, at baseline, are still naïve for game-changing medications like HEMTs. Therefore, in the present study, we prospectively assessed AS changes on a daily basis before and immediately after the initiation of a new, highly effective CFTR-modulating ETI therapy in 45 pwCF using a novel PROM, the novel CF-specific GI-symptom diary "CFAbd-day2day©". The development of the new PROM was based on the CFAbd-Score, the first CF-specific GI PROM designed and validated following FDA guidelines (Tabori et al., 2017a; U.S. Department of Health and Human Services Food and Drug Adminitration, 2019; Jaudszus et al., 2019). To the best of our knowledge, this is the first publication including a diary that closely records CF-specific AS, developed following FDA guidelines and COSMIN methodology for the development of a PROM (Mokkink et al., 2010; U.S. Department of Health and Human Services Food and Drug Adminitration, 2019).

Altogether, the results obtained using the CFAbd-day2day© cumulatively calculated for each fortnight, i.e., the fortnight before ETI initiation and the second fortnight after ETI initiation, were in accordance with our previously published results obtained using the CFAbd-Score in 107 pwCF from Germany and the UK: GI symptoms significantly decreased after 2–4 weeks, which was consistent with the previous findings using the 28-item CFAbd-Score prior to and 4 and 26 weeks after initiation of ETI, retrospectively capturing the burden of GI symptoms during the preceding 14 days (Mainz et al., 2023; Mainz et al., 2022). This is also evident in the resulting high correlation between the averaged CFAbd-day2day© and the retrospective CFAbd-Score© from a subgroup of pwCF who had concomitantly completed both questionnaires (Figure 4). Our new findings with the CFAbd-day2day© reveal that the initiation of ETI is followed by changes in

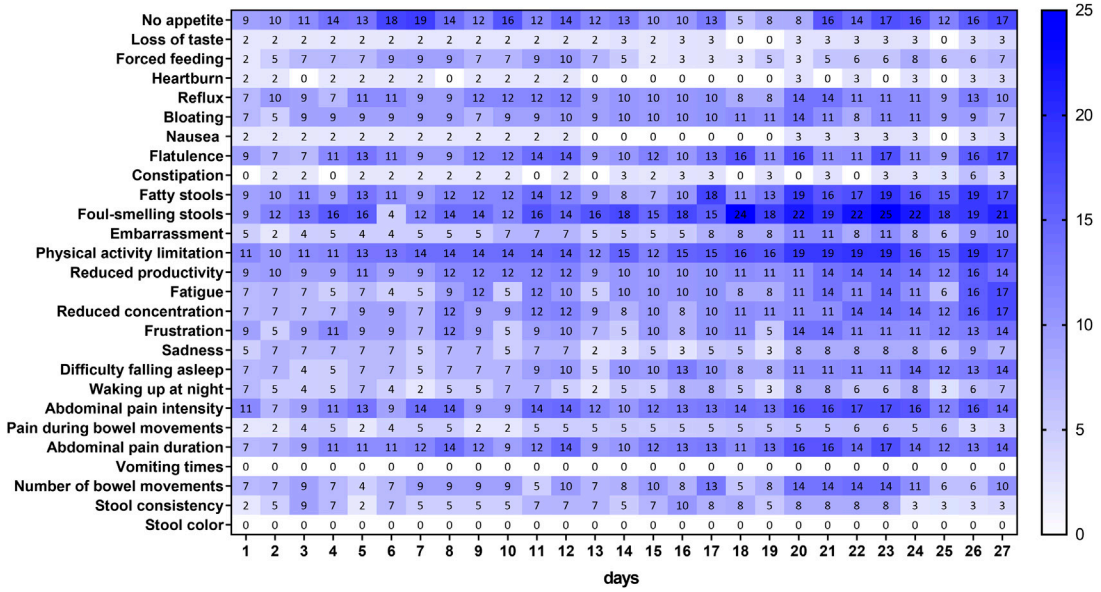

FIGURE 2
Proportion of pwCF reporting improvement for each item of the daily questionnaire with respect to their answers at baseline. Each percentage in the graph is relative to the total number of patients filling out the corresponding question on a specific day. Note that the questions regarding "Pain" and "Abdominal pain intensity" were condensed into a single conditional question.

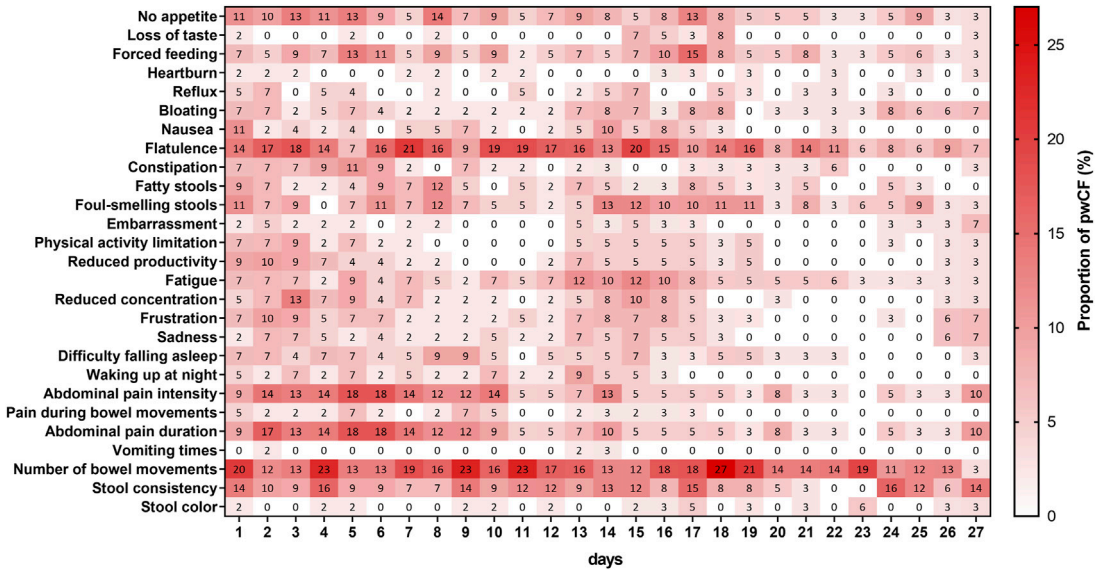

FIGURE 3
Proportion of pwCF reporting a worsening for each item of the daily questionnaire with respect to their answers at baseline. Each percentage in the graph is relative to the total number of patients filling out the corresponding question on a specific day. Note that the questions regarding "Pain" and "Abdominal pain intensity" were condensed into a single conditional question.

abdominal symptomatology according to quite individualized profiles, substantially differing from one pwCF to another receiving the new therapy. The extent of short-term changes in GI symptomatology is exemplified by the individual CFAbd-day2day© record from a single CF patient (Figure 1). In other patients included in this study, however, some of these complaints occurred less frequently during these time frames.

Despite the highly individual pattern of changes, trends in the overall cohort are visible in Figures 2, 3, revealing that some symptoms appear, increase or decline more frequently in a proportion of patients over time. For instance, abdominal pain, including its duration and intensity, increased in up to 18% of patients during the first 10 days of ETI initiation, together with an increase in flatulence in 21% of patients about day 7. Then, after

TABLE 3 Averaged absolute between-day variability in responses from patients reporting improvement or worsening in their GI symptoms after ETI initiation (see Figures 2, 3). The post-ETI therapy period was split into two 2-week time frames: 1–14 and 15–27 days after ETI therapy. Here, p1 indicates statistical significance for the comparisons between before and 1–14-day medians; p2 indicates statistical significance for the comparisons between before and 15–27-day medians; and p3 indicates statistical significance for the comparisons between 1–14-day and 15–27-day medians. No correction regarding multiple testing was conducted.

Averaged absolute between-day variability							
Item name	n	T_0: 14 days before ETI [median, IQR]	T_1: days 0–14 during ETI [median, IQR]	T_2: days 15–28 during ETI [median, IQR]	p_1 (T_0–T_1)	p_2 (T_0–T_2)	p_3 (T_1–T_2)
No appetite	17	0.2 [0–0.7]	0.4 [0.1–0.6]	0.1 [0–0.3]	0.82	**0.02**	**0.02**
Loss of taste	7	0 [0–0.2]	0 [0–0.1]	0.2 [0–0.2]	1	0.83	0.35
Forced feeding	12	0.3 [0–0.4]	0.4 [0.3–0.6]	0.3 [0–0.3]	0.4	0.33	0.07
Heartburn	7	0.3 [0.3–0.3]	0 [0–0.4]	0.3 [0.1–0.5]	1	1	0.58
Reflux	13	0.2 [0.2–0.5]	0.2 [0.1–0.3]	0 [0–0.3]	0.69	**0.02**	0.06
Bloating	14	0.2 [0–0.5]	0.2 [0–0.4]	0.1 [0–0.3]	0.31	0.72	0.84
Nausea	13	0 [0–0.2]	0.2 [0.1–0.3]	0 [0–0.1]	0.2	0.29	0.04
Flatulence	30	0.3 [0–0.7]	0.4 [0.1–0.6]	0.3 [0–0.5]	0.89	0.49	0.59
Constipation	13	0 [0–0.1]	0.2 [0.1–0.5]	0 [0–0.1]	**0.04**	0.59	0.23
Fatty stools	19	0.3 [0–0.6]	0.2 [0.1–0.7]	0.3 [0.2–0.6]	1	0.66	1
Foul-smelling stools	21	0.2 [0–0.6]	0.3 [0.1–0.6]	0.4 [0.1–0.5]	0.26	0.68	0.85
Embarrassment	10	0.4 [0.1–0.5]	0.1 [0–0.4]	0 [0–0.4]	0.53	0.14	0.37
Physical activity limitation	16	0.3 [0.1–0.6]	0.2 [0–0.3]	0 [0–0.2]	0.23	**0.03**	0.26
Reduced productivity	16	0 [0–0.2]	0.2 [0.1–0.3]	0 [0–0]	0.1	0.07	**0.01**
Fatigue	16	0.3 [0–0.5]	0.5 [0.2–0.8]	0.2 [0–0.4]	0.06	0.23	**0.01**
Reduced concentration	15	0.3 [0–0.4]	0.3 [0.2–0.5]	0.2 [0–0.3]	0.41	0.2	**0.03**
Frustration	12	0.2 [0–0.7]	0.2 [0.1–0.3]	0.1 [0–0.3]	0.73	0.08	0.11
Sadness	11	0.1 [0–0.4]	0.2 [0.1–0.4]	0.1 [0–0.2]	0.69	0.27	0.39
Difficulty falling asleep	14	0.1 [0–0.4]	0.2 [0.1–0.5]	0 [0–0.2]	0.36	0.14	**0.004**
Waking up at night	13	0 [0–0.3]	0.3 [0.3–0.5]	0 [0–0]	0.23	0.09	**0.01**
Abdominal pain intensity	23	0.5 [0.1–0.9]	0.4 [0.3–0.9]	0.1 [0–0.3]	0.59	**0.01**	**0.01**
Pain during bowel movements	7	0.1 [0–0.6]	0.2 [0.1–0.5]	0 [0–0]	0.69	0.1	**0.04**
Abdominal pain duration	23	0.4 [0.1–0.6]	0.4 [0.1–0.8]	0.1 [0–0.3]	0.81	**0.003**	**0.003**
Vomiting times	2	0 [0–0.1]	0.4 [0.4–0.5]	0 [0–0]	0.5	1	0.5
Number of bowel movements	34	0.5 [0.1–0.8]	0.5 [0.3–0.8]	0.3 [0.2–0.7]	0.85	0.32	0.09
Stool consistency	32	0.2 [0–0.7]	0.3 [0–0.8]	0.4 [0–0.7]	0.94	0.41	0.5
Stool color	9	0 [0–0.1]	0 [0–0.5]	0.2 [0–1]	0.28	0.14	0.37

p-values in bold represent statistically significant differences.

11–15 days of ETI initiation, the proportion of patients reporting pain symptoms markedly decreased to 0%–10%. Furthermore, flatulence appeared to improve during the last observational week (days 15–27) in many patients, together with a higher number of patients reporting a decrease in fatty and foul-smelling stool.

"Between-day variability" and "maximal absolute deviations from the median" in CFAbd-day2day© items reveal some statistically significant changes in the patterns of variability. Again, the crucial symptoms of abdominal pain, including its intensity and duration, as well as pain during bowel movements,

TABLE 4 Comparison of maximal absolute deviations from the median observed in each patient reporting the changes observed in Figures 2, 3 within the time frames prior to ETI therapy and after 14 and 28 days of ETI therapy initiation. The post-ETI therapy period was split into two 2-week time frames. Here, p_1 indicates statistical significance for the comparisons between before and 14-day medians; p_2 indicates statistical significance for the comparisons between before and 28-day medians; and p_3 indicates statistical significance for the comparisons between 14-day and 28-day medians. No correction regarding multiple testing was conducted.

Maximal absolute deviations							
Item name	N	T_0: 14 days before ETI [median, IQR]	T_1: days 0–14 during ETI [median, IQR]	T_2: days 15–28 during ETI [median, IQR]	p_1 (T_0 –T_1)	p_2 (T_0–T_2)	p_3 (T1–T_2)
No appetite	17	1 [1–2]	2 [1–3]	1 [0–1]	**0.03**	0.09	**0.001**
Loss of taste	7	0 [0–0.5]	0 [0–1]	1 [0.5–1.5]	1	0.3	0.3
Forced feeding	12	1 [0–2]	2 [1–3]	1 [0–2]	0.2	0.7	**0.05**
Heartburn	7	1 [0–2]	1 [0–1]	1 [1–1]	1	1	0.8
Reflux	13	1 [1–2]	1 [1–2]	0 [0–1]	0.8	0.07	0.1
Bloating	14	0.2 [0–1]	1 [1–2]	0.5 [0–2]	0.4	1	0.3
Nausea	13	1 [0–3]	2 [1–3]	0 [0–0]	0.3	0.2	**0.03**
Flatulence	30	1 [0–2]	1 [1–2]	1 [0–2]	0.6	0.7	0.4
Constipation	13	0 [0–1]	1 [1–2]	0 [0–1]	**0.02**	0.8	**0.02**
Fatty stools	19	1 [0–2]	1 [0.5–2]	1 [1–2]	0.3	0.1	0.7
Foul-smelling stools	21	1 [0–1]	2 [1–2]	1 [1–2]	**0.02**	0.2	0.6
Embarrassment	10	1 [0.1–2]	1 [0–2]	0.5 [0–2.5]	0.5	0.9	0.9
Physical activity limitation	16	1 [0.4–2]	1 [0–2]	0 [0–1]	0.6	0.2	0.08
Reduced productivity	16	1 [0–2]	2 [1–2]	0 [0–0.2]	0.2	0.5	**0.04**
Fatigue	16	1 [0.7–1.2]	2 [1–3]	1 [0–1]	**0.03**	0.5	**0.01**
Reduced concentration	15	1 [0-5–2.5]	1.5 [1–2]	1 [0–2.5]	0.9	0.5	0.3
Frustration	12	1 [0–2]	1 [1–2]	1 [0–1]	0.2	0.7	0.2
Sadness	11	1 [0–1.5]	1 [1–2.5]	0 [0–2.5]	0.2	0.8	0.2
Difficulty falling asleep	14	1 [0–1]	1.5 [1–2]	0.5 [0–1]	0.06	0.6	**0.02**
Waking up at night	13	0 [0–1]	2 [1–2]	0 [0–0]	**0.01**	0.3	**0.005**
Abdominal pain intensity	23	2 [0.5–2]	2 [1–3.5]	0 [0–1.5]	0.07	0.1	**0.007**
Pain during bowel movements	7	0.5 [0–2]	2 [1–2]	0 [0–0]	0.09	0.4	**0.03**
Abdominal pain duration	23	1 [0.5–2]	2 [1–3]	0 [0–2]	**0.02**	0.2	**0.004**
Vomiting times	2	1 [0.5–1.5]	3.5 [3–4]	0 [0–0]	1	1	0.5
Number of bowel movements	34	2 [1–2]	2 [1–2]	1 [0.2–2]	0.7	0.1	0.08
Stool consistency	32	1 [0–3]	1.5 [0–3]	1 [0–3]	0.3	0.6	0.3
Stool color	9	0 [0–0]	0 [0–3]	3 [0–3]	0.3	0.1	0.7

p-values in bold represent statistically significant differences.

reveal a significant reduction in the variability over the late observational period.

Notably, the present study included a relatively high proportion of younger pwCF, due to the approval of ETI in pwCF between 6 and 12 years of age carrying a F508del mutation during the study period (median age: 10 years, range: 6–55 years). According to our previous studies with the CFAbd-Score, children report significantly more often on abdominal pain, whereas adults complain significantly more often about gastroesophageal reflux (Tabori et al., 2017a; Jaudszus et al., 2019). Accordingly, including a higher percentage

TABLE 5 Changes in averaged CFAbd-day2day© responses after ETI therapy initiation, comparing the cumulative burden of symptoms during the 14-day period prior to (T_0) and the second 14-day period after initiation of the new therapy (T_2: days 15–28).

		T_0: 14 days before ETI [median, IQR]	T_2: days 15–28 during ETI [median, IQR]	*p*
Total cumulative CFAbd-day2day© scores		8.9 [2.8–15.7]	4.7 [1.3–10]	**0.00001**
Five domains of the CFAbd-Score	Pain	6.7 [0–20]	0.0 [0–6.7]	**0.0003**
	GERD	0.0 [0–26.7]	0.0 [0–6.7]	**0.01**
	Disorders of bowel movement	10.0 [2.5–22.5]	7.5 [0–15]	**0.02**
	Disorders of appetite	4.0 [0–28]	0.0 [0–20]	0.1
	Quality of life impairment	0.0 [0–5]	0.0 [0–0]	**0.01**

p-values in bold represent statistically significant changes between cumulative scores before and after ETI therapy.

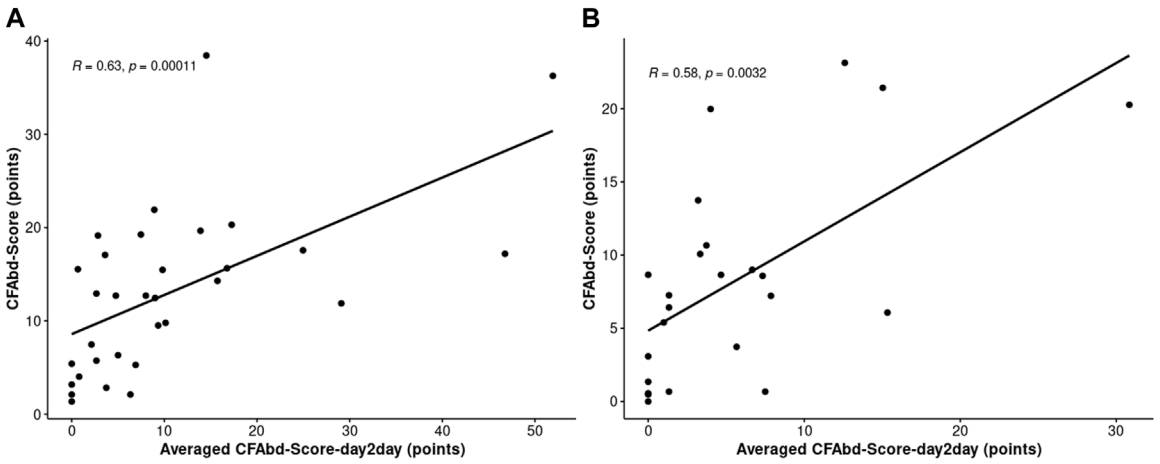

FIGURE 4
Coefficients between averaged CFAbd-day2day© responses and retrospective CFAbd-Score© covering 2 weeks prior to ETI **(A)** and 3–4 weeks after ETI initiation **(B)**. The number of patients who completed both CFAbd-day2day© and retrospective CFAbd-Score© covering 2 weeks prior to ETI was n = 32. The number of patients who completed both CFAbd-day2day© and retrospective CFAbd-Score© covering 3–4 weeks after ETI initiation was n = 24.

of adult pwCF or even patients with more advanced disease progression results in a different pattern of symptoms, specifically in regard to GERD and vomiting, which were rare symptoms in our cohort (Tabori et al., 2017a; Mainz et al., 2018; Jaudszus et al., 2019).

Calendars documenting specific patterns of symptoms are used as a golden standard in the care of patients suffering from complaints such as recurrent headaches or chronic abdominal pain, including Crohn's disease, ulcerative colitis, or irritable bowel disease. However, the pattern of AS in pwCF has been found to be rather specific due to patterns in CFTR deficiencies in the exocrine and endocrine pancreas, small and large intestines, and bile ducts (Ooi and Durie, 2016). Accordingly, PROMs developed for other non-CF-specific abdominal pathologies, like irritable bowel disease, chronic inflammatory bowel diseases, non-CF-related GER, pancreatitis, or constipation, may not be adequately sensitive to the CF-specific pattern of GI symptoms (Hayee et al., 2019). In our eyes, these limitations are reflected, for instance, in the lack of sensitivity to detect ETI effects in large multicenter trials using the PAGI-SYM, PAC-SYM, or PAC-QoL (Schwarzenberg et al., 2022). Although significant improvements in symptoms were identified therein, such changes were estimated to be too small to achieve clinical relevance, according to the authors. In contrast, CF-specific AS assessed using the CFAbd-Score declined in 107 pwCF from Germany and the UK from a mean of 14.9 to 10.6 pts during 24 weeks of ETI ($p < 0.05$), similar to the five domains of Pain, GERD, Disorders of bowel movement, Disorders of appetite, and GI-related QoL (Mainz et al., 2022). Likewise, a similar improvement was observed in 108 pwCF from Ireland and the UK, assessed using the CFAbd-Score during a new therapy with ETI (Mainz et al., 2023). Therefore, the level of changes captured with the CF-specific CFAbd-Score can be considered clinically relevant (Caley et al., 2023b).

To the best of our knowledge, the study presented here investigates for the first time AS recorded in detail after ETI initiation using a CF-specific validated diary approach. Before HEMT approval, previous studies assessing GI symptoms in pwCF focused on either abdominal pain with non-CF-specific PROMs or did not report information regarding the methodology or specific content and design of implemented diaries (Elliott et al., 1992; Obideen et al., 2006; Munck et al., 2012; Van Biervliet et al., 2018). For instance, Elliott et al. (1992) compared the effects of

different PERTs on GI complications using some type of a symptom diary. They reported pwCF to prefer microcapsules as PERT because these appeared to cause lower rates of abdominal symptoms and the dosage included fewer capsules. However, no differentiation of abdominal symptoms or further information about the employed diary was provided (Elliott et al., 1992). Two other studies analyzed abdominal pain in pwCF using a pain diary. One investigated recurrent abdominal pain in n = 8 pediatric pwCF, and the other observed the effect of nocturnal hydration on abdominal pain in n = 9 pwCF (Obideen et al., 2006; Munck et al., 2012). In the latter, the pain diary assessed the frequency, medications, and pain intensity on a visual analog scale, whereas the former used different PROMs for pain measurement (Eland Pain location, pain intensity measured by Faces Pain Scale—Revised (FPS-R), McGill Emotional Status, R-CMAS anxiety score, and health-related quality of life (CF-QOL)) at the first study visit (Obideen et al., 2006; Munck et al., 2012). In both studies, however, further information regarding the content of the diary or information about a possible validation was missing. Recently, Van Biervliet et al. (2018) used a diary to explore the effects of probiotics on GI symptoms and on intestinal flora in n = 31 pwCF. Outcomes included fecal calprotectin, pulmonary function, nutritional status, and AS assessed with a diary, which queried abdominal pain, stool frequency, and treatment changes. Again, further information about the diary was not provided.

Diaries are favourable when short term changes are expected. This applies to our setting assessing changes in AS during ETI initiation, which was motivated beforehand by numerous pwCF's reports on short-term changes in GI symptoms, often commencing hours after receiving the first dosage of HEMT. In the development process of the novel PROM, the 28 items included in the CFAbd-day2day© were identified as highly relevant by pwCF, proxies, and CF caregivers of different professions (community voice), who were repeatedly consulted. Consequently, the questionnaire was observed to have high acceptance rate.

The results presented in this publication fulfil essential steps in the validation process of the CFAbd-day2day©. They reveal that the PROM is sensitive to detect symptom changes, e.g. those caused by a newly initiated therapy. At present, further steps to validate the CFAbd-day2day© are in progress, including analyses of convergent validity with the CFAbd-Score and AS dynamics in pwCF suffering from CF-related conditions like constipation, DIOS, GERD, CF-related diabetes (CFRD), and liver disease.

In summary, implementation of the novel CFAbd-day2day© during a new therapy with the HEMT ETI provides new real-world insights relevant for the CF community and healthcare providers. Furthermore, the symptom diary can be implemented in routine care of pwCF suffering from GI symptoms in order to follow up their dynamics in daily life, as well as the effects of therapeutic interventions. Accordingly, the CF-specific CFAbd-day2day© may facilitate the identification of critical GI complications that may require the individual with CF to consult the attending CF center. Analogous to the CFAbd-Score, the PROM is being translated to other languages and will be implemented in international studies.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Prof. Dr. Kurt J.G. Schmailzl, MHB; registration number: E-01-20200519. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

Conceptualization: JM and FD. Project administration: JM, FD, and CZ. Recruitment and data acquisition: JM, PH, AB, PS, LP, LB, LN, OE, SV, PE, and UG-M. Analysis and interpretation of data: CZ, AB, PS, FD, and JM. Manuscript writing: CZ, JM, FD, PH, AB, CS, and SL. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author LN was employed by the company Universitätsklinikum Gießen-Marburg GmbH.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer MS declared a past co-authorship with the authors PE and CS to the handling editor.

Publisher's note

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